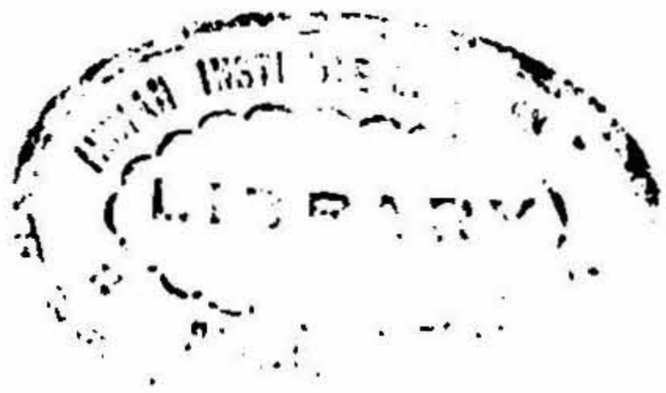
J. Indian Inst. Sci. 63 (C), Aug. 1981, pp. 111–119



Investigations on red cell adenylate kinase polymorphic system in blood and bloodstains for forensic application in India

H. JOSHI AND R. D. DUA

Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology, New Delhi 110 016

AND

S. SIVARAM*

Institute of Criminology and Forensic Science, New Delhi 110 055.

Received on February 12, 1981. Revised on August 4, 1981.

Abstract

Identification of red cell adenylate kinase (AK) polymorphic variants in fresh blood as well as in dried bloodstains and their survival in tropical Indian climatic conditions has been studied. Gene frequencies of 0·937 and 0·063 for AK^1 and AK^2 alleles were observed in the population sample of Delhi. In experimental bloodstains stored at room temperature, the enzyme activity persisted for seven months and if stored in refrigerated or in frozen conditions (-20° C) the activity persisted much longer. Stains of post-mortem blood showed typable activity for seven months when stored at room temperature. Correct identification of adenylate kinase variants was possible in about 56% of the six-month old exhibits of actual criminal cases. It can thus be concluded that the AK system is suitable for typing forensic exhibits in India.

Key words: Enzyme polymorphism—AK, Delhi population sample, blood and bloodstains.

1. Introduction

Adenylate kinase (AK) is an ATP: AMP phosphotransferase (EC. 2.7.4.3). It catalyses the reversible transfer of a high energy phosphate group from one molecule of adenosine diphosphate to another, producing one molecule each of adenosine triphosphate and adenosine monophosphate.

$$2\ ADP \xrightarrow[Mg^{++}]{AK} ATP + AMP$$

The polymorphism of human red cell adenylate kinase was first demonstrated by Fildes and Harris[1] by using starch gel electrophoresis. The polymorphic forms AK

* Present address: Central Forensic Science Laboratory, C.B.I., R.K. Puram, New Delhi 110 022.

1-1, AK 2-1 are common but AK 2-2 is rare. Other rarer forms of the enzyme are AK 3-1, AK 4-1 and AK 5-1. All these forms are identifiable by electrophoresis of the red cell lysates. In the electrophoretogram, AK 1-1 is represented by one major component at the origin and another smaller component towards the anode. AK 2-2 is composed of one major band towards the cathode and one minor band at the origin while the heterozygous AK 2-1 is identified by the presence of all the three components. The bands at the origin and towards the cathode are of equal intensity and size while the anodal band is smaller in size.

It has been established that adenylate kinase is polymorphic in all the populations of the world except Negroes. In the different ethnic groups in India, frequencies of the AK^2 allele are in the range of 0·02 to 0·127[1-4]. Studies by anthropologists have shown that Indians have a higher incidence of AK^1 gene in comparison to other populations of the world. While studies abroad have shown the suitability of this enzyme system for the individualization of dried bloodstains for forensic examinations[5-11], no such studies have been done in India where climatic conditions are so different from those prevailing in the Western countries.

Therefore, the distribution frequency of AK variants in the Delhi population and persistence of enzyme activities under simulated varied Indian climatic conditions[11] were studied. For the present paper, bloodstains of fresh and post-mortem blood were stored under actual climate of Delhi and analyzed for persistence of AK activity. AK variants were also identified in actual case exhibit bloodstains received from various forensic science laboratories of the country. These studies indicate that typing of AK activity in bloodstain is feasible for reasonable length of time and hence can form the basis of forensic examination in India.

2. Material and methods

2.1. *Chemical*

Citric acid, TRIS (tris hydroxy methyl amino methane), magnesium chloride, glucose/ sodium hydroxide and hydrochloric acid were of AR/GR grade (E. Merck/BDH).

