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IISc THESES ABSTRACTS

Thesis Abstract (Ph.D.)

Thermostability studies on some components of the protein synthesizing machinery of a thermophilic fungus *Humicola Lanuginosa* by Anil Kumar Joshi.
Research supervisor: Joseph D. Cherayil.
Department: Biochemistry.

1. Introduction

Thermophily or life at high temperatures has been a subject of interest and investigation for many years[1]. Over the years three main theories have been put forward to explain this phenomenon. These are the "kinetic"[2], the "lipid"[3] and the "macromolecular"[4] theories. The kinetic theory ascribes thermophily to a special metabolic state, characterized by high rates of macromolecular turnover. According to the lipid theory the thermophilic growth requires protective action of high melting lipids on macromolecules and subcellular structure. The macromolecular theory explains thermophily on the basis of physico-chemical differences in the stability, structure and function of important macromolecules in thermophiles and mesophiles. Although a great deal of information is available regarding thermophilic prokaryotes, very few biochemical studies have been conducted on eukaryotic thermophiles.

The present study was conducted in view of lack of information regarding the mechanisms of thermophily in the eukaryotic organisms. Some aspects of the protein synthesizing machinery have been investigated in the thermophilic fungus *Humicola lanuginosa*. It is one of the most commonly found thermophilic eukaryotes with a growth temperature range of 30° to 60°C (T_{opt} 45–50°C). Thus, it is one of the few eukaryotic thermophiles capable of growing at temperatures as high as 60°C.

2. Experimental programme

The experimental programme was to prepare a few components of the protein synthesizing machinery such as tRNA, ribosomes and aminoacyl tRNA synthetases from *H. lanuginosa* and compare them with that of mesophilic yeast, *Candida utilis*. Total tRNA from both the organisms was prepared by the usual phenol extraction procedure[5] and chromatography on DEAE-cellulose. Ribosomes were prepared by differential centrifugation of the cell extract. Change in absorbance was recorded in a Beckman DU 8 spectrophotometer. Thermal melting and the rate of digestion by RNase A of tRNA and ribosome samples were compared by noting the change in absorbance at 260 nm. Valine tRNA synthetase was prepared in bulk by ammonium sulphate fractionation and chromatography on Sephadex CM-50 and Sepharose-4B columns[6]. Final purification of the synthetase was effected by affinity chromatography on L-valine-AH-Sepharose-4B column. Purity of samples was checked by electrophoresis on polyacrylamide gels.

^{35}S-Labelled tRNA was prepared by growing the organism in a medium containing radioactive sulphate and the labelled tRNA was used for the study of thionucleotides. Conversion of tRNA to mononucleotides was effected by digestion with RNase T_2 and these were separated by high voltage paper electrophoresis or two-dimensional thin-layer chromatography. Radioactive spots were detected by autoradiography. Radioactivity measurements were made in a liquid scintillation counter.

3. Results and conclusions

Total transfer RNA isolated from *H. lanuginosa* was compared for its thermostability with that from the mesophilic yeast *Candida utilis*. The tRNAs were found to have similar thermal transitions with Tms around 40°C in the absence of any added Mg^{++}. However, spermine and Mg^{++} drastically enhanced the thermostability of both the tRNAs. As polyamines and magnesium ions are ubiquitously present in all the living cells, it was concluded from these results that *H. lanuginosa* tRNA does not possess the requisite intrinsic thermostability for growth at high temperatures but can be stabilized *in vivo* by Mg^{++}, polyamines and other intracellular factors. Total tRNA from *H. lanuginosa* was found to be comparatively more resistant to pancreatic ribonuclease A (RNase A) digestion than that from *C. utilis*. This probably reflects a relatively higher ribose methylation in the tRNA from the thermophile.

Comparative studies on the thermostability of the ribosomes from the two organisms showed the ribosomes from *H. lanuginosa* to be slightly more resistant to heat denaturation. Ribosomes from the thermophile were stable up to 50°C, the T_{opt} with a T_m of about 60°C, the T_{max} of the organism. Thus, ribosome stability *in vivo* might be important in determining the upper growth temperature of the organism. As in the case of tRNA, ribosomes from *H. lanuginosa* were also found to be more resistant to RNase A digestion.

Presence of the thioribonucleotide, 5-methyl-2-thiouridine (m^5s^2U) is known to increase the thermostability of tRNA in the thermophilic bacteria *Thermus thermophilus*[7]. Thionucleotides from *H. lanuginosa* tRNA were investigated, in view of the above information. The results of these studies showed that the fungal tRNA does not contain any significant amounts of m^5s^2U. However, two unusual thionucleotides were observed in this tRNA and attempts were made to characterise these. These have been tentatively identified as uridine derivatives.

Aminoacyl tRNA synthetases play a very crucial role in protein biosynthesis. A number of synthetases have been studied in thermophilic bacteria and like most of the proteins from thermophiles, these enzymes have been found to have higher thermostability as well as higher temperature optima than the corresponding enzymes from mesophiles. Preliminary observations with partially purified preparations of total aminoacyl tRNA synthetases from the fungus, however, indicated most of these enzymes to be optimally active around 37°C. During further fractionation of synthetases, from the fungus, valyl tRNA synthetase activity was found to split into two peaks. Both of these proteins have been purified extensively, one of them to homogeneity. The two enzymes were found to be very similar in their molecular weights, subunit structure and various kinetic properties. Maximal activity with both the enzymes was obtained between 40 and 45°C and both were found to rapidly lose activity beyond 45°C. Further detailed studies with the homogeneous enzyme showed that the purified protein was quite thermolabile. However, substrates and polyamines were found to protect the enzyme against heat denaturation. From these results it was concluded

that *H. lanuginosa* valyl tRNA synthetase does not possess the requisite intrinsic thermostability to function optimally at the growth temperatures of the organism. Thus *in vivo* the enzyme, most probably, requires substrates and other intracellular environmental factors for proper activity and thermostability. Further, total protein turnover rate in the fungus was found to increase with increase in the growth temperature. Thus, it is possible that some of the crucial proteins that are not sufficiently thermostable, are replaced through increased protein turnover.

Taken together, these results indicate that unlike in most thermophilic bacteria, all the components of the translation machinery in *H. lanuginosa* do not show high intrinsic thermostability. Intracellular microenvironment of the organism and probably increased macromolecular turnover may play a crucial role in the proper functioning of the various components at the growth temperatures of the organism.

