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Deviation behaviour of charged and aromatic molecules on highly cross-linked dextran and polyacrylamide gels

P. GOPALAKRISHNA BHAT, G. P. KRISHNAMOORTHY AND
T. N. PATTABIRAMAN
Department of Biochemistry, Kasturba Medical College, Manipal 576 119, S. India.

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Abstract

Glucuronic acid and glutamic acid were excluded more strongly from Bio-Gel P-2 than from Sephadex G-15. Lysine and arginine were more strongly retarded on Bio-Gel P-2. Phosphate buffer (pH 7·0, 0·05 M) was found to be superior to 0·05 M Tris-HCl and 0·05 M NaCl solutions in suppressing electrostatic interaction on these gels. Complete normalization of the deviation patterns of charged molecules on these gels could not be achieved in all cases with different salt solutions as eluants. Arginine and lysine could be separated due to their differing degree of retention on Sephadex G-15 and Bio-Gel P-2. Retention of aromatic compounds was stronger on the cross-linked dextran gel.

Key words: Different deviation behaviour, dextran gels, polyacrylamide gels, aromatic interaction.

1. Introduction

Gel permeation chromatography has come to stay as an effective tool in analytical studies in biochemistry. Abnormal elution behaviour of charged and aromatic compounds during gel chromatography on cross-linked dextran gels was recognized soon after the introduction of these gel materials as molecular sieves. The early elution of negatively charged compounds and abnormal retardation of basic compounds during gel chromatography have been reported by several workers[1–3]. The abnormal behaviour has been attributed to the ionic interaction between the charged groups on the substances and carboxylate groups on the gel matrices[1–3]. Gelotte[1] reported that the ionic interaction could be completely suppressed by using dilute salt solution as eluant. The presence of carboxylate groups on cross-linked polyacrylamide gels has been reported[4]. Engel[5] reported anomalous behaviour of charged compounds on Bio-Gel P-2.

The retardation of aromatic compounds on dextran gels has been reported by several workers[1, 2, 6, 7]. The extent of retardation of aromatic compounds was found to be relatively less on polyacrylamide gels.

In this communication, we report the results of a comparative study on the deviation behaviour of acidic, basic and aromatic substances on highly cross-linked dextran and polyacrylamide gels.

2. Materials and methods

Cross-linked dextrans, Sephadex G–15 and G–50 and K9/60 acrylic plastic columns were procured from Pharmacia Fine Chemicals, Sweden. Cross-linked polyacrylamides, Bio–Gel P–2, P–10 and P–30 were obtained from Bio-Rad Laboratories, U.S.A. Sodium glucuronate (GA) was purchased from Fluka A.G., Switzerland. L-Phenylalanyl L-tryptophan (phe-trp), L-phenylalanyl L-tyrosine (phe-tyr), β-naphthyl α-D-glucopyranoside (NG), ethyl N-acetyl L-tyrosinate (EAT) and N-acetyl L-tyrosine (AT) were supplied by Sigma Chemical Company, U.S.A. *p*-Nitrophenyl β-D-glucuronide was the product of CalBiochem, U.S.A. All other chemicals used were of analytical grade.

Cross-linked dextrans and polyacrylamide gels were allowed to swell in water and the columns were packed in the conventional manner. To ensure uniformity, all the chromatographic runs were performed at 25° C. The bed volume in all cases was 37·5 ml (0·9 × 59 cm) and the flow rate was 8–10 ml per hr unless mentioned otherwise.

Glucose, GA and NG were estimated by phenol-sulphuric acid method[8]. Differential estimation of GA in the presence of glucose was carried out by modified carbazole method[9]. Amino acids were estimated by ninhydrin method[10]. EAT and AT were determined by the method of Lowry *et al*[11] with corresponding standard.

3. Results and discussion

Sephadex G–15 and Bio–Gel P–2 were used in these studies since both the gels had comparable fractionation range and water regaining capacity. Both the gels had same Vo value (13·5 ml) when the bed volume was 37·5 ml.

The elution volumes for glucose, GA, alanine, glutamic acid, lysine and arginine with water, 0·05 M NaCl, 0·05 M Tris–HCl buffer pH 7·0 or 0·05 M phosphate buffer pH 7·0 are presented in Table I. The data indicate that glucose was eluted from Bio–Gel P–2 as well as from Sephadex G–15 with constant Ve values, 29 ml and 25 ml respectively with all the eluants. Similarly, alanine was eluted with constant Ve values irrespective of the eluants used. GA and glutamic acid were eluted earlier than glucose with water as eluant. Exclusion of acidic compounds like glutamic acid and GA was more prominent on Bio–Gel P–2 than on Sephadex G–15.

When 0·05 M NaCl, 0·05 M Tris–HCl buffer pH 7·0 or 0·05 M phosphate buffer pH 7·0 were used as eluants (Table I) the exclusion (earlier anomalous elution) of

Abbreviations : Tris—Tris hydroxy-methyl amino methane; Ve–Elution volume; Vo–Void volume.

