Differential elution from a Sephadex G-15 column of sodium and phosphate ions of sodium phosphate with sodium or potassium phosphate buffer

Toshihiko Okada

First Department of Biochemistry, Kanazawa Medical University, Uchinada, Ishikawa 920-02 (Japan)

Minoru Miyakoshi and Masao Inoue

Central Research Laboratory, Kanazawa Medical University, Uchinada, Ishikawa 920-02 (Japan)

Mayumi Miyanabe, Yasuko Ueno and Yasuko Nakabayashi

Central Clinical Laboratory, Kanazawa Medical University Hospital, Uchinada, Ishikawa 920-02 (Japan)

ABSTRACT

When a sample solution containing sodium-22-labelled sodium chloride and carrier-free phosphorus-32-labelled phosphoric acid was eluted from a Sephadex G-15 column with either 0.025 M sodium or potassium phosphate buffer (pH 7.0), the labelled phosphate ion was eluted earlier than the sodium-22. The presence of cold 0.72 M sodium chloride with the sodium-22-labelled sodium chloride in the sample did not affect the elution sequence. When 1 M monosodium phosphate was eluted with distilled water from fresh and phosphate-treated Sephadex columns, the sodium and phosphate ions were eluted together in approximately the same fractions in both instances. From these observations, it is concluded that sodium ion repeatedly exchanges its partner phosphate ion with that in the eluent during its elution from Sephadex.

INTRODUCTION

When inorganic compounds are eluted from Sephadex, they sometimes do not obey the rule of steric exclusion but are affected by various side-effects (such as solute–gel matrix interactions and solute–solute interactions) which alter the elution volumes predicted from the sizes of the hydrated ions [1].

In previous work [2], we studied the elution of sodium or potassium chloride from a Sephadex G-15 column with 0.025 M sodium or potassium phosphate buffer (pH 7.0). The elution profile of the ions showed that the sample cation was accompanied by the phosphate ion from the eluent and was eluted in early fractions, whereas the chloride ion from the sample was accompanied by the cation of the eluent and was eluted in late fractions.

The following mechanism for the ion-exchange reaction was assumed: the phosphate ion from the eluent was eluted more rapidly than the chloride ion from the sample, so a cation-exchange reaction occurred between the sample and the eluent until all the cations of the ion pair from the sample had been replaced by cations from the eluent. The cation (from the sample)–phosphate ion pair thus formed

Correspondence to: Dr. T. Okada, First Department of Biochemistry, Kanazawa Medical University, Uchinada, Ishikawa 920-02, Japan.

was eluted earlier than the cation (from the eluent)–chloride ion pair.

In this study, we examined whether the cation and phosphate ion of the cation (from the sample)–phosphate ion pair were eluted together or separated during the elution. For this, a mixture of sodium-22-labelled sodium chloride and phosphorus-32-labelled phosphoric acid in sodium or potassium phosphate buffer was eluted with the same buffer and the elution profiles of the sodium-22 and phosphorus-32 were examined.

EXPERIMENTAL

Chemicals

Sodium chloride (NaCl), monosodium phosphate (NaH$_2$PO$_4\cdot$2H$_2$O), disodium phosphate (Na$_2$HPO$_4\cdot$12H$_2$O), monopotassium phosphate (KH$_2$PO$_4$) and dipotassium phosphate (K$_2$HPO$_4$) were of analytical-reagent grade from Wako (Osaka, Japan). Blue dextran 2000, a product of Pharmacia (Uppsala, Sweden), was purchased from Seikagaku Kogyo (Tokyo, Osaka, Japan). Sodium-22-labelled sodium chloride (^{22}NaCl, 61.60 mCi/mg, 99% pure) was obtained from New England Nuclear (Boston, MA, USA). Carrier-free phosphorus-32-labelled phosphoric acid in 0.08 M hydrochloric acid solution (H$_3$^{32}PO$_4$), produced by the Japan Atomic Energy Research Institute, was obtained from Japan Radioisotope Association (Tokyo, Japan).

