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EXCHANGE OF THE CATION AND ANION OF THE SAMPLE (SODIUM OR POTASSIUM CHLORIDE) WITH THE CATION AND ANION OF THE ELUENT (SODIUM OR POTASSIUM PHOSPHATE BUFFER) AND THEIR ELUTION, FROM A SEPHADEX G-15 COLUMN, IN SEPARATE FRACTIONS

TOSHIHIKO OKADA* and KEIKO SUGATA
The First Department of Biochemistry, Kanazawa Medical University, Uchinada, Ishikawa 920-02 (Japan)
YASUKO NAKABAYASHI and KOHEI TERAOKA
Central Clinical Laboratory, Kanazawa Medical University Hospital, 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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SUMMARY

Various concentrations of sodium and potassium chloride were eluted with sodium or potassium phosphate buffer (0.025 *M*, pH 7.0) in various sample–buffer combinations from a Sephadex G-15 column. A more acidic buffer (pH 6.0) or a more concentrated buffer (0.125 *M*) were also used as the eluent. By observing the elution behaviour of all ions in the eluate, it was found that the cation from the sample accompanied by phosphate ion from the eluent was eluted in earlier fractions, and the chloride ion from the sample accompanied by the eluent's cation was eluted in later fractions in all homocationic and heterocationic sample–eluent combinations employed. The mechanism assumed was that chloride ion from the sample was eluted slowly and the phosphate ion from the eluent rapidly, resulting in the occurrence of cation-exchange reactions between the sample and the eluent.

INTRODUCTION

The chromatographic behaviour of inorganic compounds has been explained in terms of steric exclusion or the sieving effect in the gel phase. The size of the hydrated ions or lyotropic number affects the steric exclusion; the larger the solute molecule, the smaller is the elution volume[1–3]. However, many workers reported that, when inorganic compounds are eluted from Sephadex, various side effects are observed; they include both solute–gel matrix interactions, such as ion exchange[4–6] and adsorption[5,7–9] as well as solute–solute interactions[5,6,10] such as complexation[4,7] and ion exclusion (the Donnan exclusion effect)[4,5,11] (for a review see ref. 1). These effects alter the elution volumes predicted from the size of the hydrated ions. Such phenomena were also observed with cellulose gel (Cellulose-20)[12] and Bio-Gel P-2[4,13].

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Experiments on the elution behaviour of inorganic compounds on Sephadex were extensively carried out during the period from early 1960 to late 1970. However, pure water was used as an eluent in some experiments and in others the sample and eluent differed either in the cation or anion. When samples and eluents which differed in both the cation and anion were used, only the refractive index curves or the elution behaviours of either the anion or the cation were observed, thus the eluates were not totally characterized. Therefore, side effects in the elution behaviour on Sephadex were not completely elucidated.

In our experiments, when 0.20 *M* potassium chloride was eluted with 0.025 *M* sodium-phosphate buffer (pH 7.0) (heterocationic and heteroanionic system) from a Sephadex G-15 column, a complex refractive index curve of the eluate was observed. We then determined the concentration distribution of all of the ions in the eluate and found that the potassium and chloride ions from the sample were eluted in different fractions, *i.e.*, the potassium ion from the sample was accompanied by the phosphate ion from the eluent and eluted in the earlier fractions, and the chloride ion from the sample was accompanied by sodium ion from the eluent and eluted in the later fractions. Therefore, we examined the behaviour of each ion in relation to the counter ion in all combinations of sample (potassium chloride and sodium chloride) and eluent (potassium phosphate and sodium phosphate) under various conditions. This report describes the results and discusses the mechanism of the phenomenon observed.

EXPERIMENTAL

Chemicals

All reagents were of analytical grade from Wako Pure Chemical Industries (Osaka, Japan). Sodium-22 chloride (^{22}NaCl, 61.60 mCi/mg; 99% pure) was obtained from New England Nuclear (Boston, MA, U.S.A.).

Samples

Various concentrations of sodium chloride and/or potassium chloride dissolved in the eluent were used as sample solutions. In some experiments, the sample was ^{22}NaCl (see Chemicals) sometimes diluted in cold NaCl. The radioactivity of ^{22}NaCl was 0.03 μCi in 0.6 ml of sample solution applied.