References

1. FISCHER, F., ZILLIG, W., STETTER, K.O. AND SCHREIBER, G. — Chemolithoautotrophic metabolism of anaerobic extremely thermophilic archaebacteria, *Nature (Lond.)*, 1983, **301**, 511–513.
2. STENESH, J. AND MADISON, J.B. — Stability of bacterial mRNA in mesophiles and thermophiles, *Biochim. Biophys. Acta*, 1979, **565**, 154–160.
3. MCELHANEY, R.N. SOUZA, K.A. — The relationship between environmental temperature, cell growth and the fluidity and physical state of the membrane lipids in *Bacillus stearothermophilus, Biochim. Biophys. Acta*, 1976, **443**, 348–359.
4. LJUNGDAHL, L., BREWER, J.M., NEECE, S.H. AND FAIRWELL, T. — Purification, stability, and composition of formyltetrahydrofolate synthetase from *Clostidium thermoaceticum*, *J. Biol. Chem.*, 1970, **245**, 4791–4797.
5. VON EHRENSTEIN, G. — Isolation of sRNA from intact *E. coli* cells, *Methods Enzymol.*, 1967, **12A**, 588–596.
6. VON DER HAAR, F. — Purification of aminoacyl-tRNA synthetases, *Methods Enzymol.*, 1979, **59**, 257–267.
7. WATANABE, K., OSHIMA, T., HANSSKE, F. AND OHTA, T. — Separation and comparison of 2-thioribothymidine-containing tRNA and ribothymidine-containing counterpart from cells of *Thermus thermophiles* HB8, *Biochemistry*, 1983, **22**, 98–102.

Thesis Abstract (Ph.D.)

Studies on peptides of conformational and biological interest: Chemotactic peptide analogs, acyclic cystine peptides and fragments of bacteriorhodopsin and emerimicin by P. Antony Raj.
Research supervisor: P. Balaram.
Department: Molecular Biophysics Unit.

1. Introduction

Studies on the physical and conformational properties of oligopeptides have been stimulated by their widespread occurrence, impressive range of biological functions and wide array of structural motifs. In this study, peptides which assume specific conformational features such as helices, sheets and turns have been synthesised on the one hand and on the other, attention has been focussed on a few biologically active peptides to understand the relation between spectroscopically determined structures and biological activity. The peptides chosen for investigations are the chemotactic tripeptide analogs, cystine peptides, fragments of the bacteriorhodopsin G-helix and the fragments of the antibiotics,emerimicin III and IV.

2. Experimental procedures

All peptides chosen for the investigation were synthesised by solution-phase procedures using dicyclohexylcarbodimide (DCC) or 1-hydroxybenzotriazole (HOBt)/DCC-mediated couplings. Detailed characterisation of peptides at every stage of synthesis was performed by 60 MHz, 80MHz and 270 MHz ^{1}H NMR. All final peptides were purified by silica gel column chromatography using $CHCl_3$ and 2% Me OH in $CHCl_3$ as eluents. Peptides were found to be homogeneous by HPLC.

The conformational studies of the peptides were carried out by using ^{1}H and ^{13}C NMR, Nuclear Overhauser Effects (NOE), IR and CD methods. The biological activity of the chemotactic peptides was determined in terms of their ability to release lysozyme from rabbit neutrophils. The activity of the fragments of emerimicin was examined by comparing their ability to function as uncouplers of oxidative phosphorylation in rat liver mitochondria.

3. Main results and conclusions

The synthesis of chemotactic peptide analogs and their ability to release lysozyme from rabbit neutrophils have shown that the analogs containing α-aminoisobutyric acid (Aib) and 1-aminocyclopentanecarboxylic acid (Acc^5) at position 2 of the parent chemotactic peptide, For-Met-Leu-Phe-OH, exhibit high biological activity[1]. The spectroscopic analysis of these conformationally constrained analogs, For-Met-Aib-Phe-Ome and For-Met-Acc^5-Phe-OMe and studies on the crystal structure and solution conformation of the related peptide, Boc-Met-Aib-Phe-OMe[2] have established that these analogs favour Type II β-turn conformations, in solution.

These observations suggest that folded conformations may be important for biological activity. This conclusion is at variance with the earlier suggestion that extended conformations are necessary for interaction with the neutrophil chemotactic peptide

receptor[3,4]. It has been reported that N-formylated chemotactic peptides bind specifically to an immunoglobulin light chain (Mcg, Bence-Jones) dimer in a folded conformation[5]. The similarity in the ligand binding properties of the neutrophil chemotactic peptide receptor and the Mcg dimer suggests that folded peptide structures may indeed be functionally important. A model for the chemotactic peptide receptor topology has been proposed (fig. 1). The spectroscopic studies of two chemotactic peptide analogs, For-Met-Leu-Phe-OMe and For-Met-Aib-Phe-OMe, which have sequences with markedly different tendencies to adopt folded conformation, suggest that peptide aggregation is a more facile process in an extended conformation, as compared to a folded peptide conformation[6].

The synthesis and spectroscopic studies of several acyclic cystine peptides suggest that the cystine residue acts as a strong constraint on the peptide backbone, even in acyclic systems and stabilises intramolecular antiparallel β-sheet structures (fig. 2).

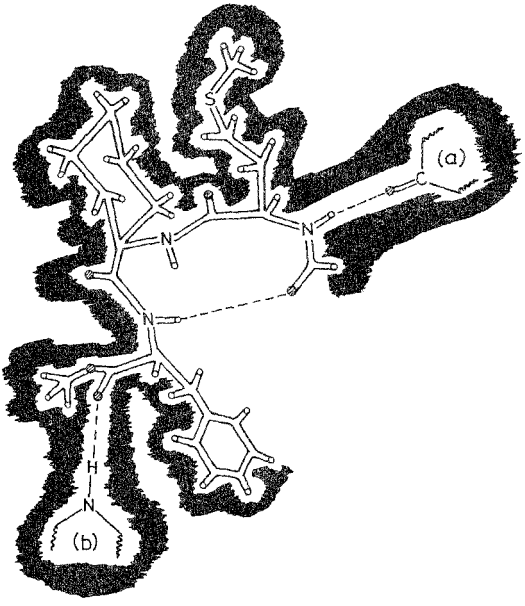
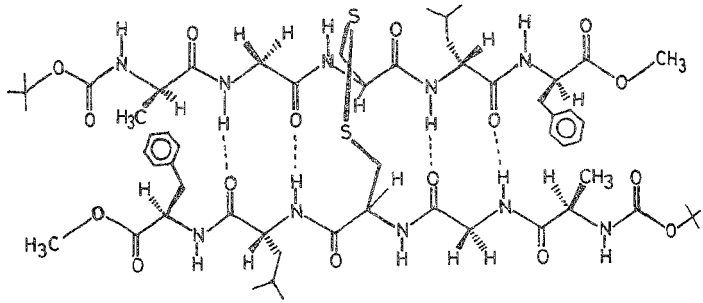
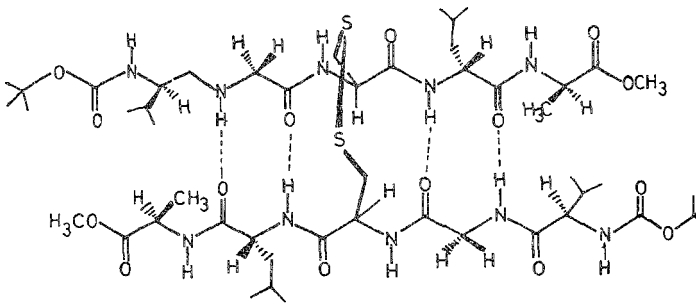


FIG. 1. Hypothetical interaction of folded For-Met-Acc^6-Phe-OMe with the chemotactic peptide receptor in rabbit neutrophils. *(a)*, *(b)* are the two proposed sites of hydrogen bonds from the receptor.