DL-Histidine, adenosine diphosphate, nicotinamide adenine dinucleotide phosphate hexokinase, glucose-6-phosphate dehydrogenase, MTT tetrazolium salt and phenazine methosulfate were purchased from Sigma Chemical Co., U.S.A., while hydrolzed starch was from Connaught Medical Research Laboratory, Canada and Agar from Oxford, U.K. or Difco, U.S.A.

2.2. *Material*

Blood samples

Blood samples were collected from the donors and made ready for electrophoresis as described earlier[12].

Bloodstains

Stains from fresh free-flowing intravenous blood were prepared and selected for investigation as described earlier[12]. A minimum of five bloodstains were typed in each set of conditions except where otherwise mentioned. Most of the stains selected were of AK 1-1 and AK 2-1 types but one AK 2-2 stain was also studied.

It is well known that persistence of enzyme activities in blood stains is affected by various factors such as temperature, humidity, sunlight, etc. Therefore, to study the effect of these parameters on the persistence of AK enzyme activity in dried stain form, experimental bloodstains were subjected to the following sets of conditions:

1. Bloodstains were kept at room temperature (experiment was performed during October-May having day-temperature of 22° to 40° C ± 5° C with relative humidity 30% to 65% ± 10% and night temperature of 10° to 30° C ± 5° C with relative humidity from 55% to 85% ± 10%).

2. Bloodstains were stored at 2° to 8° C wrapped in a polythene bag to prevent any direct dripping of water on to the stains.

3. Bloodstains were stored at −20° C in a deep freeze wrapped in a polythene bag to avoid their wetting.

4. Stains of post-mortem blood were stored at room-temperature (experiment was performed during October-May having the day-temperature of 22° to 40° C ± 5° C with relative humidity 30% to 65% ± 10% night temperature of 10° to 30° C ± 5° C with relative humidity from 55% to 85% ± 10%).

5. Cuttings from exhibits of original cases were obtained from various forensic science laboratories of the country.

For studying the effect of storage on bloodstains, typing was started after longer time intervals in the case of stains stored at lower temperatures than in case of those stored athigher temperatures, as the possibility of persistence of the enzyme activity was higher In the former cases.

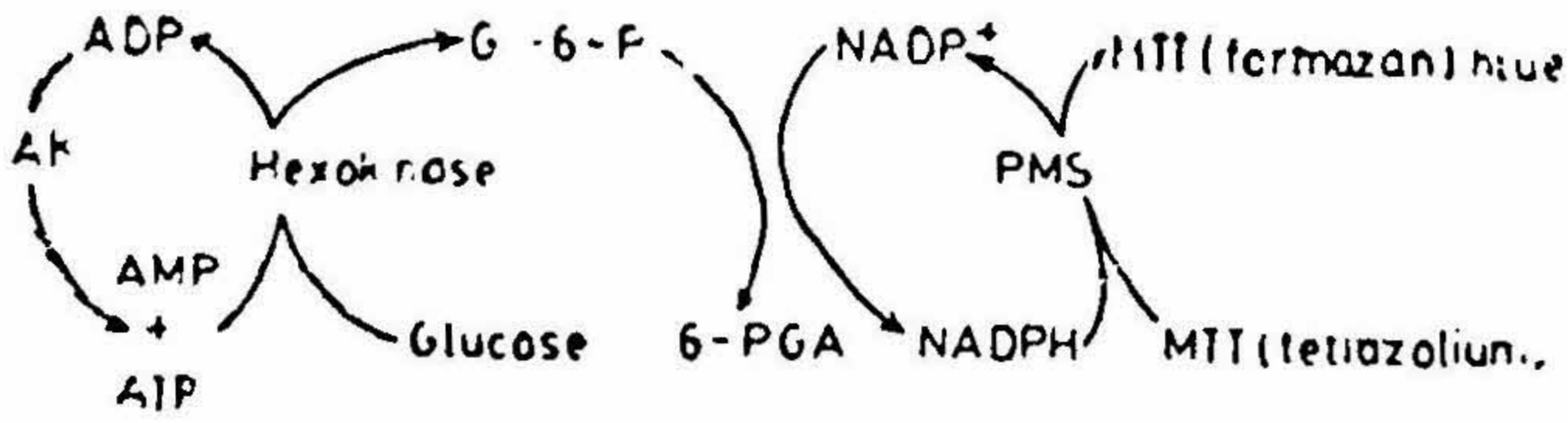
3. Typing of adenylate kinase activity

The enzyme was typed by the method of Culliford[5]. Starch gel slabs were prepared with 13% hydrolyzed starch. Threads soaked in lysate or threads of bloodstained cloth soaked in gel buffer were inserted at the centre of the gel.