Eluents

Distilled water and the following buffers were used as eluents. A 0.025 *M* sodium or potassium phosphate buffer (pH 7.0) was used for most experiments. An acidic buffer, 0.025 *M* sodium phosphate pH 6.0 and a concentrated pH 7.0 buffer, 0.125 *M* sodium phosphate, were also used.

Procedure

Sephadex G-15 (Pharmacia; dry particle diameter, 40–120 μm) was packed according to a standard procedure in two kinds of glass tube: column I (300 × 13 mm; bed height, 25 cm; glass fibre support) and column II (Excel Type SE-1000, 1000 × 19 mm; bed height, 90 cm; porous polystyrene support). Sephadex G-25 (Pharmacia; dry particle diameter, 50–150 μm) packed in the longer glass tube was also used (Fig. 8).

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 (12 ml/h). Volumes of 0.1 and 0.6 ml of the sample solution were applied to the tops of columns I and II respectively, and the eluate was continuously monitored with a Refractomonitor (Mitsumi Scientific Co., Japan; Model SF-1107). The eluate was collected in 10-min fractions using an LKB7000 Ultrorac fraction collector. All columns were operated at 4°C.

Four sample–eluent systems, two homocationic (sodium chloride–sodium phosphate buffer, and potassium chloride–potassium phosphate buffer) and two heterocationic (sodium chloride–potassium phosphate buffer, and potassium chloride–sodium phosphate buffer), were employed.

Quantitation of ions

The amounts of sodium ion (Na^+) and potassium ion (K^+) were determined in a Na–K flame photometer (Corning Eel Co., U.K.; Model 450). Chloride ion (Cl^-) was measured with a Chloride-Meter (Corning Eel, Model 920). Phosphate ion (P^-) was determined by the method of Fiske and Subbarow[14]. $^{22}Na^+$ was counted in an Auto-Well Gamma System Model JDC-751 (Aloca, Japan).

RESULTS

NaCl and/or KCl eluted with distilled water

When 0.68 *M* NaCl or 0.54 *M* KCl was eluted from column I with distilled water, the refractive index curves showed single peaks which were found to contain both the anion and cation from the sample. The elution volume of NaCl was slightly less than that of KCl. When a mixed solution of NaCl and KCl was applied to the column, the elution volume of each salt did not change appreciably and a divided peak appeared (data not shown). In order to analyze the elution profile of each ion, the mixed solution was eluted with distilled water from column II (Fig. 1). The earlier peak consisted mainly of Na^+, and the later peak mainly of K^+. The Na^+ was a little skewed toward the latter, while a portion of the K^+ accumulated in the earlier fractions, possibly because of ion–ion interaction. The Cl^- was eluted in two peaks, and accompanied both the Na^+ and K^+.

The Na^+ was eluted earlier than K^+, because hydrated Na^+ (3.58 Å) is larger than K^+ (3.31 Å)[2]. It is not likely to be due to stronger adsorption of K^+ to contaminating substances in the gel, because gel washed sequentially with Tween 80, distilled water, 1,4-dioxane and ethanol gave the same elution profile.

Elution profiles of ions in homocationic systems

When 0.68 *M* NaCl was eluted from column I with sodium phosphate buffer, the refractive index curve showed two peaks (Fig. 2A). The later (higher) peak consisted of almost all of the Na^+ and Cl^-, and the earlier (lower) peak contained some of the Na^+ accompanied by P^-.

When 0.54 *M* KCl was eluted with potassium phosphate buffer, similar refractive index curves and elution profiles were obtained (Fig. 2B), *i.e.*, the later peak consisted of most of the K^+ and Cl^- while the earlier peak contained the remaining K^+ and P^-. Thus, in both homocationic systems, most of the cation and anion from the sample are apparently eluted together.

