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The selectivity of polyacrylamide gels for sugars

Employing the usual techniques of gel permeation chromatography, it was shown[1] that polyacrylamide gels allow the separation of the cellodextrins, glucose–cellohexaose. The separation is based mainly on molecular size but interactions between solute and the gel matrix become progressively more important with increasing molecular weight.

The present work was undertaken to investigate the extent to which the structure of the monomeric sugar influences the distribution coefficient, K_D, in this type of gel.

Experimental

Column preparation. The column was packed with polyacrylamide P-2, 200–400 mesh (Bio-Rad Laboratories, Richmond, Calif.). An accurately weighed quantity of dry gel was allowed to swell in distilled water for 24 h. The slurry was de-gassed under high vacuum and the column packed under gravity with continual addition of slurry to avoid defects in layering. Subsequent to preparation, the column was washed until a constant value of refractive index was obtained for the eluent. The final column dimensions are summarized in Table I. The pressure head (Mariotte flask) was adjusted to provide a flow rate of about 2 ml/h at which it was judged that equilibrium conditions on the column would be approximated. The column was thermostated to 25 ± 0.1°.

TABLE I

DIMENSION OF THE COLUMN (POLYACRYLAMIDE P-2)

Column height: ~ 60 cm; cross-sectional area: 0.785 cm²; flow rate: 2 ml/h; sample volume: 0.1 ml; sample concentration: 1 mg/ml.

Settled bed volume	47.7 ml
Void volume (Blue dextran) V_0	17.1 ml
Internal volume[a] V_I	28.7 ml
Wt. of dry gel	~15 g

[a] Determined as the ordinate intercept of a plot of $(V_e - V_0)$ *vs.* molecular weight for the cellodextrin series; this value agreed closely with that obtained from the elution of NaCl.

Sample application and detection. The sample solution (0.1 ml containing 0.1 mg solute) was applied to the gel surface with a micropipette in a layer 1 mm deep. The solution was allowed to enter the bed, was washed with successive portions of solvent and the column was connected to the constant head device. The eluent was collected and the volume recorded to within ± 0.02 ml at regular intervals. A Waters Associates Model R4 differential, automatically recording refractometer was operated at ×8 attenuation. Under these conditions 90 % full-scale deflection was obtained at the sample maximum. The dead volume between the end of the column and the refractive index cell was approximately 0.3 ml.

Results and discussion

Table II lists distribution coefficients for a number of common sugars (reproducibility in K_D is $\pm$ 0.002). Comparison of the figures for mannose and rhamnose (6-deoxy-L-mannose) shows that replacement of the primary hydroxyl at C_6 with H results in a lower K_D value. The reactions of the 6-deoxy-aldohexoses are known to be the same as those of the corresponding aldohexose except where the primary hydroxyl group is involved in the latter. Consequently, this means that the carbinol group plays a significant part in the interaction between solute and gel matrix, as the order would be reversed if molar volumes predominate.

TABLE II

K_D VALUES FOR SOME SUGARS ON POLYACRYLAMIDE P-2 WITH DEIONIZED WATER AS ELUENT

Compound	K_D[a]
D-Glucose	0.921
L-Glucose	0.920
Mannose	0.930
Rhamnose	0.879
Glucuronic acid	0.415
Galactose	0.917
Fructose	0.929
Xylose	0.931
Sucrose	0.854
Raffinose	0.750
Stachyose	0.715

[a] $K_D = \frac{V_e - V_0}{V_t - V_0}$

V_0 = void volume
V_t = total solvent volume in column
V_e = elution volume for a given solute.

The K_D values for glucose and glucuronic acid show that replacement of the carbinol group with a carboxyl group leads to a striking reduction in K_D. The probable explanation is ionic exclusion[2] due to coulombic interactions between the solute and the small number of residual carboxyl groups present in the gel. One would also expect a Donnan effect at low sample concentrations causing a reduced concentration in the internal volume of the gel; this in turn would cause earlier elution of the charged solute compared with the neutral counterpart.

D- and L-glucose have identical K_D values, as observed by MARSDEN[3] for a number of enantiomers on Sephadex gels. The similar K_D values for glucose and galactose show that the configuration of the hydroxyls at the C_4 position (erection from the equatorial to the axial position) is unimportant. This is understandable as the group will be equally accessible sterically in either position. However, erection of the hydroxyl at the C_2 position as in mannose leads to a small but significant increase in K_D compared with the value for glucose.