Table I

Ve values of compounds on Bio-Gel P-2 and Sephadex G-15

Aliquots of 0·5 ml from aqueous solution of compounds (2 mg/ml) adjusted to pH 7·0 was applied to Bio-Gel P-2 and Sephadex G-15 columns (0·9 × 59 cm, bed volume 37·5 ml). Elution was performed with water, 0·05 M NaCl, 0·05 M Tris-HCl buffer pH 7·0 or 0·05 M phosphate buffer pH 7·0. One ml fractions were collected and assayed.

Compound	Bio-Gel P-2				Sephadex G-15			
	Water	0·05 M NaCl	0·05 M Tris-HCl pH 7·0	0·05 M phosphate pH 7·0	Water	0·05 M NaCl	0·05 M Tris-HCl pH 7·0	0·05 M phosphate pH 7·0
Glucose	29	29	29	29	25	25	25	25
Glucuronate	16	23	21	26	19	22	20	22
Alanine	26	26	26	26	24	24	24	24
Glutamic acid	16	20	20	21	19	22	21	22
Lysine	exchanged	31	34	26	exchanged	22	24	21
Arginine	,,	38	42	31	,,	26	28	22

GA and glutamic acid was found to be suppressed to different extent on Bio-Gel P-2 and Sephadex G-15. The exclusion of GA was not completely abolished on Bio-Gel P-2 and Sephadex G-15 with the three different eluting systems. Inclusion of 2·0 M NaCl in 0·05 M phosphate system did not cause any further suppression of charge interaction as evidenced by similar Ve values for GA.

Lysine and arginine were strongly retarded (anomalous later elution) on both the gels and could not be eluted with distilled water. Elution with 0·05 M NaCl, 0·05 M Tris-HCl buffer pH 7·0 or 0·05 M phosphate buffer pH 7·0 suppressed the retardation of these basic amino acids to different extent as indicated by their Ve values (Table I). Retardation of basic amino acids was found to be stronger on Bio-Gel P-2 than on Sephadex G-15. Only phosphate buffer (0·05 M, pH 7·0) was effective in complete suppression of the deviation behaviour of the basic amino acids on Sephadex G-15. The retardation of arginine on Bio-Gel P-2 could not be abolished completely by any of the eluants used in these studies. Gelotte[1] reported that the abnormal elution of acidic and basic compounds could be normalized by using 0·05 M NaCl as eluant on Sephadex G-25. The present studies indicate that complete normalization of the elu-

tion of acidic and basic compounds could not be obtained with 0·05 M NaCl on Sephadex G–15 and Bio–Gel P–2. The difference could be due to the fact that the gels used in these studies are more cross-linked than Sephadex G–25 and hence the electrostatic interactions are too strong on these gels to be completely suppressed by salt solutions.

The binding capacities for lysine and arginine on Bio–Gel P–2 and Sephadex G–15 were estimated and the values are presented in Table II. Binding capacity on Bio-Gel P–2 for lysine and arginine were comparable. Similarly Sephadex G–15 exhibited comparable binding capacity for these amino acids. However, the capacity of Bio-Gel P–2 for the basic amino acids were 6–7 times greater than those of Sephadex G–15. This might be due to a higher negative charge density on Bio–Gel P–2 than on Sephadex G–15. The binding capacity of Sephadex G–50 for basic amino acids was significantly lower than that of Sephadex G–15. Similarly, binding capacities of Bio-Gel P–10 and P–30 were less than those of Bio–Gel P–2 indicating reduction in the binding capacities with decrease in the degree of cross-linkages. This observation is in agreement with the observations of Ortner and Pacher[12] that the carboxylate group content of Sephadex gels decreased with the decrease in the degree of cross-linkages.

The differential elution behaviour of lysine and arginine on both the gels was confirmed by subjecting a mixture of lysine and arginine to chromatography. Lysine and arginine were separated on both the gels and separation was found to be better on Bio-Gel P–2 than on Sephadex G–15.

Table II

Binding capacities of the gels

Ten mg of arginine or lysine dissolved in 0·5 ml of distilled water was applied to a 5 ml column (1·2 × 4 cm) of Sephadex G–15 or Bio–Gel P–2. The column was washed with 15 ml of water followed by 15 ml of 0·05 M NaCl at a flow rate of 5 ml per hr. Five ml fractions were collected. Amino acid in the fractions was estimated by ninhydrin method using corresponding standards. In the case of Bio–Gel P–10, P–30 and Sephadex G–50, 2 mg of amino acid in 0·5 ml aqueous solution was applied. Other details were as described above.