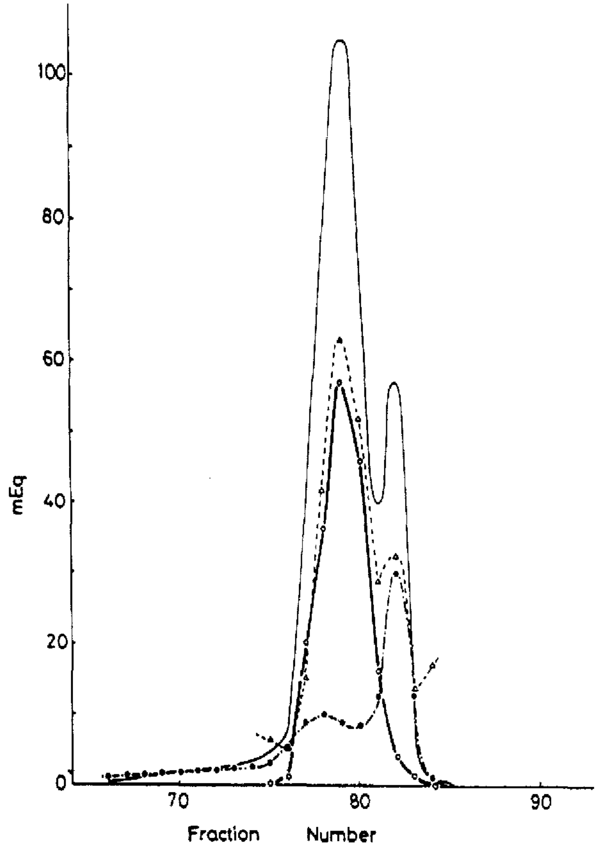

Fig. 1. Refractive index curve (—) and elution profiles of Na^+ (○), K^+ (●) and Cl^- (△) when a mixed solution of 0.72 *M* NaCl and 0.56 *M* KCl was eluted with distilled water from Sephadex G-15 column II.

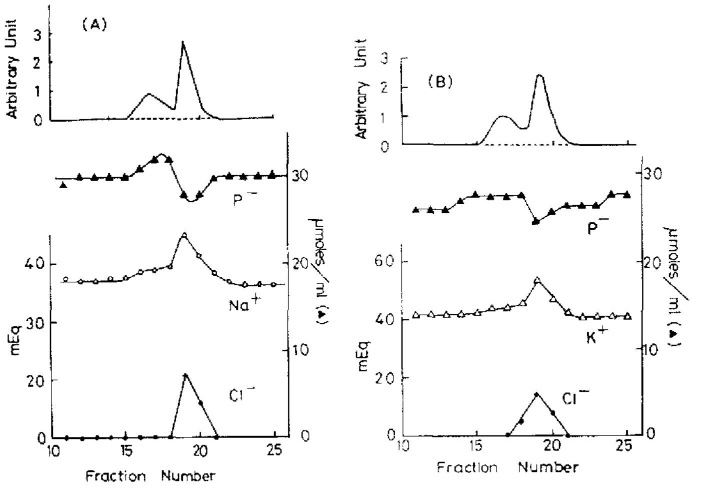

Fig. 2. Refractive index curves and elution profiles of each ion from column I, (A) when 0.68 *M* NaCl was eluted with sodium phosphate buffer, and (B) when 0.54 *M* KCl was eluted with potassium phosphate buffer.

Elution profiles of ions in heterocationic systems

When 0.68 *M* NaCl was eluted from column I with potassium phosphate buffer, the refractive index curve of the eluate showed two peaks with a negative peak in between. Contrary to the results obtained in homocationic systems, the elution profiles of each ion showed that the Na^+ and Cl^- from the sample were eluted in different fractions. The Na^+ accompanied by P^- was eluted first followed by K^+ and Cl^- (Fig. 3A).

When 0.20 *M* KCl was eluted from column I with sodium phosphate buffer, the refractive index curve showed one small negative peak followed by two positive peaks (Fig. 3B). The elution profiles of each ion revealed that the K^+ and Cl^- from the sample were completely separated from each other. The K^+ accompanied by P^- appeared in the first positive peak; Na^+ and Cl^- appeared in the second. Thus, in both heterocationic systems, the cation from the sample was accompanied by the anion (P^-) from the eluent and was eluted in early fractions; the anion (Cl^-) from the sample was accompanied by the cation from the eluent and was eluted in late fractions. This elution profile, which was quite different from that obtained by the use of distilled water as an eluent (Fig. 1), indicated that the differential elution of the cation and anion from the sample was caused by the interaction between the ions derived from the sample and eluent.

