# Differential elution of sodium or potassium dihydrogen- and hydrogenphosphate ions from a Sephadex G-15 column with sodium or potassium chloride solution

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## Abstract

When a mixed solution of sodium or potassium dihydrogenphosphate and disodium or dipotassium hydrogenphosphate was eluted from a Sephadex G-15 column with either a sodium or potassium chloride solution, the elution profiles of ions showed that the hydrogenphosphate ion was eluted more rapidly than the dihydrogenphosphate ion. When the sample solutions containing potassium dihydrogenphosphate and/or dipotassium hydrogenphosphate, all of which were supplemented with phosphorus-32-labelled potassium dihydrogenphosphate, were eluted with sodium chloride solution, the elution profiles of radioactivity showed that the dihydrogenphosphate ion changed to hydrogenphosphate ion and vice versa, depending on the pH values of the sample solution and the availability of the cation of the eluent during elution for the phosphate ion to pair with.  © 2001 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Sephadex G-15 is a bead-formed, dextran gel chromatographic material capable of separating substances with molecular masses less than 1500. However, various side reactions such as solute–gel matrix and solute–solute interactions which alter the elution volume had been reported [1–3].

We observed previously a few new ion–ion inter-actions [4–7]. Firstly, when sodium or potassium chloride solution was eluted from a Sephadex G-15 column with either sodium or potassium phosphate buffer (0.025 *M*, pH 7.0) in various sample–eluent systems, the elution profiles of ions showed that the cation from the sample was associated with the phosphate ion of the eluent and eluted in early fractions, whereas the anion (chloride ion) from the sample was associated with the cation of the eluent and eluted in late fractions [4]. This phenomenon occurred because the phosphate ion from the eluent was eluted more rapidly than the chloride ion from the sample, so that the cations of the ion pairs from the sample and the cations of the ion pairs of the eluent were exchanged until all cations of the cation–

chloride ion pairs from the sample have been replaced by the cations of the cation–phosphate ion pairs of the eluent. We called this phenomenon ''(chromatographic) ion-exchange reaction''.

Secondly, it was observed that the cation and phosphate ions of the cation–phosphate ion pair were not eluted together, but separately. This conclusion came from the observation that when a sample solution containing sodium-22-labelled sodium chloride and phosphorus-32-labelled phosphoric acid was eluted from a Sephadex G-15 column with either sodium or potassium phosphate buffer (0.025 $M$, pH 7.0), sodium-22 ion was eluted in the latter fractions than phosphorus-32 ion, but in the earlier fractions than the sodium and chloride ion pair which was formed by an ion-exchange reaction [5]. This phenomenon was explained as follows: the cation of the cation–phosphate ion pair was pulled down by the partner phosphate ion, but released gradually and bound to other phosphate ion flowing through later. This reaction occurred repeatedly during passage through the column. We called this phenomenon ''change-partner reaction''. The results we observed were reviewed [6].

Thirdly, when a solution containing sodium or potassium dihydrogenphosphate or disodium or dipotassium hydrogenphosphate was eluted from a Sephadex G-15 column with either sodium or potassium chloride solution in various sample-eluent systems, it was observed that a negative peak of chloride ion coeluted with a positive peak of phosphate ion, in all sample–eluent systems employed. The mechanism of this phenomenon was assumed to occur by the coupled reaction of the ion-exchange and change-partner reactions including ion exclusion [7].This paper describes the elution profiles of ions when mixed solutions of sodium or potassium dihydrogenphosphate and disodium or dipotassium hydrogenphosphate were eluted from a Sephadex G-15 column with either sodium or potassium chloride solution. The elution profiles of the radioactivity is also described when the sample solutions containing potassium dihydrogenphosphate and/or dipotassium hydrogenphosphate which were supplemented with the phosphorus-32-labelled potassium dihydrogenphosphate were eluted with sodium chloride solution.

## 2. Experimental

### 2.1. Chemicals

Sodium chloride (NaCl), potassium chloride (KCl), sodium dihydrogenphosphate (NaH$_2$PO$_4$·2H$_2$O), disodium hydrogenphosphate (Na$_2$HPO$_4$·2H$_2$O), potassium dihydrogenphosphate (KH$_2$PO$_4$) and dipotassium hydrogenphosphate (K$_2$HPO$_4$) were analytical-reagent grade from Wako (Osaka, Japan).

