

Potassium and sodium chloride ion pairs are presumed to constitute a complex during elution from a Sephadex G-15 column with sodium phosphate buffer

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Abstract

When a mixed solution of 0.72 M potassium and sodium chloride was eluted from a Sephadex G-15 column with 0.025 M sodium phosphate buffer (pH 7.0), the elution profiles of ions showed that the potassium and chloride ion pair from the sample and the sodium and chloride ion pair produced by ion-exchange reaction, were eluted in the same fractions as if they constituted a complex. When a mixed solution of different concentrations of potassium and sodium chloride was eluted with the same buffer, the excess amount of one ion pair over the other was eluted freely from the presumed complex.

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1. Introduction

Sephadex G-15 is a dextran gel chromatographic material, capable of fractionating substances with molecular masses less than 1500. The chromatographic behaviours of inorganic ions in this Sephadex G-15 column were extensively studied by many workers from the early 1960s to the late 1970s [1]. They reported that the elution volume of ions was altered by the side effects such as solute–gel and solute–solute interactions. However, they determined either the cation or anion of the cation and anion ion pair from a sample and assumed that the counter ion accompanied the ion [1]. Therefore, the side effects were not completely elucidated.

In 1979, we double-checked the data described in a booklet published by Pharmacia, which showed on p.14 that when a solution of potassium chloride (KCl) was eluted from a Sephadex G-15 column with 0.025 M sodium phosphate buffer (pH 7.0), potassium and chloride ions (K^+ and Cl^-) from the sample were eluted together in a single peak [2]. However, when we determined the concentration distribution of all of the ions we employed in the eluate, it was found that K^+ was eluted in the early fractions and Cl^- in the late fractions, apparently against the electrical neutrality.

This phenomenon was found to occur by the following mechanism, i.e., because of the larger ionic size of the phosphate ion (P^-) than that of Cl^- , P^- accompanied by sodium ion (Na^+) (Na^+P^- ion pair) from the eluent was eluted more rapidly than the Cl^- accompanied by K^+ (K^+Cl^- ion pair) from the sample. When they passed each other, the ions

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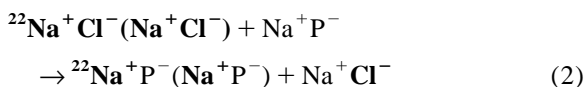
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were exchanged between them, producing the $\mathbf{K}^+\mathbf{P}^-$ and $\mathbf{Na}^+\mathbf{Cl}^-$ ion pairs until all of the \mathbf{K}^+ of the $\mathbf{K}^+\mathbf{Cl}^-$ ion pair from the sample were replaced by \mathbf{Na}^+ of the $\mathbf{Na}^+\mathbf{P}^-$ ion pair from the eluent during elution. This reaction can be formulated as follows (the bold face letters indicate the ions from the sample):



We called this phenomenon ‘(chromatographic) ion-exchange reaction’ [3].

The same phenomenon was observed in homocationic system, i.e., when a solution of sodium chloride (NaCl) supplemented with sodium-22-labelled sodium chloride ($^{22}\text{NaCl}$) was eluted with sodium phosphate buffer, $^{22}\text{Na}^+$ (and Na^+ from the sample) \mathbf{P}^- and \mathbf{Na}^+ (from the eluent) \mathbf{Cl}^- ion pairs were produced by ion-exchange reaction between the ion pairs from the sample and the eluent. Thus, the radioactivity of sodium-22-labelled sodium ion ($^{22}\text{Na}^+$) was eluted in the early fractions, because of being accompanied by \mathbf{P}^- . This reaction is formulated as follows (the bold face letters indicate the ions from the sample):



Then, in order to know whether the \mathbf{Na}^+ and \mathbf{P}^- of the $\mathbf{Na}^+\mathbf{P}^-$ ion pair was eluted together or separately, the mixed solution of $^{22}\text{NaCl}$ and phosphorus-32-labelled phosphoric acid ($\text{H}_3^{32}\text{PO}_4$) was eluted with 0.025 M sodium or potassium phosphate buffer [4]. The elution profiles of the radioactivities showed that the $^{22}\text{Na}^+$ from the sample was eluted in the later fractions than the phosphorus-32-labelled phosphate ion ($^{32}\text{P}^-$) from the sample, but in the earlier fractions than the $\mathbf{Na}^+\mathbf{Cl}^-$ ion pair produced by ion-exchange reaction. This phenomenon indicated that the \mathbf{Na}^+ of the $\mathbf{Na}^+\mathbf{P}^-$ ion pair was pulled down by the partner \mathbf{P}^- during elution, released gradually from the \mathbf{P}^- and paired with the other \mathbf{P}^- which flowing through later. In other words, the \mathbf{Na}^+ of the $\mathbf{Na}^+\mathbf{P}^-$ ion pair changed the partner \mathbf{P}^- repeatedly during elution. This reaction was called as ‘change-partner reaction’ [4]. These results described above were reviewed [5]. The change-partner reaction possibly occurs in general when the cation of the