Effect of sample concentrations on the elution profiles of ions in heterocationic systems

When various concentrations of NaCl were eluted from column II with potassium phosphate buffer, the refractive index curves of the eluates were complicated and varied with the concentration of the sample (Fig. 4A). The higher the concentration, the later the fractions to which Na^+ spread. The K^+ from the eluent was apparently excluded from the earlier sodium fractions to the late fractions where it made a peak. At high sample concentrations (2.16, 2.87*M*), a sharp trailing spike of Na^+ appeared in fractions a little bit earlier than those in which the potassium peak occurred (Fig.

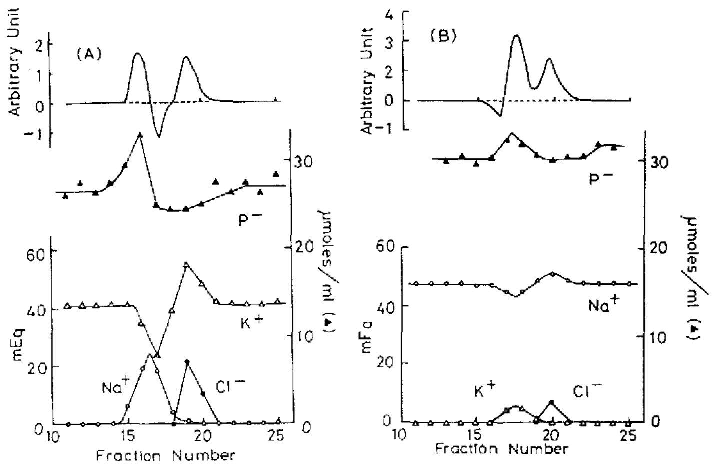

Fig. 3. Refractive index curves and elution profiles of each ion from column I, (A) when 0.68 *M* NaCl was eluted with potassium buffer, and (B) when 0.20 *M* KCl was eluted with sodium phosphate buffer.