Eluents and samples

The eluents were 0.025 M sodium and potassium phosphate buffers (pH 7.0) and distilled water.

The sample salts were 0.03 μCi of ^{22}NaCl and 0.03 μCi of H$_3$^{32}PO$_4$ dissolved in 0.6 ml of phosphate buffer, the eluent. In some experiments, the sample was added to cold 0.72 M NaCl solution. Monosodium phosphate solution (1 M) was also used.

Procedure

Sephadex G-15 (Pharmacia) (dry particle diameter 40–120 μm) was packed according to a standard procedure in a glass column (Excel type SE-1000, 1 m × 19 mm I.D.; bed height 90 cm, porous polystyrene support). A peristaltic pump (LKB) (gear ratio 3:250) was connected between the eluent reser-

voir and the top of the column to maintain a constant flow-rate (12 ml/h).

Previous experiments [3] showed that phosphate ion (P$^-$) was bound on the gel tightly and did not exchange with the P$^-$ in the eluent under the conditions used in these experiments. Therefore, to prevent adsorption of the phosphorus-32-labelled phosphate ion (^{32}P$^-$) on the gel, we pre-equilibrated the gel with phosphate buffer (the eluent) and dissolved carrier-free H$_3$^{32}PO$_4$ (the sample salt) in the eluent.

A sample of 0.6 ml of solution was applied to the top of the column. The eluate was collected in 10-min fractions with an LKB 7000 Ultrorac fraction collector. All columns were operated at 4°C.

Two sample–eluent systems, ^{22}NaCl · H$_3$^{32}PO$_4$–sodium phosphate buffer and ^{22}NaCl · H$_3$^{32}PO$_4$–potassium phosphate buffer, were employed. In each system, the sample in 0.72 M NaCl was also used. In one experiment, 1 M monosodium phosphate was eluted with distilled water.

Determination of ions

The amounts of sodium ion (Na$^+$) and potassium ion (K$^+$) were determined with a Corning Model 480 flame photometer (Corning Medical, Sudbury, UK) and chloride ion (Cl$^-$) was measured in a Corning Model 925 chloride analyser. Phosphate ion (P$^-$) was determined by the method of Fiske and Subbarow [4]. Sodium-22 (^{22}Na$^+$) was counted in a Model JDC-751 Auto well gamma system (Aloka, Tokyo, Japan), and phosphorus-32-labelled phosphate ion (^{32}P$^-$) in a Model LSC-900 liquid scintillation counter (Aloka). The Auto well gamma system counts ^{32}P$^-$ about 1.2% as efficiently as the liquid scintillation counter, and the liquid scintillation counter counts ^{22}Na$^+$ about 5% as efficiently as the Auto well gamma system. Hence the small ^{32}P$^-$ peak appearing within the ^{22}Na$^+$ fractions might actually result from counting ^{22}Na$^+$ by liquid scintillation.

RESULTS

Elution with sodium phosphate buffer

When a sample containing ^{22}NaCl and H$_3$^{32}PO$_4$ was eluted with sodium phosphate buffer (^{22}NaCl · H$_3$^{32}PO$_4$–sodium phosphate buffer system), the elution profiles showed that the peak fraction of the
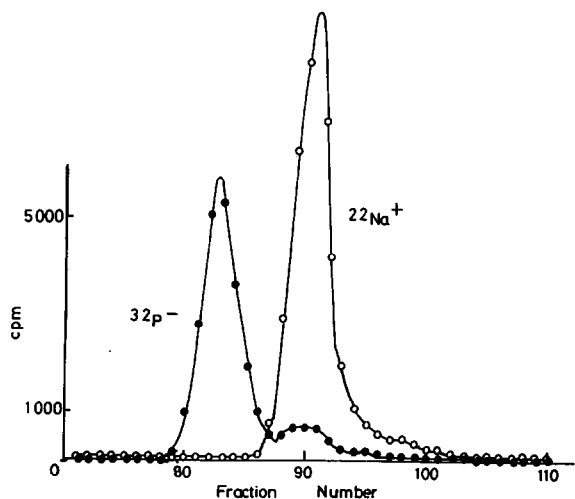
Fig. 1. Elution profiles of ^{22}Na$^+$ and ^{32}P$^-$ from a sample solution of ^{22}NaCl and H$_3$^{32}PO$_4$. Eluent: sodium phosphate buffer.