Carrier-free phosphorus-32-labelled potassium dihydrogenphosphate (KH$_2^{32}$PO$_4$) in 0.08 $M$ hydrochloric acid solution, produced by the Japan Atomic Energy Research Institute, was obtained from the Japan Radioisotope Association (Tokyo, Japan).

### 2.2. Samples and eluents

The sample solutions employed were the mixtures of equal volumes of two kinds of phosphate solutions dissolved in the eluent; 1 $M$ KH$_2$PO$_4$ and 1 $M$ K$_2$HPO$_4$ (KP+K$_2$P), 1 $M$ NaH$_2$PO$_4$ and 0.125 $M$ Na$_2$HPO$_4$ (NaP+Na$_2$P) and 1 $M$ NaH$_2$PO$_4$ and 1 $M$ K$_2$HPO$_4$ (NaP+K$_2$P). Therefore, the concentrations of each one of the phosphate salts in the sample solutions were half of those described above. Since Na$_2$P was not dissolved completely in the eluent at 0.25 $M$ or more at 4°C, but dissolved completely at 0.125 $M$, 0.125 $M$ Na$_2$P was used. In the radioactive experiments, sample solutions containing KP and/or K$_2$P supplemented with 1.11 kBq of KH$_2^{32}$PO$_4$ (K$^{32}$P) were eluted.

The eluent was 0.05 $M$ NaCl or KCl solutions.

Six sample–eluent systems were employed; KP·K$_2$P–NaCl, NaP·Na$_2$P–KCl and NaP·K$_2$P–NaCl systems in the cold experiments, and KP·K$_2$P·K$^{32}$P–NaCl, KP·K$^{32}$P–NaCl and K$_2$P·K$^{32}$P–NaCl systems in the radioactive experiments.

### 2.3. Procedure

Sephadex G-15 (Pharmacia, Uppsala, Sweden; dry particle diameter, 40–120 μm) was packed according to a standard procedure in a glass column (Excel type SE-1000; 1000×19 mm I.D.; bed height, 90 cm; porous polystyrene support). Care was taken to make the gel surface flat as an irregular surface causes the

appearance of a small artificial peak in the earliest part of the elution profile. A peristaltic pump (LKB Instruments, Rockville, USA; gear ratio 3:250) was inserted between the eluent reservoir and the top of the column to maintain a constant flow-rate (12 ml/h).

As phosphate ion ($P^-$) binds to the gel tightly and scarcely exchanges with $P^-$ in the eluent under the test conditions [8], before use we equilibrated the gel with 0.025 $M$ sodium phosphate buffer (pH 7.0) to prevent adsorption of $P^-$ from the sample to the gel. We then washed and equilibrated the gel with the eluent. A sample of 0.6 ml of solution was applied to the top of the column. The eluate was continuously monitored with a Refractomonitor Model SF1107 (Mitsumi Scientific, Tokyo, Japan) and collected in 10-min fractions using a LKB7000 Ultrorac fraction collector (LKB Instruments).

Elutions were preformed in a cold room at 4°C to avoid change of the elution behavior of ions by temperature shift.

## 2.4. Quantitation of ions

The amounts of sodium and potassium ions ($Na^+$ and $K^+$) were determined in a Corning Model 480 flame photometer (Corning Medical, Sudbury, UK), and chloride ion ($Cl^-$) was measured in a Corning Model 925 chloride analyzer (Corning Medical). The phosphate ion ($P^-$), irrespective of dihydrogen ($H_2PO_4^{1-}$) or hydrogen ($HPO_4^{2-}$), was determined by the method of Fiske and Subbarow [9]. However, when it is necessary to specify for the $P^-$ whether it is $H_2PO_4^{1-}$ or $HPO_4^{2-}$, they are abbreviated as $P^{1-}$ and $P^{2-}$ respectively, in this paper. The radioactivity of phosphorus-32-labelled phosphate ion ($^{32}P^-$) was counted in a Model LSC-900 liquid scintillation counter (Aloka, Japan).

## 2.5. Determination of pH

The pH of the eluates were measured at about 28°C in a pH meter Model H-7 (Horiba, Japan).

## 2.6. Drawing of the figures

In order to see the ion–ion interactions between

the ions from the sample and the eluent more easily in a figure, the zero level of the concentration of ions from the sample was adjusted to 50 μmol per ml (0.05 $M$) of ions from the eluent (Figs. 1–3).