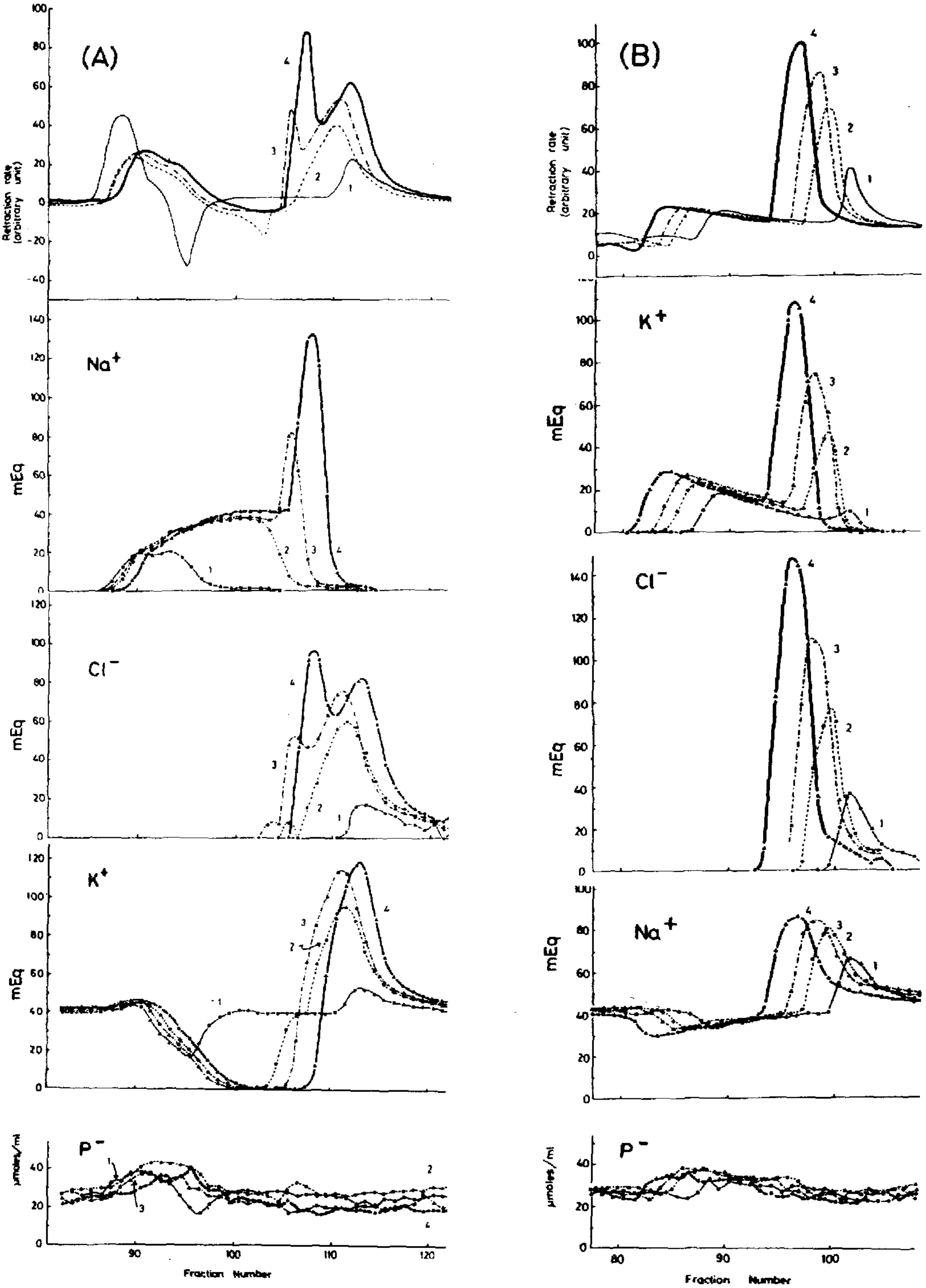

Fig. 4. Refractive index curves and elution profiles of each ion from column II, (A) when various concentrations of NaCl were eluted with potassium phosphate buffer and (B) when various concentrations of KCl were eluted with sodium phosphate buffer. Concentrations (*M*): NaCl: 0.72 (1), 1.44 (2), 2.16 (3), 2.87 (4); KCl: 0.56 (1), 1.13 (2), 1.69 (3), 2.25 (4).

4A). The Cl^- was thus split into two peaks: one which accompanied the potassium peak and the other which accompanied the Na^+ in the spike.

A similar experiment was performed with various concentrations of KCl eluted with sodium phosphate buffer (Fig. 4B). The refractive index curves differed from those obtained in the previous system. At the lowest sample concentration (0.56 *M*), the K^+ was distributed over many fractions and made a small broad spike where the Cl^- accompanied it. Thus, most of the K^+ and Cl^- from a sample were separated. The K^+ in the earlier fractions was accompanied by P^-. The Na^+ from the eluent was apparently excluded from the earlier potassium area to the later fractions where it made a peak. When the sample concentration increased, the elution volume of the K^+ decreased and the potassium spike became higher. In this system, the latter spike appeared in about the same fractions as the sodium peak. Therefore, the Cl^- accompanied both the potassium spike and the sodium peak.

Effects of pH and buffer concentration on the elution profile

When 0.56 *M* KCl was eluted from column II with sodium phosphate buffer (pH 6.0) the elution volume of the K^+ increased (Fig. 5B), compared with that obtained when the same sample was eluted with sodium phosphate buffer (Fig. 5A).

When 0.56 *M* KCl was eluted with 0.125 *M* sodium phosphate buffer, the K^+ did not spread and the elution volume of the Cl^- increased. The K^+ and Cl^-, therefore, were completely separated from each other (Fig. 5C).