Fig. 3. Elution profiles of ^{22}Na$^+$ and ^{32}P$^-$ from the same sample as in Fig. 1 eluted with potassium phosphate buffer.

^{32}P$^-$ was No. 83 or seven fractions earlier than that of ^{22}Na$^+$ (Fig. 1). The small ^{32}P$^-$ peak within the ^{22}Na$^+$ fractions may not have been due to ^{32}P$^-$ but to ^{22}Na$^+$ counted in the liquid scintillation counter, as described under Experimental.

When the same sample in 0.72 M NaCl was eluted with sodium phosphate buffer, the ^{22}Na$^+$ spread because of the high concentration of cold NaCl. The peak fraction of the ^{32}P$^-$ was No. 80, which was ten fractions earlier than that of ^{22}Na$^+$ (Fig. 2).

Elution with potassium phosphate buffer

When a sample containing ^{22}NaCl and H$_3$^{32}PO$_4$ was eluted with potassium phosphate buffer

(^{22}NaCl · H$_3$^{32}PO$_4$–potassium phosphate buffer system), the elution profiles showed that the peak fraction of the ^{32}P$^-$ was No. 83, or two fractions earlier than that of ^{22}Na$^+$ (Fig. 3).

When the same sample in 0.72 M NaCl was eluted with potassium phosphate buffer, the ^{22}Na$^+$ spread. Again the peak fraction of the ^{32}P$^-$ was No. 83, which was earlier than that of ^{22}Na$^+$ (Fig. 4).

Elution with distilled water

First, 1 M monosodium phosphate was eluted with distilled water from a fresh Sephadex G-15 column, and then from a phosphate-treated column, to
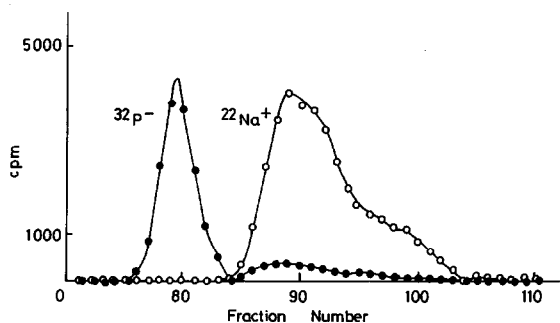

Fig. 2. Elution profiles of ^{22}Na$^+$ and ^{32}P$^-$ from the same sample as in Fig. 1 but in cold 0.72 M NaCl. Eluent: sodium phosphate buffer.
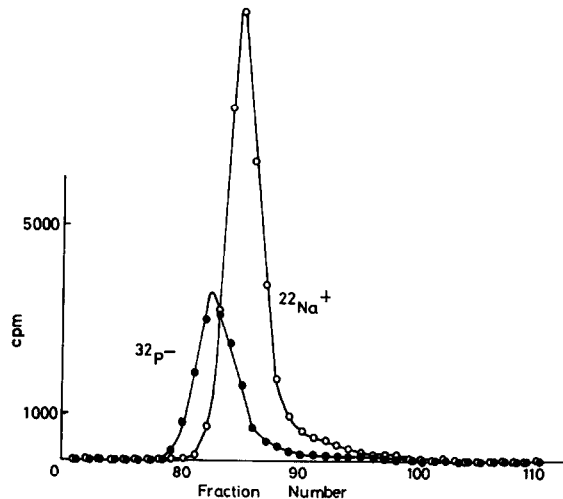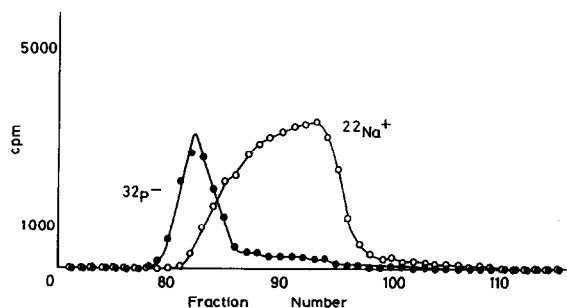