Electrophoresis was performed at 120 volts for 2 hr for a gel plate of dimensions 12 × 20 × 0·1 cm, the shorter side (12 cm) being the distance between anode and cathode. AK bands as seen in fig. 1 were detected on the cathodal side of the origin as blue

FIG. 1. A starch gel electrophoretogram showing the three polymorphic variants of adenylate kinase (From left : sample nos. 1 and 4 are AK 2–2 ; 2, 5, 8 and 9 are AK 2–1 and 3, 6 and 7 are AK (1 1). Tank buffer : Citrate buffer pH 7·1. Gel buffer : Histidine buffer pH 7·1. Electrophoresis at 120 volts at 4° C for 2 hr on a gel plate of size 12 × 20 × 0·1 cm with electrodes on either end of the 12 cm side.

formazan complex on agar overlay on incubation at 37° C with the following reaction mixture :



4. Results and discussion

4.1. *Blood samples*

Table I gives the data on frequency distribution of AK in the present study sample. Of the total of 284 red cell lysates analyzed for AK polymorphic variants, 250 showed AK 1–1, 32 showed AK 2–1 and 2 showed AK 2–2 pattern. No rare variants were observed. The gene frequencies of 0·937 for AK^1 and 0·063 for AK^2 calculated from the above figures fall within the range of gene frequencies reported for the Indian ethnic groups. Statistical comparison with the hypothetical expected numbers showed no significant difference.

Table I

Distribution of adenylate kinase polymorphic variants in population sample of Delhi

Phenotype	Observed		Expected		Gene frequencies
	No.	%	No.	%	
1-1	250·00	88·00	249·10	87·70	
2-1	32·00	11·30	33·80	11·90	$AK^1 = 0·937$
2-2	2·00	0·70	1·10	0·40	$AK^2 = 0·063$
Total	284·00	100·00	284·00	100·00	

($\chi^2 = 0·7416$, $df = 1$, $0·75 > P > 0·50$).

4.2. *Bloodstains*

Results of the persistence of adenylate kinase activity in bloodstains stored at various storage conditions are given in Table II.

In the case of bloodstains stored at room temperature during October-May, typable AK activity was observed even in seven-month old bloodstains in 100% cases (Table IIA).

In the second set of bloodstains kept at 2°–8° C, typable AK activity was retained in 100% cases at the end of 22 month and thereafter their study was discontinued (Table IIB).

In the third set of bloodstains stored at – 20° C (frozen), typable AK activity was preserved even longer than 22 months (Table IIC). Hence, it is clear that samples can be safely stored in the frozen or refrigerated state for AK typing in the forensic laboratories.

The results obtained on the stains of post-mortem blood stored at room temperature during October-May (temperature and relative humidity as given under 'Materials') have shown that good typable activity of AK is present in 100% of the stains even after 7 months (Table IID).

It is observed from the data obtained from cuttings of actual criminal case exhibits that satisfactory identification of AK variants is possible in 56% cases in six month-old bloodstains (Table IIE). The apparently anomalous situation of a higher percentage of typable results in older stains (56% in six-month old and 38·5% in three-month old) can be explained by the fact that the bloodstained-exhibits came from forensic laboratories in different parts of the country and had, therefore, been subjected to diverse

Table II

Persistence of typable adenylate kinase activity in bloodstains under various conditions of storage

A. *Stains of fresh blood*

Storage condition = Room temperature (maximum day temperature ranging from 22° to 40° C, minimum night temperature ranging from 10° to 30° C ± 5° C and relative humidity ranging from 55% to 85% ± 10% to 30% to 65% + 10%)

Age of stains	Number of stains tested	Typable results	% correctly typed stains
3 days	5	5 +	100
3 month	5	5 +	100
5 months	5	5 +	100
6 months	5	5 +	100
7 months	5	5 +	100

B. *Storage condition = Temperature* (2° *to* 8° C)

4 days	5	5 +	100
3 months	5	5 +	100
17 months	5	5 +	100
22 months	5	5 +	100

C. *Storage condition = Temperature* (− 20° C)

1 week	5	5 +	100
3 months	5	5 +	100
10 months	5	5 +	100
16 months	5	5 +	100
20 months	5	5 +	100
24 months	5	5 +	100

D. *Stains of post-mortem blood*

Storage conditions

Temperature		Relative humidity
Day:	22° to 30° C ± 5° C	30% to 55 % ± 10%
Night:	10° to 15° C ± 5° C	55% to 85 % ± 10%

5 days	6	6 +	100
3 months	6	6 +	100
4 months	6	6 +	100
5 months	6	6 +	100
6 months	6	6 +	100
7 months	6	6 +	100

E. *Stains of case exhibits*

2 months	118	61	51·70
3 months	52	20	38·46
4 months	39	17	43·60
6 months	29	17	56·00

climatic conditions. Case exhibits from the colder and drier regions gave more positive results even in older stains than those from hot and humid climate regions of fresher origin.