S,S'- bis(Boc -Ala - Gly - Cys - Leu - Phe - OMe)



S,S'- bis(Boc - Val - Gly - Cys - Leu - Phe - OMe)

FIG. 2 Proposed antiparallel β-sheet conformations of acyclic cystine peptides.

The synthesis and spectroscopic studies of the G-helix of the membrane protein, bacteriorhodopsin, Boc-Gly-Ile-Val-Pro-Leu-OMe (197-201) Boc-Gly-Ala-Gly-Ile-Val-Pro-Leu-OMe (195-201) and Boc-Gly-Ala-Gly-Ile-Val-Pro-Leu-Asn-Ile-OMe (195-203) suggest the structural flexibility of these peptides in polar and non-polar solvents and corroborate previous reports on similar molecules[7-9].

An attempt at the synthesis of a 15-residue peptide which is an analog of emerimicin IV
1 5 10 15
(Boc-Phe-$(Aib)_3$-Val-Gly-Leu-$(Aib)_2$-Hyp-Gln-Aib-Hyp-Aib-Phol) wherein Iva at position 12 has been replaced by Aib has been described. The synthesis of 1-10 and 11-15 fragments has also been described.

The conformational studies of emerimicin III and IV fragments illustrate an important correlation of sequence and conformation. The β-turn structures in shorter fragments, promoted by Aib residues, serve to nucleate 3_{10} helical structures in longer peptides. The ability of the fragments to uncouple oxidative phosphorylation in mitochondria correlates with tendencies to adopt helices. The solvent dependent conformational change observed in these fragments by spectroscopic studies has been interpreted to be a 3_{10}– to α–helical transition.

The results emphasise the utility of stereochemically constrained analogs of biologically active peptides, in delineating structure activity correlations. The studies reported in this thesis provide the first evidence that short emerimicin fragments can exhibit membrane modifying activity.

References

1. SUKUMAR, M., ANTONY RAJ, P., BALARAM, P. AND BECKER, E. L. — *Biochem. Biophys. Res. Commun.*, 1985, **128**, 339–344.
2. BARDI, R., PIAZZESI, A.M., TONIOLO, C., ANTONY RAJ, P., RAGOTHAMA, S. AND BALARAM, P. — *Int. J. Peptide Protein Res.*, 1985 (in press).
3. FREER, R.J., DAY, A.R., MUTHUKUMARASWAMY, N., PINON, D., WU, A., SHOWELL, H.J. AND BECKER, E.L. — *Biochemistry*, 1982, **21**, 257–263.
4. TONIOLO, C., BONORA, G.M. SHOWELL, H.J., FREER, R.J. AND BECKER, E.L. — *Biochemistry*, 1984, **23**, 698–704.
5. EDMUNDSON, A.B. AND ELY, K.R. — *Mol. Immunology*, 1985, **22**, 463.
6. ANTONY RAJ, P. AND BALARAM, P. — *Biopolymers*, 1985, **24**, 1131–1146.
7. KATAKAI, R. AND IIZUKA, Y. — *J. Am. Chem. Soc.*, 1984, **106**, 5715–5718.
8. SHINNAR, A.E. AND KAISER, E.T. — *J. Am. Chem. Soc.*, 1984,**106**, 5006–5007.

9. BRIGGS, M.S. AND GIERASCH, L.M. *Biochemistry*, 1984, 23, 3111–3114.

Thesis Abstract (Ph.D.)

Characterisation of rinderpest virus and mechanism of its persistence in vero cells
by K.V. Kesari.
Research supervisor: M.S. Shaila.
Department: Molecular and Cell Biology Laboratory.

1. Introduction

Despite the fact that enormous amount of work is being carried out on measles and canine distemper viruses, very little information is available on structure-function relationship and persistence of rinderpest virus *in vivo* and *in vitro*. Preliminary characterisation of rinderpest virus in relation to the virus-specific macromolecular synthesis and the effect of actinomycin D on its replication has been done in our laboratory.

The present study is undertaken to understand the mechanisms of rinderpest virus persistence *in vitro*. A knowledge of the structure-function relationship of the virus is essential for in-depth study of the course of its multiplication in acute and persistence infection.

2. Experimental programme

The work is divided into two parts. The first part deals with the structure-function relationship of the virus. This involves, morphological and biochemical characterization of the virus. This was experimentally approached by infection of the Vero cell in culture with the virus, purification of the virus and purification of the structural proteins following disruption of the virus. With the help of monospecific antibodies to these structural proteins, the location and functional aspects of the proteins have been delineated.

The second part describes establishment of rinderpest virus persistent infection in Vero cells and characterization of the persistently infected cell line. Various aspects of persistent infections presented include, biological characterization of cell line, structural alterations in plasma membrane, expression of the viral antigens and mode of their synthesis *in vivo* and *in vitro*. Ultrastructural studies have been carried out to find out the differences, if any, in the maturation process of the virus during acute and persistent infection. The conformation of the purified nucleocapsids in both the systems has been studied by way of electron microscopy. Using RNA-RNA hybridization technique, the proportion of plus and minus strand RNAs associated with the nucleocapsids in acute and persistent infection has been determined.

3. Results and conclusion

Kabete 'O' strain of rinderpest virus (RV) has been purified. The viral polypeptides have been identified by immunoprecipitating (^{35}S) methionine-labelled virus with the antiserum raised in rabbit against purified virus and with the sera from convalescent animals. The five structural proteins, as identified by this method are, a haemogglutinin-related protein (H),

74000 Mr; nucleocapis protein (N), 62,000 Mr; a phosphoprotein (P), 70,000 Mr; fusion protein (fi), 46,000 and a matrix protein (M) of molecular weight 38,000. The proteins N and P are phosphosylated, whereas, the protein H is present in glycosylated form. The proteins H, N, F, and M have been purified using various protein purification techniques. Monospecific antibodies have been raised against purified H, N, and F, proteins.

