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Note

Adsorbability to and desorbability from Sephadex G-15 of sodium and phosphate ions

TOSHIHIKO OKADA*
The First Department of Biochemistry, Kanazawa Medical University, Uchinada, Ishikawa 920-02 (Japan)
and
MINORU MIYAKOSHI and MASAO INOUE
Central Research Laboratory, Kanazawa Medical University, Uchinada, Ishikawa 920-02 (Japan)
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Sephadex G-15 is a well known gel having a molecular sieving function. However, the elution volume of an inorganic ion sometimes deviates from the expected value because of ion–gel matrix and ion–ion interactions[1]. One ion–gel matrix interaction is the adsorption of the ion to the gel; this phenomenon has been reported by many workers[2–11]. However, no quantitative analysis of ion adsorption to the gel has been reported. This report describes the results of quantitative adsorption experiments using sodium-22 labelled sodium chloride and phosphorus-32 labelled phosphoric acid.

EXPERIMENTAL

Chemicals

Sodium chloride, monosodium phosphate, $NaH_2{}^{32}PO_4 \cdot 2H_2O$, and disodium phosphate, $Na_2HPO_4 \cdot 12H_2O$, were of analytical grade from Wako (Osaka, Japan). Sodium-22 chloride (61.60 mCi/mg, 99% pure) and phosphorus-32 labelled phosphoric acid (carrier free, 99% pure) in 0.02 *M* HCl were obtained from New England Nuclear (Boston, MA, U.S.A.).

Eluents and samples

Distilled water and 0.025 *M* sodium phosphate buffer (pH 7.0) were used as the eluents.

The sample was $^{22}NaCl$ dissolved in distilled water. Unlabelled NaCl was added at various concentrations to the sample when necessary. Another sample was 0.218 μCi of $H_3{}^{32}PO_4$ ($7.5 \cdot 10^{-18}$ mmol) in either distilled water or sodium phosphate buffer.

Procedures

Sephadex G-15 (Pharmacia; dry particle diameter, 40–120 μm) was packed according to standard procedures in two kinds of glass tubes: a long column (Excel column Type SE-1000, 1000 mm × 19 mm; bed height, 90 cm; porous polystyrene

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support) and a short one (100 mm × 6 mm; bed height, 6 cm; glass wool support). The former contains 85 g of the gel and the latter 0.5 g for the experiments with $^{22}NaCl$, 0.43 g with $H_3{}^{32}PO_4$.

When the long column was used, a peristaltic pump (LKB; gear-box, 3:250) was inserted between the eluent reservoir and the top of the column to maintain a constant flow-rate of 12 ml/h. A 0.6-ml volume of sample solution was applied to the top of the column, and the eluate was collected in 10-min fractions using an LKB7000 Ultrorac fraction collector. The column was operated at 4°C.

A peristaltic pump was not used with the short column. A 1.0-ml volume of sample solution was applied to the column and was collected in 1.0-ml fractions. The column was operated at room temperature.

Quantitation of ions

The sodium-22 ion ($^{22}Na^+$) was counted in an Auto Well Gamma system (Model JDC-751, Aloca), and phosphorus-32 labelled phosphate ion was counted in a liquid scintillation counter (Model LSC-900, Aloca).

RESULTS AND DISCUSSION

Adsorption and desorption of $^{22}Na^+$

When 0.06 μCi of $^{22}NaCl$ ($8.3 \cdot 10^{-9}$ mmol) in distilled water were applied to the long column and eluted with distilled water no radioactivity was found in the eluate. This indicated that almost all of the applied radioactivity was adsorbed to the column. The adsorbed radioactivity was recovered in a single peak using sodium phosphate buffer as an eluent (Fig. 1). On the other hand, when 0.02 μCi of $^{22}NaCl$ in the same buffer were applied to the long column and eluted with buffer, almost all of the radioactivity appeared in a peak (data not shown).