## 3. Results

### 3.1. Elution profiles of ions

The mixed solutions of KP or NaP and $K_2P$ or $Na_2P$ were eluted with either NaCl or KCl solution and the elution profiles of ions were observed.

### 3.1.1. KP·$K_2P$–NaCl system (Fig. 1)

In this system, two $Na^+P^-$ peaks and one $K^+Cl^-$ peak were produced in the early and late fractions, respectively, indicating that the ions were exchanged between the ion pairs from the sample and the eluent. The two $P^-$ peaks were of approximately the same size, but the $Na^+$ peak was larger than the $P^-$ peak in the earlier $Na^+P^-$ peak and smaller in the latter, suggesting that the earlier peak mainly consisted of $2Na^+P^{2-}$ ion pair and the latter, of $Na^+P^{1-}$ ion pair.

Two negative peaks of $Cl^-$ coeluted with the two positive peaks of $P^-$.
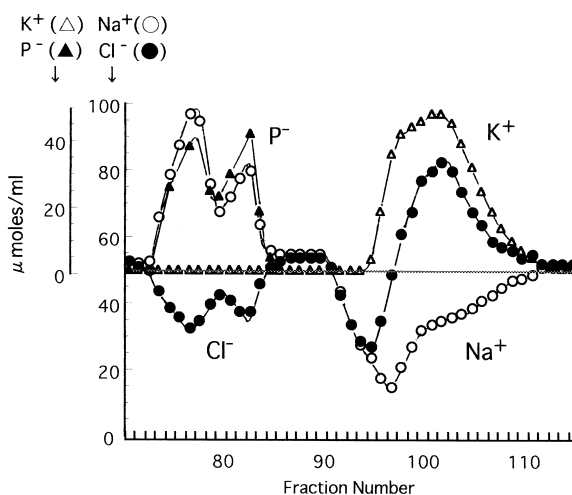


Fig. 1. Elution profiles of ions when a solution containing 1 $M$ $KH_2PO_4$ and 1 $M$ $K_2HPO_4$ was eluted with 0.05 $M$ NaCl solution (KP·$K_2P$–NaCl system).
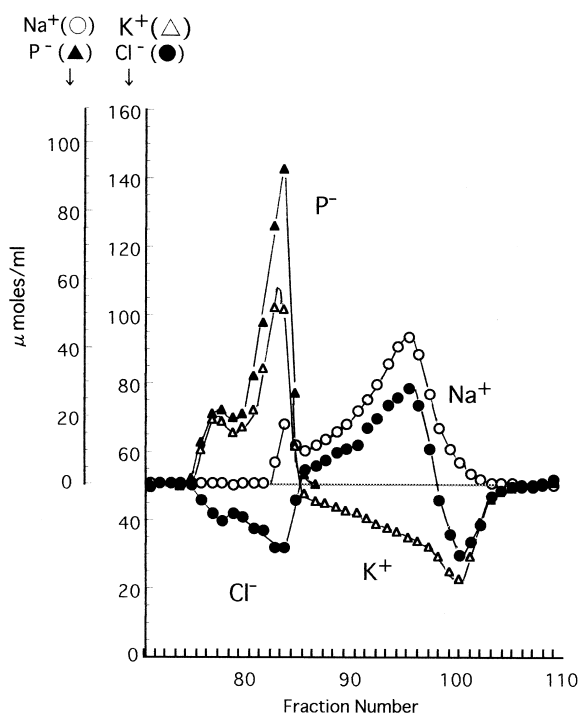
Fig. 2. Elution profiles of ions when a solution containing 1 *M* NaH$_2$PO$_4$ and 0.125 *M* Na$_2$HPO$_4$ was eluted with 0.05 *M* KCl solution (NaP·Na$_2$P·KCl system).



Fig. 3. Elution profiles of ions when a solution containing 1 *M* NaH$_2$PO$_4$ and 1 *M* K$_2$HPO$_4$ was eluted with 0.05 *M* NaCl solution (NaP·K$_2$P–NaCl system).

### 3.1.2. NaP·Na$_2$P–KCl system (Fig. 2)

In this system, two K$^+$P$^-$ peaks appeared in the early fractions, but the earlier K$^+$P$^-$ peak was extremely small, reflecting the low concentration of Na$_2$P (0.125 *M* instead of 1 *M*) employed for the sample solution, suggesting that the earlier P$^-$ peak consisted of 2K$^+$P$^{2-}$ ion pair and the later, of K$^+$P$^{1-}$. The peaks of K$^+$ and P$^-$ in the earlier K$^+$P$^-$ peak were similar in size, but the K$^+$ peak in the later K$^+$P$^-$ peak was smaller than the P$^-$ peak.