cation and anion ion pair is eluted more slowly than the anion of the ion pair or vice versa.

This paper describes a novel elution profile of inorganic ions observed when the mixed solutions of the identical or different concentrations of KCl and NaCl were eluted from Sephadex G-15 column with 0.025 M sodium phosphate buffer.

2. Experimental

2.1. Chemicals

Potassium and sodium chloride (KCl and NaCl), potassium and sodium dihydrogenphosphate (KH_2PO_4 and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), dipotassium and disodium hydrogenphosphate (K_2HPO_4 and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) were of analytical grade from Wako (Osaka, Japan). The sodium-22-labelled sodium chloride ($^{22}\text{NaCl}$) was obtained from New England Nuclear (Boston, MA, USA) (1000 $\mu\text{Ci}/\text{ml}$, 61.60 mCi/mg, 99% pure).

2.2. Samples and eluents

The following solutions were employed as samples, i.e., the mixed solution of 0.72 M KCl and 0.72 M, 0.48 M or 0.24 M NaCl, and 0.48 M or 0.24 M KCl and 0.72 M NaCl. In an experiment, the mixed solution of 0.72 M KCl and 0.72 M NaCl supplemented with $^{22}\text{NaCl}$ (about 8.5×10^{-9} M and 1.11 kBq in 0.6 ml of the sample) was eluted. As controls, 0.72 M KCl and 0.72 M NaCl solutions were also employed. All salts employed were dissolved in the eluent.

The eluents used were 0.025 M potassium or sodium phosphate buffer (pH 7.0).

2.3. Sample and eluent systems

For example, when a mixed solution of 0.72 M KCl and 0.72 M NaCl was eluted with 0.025 M sodium phosphate buffer, it is referred to 0.72 M KCl·0.72 M NaCl:NaP-buffer system in this paper.

The following sample:eluent systems were employed. The 0.72 M KCl·0.72 M NaCl:NaP-buffer, 0.72 M KCl·0.72 M NaCl· $^{22}\text{NaCl}$:NaP-buffer, 0.72

M KCl· $0.48 M$ (or $0.24 M$) NaCl:NaP-buffer, and $0.48 M$ (or $0.24 M$) KCl· $0.72 M$ NaCl:NaP-buffer systems. As controls, $0.72 M$ KCl:NaP-buffer (heterocationic), $0.72 M$ NaCl:NaP-buffer (homocationic) and $0.72 M$ KCl· $0.72 M$ NaCl:KP-buffer systems were employed (where KP=potassium phosphate).

2.4. Procedures

The following procedure was employed as was in our previous experiments [4,5]. 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 prevent irregularity of the gel surface as this 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 (10.8 ml/h).

A 0.6-ml volume of sample 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 Ultrac fraction collector (LKB Instruments).

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

2.5. Quantitation of ions and counting radioactivity

Potassium and sodium ions (K^+ and Na^+) in the eluates were determined in a Corning model 480 Na-K 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^-) was not determined. The radioactivity of the sodium-22 ion ($^{22}\text{Na}^+$) was counted in an Auto-well γ system model JDC-751 (Aloca, Tokyo, Japan).

3. Results

3.1. $0.72 M$ KCl· $0.72 M$ NaCl:NaP-buffer and $0.72 M$ KCl· $0.72 M$ NaCl· $^{22}\text{NaCl}$:NaP-buffer systems (Fig. 1)

The mixed solutions of $0.72 M$ KCl and $0.72 M$ NaCl with or without supplement of $^{22}\text{NaCl}$ were eluted with NaP-buffer independently and the elution profiles of the radioactivity and of ions were observed and are shown in Fig. 1.