Fructose, having predominantly the pyranose form in free solution but the furanose form when linked to other sugars, has the same K_D value as mannose. It would appear to be immaterial whether the carbinol group is situated at C_2 or C_5 unless

the differing configurations at C_3 counterbalance a possible effect; erection of the hydroxyl at C_3 should lead to an increase in K_D.

It is open to speculation whether the differences in K_D arise mainly from the relative abilities of the solutes to fit into the water lattice or whether they are primarily a function of the accessibility of the hydroxyls to the gel. KABAYAMA AND PATTERSON[4] suggest that β-D-glucose, having all hydroxyls equatorially displaced, can fit into the water lattice with unstrained hydrogen bonds. This would lead to a higher affinity for the water phase than the gel phase. Alternatively, one might reason that axial hydroxyls facilitate interaction with the gel.

On the basis of molar volumes, one would expect xylose to elute at a greater volume than glucose, as observed. The data for the cellodextrins and xylodextrins[5] (up to the heptamers) fall on a common line in a plot of K_D as a function of molecular weight.

The significance of the structure of the group at C_5 in the pyranose ring may be inferred from the K_D values for methyl alcohol, formic acid and formamide (Table III).

TABLE III

K_D VALUES FOR SOME SIMPLE COMPOUNDS ON POLYACRYLAMIDE P-2 WITH DEIONIZED WATER AS ELUENT

Compound	K_D
Formic acid	0.674
Formamide	0.939
Methanol	0.839
n-Propanol	0.820
Isopropanol	0.777

Formic acid and formamide are respectively accelerated and retarded in relation to methanol by charge effects owing to the residual carboxyl groups in the gel. These differences should be eliminated if an electrolyte solution is used as the eluent. Methanol has a higher K_D value than *n*-propanol in correspondence with the molar volumes. However, MARSDEN[3] has observed with Sephadex gels that the increasing hydrophobicity of *n*-alcohols of longer chain length leads to a progressive retardation on elution, which outweighs the molecular size effect. That a greater weight is to be allotted to the affinity of a given alcohol for water (or conversely for the gel matrix) rather than molar volume is shown by the lower K_D value for isopropanol than *n*-propanol; the former is the more hydrophilic.

The series glucose–sucrose–raffinose–stachyose separate linearly in a plot of chain length *versus* K_D as observed for the cellodextrins glucose–cellotetraose[1]. The plots are in fact superimposable. This is not unexpected as galactose, glucose and fructose have similar K_D values.

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The isolation, separation and determination of isopropylnoradrenaline and its O-methyl derivative in blood serum and tissue homogenate

The assay procedure was essentially that of COHEN AND GOLDENBERG[1] commonly applied for the estimation of epinephrine and norepinephrine using the final evaluation of the isoproterenol and O-methylisoproterenol in the form of corresponding adrenolutines. For the estimation in serum the procedure of KAHANE AND VESTERGAARD[2] was modified, and for the determination of the isoproterenol level in tissue homogenates the original procedure of BERTLER *et al.*[3] was applied. The final part of the analytical procedure was the chromatographic separation of isoproterenol and its O-methyl derivative from adrenalin and noradrenalin. For this purpose tandem chromatography on cellulosophosphate and carboxymethylcellulose was used.

Experimental

Isolation of isoproterenol and the corresponding O-methylderivative from plasma. A 15-ml plasma sample was incubated with 0.5 g of aluminium oxide under vigorous shaking for 3 min. The suspension of aluminium oxide was spun off at 700 × *g*, and the sediment was resuspended in an equal volume of double-distilled water and spun off again. The whole procedure was repeated twice. In the last phase the resuspended mixture of aluminium oxide (containing adsorbed catecholamines including isoproterenol and O-methylisoproterenol) were poured into a small column (0.8 × 15 cm) and eluted with distilled water; the eluate was discarded. The catecholamine mixture was eluted with 5 ml of 0.4 *M* acetic acid. The eluate was taken to dryness and repurified by chromatography on a Dowex 50 X2 column. The ion-exchange column was prepared as follows. A column (1.2 × 4 cm) was filled with swollen ion exchanger