### 3.1.3. NaP·K$_2$P–NaCl system (Fig. 3)

In this system, the elution profiles of ions were similar to those observed in the KP·K$_2$P–NaCl system (Fig. 1). However, a small Na$^+$ peak appeared in the earliest part of the fractions of the K$^+$Cl$^-$ peak. The small Na$^+$ peak was supposed to be produced by ion-exchange reaction between the Na$^+$P$^-$ ion pair from the sample and the Na$^+$Cl$^-$ ion pair from the eluent. The Na$^+$ peak in the earlier Na$^+$P$^-$ peak was larger than the P$^-$ peak but smaller in the latter Na$^+$P$^-$ peak, indicating again that the earlier P$^-$ peak consisted of 2Na$^+$P$^{2-}$ ion pair and the later, of Na$^+$P$^{1-}$ ion pair.
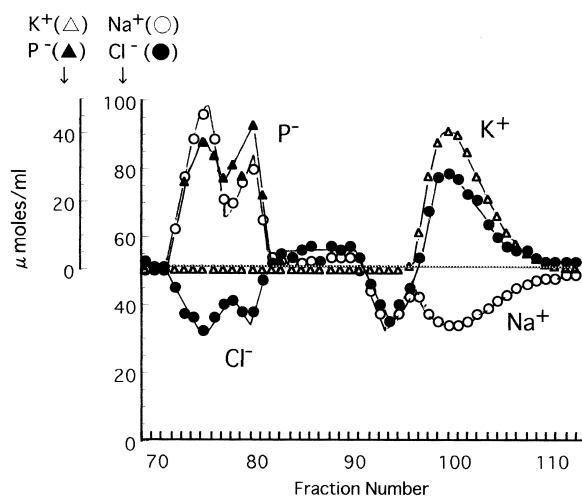
### 3.2. Elution profiles of radioactivity (Fig. 4)

When the sample solutions containing KP and/or K$_2$P which had been supplemented with KH$_2^{32}$PO$_4$, were eluted with NaCl solution, the elution behaviours of the radioactivity in the three systems were quite different from each other.

In the KP·K$_2$P·K$^{32}$P–NaCl system (Fig. 4A), two $^{32}$P$^-$ peaks of approximately the same size were produced, as was observed in the KP·K$_2$P–NaCl system (Fig. 1). Since the earlier peak was assumed to consist of 2Na$^{+32}$P$^{2-}$ ion pair and the later, of Na$^{+32}$P$^{1-}$, it was concluded that approximately half the amounts of the $^{32}$P$^{1-}$ in the sample solution changed to $^{32}$P$^{2-}$.

In the KP·K$^{32}$P–NaCl system (Fig. 4B), a peak of $^{32}$P$^-$ appeared in the later fractions which trailed to the earlier fractions and made a small peak. This elution profile indicated that a small amount of $^{32}$P$^{1-}$ in the sample solution changed to $^{32}$P$^{2-}$.
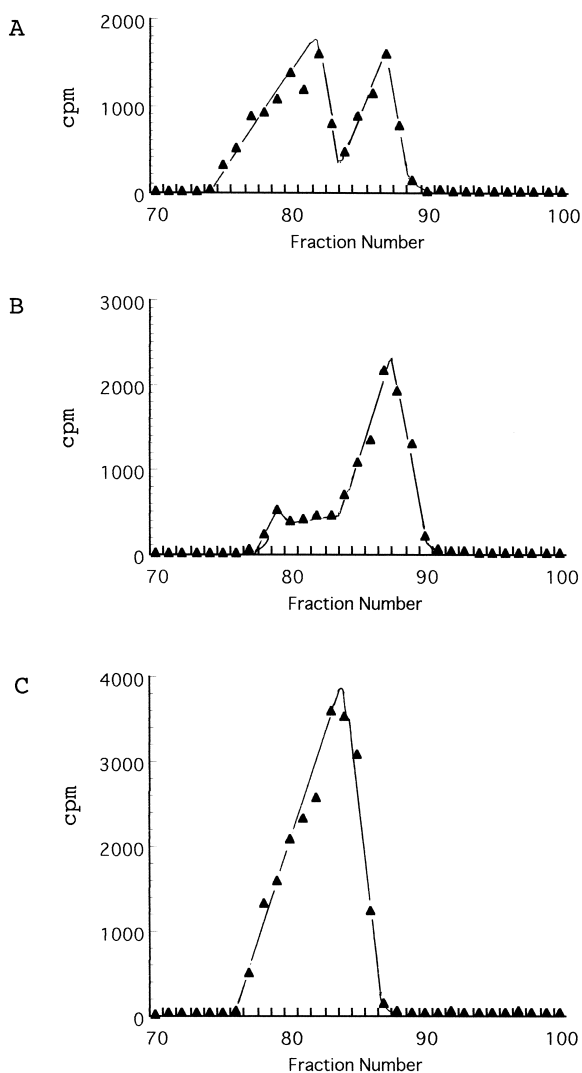
In the K$_2$P·K$^{32}$P–NaCl system (Fig. 4C), a large