The phenomenon that the radioactivity of $^{22}\text{Na}^+$ was eluted in the early fractions indicated that ion-exchange reaction occurred between the $^{22}\text{Na}^+\text{Cl}^-$ (and Na^+Cl^-) ion pair from the sample and Na^+P^- (and Na^+P^-) ion pair from the eluent, producing $^{22}\text{Na}^+\text{P}^-$ (and Na^+P^-) and Na^+Cl^- ion pairs (see Eq. (2)). However, the K^+Cl^- ion pair from the sample was not subjected to ion-exchange reaction, while it was subjected to the reaction in $0.72 M$ KCl:NaP-buffer system (cf. Fig. 2a). The reaction was formulated as follows (the bold face letters indicate the ions from the sample);

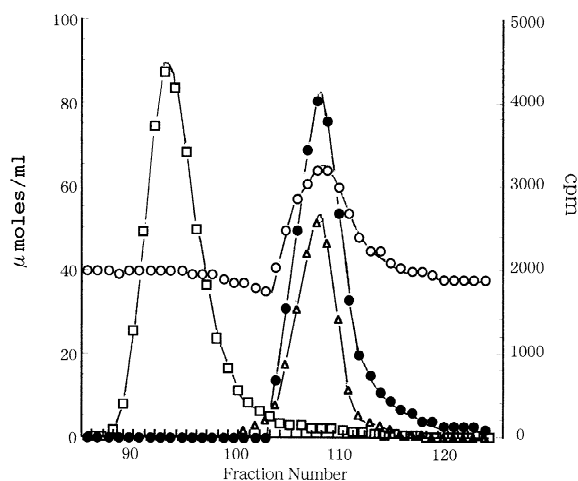
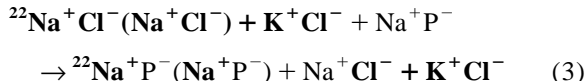


Fig. 1. Elution profiles of ions observed in $0.72 M$ KCl· $0.72 M$ NaCl:NaP-buffer system and that of radioactivity observed in $0.72 M$ KCl· $0.72 M$ NaCl· $^{22}\text{NaCl}$:NaP-buffer system were combined in a figure. Na^+ (\circ), K^+ (Δ), Cl^- (\bullet), $^{22}\text{Na}^+$ (\square).

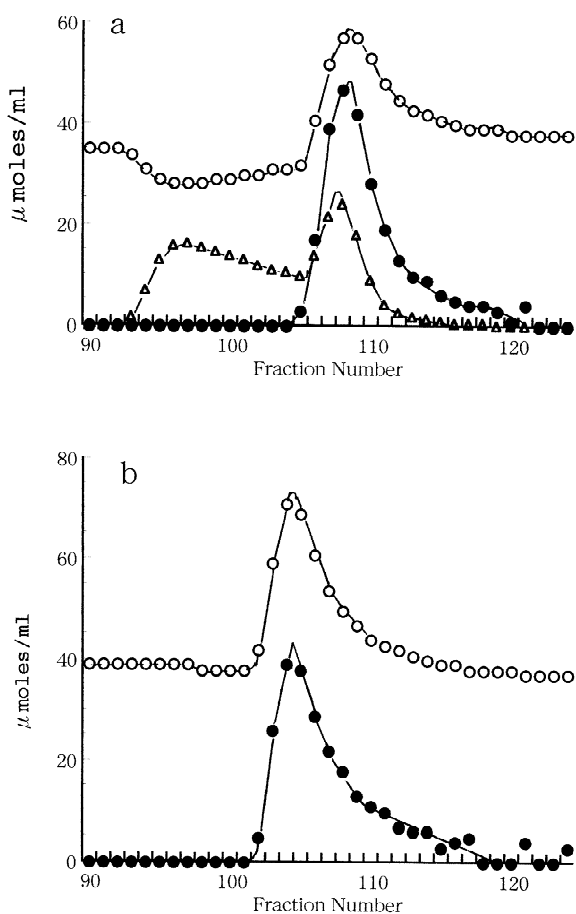


Fig. 2. Elution profiles of ions observed. (a) 0.72 M KCl:NaP-buffer system. (b) 0.72 M NaCl:NaP-buffer system. Na^+ (○), K^+ (Δ), Cl^- (●).