	Lysine μmoles/g dry weight	Arginine μmoles/g dry weight
Bio–Gel P–2	38·4	34·4
Bio–Gel P–10	4·0	8·5
Bio–Gel P–30	0·8	2·0
Sephadex G–15	4·6	6·5
Sephadex G–50	0·6	0·8

In Table III, VelVo values of some aromatic compounds during chromatography on Sephadex G–15 and Bio–Gel P–2 are given. Ve/Vo values of glucose and alanine are given in Table III for comparison. Among the aromatic compounds NG was retarded most on both the gels. The extent of retardation of NG was more on Sephadex G–15 than on Bio–Gel P–2. Similarly, phe-trp and tryptophan were retarded more on Sephadex G–15. Phe-trp was retarded more than either phenylalanine or tryptophan on Sephadex G–15. But phe-trp was retarded more than phenylalanine and less than tryptophan on Bio–Gel P–2. The stronger retardation of NG and tryptophan on Bio–Gel P–2 and Sephadex G–15 than phenylalanine, tyrosine and phe-tyr suggests that the size of the aromatic ring is the decisive factor in determining the retardation of the compounds. Phenylalanine, tyrosine and phe-tyr were retarded to comparable extents on Bio–Gel P–2 and Sephadex G–15 (Table III). But stronger retardation of NG, tryptophan and phe-trp on Sephadex G–15 than on Bio–Gel P–2 suggests that more than one factor contribute to the retardation of aromatic and heterocyclic compounds. It is probable that sorption due to the ring system is a minor factor whereas the π electron interaction could be a dominant factor affecting retention. The latter could be a stronger factor on Sephadex G–15 than on Bio–Gel P–2.

The retardation of aromatic and heterocyclic compounds on cross-linked dextran and polyacrylamide gels have been observed by several workers[1, 3–7]. Hydrophobic inter-

Table III

Ve values of aromatic compounds

One mg each of NG, phe-tyr, phe-trp were dissolved in 1 ml of 50% ethanol. Other compounds were dissolved in distilled water. One ml of the solution was applied to 37·5 ml column (0·9 × 59·0 cm) of Sephadex G–15 or Bio–Gel P–2 and eluted with water at a flow rate of 7–8 ml/hr and one ml fractions were collected. NG was estimated by phenol sulphuric acid method. Amino acids and dipeptides were estimated by ninhydrin method.

Compound	Bio–Gel P–2	Sephadex G–15
NG	4·4	9·6
Tryptophan	3·5	5·0
Tyrosine	2·8	2·8
Phenylalanine	2·4	2·4
Phe-trp	3·0	7·0
Phe-tyr	2·8	2·8
Alanine	1·9	1·8
Glucose	2·1	1·9

actions[13], hydrogen bonding[14] and π-electron interactions[15] have been suggested to be involved in the retardation of heterocyclic and aromatic compounds. The elution volumes of NG, tryptophan and phe-trp were estimated on Bio–Gel P–30 column, a less cross-linked polyacrylamide gel. The Ve/Vo values of tryptophan (3·5) and phe-trp (3·0) on Bio–Gel P–30 were not different from those on Bio–Gel P–2. But NG had a lower Ve/Vo value (3·4) on Bio–Gel P–30 than on Bio–Gel P–2, indicating that degree of cross-linkage affects the retardation of NG.

EAT, a neutral aromatic ester, was eluted with a Ve value of 32 ml from Bio–Gel P–2, which was slightly higher than that of glucose (Ve, 29 ml). On Sephadex G–15 EAT was significantly retarded (Ve, 49 ml). The negatively charged AT was excluded from Bio–Gel P–2 (Ve, 16 ml) as in the case of GA. On Sephadex G–15, the elution volume of AT (25 ml) was same as that of glucose.

p-Nitrophenyl glucuronide was eluted earlier from Bio–Gel P–2 with water as eluant and the Ve value (16 ml) was comparable to that of GA. On Sephadex G–15, *p*-nitrophenyl glucuronide was eluted (Ve, 21 ml) later than GA (Ve, 19 ml) and earlier than glucose. This could be attributed to the net effect of two opposing factors affecting the elution of *p*-nitrophenyl glucuronide, namely, exclusion effect due to the negative charge and the adsorption effect due to *p*-nitrophenyl group on Sephadex G–15. In the case of AT the adsorption effect and exclusion effect were mutually compensated. On Bio–Gel P–2 the exclusion effect appeared to be the dominant factor both in the case of AT and *p*-nitrophenyl glucuronide as indicated by their early elution.

Thus it is evident that the ionic interactions are more prominent on Bio–Gel P–2 than on Sephadex G–15. On the other hand the adsorption of compounds due to the presence of aromatic and heterocyclic groups is more on Sephadex G–15 than on Bio–Gel P–2. The cross-linked dextrans are suitable for the separation of charged aromatic substances from their neutral derivatives, whereas cross-linked polyacrylamide gels could be more effective in the separation of non-aromatic organic acids from their neutral derivatives.

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