Rinderpest virus persistent infection has been established in Vero cells using 'cell survivor' technique and a clone designated as Pi-2 has been isolated. The average titer of the virus shed by Pi-2 cells was extremely low and barely detectable after 200 days of persistence. Pi-2 cells are totally resistant to the superinfection by RV but partially to measles and canine distemper viruses. The viral antigens could be detected in the plasma membrane and cytoplasm using monospecific antibodies. Antibodies against the N protein give granular fluorescence of cytoplasm, whereas the proteins H and F1 are poorly expressed in the plasma membrane of Pi-2 cells.

Undiluted passage of standard RV-produced defective interfering particles. A similar activity, although at a lower level, is found in the medium in which Pi-2 cells are grown.

Acute infection of Vero cells by RV is characterized by fusion of cells from within. However, Pi-2 cells did not show fusion at any stage during maintenance, but they fused spontaneously with Vero cells. In the initial stages of persistent infection, the fusion of Pi-2 cells with Vero cells was followed by release of the virus in significant quantity. In the later stages, although the capacity of Pi-2 cells to fuse with the normal cells is retained, the virus is not released. In order to understand the role of plasma membrane structure in favouring or preventing the fusion, a fluorescent probe, diphenyl hexatriene (DPH) was used. Polarization of DPH in the plasma membrane of Vero, acutely infected (AI) and Pi-2 cells was monitored at different temperatures. The flow activation-energy (ΔE) for all three cell types has been determined. The ΔE value for Vero cells is higher than that for AI cells but is significantly lower than that for Pi-2 cells, indicating decreased fluidity of Pi-2 plasma membrane.

Long term labelling of acutely infected and Pi-2 cells did not show any difference in the profiles of immunoprecipitated proteins. However, pulse-chase experiments revealed relative instability of the M protein in Pi-2 cells. The virious shed by Pi-2 cells are unaltered in protein composition. The abundance of the viral Poly (A^+) RNA is five-fold lower in Pi-2 cells when compared to that in AI cells, as judged by *in vitro* translation experiments.

The viral nucleocapsids (NC) have been purified from AI & Pi-2 cells. The average length of the nucleocapsids is 1156 nm. Although, most of the structural parameters of the NCs like external diameter, subunit angle, protein subunit molecular weight are unaltered during persistence, a significant difference is seen in the pitch of the helix. While the NCs isolated from AI cells have an average pitch value of 6.5 nm, those from Pi-2 cells are compressed (average pitch 5.5 nm). The AI cells have large inclusion bodies in the cytoplasm and distinct arrays of NCs underneatn the plasma membrane, whereas, the NCs are seen scattered in the cytoplasm of Pi-2 cells.

Experiments involving self-annealing and hybridization of purified nucleocapid RNA from AI and Pi-2 cells with Poly $(A)^+$ RNA from virus-infected cells reveal predominant presence of negative strand RNAs in AI cells, whereas the proportion seems to have changed in favour of positive strand RNA in Pi-2 cells. These experiments are indicative of an aberrent replicative process in RV persistence.

References

1. PRAKASH,K., ANTONY,A. AND RAMAKRISHNAN, T. Host-cell macromolecular synthesis and viral RNA synthesis in rinderpest virus-infected cells, *Curr. Sci.*, 1978, **47**, 42–44.
2. PRAKASH,K., ANTONY, A. AND RAMAKRISHNAN, T. Characterisation of rinderpest virus RNA and the action of actinomycin D on its replication, *J. Biosci.*, 1979, **1**, 307–316.
3. PRAKASH,K., ANTONY, A. AND RAMAKRISHNAN, T. Polypeptides of rinderpest virus and virus-specific polypeptide synthesis in infected cells, *Indian J. Expl., Biol.*, 1979, **17**, 1287–1289.

Thesis Abstract (Ph.D.)

Synthetic studies in terpenoids by D. Murali.
Research supervisor: G. S. Krishna Rao.
Department: Organic Chemistry.

The syntheses of several naturally occurring terpenoids are reported herein.

The first total synthesis of **8**, a norsesquiterpene occurring in the plant *Isocoma wrightii* has been achieved[1]. Succinoylation of 4-*t*-butyl-*o*-xylene, under Friedel-Crafts conditions, gave a mixture of keto acids **1** and **3**, which were separated as their methyl esters **2** and **4** respectively. Ester **5**, obtained from **2** by de-*t*-butylation, gave on modified Clemmensen reduction the arylbutanoate **6**. Grignard reaction of **6** with MeMgI, followed by cyclodehydration of the resulting tertiary alcohol, produced the natural product **8**.

The norsesquiterpene **8** was converted to chrysolic acid[2] (**13**) a diterpenoid isolated[3] from the desert plant *Chrysothamnus paniculatus*. Tetralin **8** on chloromethylation gave **9**. Condensation of **9** with ethyl sodioacetoacetate gave the keto ester **10**, which on decarbethoxylation afforded **11**. Modified Reformatsky reaction of **11** with *t*-butyl bromoacetate, followed by thermal treatment of the product (**12**), furnished chrysolic acid (**13**).

The synthesis of two isomeric sesquiterpenic metabolites (**21** and **25**) isolated[4] from tobacco leaves inoculated with tabacco mosaic virus (TMV) was achieved through two different routes. Stobbe condensation of 2,3-dimethylbenzaldehyde (**15**) (prepared from *o*-xylene by a novel route *via* the *t*-butyl compound **14**) with diethyl succinate gave the half-ester **16**. Simultaneous reduction and hydrolysis of **16** gave the diacid **17**. Its cyclodehydration to the tetralone acid **18**, followed by its Clemmensen reduction gave **19**. Grignard reaction of its methyl ester **20**, with MeMgI afforded metabolite **21**.

In the synthesis of the second metabolite **25**, tetralone **22** the cyclodehydration product of **7** derived from **6** already referred to served as the key synthon. Carbethoxylation of **22** produced the keto ester **23**. Modified Clemmensen reduction of **23**, followed by the action of MeMgI on the resulting ester **24**, furnished **25**.