Unexpected phenomena observed were: (1) K^+Cl^- ion pair from the sample was not subjected to ion-exchange reaction, and (2) the Na^+Cl^- ion pair produced by the ion-exchange reaction was eluted in the same fractions of the K^+Cl^- ion pair in this system, although Na^+ was usually eluted more rapidly than K^+ (cf. Fig. 4).

In other words, the K^+Cl^- ion pair from the sample and the Na^+Cl^- ion pair produced by ion-exchange reaction were eluted in the same late fractions, as if they constituted a complex.

We recognized that a similar phenomenon was also observed in KCl:NaP-buffer system (Fig. 2a), i.e., Na^+Cl^- ion pair produced by ion-exchange

reaction was eluted in the peak fractions of K^+Cl^- ion pair.

3.2. 0.72 M KCl·0.48 M (or 0.24 M) NaCl:NaP-buffer system (Fig. 3)

When a mixed solution of 0.72 M KCl and 0.48 M NaCl was eluted with NaP-buffer, a small amount of the K^+ spread to the earlier fractions, presumably accompanied by P^- (Fig. 3a). This phenomenon indicated that a small amount of K^+Cl^- ion pair

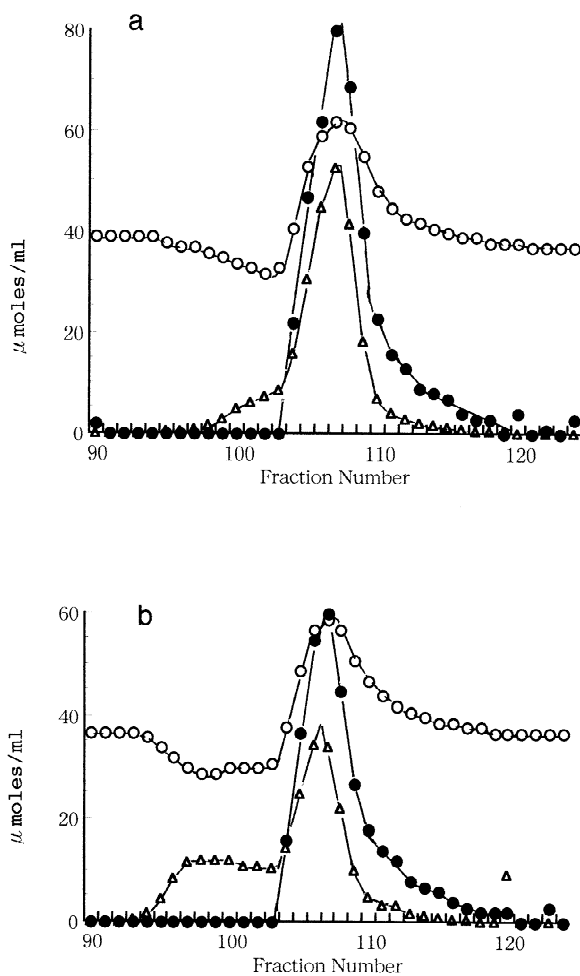


Fig. 3. Elution profiles of ions observed. (a) 0.72 M KCl-0.48 M NaCl:NaP-buffer system. (b) 0.72 M KCl-0.24 M NaCl:NaP-buffer system. Na^+ (○), K^+ (Δ) and Cl^- (●).

from the sample was subjected to ion-exchange reaction.

When a mixed solution of 0.72 M KCl and 0.24 M NaCl was eluted with NaP-buffer, much of the K^+ spread to the earlier fractions (Fig. 3b). These phenomena seemed to show that the excess amount of K^+Cl^- ion pair from the sample over the Na^+Cl^- ion pair produced by ion-exchange reaction was subjected to ion-exchange reaction.

3.3. 0.48 M (or 0.24 M) KCl·0.72 M NaCl:NaP-buffer system (Fig. 4)

When a mixed solution of 0.48 M KCl and 0.72 M NaCl was eluted with NaP-buffer, the elution profiles of ions showed that a small Na^+ peak appeared in the earlier fractions than that of K^+ (Fig. 4a).

