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Gel filtration behaviour of sulphur and selenium-containing nicotinamide-analogues on Sephadex G-25

Most of the procedures described for the separation and purification of NAD(P)-analogues, *e.g.* ion-exchange chromatography, paper-chromatography and high voltage electrophoresis, possess certain inherent disadvantages, especially when working with labile nucleotides. Recently convenient procedures have been published utilising Sephadex or DEAE-Sephadex[1,2]. In the course of investigations with the relatively labile thio-$NADP^+$ and seleno-$NADP^+$ (refs. 3–5) we found that Sephadex was a suitable material for the purification of enzymatically synthesised $NADP^+$-analogues. A complete separation of small amounts of thionicotinamide and selenonicotinamide from the corresponding $NADP^+$-analogues was achieved by chromatography on Sephadex G-10 and G-25 using distilled water as eluant. During these experiments we observed an increase in the adsorption to the bed material if oxygen is replaced by sulphur or selenium in the carbamoyl-group of the nicotinamide.

Experimental

Materials. Nicotinamide was obtained from E. Merck, Darmstadt, G.F.R. Thionicotinamide was a product of Aldrich Chemical Co., Milwaukee/Wisc., U.S.A. Selenonicotinamide was prepared according to the method described previously[3]. Selenobenzamide was synthesised in a similar way from benzonitrile and hydrogen selenide. Thionicotinamide was recrystallised from water and the selenoamides, mentioned above, from *n*-propanol. Blue Dextran 2000 and Sephadex G-25 fine (particle size 20–80 μ) were purchased from Pharmacia, Uppsala, Sweden.

Methods. The dry Sephadex (20 g) was allowed to swell in distilled water overnight. The gel was sedimented in a 2 × 90 cm glass column to a height of 29.5 cm. After equilibration of the column with distilled water, the substances to be tested were put on the column in a volume of 2.0 ml. Elution was then started with distilled water with a hydrostatic pressure of 60 cm. This gave a flow rate of 2.6 ml per min. The effluent was collected in fractions of 5.0 ml in an automatic fraction collector. The column was alcohol jacketed and held at constant temperature (+4°) by means of a cryostat. The fractions were analysed by measurement of their visible or UV absorption with a Zeiss spectrophotometer, model PMQ II. Wavelengths used were: 625 nm for Blue Dextran, 260 nm for nicotinamide, 278.5 nm for thionicotinamide, 327 nm for selenonicotinamide and 323 nm for selenobenzamide. The substances tested were separately filtered through the column.

Results and discussion

A substance submitted to gel filtration is preferentially characterised by its K_D value, which is calculated from the expression

$$K_D = \frac{V_e - V_o}{V_i}$$

The elution volume, V_e, was determined by measuring the effluent volume from the time of addition of the test solution to the point where the concentration gradient

of the eluted substance is maximum. V_o, the outer volume was experimentally determined as the elution volume for Blue Dextran. The inner volume, V_i, was calculated from a reference value for Sephadex G-25, $V_i = 2.5$ ml per g dry weight[6].

TABLE I

NICOTINAMIDE ANALOGUES FILTERED THROUGH A COLUMN OF SEPHADEX G-25

Substance	*Molecular weight*	*Quantity (mg)*	*Eluant*	K_D
Nicotinamide	122.13	2	Distilled water	1.4
Thionicotinamide	138.20	2	Distilled water	2.0
Selenonicotinamide	185.08	3.5	Distilled water	2.2
Selenobenzamide	184.09	approx. 4–5	Distilled water	3.0

The nicotinamide analogues were eluted almost quantitatively, but selenobenzamide underwent partial decomposition on the top of the gel bed, producing red selenium. The K_D value of nicotinamide (Table I) is in good agreement with the one reported by GELOTTE[7]. This author demonstrated in detail that retention of heterocyclic and aromatic substances occurs when distilled water is used as eluant. As can be noticed in Table I there is a shift to stronger adsorption to the bed material on going from nicotinamide and thionicotinamide to selenonicotinamide. Furthermore, selenobenzamide which has practically the same molecular weight as selenonicotinamide is adsorbed more strongly than the latter. It is thought that perhaps there is a connection between the adsorption behaviour of nicotinamide analogues and the fact that thionicotinamide is in equilibrium with its tautomeric thiol structure $S{=}\overset{1}{C}{-}NH_2 \rightleftharpoons HS{-}\overset{1}{C}{=}NH$ thus increasing the tendency to interact with the gel matrix.

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