In order to analyze quantitatively the adsorbability of Na^+ to the gel, 10 ml containing 0.02 μCi of $^{22}NaCl$ in 0.5 m*M* cold NaCl were applied to two short columns and eluted with distilled water. No radioactivity appeared in the eluates, and

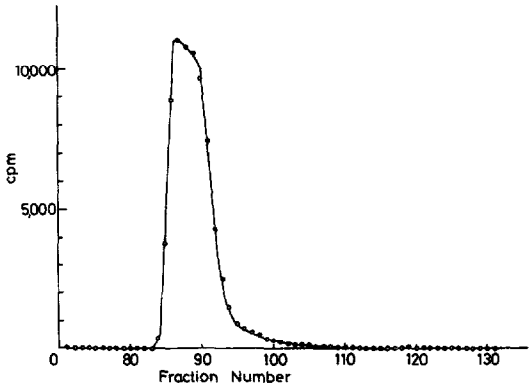

Fig. 1. Elution profile of $^{22}Na^+$ which had been adsorbed to Sephadex G-15. When 0.06 μCi of $^{22}NaCl$ in distilled water were eluted with distilled water from the long column no radioactivity was detected in the eluate. The adsorbed radioactivity was eluted with sodium phosphate buffer.

the gel was taken out from one of the columns and counted. From a count of 35 486 cpm, it was calculated that $3.2 \cdot 10^{-3}$ mmol of Na^+ were adsorbed to 1.0 g of gel. Assuming that all of the adsorbed sodium ion binds to the stray –COOH groups in the gel, the number of such groups to which Na^+ is adsorbed was calculated to be approximately $1.9 \cdot 10^{18}$ per gram of the gel. When the Na^+ adsorbed to the gel in the other short column was eluted with sodium phosphate buffer the eluate gave 40 055 cpm. This means that at least 88% of the radioactivity adsorbed was in the gel, not on the column glass.

Adsorption and desorption of phosphorus-32 labelled phosphate

When carrier-free labelled phosphate (0.218 μCi) in distilled water was applied to the long column and eluted with distilled water no radioactivity appeared in the eluate. This indicated again that the radioactivity was adsorbed to the column. In contrast to the case of Na^+, the adsorbed radioactivity was not recovered appreciably by eluting with either distilled water or sodium phosphate buffer. Therefore, the labelled phosphate seemed to bind tightly to the gel and was exchanged scarcely with the unlabelled phosphate in the eluent.

Pretreatment of the gel with buffer prevents the adsorption of phosphate. Of the 13 229 cpm of labelled phosphate in distilled water applied to the pretreated short column, only 945 cpm were adsorbed (Table I). This value is far less than that obtained (4365 cpm) with the non-treated column. However, when the labelled phosphate dissolved in buffer was applied to the treated short column and eluted with distilled water no radioactivity was adsorbed to the gel (Table I), indicating that unlabelled phosphate from the buffer prevented the adsorption of the labelled phosphate.

In order to determine quantitatively the adsorbability of the labelled phosphate to the gel, 100 μl of $H_3{}^{32}PO_4$ (13 229 cpm) solution were added to 1.0 ml of the buffer which had been diluted 0-, 100-, 1000- or 10 000-fold in distilled water. The samples were eluted with distilled water from the short columns. In every case almost all of the applied radioactivity was recovered in the eluate (Table I). Therefore, 100 μl of $H_3{}^{32}PO_4$ were added to 1.0 ml of distilled water when the solution was applied to the short column and eluted with distilled water, 4365 cpm (33%) were lost in the column

TABLE I

ELUTION BEHAVIOUR OF LABELLED PHOSPHATE ELUTED WITH DISTILLED WATER FROM A SHORT SEPHADEX G-15 COLUMN WHICH WAS EITHER NOT TREATED OR PRETREATED WITH BUFFER

	Non-treated		*Pretreated*	
	Distilled water	*Phosphate buffer[a] (2.5 μM)*	*Distilled water*	*Phosphate buffer*
Sample	13 229	13 229	13 229	13 229
Eluate	8 864	13 611	12 284	13 776
Difference	−4 365	+382	−945	+547

[a] Sodium phosphate buffer diluted 10 000-fold.

(Table I). Since the gel itself gave 4335 cpm, essentially all the labelled phosphate was adsorbed to the gel and not to the glass, *i.e.*, $6.3 \cdot 10^{-12}$ mmol ($3.7 \cdot 10^{9}$ atoms) per gram of gel.

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