Fig. 4. Elution profiles of ^{22}Na$^+$ and ^{32}P$^-$ from the same sample as in Fig. 1 but in cold 0.72 M NaCl eluted with potassium phosphate buffer.
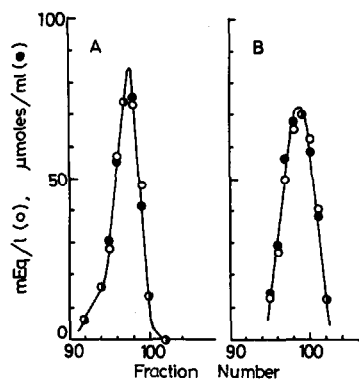
Fig. 5. Elution profiles of Na$^+$ (O) and P$^-$ (●) from 1 M monosodium phosphate solution eluted with distilled water from (A) a fresh Sephadex G-15 column and (B) a phosphate-treated column. mEq = milliequivalent.

determine whether Na$^+$ and P$^-$ from sodium phosphate in the sample were eluted together or separately. The two ions were eluted together in approximately the same fractions in both instances (Fig. 5).

DISCUSSION

To determine whether the Na$^+$ and P$^-$ of the Na$^+$P$^-$ ion pair formed by an ion-exchange reaction [2] are eluted together or separately, we prepared a sample solution containing ^{22}NaCl and H$_3$^{32}PO$_4$ in 0.025 M sodium (or potassium) phosphate buffer. We assumed that because of the much lower concentrations of ^{22}Na$^+$ and ^{32}P$^-$ than those of Na$^+$ (or K$^+$) and P$^-$ of sodium (or potassium) phosphate in the sample, the ^{22}Na$^+$ and ^{32}P$^-$ would promptly associate with the P$^-$ and Na$^+$ (or K$^+$), respectively, to form ^{22}Na$^+$P$^-$ and Na$^+$^{32}P$^-$ (or K$^+$^{32}P$^-$) ion pairs. Saunders and Pecsok [5] also observed similar ion exchange. The ^{22}Na$^+$Cl$^-$ ion pair in the sample, if it existed, would also be subjected to the ion-exchange reaction during elution with 0.025 M sodium (or potassium) phosphate buffer, the eluent, to form a ^{22}Na$^+$P$^-$ ion pair. As ^{22}Na$^+$ and ^{32}P$^-$ are eluted at the same velocity as Na$^+$ and P$^-$, respectively, the elution behaviours of ^{22}Na$^+$ and ^{32}P$^-$ should indicate whether the ^{22}Na$^+$ and P$^-$ of the ^{22}Na$^+$P$^-$ ion pair formed are eluted together or not.
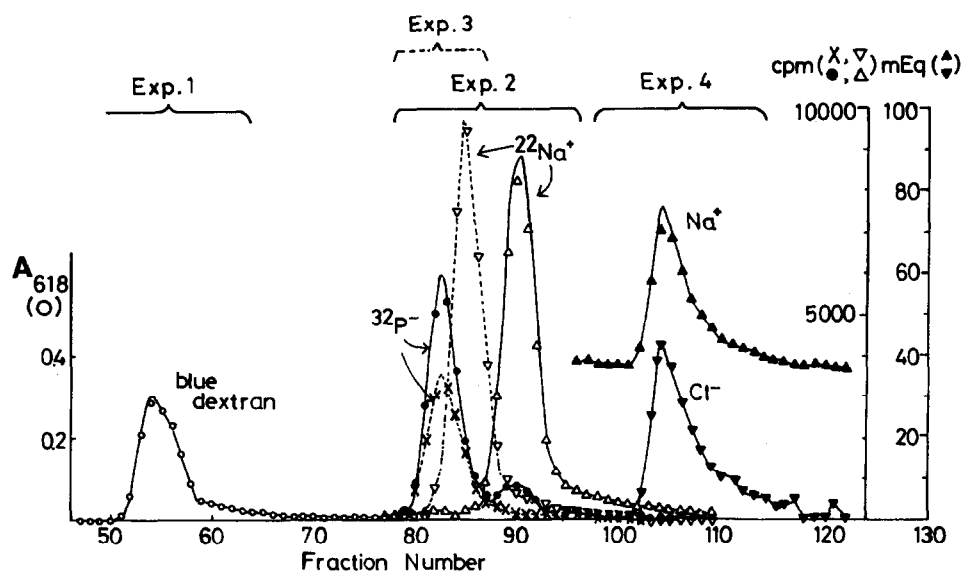