Fig. 4. Elution profiles of the radioactivity when the sample solutions containing $KH_2PO_4$ and/or $K_2HPO_4$, which were supplemented with $KH_2^{32}PO_4$, were eluted with 0.05 $M$ NaCl solution. (A), $KP \cdot K_2P \cdot K^{32}P$–NaCl system; (B), $KP \cdot K^{32}P$–NaCl system; (C), $K_2P \cdot K^{32}P$–NaCl system.

### 3.3. pH of the eluates in the $KP \cdot K_2P$–NaCl system (Fig. 5)

The elution profile of radioactivity observed in the $KP \cdot K_2P \cdot K^{32}P$–NaCl system (Fig. 4A) was compared with the pH value of the eluates determined in the $KP \cdot K_2P$–NaCl system (Fig. 5). In Fig. 5A, a, b, c, and d are the fractions corresponding to those in Fig. 5B. The result showed that the earlier $P^-$ peak was alkaline and the latter one acidic, indicating that the earlier $P^-$ peak mainly consisted of $P^{2-}$ and the later of $P^{1-}$. Fig. 6 shows the pH of the solutions of each one of the four kinds of phosphate salts
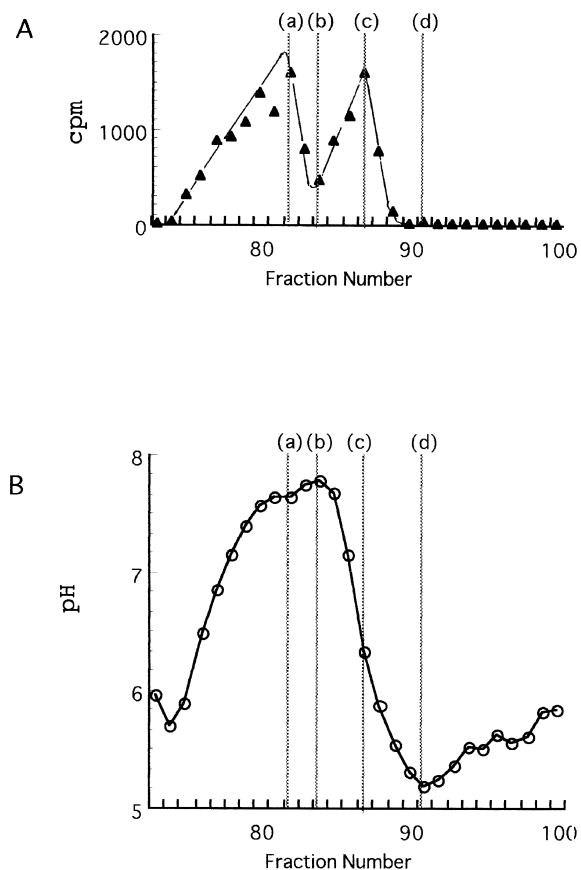


Fig. 5. (A), Elution profile of the radioactivity in $KP \cdot K_2P \cdot K^{32}P$–NaCl system; (B), pH values of the eluate in $KP \cdot K_2P$–NaCl system. The fractions (a), (b), (c) and (d) in (A) correspond to those in (B).