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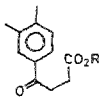
2, R = Me



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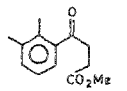

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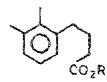
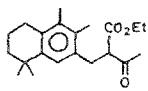


6, R = Me
7, R = H

8, R = H
9, R = CH_2Cl



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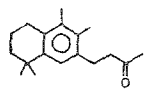

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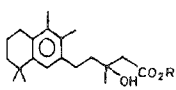
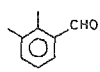


12, R = t - Bu
13, R = H

14



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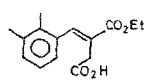

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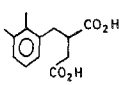

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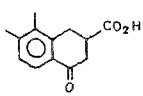

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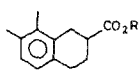
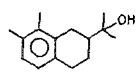


19, R = H
20, R = Me



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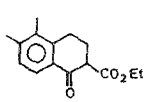

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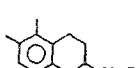

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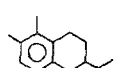

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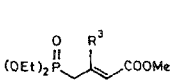

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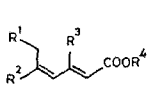

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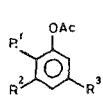

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Chart 1

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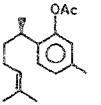

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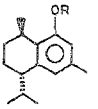
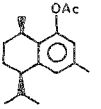


36, R = Ac
37, R = H
38, R = Me



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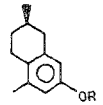
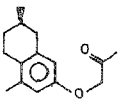


41, R = Ac
42, R = H



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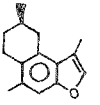

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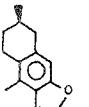

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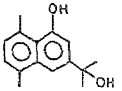

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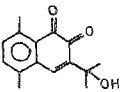

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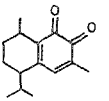

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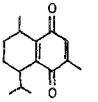

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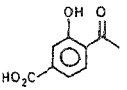
50, R = H
51, R = Me



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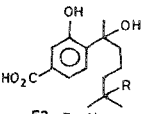
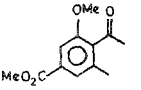


53, R = H
54, R = OH



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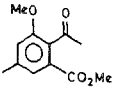

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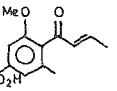

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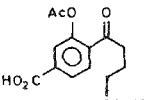

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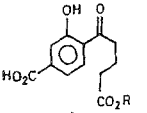


59, R = Me
60, R = H

Chart 2

A simple benzoannulation procedure involving cyclization of 2,4-dienoic acids **28**, (R^4 = H) has been developed and successfully employed in the synthesis of terpenoids (–)-(R)-cur-cuphenol acetate (**33**), (–)-(1R, 4S)-8-hydroxy- and 8-methoxycalamenenes (**37** and **38**) and (+)-(R)-dihydropyrocurzerenone (**44**). The 2,4-dienoic acids **28** (R^4 = H) underwent cyclization, in the presence of sodium acetate in acetic anhydride under reflux, to give the phenolic acetates **29**. The 2,4-dienoic acid **28** (R^4 = H) were prepared by the basic hydrolysis of the corresponding esters **28** (R^4 = Me), which in turn were obtained by the Wittig-Horner reaction of the carbonyl compounds **26** (R^1, R^2 = H or alkyl), with the phosphonate **27** (R^3 = H or Me). The generality of this benzoannulation procedure was established by a variety of successful transformations, *viz.*, acetaldehyde to phenyl acetate; propion-aldehyde to *o*-cresyl acetate; acetone to both *m*-cresylacetate (**29**, $R^1 = R^3$ = H; R^2 = Me) and 3,5-dimethylphenyl acetate (**29**, R^1 = H; $R^2 = R^3$ = Me); and cyclohexanone to **30**. Further examples are the conversion of isovaleraldehyde to the monoterpene thymol acetate (**31**), (+)-(R)-pulegone (**34**), to the acetates **36** and **39**. Acetate **36** on hydrolysis gave (–)-(1R, 4S)-8-hydroxycalamenene (**37**) and methylation of **37** gave (–)-(1R, 4S)-8-methoxycalamenene (**38**). Both **37** and **38** are enantiomers of naturally occurring 8-hydroxy[5] and 8-methoxycalamenenes[6] (constituents of the sea weed *Dysoxylum acutangulum* and the horny coral *Subergorgia hicksoni*, respectively). Hydrolysis of **39** gave (+)-(1R, 4R)-8-hydroxycalamenene, a natural product[7] from the liverwort *Bazzania trilobata*. (+)-(R)-Pulegone (**34**) on Wittig-Horner condensation with triethyl phosphonoacetate, followed by basic hydrolysis of the product, gave a mixture of 2,4- and 3,5-dienoic acids **40**. Treatment of the mixture **40** with sodium acetate-acetic anhydride under reflux, led to cyclization to give the bicyclic acetate **41** in high yield. The tetrahydronaphthol **42**, obtained from **41**, was converted to the acetonate **43**, which on cyclodehydration gave (+)-(R)-dihydropyrocurzerenone (**44**, the enantiomer of the natural product isolated[8] from the roots of *Chloranthus serratus*), along with a small amount of the linear furan **45**.

The iodoxybenzene oxidation of 1-naphthols has been studied in detail. Oxidation of substituted, 3-methyl-, 7-methyl-, 6-methoxy-, 7-methoxy- and 8-methoxy-1-naphthols, using iodoxybenzene in aqueous acetonitrile gave the corresponding 1,2- and 1,4-naphthoquinones. Similar oxidation of naphthol **46**, prepared according to a published procedure, gave emmotin H (**47**, one of the emmotins reported[9] from the trunk-wood of *Emmotum nitens*) as the sole product. Iodoxybenzene oxidation of (–)-(1R, 4S)-8-hydroxycalamenene (**37**) gave mansonone A[10] (**48**, an allergen from the heart-wood of *Mansonia altissima*) and the corresponding *p*-quinone **49**.

The study on selective benzylic oxidation of 4-acyltoluenes, using benzyltriethylammonium permanganate (BTAP) led to the synthesis of sydonic acid[11] **53** (a sesquiterpenic metabolite from the culture filtrates of *Aspergillus sydowi*) and an ester **55**, an intermediate in the synthesis of pyrenochaetic acid A[12] **57** (a phytotoxin from the culture filtrates of *Pyrenochaeta terrestris*, the causal fungus of onion pink root disease). Oxidation of the acetoxy ketone **50** using BTAP in aqueous acetic acid, gave the phenolic keto acid **52**. Treatment of **52** with excess of isohexylmagnesium bromide gave sydonic acid **53**. Oxidation of **51** by BTAP, followed by methylation of the resulting mixture of acids gave the methoxy methyl keto esters **55**, **56** and methyl 4,6-dimethyl-2-methoxybenzoate. Oxidation of **58** by BTAP gave an inseparable mixture of acids **59** and **60**, the former being the one required for further elaboration of hydroxysydonic acid **54**.