Culliford and Wraxall[5] reported the presence of typable AK activity in three- month old bloodstains in England. Older stains were not attempted. Rothwell[6] had also found the typable AK activity in one-month old bloodstains which persisted in a few cases even till the end of four months. Saha and Kirk[13] while working with bloodstains on filter paper had reported the stability of AK activity for typing bloodstains for four weeks when stored at room temperature (20° C) and for longer periods when stored in the cold. Saenger *et al*[7] claimed typable activity of AK even in six month old bloodstains. Stombaugh's[8] investigations on the enzyme showed that bloodstains stored below 25° C retain the AK activity for 18 months and those stored at 37° C for three months.

5. Conclusion

The present study has indicated that the AK enzyme has reasonably good frequency distribution in Indian population. Activity could be detected in stains of fresh as well as post-mortem blood even after seven months when stored at room temperature. AK activity could withstand the extreme weather conditions prevailing in Delhi. AK activity when determined in actual cuttings from two and six month-old case exhibits was successful in reasonable percentage. This establishes the suitability of this system for forensic examination even in India where exhibits from criminal cases reach forensic laboratories quite late and under adverse storage conditions.

Acknowledgement

The authors are grateful to the Director and the Deputy Director, ICFS, New Delhi, for their kind encouragement and to the Director, I.I.T., Delhi, for providing the laboratory facilities.

The authors' thanks are due to the officer-in-charge, Blood Bank, S.J. Hospital, New Delhi, for arranging the fresh blood samples, to the Professor, Forensic Medicine, A.I.M.S., Delhi, for the post-mortem blood samples and to the Directors of the various Forensic Science Laboratories in India, for case exhibit-cuttings. Thanks are also due to the B.P.R. & D., M.H.A., Government of India, and C.S.I.R., New Delhi, for financial assistance to one of us (HJ).

References

1. FILDES, R. A. AND HARRIS, H. — Genetically determined variation of adenylate kinase in man, *Nature*, 1966, 209, 261–263.
2. ROY CHOUDHARY, A. K. — The distribution of gene frequencies of proteins and enzymes in India, *Trans. Bose Res. Inst.*, 1975, 38 (1–2), 9–48.
3. GHOSH, A. K., KIRK, R. L., JOSHI, S. R. AND BHATIA, M. H. — A population genetic study of the Kota in the Nilgiri Hills, South India, *Hum. Hered.*, 1977, 27, 225–241.
4. SAHA, N., KIRK, R. L., SHANBERG, S., JOSHI S. R. AND BHATIA, H, M. — Population genetics studies in Kerala and Nilgiri (South West India), *Hum. Hered.*, 1976, 26, 175–197.
5. CULLIFORD, B. J. AND WRAXALL, B. G. D. — Adenylate kinase (AK) types in bloodstains, *J. Forens. Sci. Soc.*, 1968, 3, 203.
6. ROTHWELL, T. J. — The effect of storage upon the activity of phosphoglucomutase and adenylate kinase enzymes in blood samples and bloodstains, *Med. Sci. Law*, 1970, 10, 230–234.
7. SAENGER, M. S. AND YATES, R. G. — A cellulose acetate membrane technique for the determination of adenylate kinase types in bloodstains, *J. Forens. Sci.*, 1975, 20 (4), 643.

8. STOMBAUGH, F. H. AND KEARNEY, J. J. Determination of adenylate kinase variants in two Washington, D.C. population samples : A microcellulose acetate procedure *J. Forens. Sci.*, 1977, 22, 590–595.

9. CULLIFORD, B. J. Examination and typing of bloodstains in the crime laboratory, National Institute of Law Enforcement and Criminal Justice, Washington, 1971, pp. 129–145.

10. GRUNBAUM, B. W. Some new approach to the individualization of fresh and dried bloodstains, *J. Forens. Sci.*, 1976, 21, 488.

11. JOSHI, H., SIVARAM, S. AND DUA, R. D. Studies on three biochemical polymorphic systems PGM_1, AK and PCE_2 in human bloodstains subjected to tropical and temperate climatic conditions, *Indian J. Biochem. Biophys.*, 1980, 17, 67–71.

12. JOSHI, H., SIVARAM, S. AND DUA, R. D. Phosphoglucomutase (locus one) polymorphism in bloodstains, *J. Indian Acad. Forens. Sci.*, 1978, 17 (2), 55–67.

13. SAHA, N. AND KIRK, R. L. A simple technique for collecting blood for population studies of enzyme polymorphism and haemoglobins, *Hum. Hered.*, 1973, 23, 182–187.