$^{32}P^-$ peak appeared between the earlier and later $P^-$ peak-fractions. This elution profile seemed to indicate that the peak consisted of the mixture of $^{32}P^{1-}$ and $^{32}P^{2-}$. In other words, changing from $^{32}P^{1-}$ to $^{32}P^{2-}$ and vice versa occurs repeatedly during elution.
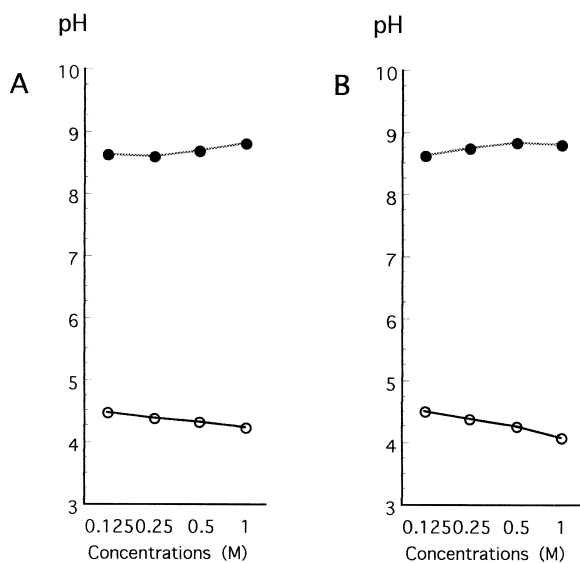
Fig. 6. The pH values of the solutions of $KH_2PO_4$ (KP) ($\circ$) and $K_2HPO_4$ ($K_2P$) ($\bullet$) (A), and $NaH_2PO_4$ (NaP) ($\circ$) and $Na_2HPO_4$ ($Na_2P$) ($\bullet$) (B) at four different concentrations.

employed (KP, $K_2P$, NaP and $Na_2P$) at four concentrations (0.125 $M$, 0.25 $M$, 0.5 $M$ and 1 $M$). The solutions of KP and NaP were acidic and those of $K_2P$ and $Na_2P$ were alkaline.

## 4. Discussion

### 4.1. Differential elution of $P^{1-}$ and $P^{2-}$

When a mixed solution of KP or NaP and $K_2P$ or $Na_2P$ were eluted with either 0.05 $M$ NaCl or KCl solution, the two $P^-$ peaks appeared in the early fractions in all of the three systems employed (Figs. 1–3). The earlier $P^-$ peak was assumed to consist of 2 cations–$P^{2-}$ ion pair and the latter, cation–$P^{1-}$ ion pair.

This assumption was supported by the following evidence. (1) The ionic size of 2 cations–$P^{2-}$ ion pair is larger than the cation–$P^{1-}$ ion pair. (2) In the $KP \cdot K_2P$–NaCl and $NaP \cdot K_2P$–NaCl systems, two $P^-$ peaks of approximately the same size were produced, but the $Na^+$ peak was larger than the $P^-$ peak in the earlier $Na^+P^-$ peak and smaller in the latter one (Figs. 1, 3). (3) In the $NaP \cdot Na_2P$–KCl system, the earlier $K^+P^-$ peak was extremely small

reflecting the low concentration of $Na_2P$ (0.125 $M$ instead of 1 $M$) employed for the sample solution (Fig. 2). (4) The pH of the fractions of the earlier $P^-$ peak were alkaline and of the latter, acidic (Fig. 5).

Comparing the pH value of the eluate (Fig. 5) with those of the solutions containing each one of the phosphate salts (Fig. 6), it was shown that the highest pH value of the eluate ($<8.0$) was lower than those of the solutions of $Na_2P$ or $K_2P$ ($>8.0$) and the lowest pH value ($>5.0$) was higher than those of the solutions of NaP or KP ($<5.0$). Therefore, it was concluded that the earlier $^{32}P^-$ peak consisted mainly of $2Na^+P^{2-}$ ion pair but mixed with the $Na^+P^{1-}$ ion pair more and more in the later fractions, and the later $^{32}P^-$ peak consisted mainly of $Na^+P^{1-}$ ion pair, but mixed with $2Na^+P^{2-}$ less and less in the later fractions.

### 4.2. Changing of $P^{1-}$ to $P^{2-}$ and vice versa, traced by the radioactivity of $^{32}P^-$

When the sample solutions containing KP and/or $K_2P$ supplemented with $K^{32}P$ were eluted with NaCl solution, the elution profiles of the radioactivity in the three systems were quite different from each other.