References

1. Murali, D. and Krishna Rao, G.S. — *Indian J. Chem.*, 1982, **21B**, 1033.
2. Murali, D. and Krishna Rao, G.S. — *National symposium on the chemistry of natural products and felicitations to Dr. Sukh Dev*, held at the National Chemical Laboratory, Poona on Sept. 2–3, 1983.
3. Timmermann, B.N., Hoffmann, J.J., Jolad, S.D., Schram, K.H., Klenck, R.E. and Bates, R.B. — *J. Org. Chem.*, 1982, **47**, 4114.
4. Uegaki, R., Fujimori, T., Kubo, S. and Kato, K. — *Phytochemistry*, 1983, **22**, 1193.
5. Nishizawa, M., Inoue, A., Sastrapradja, S. and Hayashi, Y. — *Phytochemistry*, 1983, **22**, 2083.
6. Kashman, Y. — *Tatrahedron*, 1979, **35**, 263.
7. Konecny, K., Streibl, M., Vasickova, S., Budesinsky, M., Saman, D., Ubik, K. and Herout, V. — *Colln Czech. Chem. Comm.*, 1985, **50**, 80.
8. Takemoto, T., Uchida, M. and Kusano, G. — *Chem. Pharm. Bull.*, 1976, **24**, 531.
9. De Oliveira, A.B., De Oliveira G.G., Liberalli, C.T.M., Gottlieb, O.R. and Magalhaes, M.T. — *Phytochemistry*, 1976, **15**, 1267.
10. Dumas, M.T., Strunz, G.M., Hubbes, M. and Jeng, R.S. — *Experientia*, 1983, **39**, 1089.
11. Hamasaki, T., Nagayama, K. and Hatsuda, Y. — *Agric. Biol. Chem.*, 1978, **42**, 37.

12. SATO, H., KONOMA, K. AND SAKAMURA, S. *Agric. Biol. Chem.*, 1981, 45, 1675.

Thesis Abstract (Ph.D.)

Novel methods of synthesis of complex metal oxides (A contribution to preparative solid state chemistry) by K. Vidyasagar
Research supervisors: C. N. R. Rao and J. Gopalakrishnan.
Department: Solid State and Structural Chemistry Unit.

1. Introduction

Complex metal oxides are traditionally prepared by the ceramic method which involves reacting oxides or other decomposable salts of the component metals by repeatedly heating them at high temperatures. Ceramic reactions are often incomplete yielding inhomogeneous products. More importantly, by its very nature, the ceramic method does not lend itself to the synthesis of metastable and low-temperature phases. There has been much interest in the last few years to develop new methods for the synthesis of solids especially at low temperatures. One such method is based on the use of solid solutions of isostructural inorganic solids as precursors in the synthesis of complex metal oxides. The solid solution precursor method[1] enables continuous variation of cation ratios and hence the synthesis, in principle, of any desired composition of a given oxide system. Another means of synthesizing complex metal oxides at relatively low temperatures is by topochemical redox reactions[2] in which the starting material undergoes a minimum structural reorganization to give a product that retains all the original features of the reactant. Synthesis of a number of new and already known complex metal oxides using these two new methods is described in the thesis.

We have prepared a number of carbonate, nitrate, hydroxide, oxalate and cyanide solid solutions and used them as precursors to synthesize a number of complex metal oxides. Some of these oxides have been used as starting materials to synthesize metastable phases by the topotactic hydrogen reduction. Thus two metastable perovskite-related oxides, $La_2B_2O_5$ (B = Co or Ni) have been prepared by reducing the parent perovskites, $LaBO_3$. Synthesis of a series of quaternary perovskite-related oxides, $Ca_2Fe_{2-x}Mn_xO_5$ has been achieved by a combination of solid solution precursor route and the topotactic hydrogen reduction. A series of oxides of the general formula, $Li_xVO_2 (0<x<1)$ has been synthesized by the topochemical delithiation of $LiVO_2$ using Br_2 in $CHCl_3$. $AThV_2O_6$ (A = Ca, Sr) perovskites have been prepared by the hydrogen reduction of $AThV_2O_8$ oxides of zircon structure.

2. Experimental

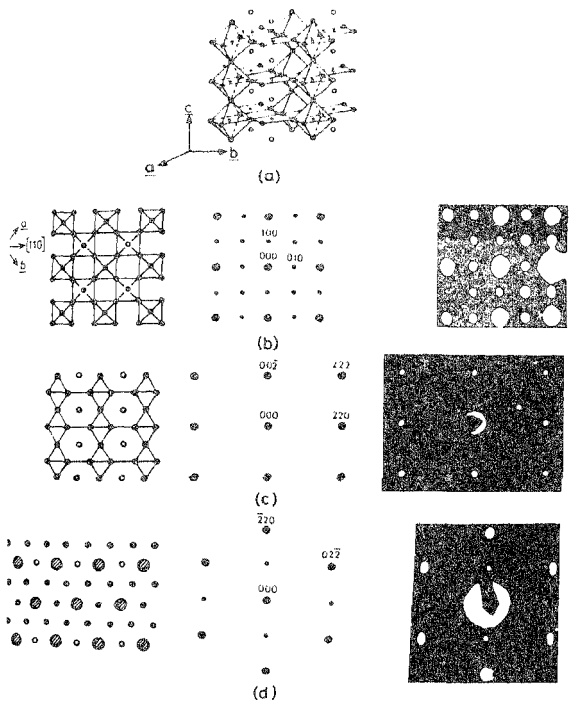
Carbonate solid solutions, $Ca_{1-x}M_xCO_3$ (M = Fe or Co), $Mn_{1-x}M_xCO_3$ (M = Mg, Co or Cd) and $Ca_{1-x-y}M'_xM''_yCO_3$ (M', M'' = Mn, Fe or Co) were prepared by adding an aqueous solution of the divalent metal nitrates to an excess of ammonium carbonate (~2M) or sodium bicarbonate (~1M) solution. Nitrate solid solutions, $A_{1-x}Pb_x(NO_3)_2$ (A = Ba, Sr)

were prepared by adding an aqueous concentrated nitrate solution of the componen metals to 13N nitric acid (AnalaR) at 5°C. Using ammonium hydroxide or sodiur hypochlorite solution as the precipitating reagent, hydroxide solid solutions of the generz formula $Ln_{1-x}M_x(OH)_3$ [Ln = La or Nd; M = Al, Cr, Fe, Co or Ni] were precipitated. Soli solutions among isostructural oxalate dyhydrates of Mn, Co, Ni and Zn were prepared b adding acetate solution of the component metals to 1M oxalic acid at 80°C. All th precursors were characterized by X-ray powder diffraction and, where essential, b thermogravimetric and IR absorption studies. They were decomposed at the lowes possible temperatures in suitable atmosphere (vacuum, oxygen, air, nitrogen or hydroger to obtain complex metal oxides. $La_2B_2O_5$ oxides (B = Co or Ni) were prepared by reducin the parent perovskites, $LaBO_3$ in a thermogravimetric balance in dilute hydrogen (H_2:N_2 i the 1:9 volume ratio). $Ca_2Fe_{2-x}Mn_xO_5$ oxides were obtained by the reduction of th corresponding fully oxidized phases in dilute hydrogen. Li_xVO_2 ($0<x<1$) phases wer prepared by room-temperature dilithiation of $LiVO_2$ using Br_2 in $CHCl_3$ as the oxidizin agent. All the oxides prepared were characterized by X-ray powder diffraction and electro diffraction. Some of the oxides were characterized by magnetic susceptibility measur ments.