Fig. 6. Elution profiles of blue dextran and ions in four independent experiments. Experiment 1, blue dextran 2000 (10 mg/ml) eluted with sodium phosphate buffer (data not shown); experiment 2, ^{22}Na$^+$ and ^{32}P$^-$ in the ^{22}NaCl · H$_3$^{32}PO$_4$–sodium phosphate buffer system (solid line, from Fig. 1); experiment 3, ^{22}Na$^+$ and ^{32}P$^-$ in the ^{22}NaCl · H$_3$^{32}PO$_4$–potassium phosphate buffer system (dotted line, from Fig. 3); experiment 4, Na$^+$ and Cl$^-$ of the Na$^+$Cl$^-$ ion pair formed by the ion-exchange reaction in the NaCl–sodium phosphate buffer system (from ref. 2).

The results obtained showed that $^{22}Na^+$ was eluted more slowly than $^{32}P^-$ (Figs. 1–4), but more rapidly than the Na^+Cl^- ion pair which was formed by the ion-exchange reaction (Fig. 6). A possible explanation of this phenomenon is that the Sephadex which had previously adsorbed P^- behaved as a cation exchanger and the $^{32}P^-$ in the sample is excluded from the gel beads (ion exclusion), whereas the $^{22}Na^+$ was adsorbed (adsorption) and retarded. However, this possibility was unlikely because the amount of P^- adsorbed on the gel was $6.3 \cdot 10^{-12}$ mmol per gram of gel [3] and 2.5 μM phosphate buffer could completely prevent the adsorption of P^- on the gel under the present conditions [3]. Therefore, the amount of adsorbed P^- may be too small to affect the elution behaviour of P^- from 0.025 M phosphate buffer. With Na^+, $3.2 \cdot 10^{-3}$ mmol of Na^+ was adsorbed on 1.0 g of gel [3]. This amount is much more than that of P^- adsorbed on the gel, but is negligible compared with the high concentration of Na^+ in the buffer. Therefore, the retardation of $^{22}Na^+$ may not be due to its adsorption on the gel.

We performed additional experiments in which 1 M monosodium phosphate was eluted with distilled water from fresh and phosphate-treated gel to ascertain whether the Na^+ and P^- from the monosodium phosphate in the sample are eluted together or separately. If P^- is excluded from the gel and Na^+ is adsorbed, P^- and Na^+ should be eluted separately. However, the results showed that they were eluted together in approximately the same fractions from both fresh and phosphate-treated columns (Fig. 5). Therefore, ion exclusion and adsorption, if they occurred, did not affect the elution behaviour of the bulk of the Na^+ and P^- in the sample.