In the $KP \cdot K_2P \cdot K^{32}P$–NaCl system (Fig. 4A), the two $^{32}P^-$ peaks of approximately the same size appeared as was observed in the $KP \cdot K_2P$–NaCl system (Fig. 1), indicating that about half the amount of the $^{32}P^{1-}$ changed to $^{32}P^{2-}$ in the neutral sample solution. In the $KP \cdot K^{32}P$–NaCl system (Fig. 4B), a large $^{32}P^{1-}$ peak appeared in the latter fractions, which trailed to the earlier fractions and made a small peak, indicating that the small amounts of the $^{32}P^{1-}$ changed to $^{32}P^{2-}$. We assumed that this change tended to occur at the foot of the $P^{1-}$ peak, where the $P^{1-}$ solution was diluted and the pH value of the solution was elevated.

In the $K_2P \cdot K^{32}P$–NaCl system (Fig. 4C), a large $^{32}P^-$ peak appeared between the $P^{1-}$ and $P^{2-}$ peak fractions. This elution profile showed that the peak consisted of the mixture of $^{32}P^{2-}$ and $^{32}P^{1-}$. This phenomenon can be explained as follows; in the alkaline sample solution containing $P^{2-}$, some, if not all, of the $^{32}P^{1-}$ changed to $^{32}P^{2-}$. When eluted with NaCl solution, some of the $^{32}P^{2-}$ could not obtain

the two $Na^+$ ions from the eluent but only one to make $Na^{+32}P^{1-}$ ion pair by chance. This might occur because of the high concentration (1 *M*) and the rapid elution velocity of the $P^{2-}$. The $^{32}P^{1-}$ could obtain two $Na^+$ ions from the eluent to make $2Na^{+32}P^{2-}$ ion pair by chance. These forward and backward reactions repeat during elution, making a mixture of $^{32}P^{1-}$ and $^{32}P^{2-}$.

On the other hand, in the $KP \cdot K_2P \cdot K^{32}P$–NaCl system, the concentration of $P^{2-}$ in the sample solution was lower (0.5 *M*), and the $^{32}P^{2-}$ could obtain two $Na^+$ ions from the eluent and produced an earlier $^{32}P^-$ peak.

## 5. Conclusion

When a mixed solution of KP or NaP and $K_2P$ or $Na_2P$ was eluted from a Sephadex G-15 column with either 0.05 *M* NaCl or KCl solution, two $P^-$ peaks appeared in the early fractions in all of the three systems employed. It was concluded from several evidences that the earlier $P^-$ peak consisted of a 2 cation–$P^{2-}$ ion pair and the later one of a cation–$P^{1-}$ ion pair.

When the sample solutions containing KP and/or $K_2P$ which were supplemented with $K^{32}P$ were eluted with NaCl solution, the elution profiles of radioactivity in the three systems were quite different from each other. In the $KP \cdot K_2P \cdot K^{32}P$–NaCl system, two $P^-$ peaks were produced in the early fractions, indicating that about half the amount of the $^{32}P^{1-}$ changed to $^{32}P^{2-}$ in the neutral sample solution. In the $KP \cdot K^{32}P$–NaCl system, the $^{32}P^{1-}$ peak was produced in the latter fractions, which trailed to the earlier fractions and made a small peak, indicating that a small amount of $^{32}P^{1-}$ changed to $^{32}P^{2-}$. In the $K_2P \cdot K^{32}P$–NaCl system, a large $^{32}P^-$ peak

appeared between the earlier and later $P^-$ peak-fractions, indicating that some, if not all, of the $^{32}P^{1-}$ in the alkaline sample solution changed to $^{32}P^{2-}$, but the $^{32}P^{2-}$ could not always obtain two cations but one from the $Na^+Cl^-$ ion pair from the eluent and changed to $^{32}P^{1-}$ by chance, because of the high concentration of the $P^{2-}$ which was eluted rapidly. The $^{32}P^{1-}$ could change to $^{32}P^{2-}$ by obtaining two $Na^+$ ions by chance. These forward and backward reactions repeated during elution, making a mixture of $^{32}P^{1-}$ and $^{32}P^{2-}$.

Therefore, it was concluded that $P^{1-}$ changes to $P^{2-}$ and vice versa depending not only on the pH value of the sample solution but also on the availability of the cation from the cation–$Cl^-$ ion pair of the eluent for the $P^-$ to pair with, during elution.

## Acknowledgements

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