When a mixed solution of 0.24 M KCl and 0.72 M NaCl was eluted with NaP-buffer, larger Na^+ peak appeared in the earlier fractions (Fig. 4b). Since the sum of K^+ and Na^+ in each fraction of the K^+ and Na^+ peaks were almost equal to the amount of Cl^- in the fractions (data are not shown), not only the K^+ of the K^+ peak but also the Na^+ of the Na^+ peak was concluded to be accompanied by Cl^- . Therefore, these elution profiles seemed to show that the excess amount of Na^+Cl^- ion pair produced by ion-exchange reaction over the K^+Cl^- ion pair from the sample was eluted freely from the presumed complex. This phenomenon also showed that the elution velocity of Na^+Cl^- ion pair is more rapid than K^+Cl^- ion pair (cf. Fig. 2b).

3.4. 0.72 M KCl·0.72 M NaCl:KP-buffer system (Fig. 5)

When a mixed solution of 0.72 M KCl and 0.72 M NaCl was eluted with KP-buffer, the elution profiles of ions were quite different from those observed when the same sample solution was eluted with NaP-buffer (cf. Fig. 1).

In this system, the K^+Cl^- ion pair from the sample was subjected to ion-exchange reaction, producing the K^+ (from the sample) P^- and K^+ (from the eluent) Cl^- ion pairs. Since the K^+P^- ion pair produced by ion-exchange reaction was the same kind as that of the eluent, no K^+P^- peak was produced. The K^+Cl^- ion pair produced by ion-

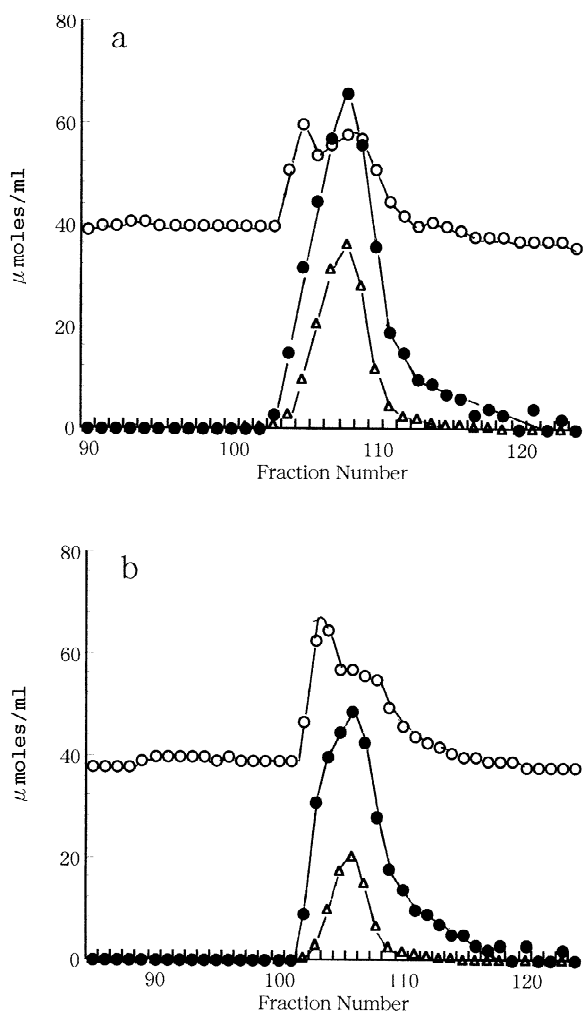


Fig. 4. Elution profiles of ions observed. (a) 0.48 M KCl·0.72 M NaCl:NaP-buffer system. (b) 0.24 M KCl·0.72 M NaCl:NaP-buffer system. Na^+ (○), K^+ (△) and Cl^- (●).

exchange reaction was eluted in the late fractions, because the K^+Cl^- ion pair was eluted more slowly than the K^+P^- ion pair from the eluent.

The Na^+Cl^- ion pair from the sample was also subjected to ion-exchange reaction with K^+P^- ion pair from the eluent, producing the Na^+P^- and K^+Cl^- ion pairs. The Na^+P^- ion pair was eluted in the early fractions producing a peak and the K^+Cl^- ion pair, in the late fractions (the bold face letters indicate the ions from the sample).