3. Results and discussion

All the solid solutions prepared by us are single-phase solids as revealed by X-ra diffraction studies. Carbonate solid solutions have yielded several interesting oxides suc as $Ca_2Co_2O_5$, $Ca_2Fe_2O_5$, Ca_2FeCoO_5 and $Ca_3Fe_2MnO_8$. While $Ca_2Co_2O_5$ adopts a vacanc ordered perovskite superstructure, similar to $Ca_2Mn_2O_5$, ith square pyramidal coordin tion around the transition metal ion[2], the other oxides belong to n = 2 or 3 members of th $A_nB_nO_{3n-1}$ series with brownmillerite type[3] of oxygen vacancy ordering. Nitrate soli solution route affords a simple way of preparing $BaPbO_3$, Ba_2, PbO_4, etc. at low temperature without any volatilization of lead. Hydroxide solid solutions have yielded many perovskit oxides, particularly $NdNiO_3$ at ambient pressure. Quaternary spinel and perovskite oxide have been prepared from oxalate and cynanide solid solutions respectively. Monoxic solid solutions of the rocksalt structure of the general formula $Mn_{1-x}MxO$ (M = Mg, Co (Cd) ($0 \leqslant x \leqslant 1$) obtained from the corresponding carbonate solid solutions have show interesting magnetic susceptibility phenomena. Antiferromagnetic ordering complete disappears when $x>0.25$ in $Mn_{1-x}M_xO$ (M = Mg or Cd) systems, whereas in $Mn_{1-x}Co_x($ T_N is found to vary linearly with x.

$La_2Co_2O_5$ and $La_2Ni_2O_5$ retain the original features of the parent pervoskite structur While $La_2Co_2O_5$ adopts a brownmillerite structure with Co^{2+} in octahedral and tetrahedr coordination, $La_2Ni_2O_5$ adopts a new perovskite related structure with Ni^{2+} in octahedr and square planar coordination as revealed by electron diffraction studies (fig. 1). Bo these phases can be oxidized back to the perovskite structure revealing the topotact nature of the reaction. $Ca_2Fe_{2-x}Mn_xO_{6-y}$ ($0<y<1$) oxides obtained from carbonate sol solutions $Ca_2Fe_{2-x}Mn_x(CO_3)_4$ have yielded, on hydrogen reduction, a series of metastab quaternary perovskite-related oxides of the formula $Ca_2Fe_{2-x}Mn_xO_5$ (x = 2/3, 1, 4/3) wi octahedral, tetrahedral and square pyramidal coordination around transition metal ion However, these phases, on heating in vacuum, transform to the brownmillerite structur This observation shows that the nature of oxygen vacancy ordering in a given oxide syste depends not only on the nature of transition metal ions but also on the method preparation.



3. 1. Vacancy ordering in perovskite related $La_2Ni_2O_5$. (a) Proposed ructure for $La_2Ni_2O_5$. Projection of the proposed structure (left), :pected E.D. pattern (centre) and E.D. pattern observed for $La_2Ni_2O_5$ ght) in (b) $[001]_c$, (c) $[110]_c$ and (d) $[111]_c$ directions [● – lanthanum; · – ckel; ○-oxygen; ○-oxygen vacancy].

Li_xVO_2 oxides ($0<x<1$) obtained by delithiation of rocksalt related $LiVO_2$ retains the ıbic close packed anion array of the parent $LiVO_2$. A sample obtained by complete ılithiation of $LiVO_2$ transforms, on heating in vaccum, to the monoclinic structure of VO_2.

The present study shows that the solid solution precursor route and topochemical redox ıactions afford convenient routes for the synthesis of many complex metal oxides, ıpecially metastable phases.

References

1. HOROWITZ, H.S. AND LONGO, J.M. *Mater Res. Bull.*, 1978, **13**, 1359.
2. POEPPELMEIER, K.R., LEONOWICZ, M.E. AND LONGO, J.M. *J. Solid St. Chem.*, 1982, **44**, 89–98.
3. GRENIER, J.C., POUCHARD, M. AND HAGENMULLER, P. *Struct. Bonding,*, 1981, **47**, 1.

Thesis Abstract (Ph. D.)

Inter-organellar interaction: Regulation of mitochondrial oxidative phosphorylation by chloroplasts by Vipin Kumar.
Research supervisor: C.K. Ramakrishna Kurup.
Department: Biochemistry.

1. Introduction

There is increasing evidence that interaction between the two energy-transducing organelles of higher plants, namely the chloroplast and the mitochondrion plays an important role in the regulation of energy metabolism during growth and development. The entire energy needs of the mature plant during illumination can be met from photophosphorylation by chloroplasts[1]. Presumably, a further increase in phosphate potential (ATP concentration) by mitochondrial oxidative phosphorylation would exert a negative feedback on both energy transducing systems. However, a decrease in the oxygen concentration of the cytoplasm facilitates efficient fixation of carbon dioxide and minimization of photorespiration. These considerations would suggest that mitochondrial oxidation in the light should proceed without being tightly coupled to ADP phosphorylation. This thesis makes an attempt at understanding the communication between the two energy-transducing membrane systems and reveals that the xanthophyll lutein may play a role in the regulation of mitochondrial oxidative phosphorylation.

2. Materials and methods

Tightly coupled mitochondria were prepared from mung bean seedlings and from rat liver. Chloroplasts were isolated from the leaves of alfalfa and peanut. Xanthophylls were isolated from chloroplasts by extraction with ether and lutein was separated and purified by chromatography. Structure was established by physical methods. The effect of lutein on mitochondrial volume changes was determined by electron microscopy and by absorbance decrease at 520 nm. Increase in permeability to calcium was monitored by chlortetracycline fluorescence[2] and by uptake of [$^{45}Ca^{2+}$].

3. Results and conclusions

Addition of chloroplasts (100 – 300 μg equivalent of chlorophyll) to rat liver or mung bean mitochondria inhibited State 3 (ADP present) and stimulated State 4 (ADP exhausted) respiration (fig. 1). The uncoupling activity was found to be associated with particles rich in photosystem II[3].