Elution profile of $^{22}Na^+$ in an heterocationic system

In order to examine whether the higher salt concentration in the sample (rather than the eluent) was responsible for the association of the sample's cation with the P^- from the eluent, a solution containing an extremely low concentration of ^{22}NaCl ($5.5 \cdot 10^{-9} M$) was eluted with potassium phosphate buffer. The radioactivity was eluted within a sharp peak in early fractions (Fig. 6). From this result and those shown in Figs. 3A and 4A, it was concluded that the phenomenon was independent of the salt concentration in the sample.

Elution profile of $^{22}Na^+$ in an homocationic system

In homocationic systems, the cation and the anion from the sample were apparently eluted together in the late fractions. However, we hypothesized that the same ion-exchange reaction that occurs in heterocationic systems also occurs in homocationic systems. To confirm this, ^{22}NaCl was added to the cold NaCl solution and eluted with sodium phosphate buffer. The Na^+ and Cl^- appeared mainly in the late fractions, but the radioactivity appeared in the early fractions (Fig. 7). Even when the low concentration of ^{22}NaCl ($5.5 \cdot 10^{-9}$ *M*) was used as a sample, the $^{22}Na^+$ was eluted in the early fractions (Fig. 6).

We conclude from these results that not only in heterocationic systems but also in homocationic systems the cation from the sample was eluted in the earlier fractions and the cation from the eluent in the later fractions. However, since the cations from the sample and the eluent were the same, the negative peak of the eluent's cation was cancelled by the positive peak of the sample's cation as illustrated in Fig. 10. Although we have never performed the experiment in the other homocationic system (KCl–potassium phosphate buffer system), the same rule would be expected to operate in that system as well.

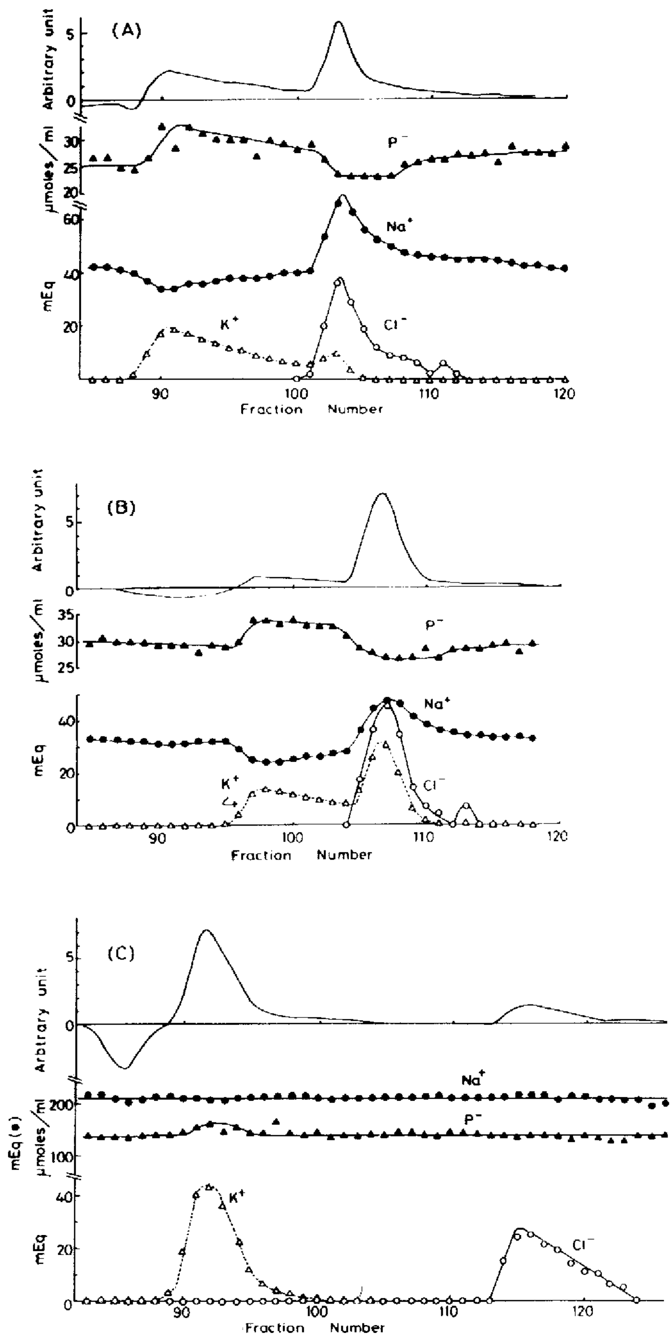

Fig. 5. Refractive index curves and elution profiles of each ion when 0.56 M KCl was eluted from column II, (A) with sodium phosphate buffer, (B) the same buffer (pH 6.0) and (C) 0.125 *M* sodium phosphate buffer.