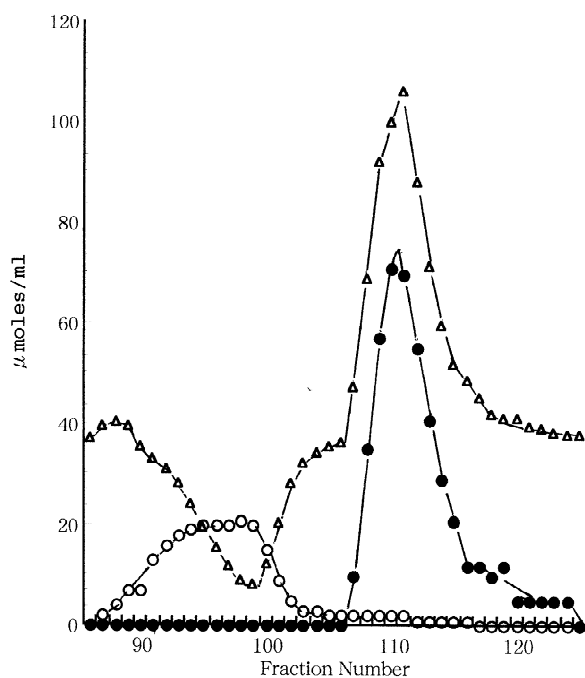
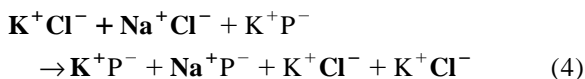


Fig. 5. Elution profiles of ions observed in 0.72 M KCl·0.72 M NaCl:KP-buffer system. Na⁺ (○), K⁺ (△) and Cl⁻ (●).



Thus, the sum of the K⁺Cl⁻ ion pairs produced by the two ion-exchange reactions made a large K⁺Cl⁻ peak in the late fractions, while a large negative peak of K⁺ was produced in the early fractions, instead. No Na⁺Cl⁻ ion pair was produced by ion-exchange reaction in this system, and no complex was formed (Fig. 5).

Therefore, the elution profiles of ions observed in this system was the combination of the elution profiles of ions which would be observed in KCl:KP-buffer and NaCl:KP-buffer systems.

4. Discussion

The elution profiles we observed in 0.72 M KCl·0.72 M NaCl:NaP-buffer system (Fig. 1) posed several questions to be answered. (1) Why was the K⁺Cl⁻ ion pair from the sample not subjected to

ion-exchange reaction in this system, although the K⁺Cl⁻ ion pair from the sample was subjected to the reaction in KCl:NaP-buffer system (Fig. 2a)? (2) Why was the Na⁺Cl⁻ ion pair produced by ion-exchange reaction eluted in the same late fractions as those of K⁺Cl⁻ ion pair in this system, although the excess amount of Na⁺Cl⁻ ion pair produced by ion-exchange reaction was eluted in the earlier fractions than those K⁺Cl⁻ ion pair from the sample in 0.72 M NaCl·0.48 M (or 0.24 M) KCl:NaP-buffer system (Fig. 3)? (3) Did the Na⁺Cl⁻ ion pair produced by ion-exchange reaction and K⁺Cl⁻ ion pair from the sample constitute a complex? And if they did, what kind of structure did the complex have? A possibility for the structure was that Na⁺ and K⁺ were exchanging their partner Cl⁻ frequently and repeatedly in such a high concentrations of ions in the fractions, and all of the three kinds of ions were eluted together. However this mechanism does not explain why the Na⁺Cl⁻ ion pair from the sample did not participate in forming the complex.

5. Conclusion

When a mixed solution of 0.72 M KCl and 0.72 M NaCl was eluted from a Sephadex G-15 column with 0.025 M NaP-buffer (pH 7.0), the K⁺Cl⁻ ion pair from the sample and the Na⁺Cl⁻ ion pair produced by ion-exchange reaction were eluted together in the same fractions, as if they constituted a complex.

When a mixed solution of 0.72 M KCl and the lower concentrations than 0.72 M of NaCl was eluted with NaP-buffer, the excess amount of K⁺Cl⁻ ion pair from the sample over Na⁺Cl⁻ ion pair was subjected to ion-exchange reaction. On the other hand, when the mixed solution of the lower concentrations than 0.72 M of KCl and 0.72 M NaCl was eluted with NaP-buffer, the excess amount of Na⁺Cl⁻ ion pair over the K⁺Cl⁻ ion pair was eluted by its own elution velocity in the earlier fractions than those of the presumed complex.

These phenomena seemed to indicate that the identical concentrations of K⁺Cl⁻ ion pair from the sample and Na⁺Cl⁻ ion pair produced by ion-exchange reaction were eluted together and the excess amount of K⁺Cl⁻ or Na⁺Cl⁻ ion pair over the other is eluted freely from the presumed complex.

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