A factor with uncoupling activity was isolated from chloroplasts and identified as the xanthophyll lutein (fig. 2). Addition of lutein to tightly coupled plant or animal mitochondria

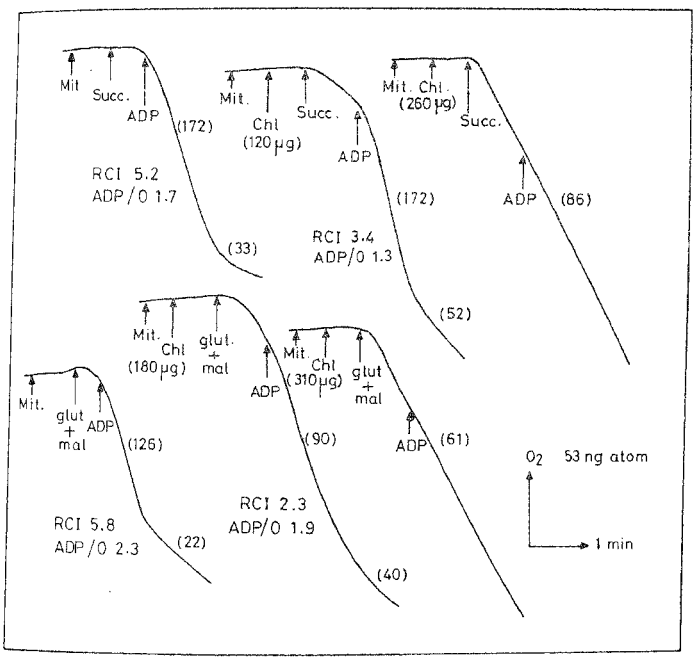


FIG. 1. Uncoupling of oxidative phosphorylation in rat liver mitochondria by groundnut leaf chloroplasts. The effect of addition of intact chloroplasts prepared from fresh leaves of groundnut on respiratory control and ADP/O ratio of rat liver mitochondria is shown. After the addition of substrate (succinate or glutamate + malate), 'State 3' respiration was initiated by the addition of 200μM ADP. Chloroplasts (concentration in chlorophyll equivalents indicated) were added before substrate. The values in parentheses represent the rate of oxygen uptake (ng atom O/min/mg protein). The values of respiratory control index (RCI) and ADP/O are also given.

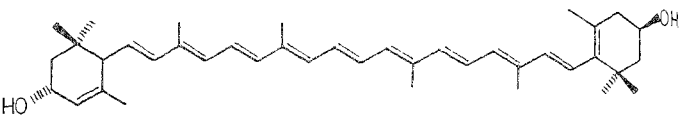


FIG. 2. Structure of lutein.

uncoupled and inhibited oxidation as was done by whole chloroplasts. Xanthophyll induced swelling in mitochondria as evidenced by light scattering and electron microscopy[3]. Consistent with its uncoupling action, lutein stimulated dormant ATPase of tightly coupled mitochondria for which the presence of Mg^{2+} ions in the reaction system was obligatory.

The properties described above suggested that perturbation of membrane organization may hold the key to the mode of action of the compound. To gain further insight, the interaction of the xanthophyll with phospholipid bilayers was examined employing a variety of physical methods. Differential calorimetric scans revealed that incorporation of lutein into aqueous dispersions of dipalmitoyl phosphatidyl choline decreased the gel-liquid crystal transition enthalpy as well as the size of the cooperative unit without affecting the transition temperature. Permeability of the bilayer of ascorbate was enhanced significantly by the presence of lutein.

Incorporation of lutein broadened the NMR peaks of both the acyl side chain $-CH_2-$ and

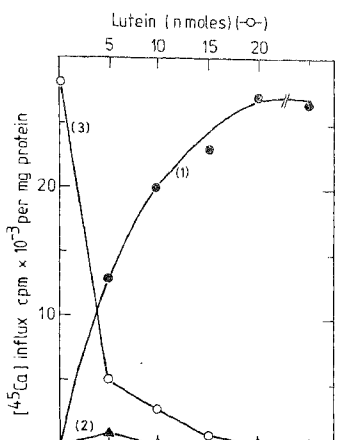


FIG. 3. Effect of lutein on energy-dependent uptake of calcium by rat liver mitochondria. Succinate-mediated uptake of $[^{45}Ca^{2+}]$ by rat liver mitochondria as a function of time in the absence (●) and in the presence of 20 μM lutein (▲) is depicted in experiments 1 and 2 respectively. In experiment 3, the uptake of $[^{45}Ca^{2+}]$ in 1 min in the presence of varying concentrations of lutein is depicted (○).

terminal $-CH_3$ protons of the phospholipid without any change in the line width of the choline head group $-CH_3$ proton signal. This indicated the ability of the compound to integrate deep into the hydrophobic regions of the phospholipid bilayer, distal to the polar regions.

Even though lutein does not bind Ca^{2+}, it increased the permeability of phospholipid vesicles to Ca^{2+} ions. Consistent with its uncoupling action, energy-dependent uptake of Ca^{2+} by mitochondria was inhibited by lutein. However, addition of lutein permitted a rapid efflux of the cation from Ca^{2+} loaded mitochondria (fig. 3).

These properties of lutein throw sufficient light on its mechanism of action. Even though highly lipophilic, the compound does not contain groups which can associate or dissociate protons[4], and as such, cannot act as a protonophore. Therefore, the mechanism of uncoupling may not involve the shuttling of protons across the mitochondrial inner membrane as demanded by the chemiosmotic hypothesis[5]. The deleterious action of lutein on mitochondrial function might reside in the ability of the lipophile to interact with the membrane bilayer and perturb the structure by the induction of 'pores'.

References

1. GRAHAM, D. — Effects of light on 'dark' respiration. In *Biochemistry of plants* (Davies, D.D. Ed.) Academic Press, New York, 1980, Vol. 2, 525–579.

2. NAGARAJ. R., MATHEW, M.K. AND BALARAM, P. — Cation translocating effects of alamethicin and its synthetic fragments in lipid membranes. Influence of peptide chain length and charge, *FEBS Lett.*, 1980, **121**, 365–368.

3. CHATURVEDI, V.K. AND KURUP, C.K.R. — *Regulation of mitochondrial oxidative phosphorylation* by chloroplasts, *Curr. Sci.*, 1985, **54**, 550–553.

4. GOODWIN, T.W. — *The biochemistry of the carotenoids*, Vol. 1. Chapman and Hall, London, 1980.

5. MITCHELL, P. — Chemiosmotic coupling in oxidative and photosynthetic phosphorylation, *Biol. Rev.*, 1966, **41**, 445–502.