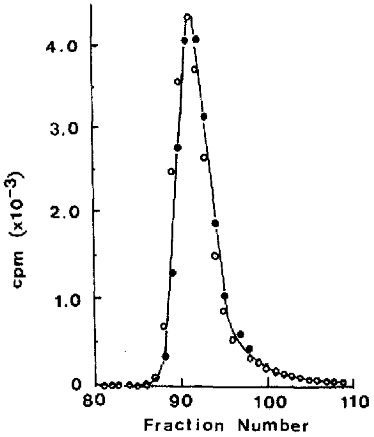

Fig. 6. Combined elution profiles of $^{22}Na^+$ obtained in two independent experiments, *i.e.*, ^{22}NaCl–potassium phosphate buffer (○) and ^{22}NaCl–sodium phosphate buffer (●) systems.

Sephadex G-25 column

When 1.13 *M* KCl was eluted with sodium phosphate buffer from a Sephadex G-25 column, the cation and anion from the sample were eluted in almost identical fractions (Fig. 8). This seems to show that the pore size of Sephadex G-15 is particularly suitable for the occurrence of the ion-exchange reaction.

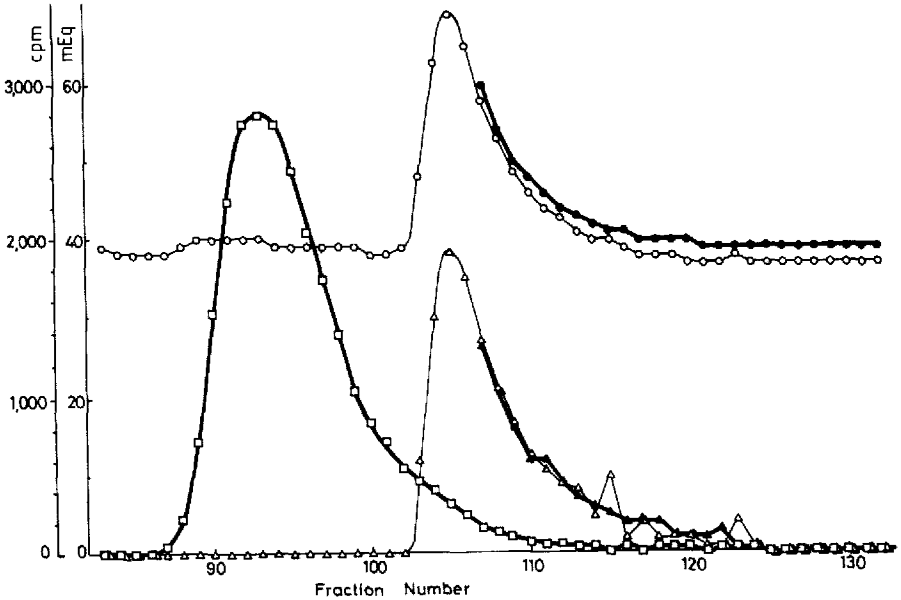

Fig. 7. Combined elution profiles of Na^+ and Cl^- obtained in two independent experiments: (1) sample, 0.72 *M* NaCl solution; eluent, sodium phosphate buffer Na^+ (○), Cl^- (△); (2) sample, ^{22}NaCl added to the same sample solution as in (1); eluent, sodium phosphate buffer, $^{22}Na^+$ (□), Na^+ (●) and Cl^- (▲) determined only for the fractions having low or no radioactivity.

