Separation of nucleic acid components on tightly cross-linked Sephadex*

Shortly after the introduction of the filtration technique on dextran gels (Sephadex, trade mark of Pharmacia, Uppsala), GELOTTE[1] described the adsorption of aromatic and heterocyclic compounds, resulting in K_D values greater than 1. Nucleic acid components were among the substances retarded. Further K_D values for purine and pyrimidine bases, nucleosides and nucleotides on Sephadex G-25 were given by ZADRAZIL *et al.*[2] and by HOHN AND POLLMANN[3]. As the sieving effect of this gel starts only at a molecular weight of 1000, the separations obtained were essentially due to the above-mentioned adsorption.

We surmised that on the more tightly cross-linked gels Sephadex G-10 and G-15 the molecular sieving effect might play an effective role, while the increased amount of

TABLE I

K_D VALUES ON VARIOUS GELS

	G-10	*G-15*	*G-25*
Adenine	6.0	4.26	2.42
Adenosine	3.36	2.92	1.87
Deoxyadenosine	3.23	2.81	1.81
AMP	0.91	1.37	1.35
dAMP	0.94	1.39	1.38
ADP	0.41	0.74	1.03
ATP	0.29	0.50	0.80
Guanine	3.35	2.80	2.02
Guanosine	2.43	2.29	1.77
Deoxyguanosine	2.66	2.40	1.73
GMP	0.8	1.07	1.25
Hypoxanthine	2.17	1.89	1.56
Inosine	1.26	1.31	1.25
Deoxyinosine	1.26	1.30	1.14
IMP	0.45	0.71	0.85
Xanthine	3.43	2.94	2.14
Xanthosine	1.83	1.95	1.56
XMP	0.60	0.79	1.09
Uric acid	3.15	2.89	2.00
Cytidine	1.43	1.26	1.29
Cytosine	0.99	1.06	1.13
Deoxycytosine	1.09	1.16	1.20
CMP	0.4	0.54	0.87
Uracil	1.56	1.50	1.18
Uridine	1.07	1.21	1.12
Deoxyuridine	1'09	1.15	1.05
UMP	0.45	0.74	0.86
Thymine	1.86	1.63	1.24
Thymine riboside	1.00	1.15	1.09
Thymidine	1.24	1.29	1.04
TMP	0.52	0.73	0.92
TDP	0.28	0.44	0.60
TTP	0.19	0.32	0.56
Orotic acid	1.31	1.22	1.22
Orotidine	0.62	0.70	0.93

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dry gel for a same bed volume would increase adsorption. The overall result would be a better separation between a base, its nucleoside and its nucleotide.

Materials and methods

The gels Sephadex G-10, G-15 and G-25 Superfine were used in 1.5 × 25 cm columns. Elution was done at 30 ml/h with 0.13 *M* ammonium formate pH 6.0 Hundred μl of a 2 mg/ml (or saturated) solution or mixture of the test substance(s) was used. The transmission at 254 nm (293 nm for uric acid) was recorded continuously. The void volume was determined with hemoglobin, high molecular weight RNA and Blue Dextran 2000.

Results and discussion

The K_D values obtained are given in Table I. They were rather independent of buffer and pH, but erratic in very dilute solutions. The calculated EHTP were approximately 0.04 cm on G-10 and G-15, 0.02 cm on G-10.

As expected the use of dextran gels with tighter cross-links than Sephadex G-25 had a double effect:

(i) an appreciable molecular sieving, as the molecular weights of the substances studied fell into the range in which the gels are operative (up to 700 for G-10, up to 1500 for G-15);

(ii) an increased adsorption of the substances already retained on G-25.

The net result is an accelerated elution of the nucleotides and a further retardation of the bases, in particular of the purine bases. The effect on the nucleosides is variable: some keep approximately the same K_D value, while it is increased for others (*e.g.* adenosine). A complete separation was obtained between thymine, thymidine and thymidylic acid, a mixture for which few good methods exist.

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