From these observations, we conclude that the differential elutions of $^{22}Na^+$ and $^{32}P^-$ in the sample were not due to ion exclusion of the P^- from and adsorption of Na^+ on the gel, but to exchange of Na^+ with the partner P^- in the eluent. In other words, acceptor P^- in the eluent is essential for exchange of the partner of Na^+, and Na^+ is pulled down by the P^-, released gradually from the P^- and bound to other P^- flowing through later (Fig. 7). This change of partners occurs repeatedly during the passage through the column. Na^+ or K^+ is separated from P^- in the column owing to their differences in penetrability into the gel particles, resulting
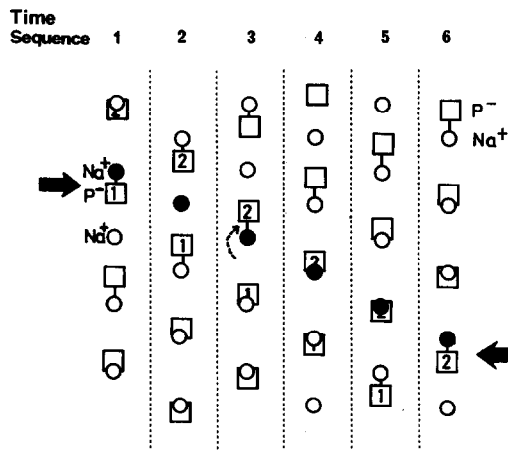

Fig. 7. Hypothetical scheme of the mechanism of changing partners. The sample was a solution containing sodium chloride labelled with ^{22}Na ($^{22}NaCl$) and phosphoric acid labelled with ^{32}P ($H_3{}^{32}PO_4$) in 0.025 M sodium phosphate buffer (the eluent). The $^{22}Na^+$ and $^{32}P^-$ promptly associated with P^- and Na^+ from the buffer to form $^{22}Na^+P^-$ and $Na^+{}^{32}P^-$ ion pairs, respectively, because the concentrations of $^{22}Na^+$ and $^{32}P^-$ were much lower than those of Na^+ and P^- in the buffer. When the sample was eluted with sodium phosphate buffer ($^{22}NaCl \cdot H_3{}^{32}PO_4$–sodium phosphate buffer system), $^{22}Na^+$ was eluted more slowly than $^{32}P^-$ but more rapidly than Na^+ of the Na^+Cl^- ion pair, which was formed by the ion-exchange reaction. As $^{22}Na^+$ and $^{32}P^-$ are eluted at the same velocity as Na^+ and P^-, respectively, the result indicates that Na^+ from the sample is gradually released from the partner P^- and binds to other P^- in later eluent. The figure shows one cycle of changing partners. The time sequence of elution is from left to right (1–6). Na^+ of the $Na^+(\bullet)$ $P^-(1)$ ion pair is gradually released from $P^-(1)$ and associates with $P^-(2)$ which comes later to form the $Na^+(\bullet)P^-(2)$ ion pair. The Na^+ in the eluent ($\bigcirc$) also changes partners by the same mechanism.

in weakening of the cation–anion bond. Hence not only the different elution velocities of the different ions in the column, but also the separation of the different ions by the Sephadex network may be involved in changing partners. The phenomenon of ion exchange also supports the changing-partner hypothesis, because if the binding of Na^+ to P^- in the Na^+P^- ion pair is tight, there will be no exchange of ions between the sample and the eluent.

Our results also showed that $^{22}Na^+$ was less separated from $^{32}P^-$ when eluted with potassium phosphate buffer (Fig. 3) than when eluted with sodium phosphate buffer (Fig. 1). As potassium phosphates are more soluble than Na_2HPO_4 in distilled water, the phenomenon is independent of the solu-

bility of the phosphates in the eluent, but might be due to the higher electronegativity of K^+ than Na^+, resulting in stronger binding of K^+ than of Na^+, and a lower efficiency of changing partners of $^{22}Na^+$ in the potassium phosphate buffer than in sodium phosphate buffer.

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