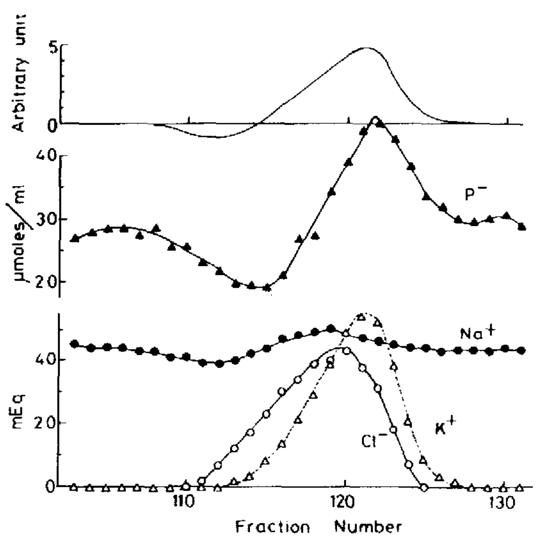

Fig. 8. Refractive index curve and elution profiles of each ion when 1.13 *M* KCl was eluted with sodium phosphate buffer from a Sephadex G-25 column.

DISCUSSION

In this study we used NaCl or KCl as a sample and sodium phosphate or potassium phosphate buffer as an eluent and observed that the cation from the sample accompanied by P^- from the eluent was eluted in earlier fractions, and Cl^- from the sample accompanied by the eluent's cation was eluted in later fractions in both homocationic and heterocationic systems (Fig. 2–7). This phenomenon was independent of the concentration of the sample salt (Fig. 6). On the other hand, when NaCl and/or KCl was eluted with distilled water, the bulk of the cation and anion from a sample salt were eluted together and the ion pair Na^+Cl^- was eluted slightly earlier than K^+Cl^- (Fig. 1). Therefore, the differential elution of the cation and anion from a sample described above was assumed to be caused by the interactions between the ions derived from the sample and the eluent.

Since Na^+ was eluted earlier than K^+ in the NaCl–potassium phosphate buffer system (Figs. 3A, 4A) and K^+ was eluted earlier than Na^+ in the KCl–sodium phosphate buffer (Figs. 3B, 4B), the elution volume of each cation was independent of the size of the molecule. Since the Na^+ from the sample was associated with the P^- in one heterocationic system (Figs. 3A, 4A), and the K^+ from the sample was associated with the P^- in the other (Figs. 3B, 4B), no preferential association of a cation with P^- was observed, and since the elution volumes of Na^+ and K^+ were reversed in the two heterocationic systems (Figs. 3, 4) no preferential adsorption of a cation to the gel matrix was observed. However, P^- was always eluted earlier than Cl^- and their elution sequence was not reversed in the two heterocationic systems (Figs. 3, 4).

We therefore tried to explain the ion-exchange reactions based on the phenomenon that P^- is eluted earlier than Cl^-. For convenience of interpretation, the gel phase can be divided into two phases according to the penetrability of P^- as described by Ogata *et al.*[7] The hydrated Na^+, K^+ and Cl^- which are smaller than hydrated P^- can penetrate into both phase I and phase II, hydrated P^- only into phase II. Thus P^- is eluted earlier than Cl^-. Before the sample is applied, some of the Na^+ or K^+ from the eluent remains in phase II contrary to the principle of electrical neutrality. Thus, some of the cation and the P^- from the eluent are separated topographically. Therefore, when the sample is applied, the Cl^- from the sample is promptly taken up by the phase I cation from the eluent; the cation from the sample associates with the P^- in phase II which has lost its counter ion. The ion pair Na^+P^- or K^+P^- thus formed is eluted in earlier fractions than Na^+Cl^- or K^+Cl^-. This mechanism explains the phenomenon we observed.

A second possibility is that, after the sample is applied to the column, Cl^- from the sample is eluted more slowly than P^- from the eluent due to some mechanism (discussed later). Then, the cation–Cl^- pair will meet the cation–P^- pair continuously during the elution and the ion-exchange reaction occurs, between the two ion pairs and continues until all of the cation from the sample is replaced by the cation from the eluent. Thus, the cation from the sample which associates with P^- would be eluted rapidly, the cation from the eluent which associates with Cl^- more slowly (Fig. 9). This mechanism also explains the phenomenon we observed. It is more plausible, because the cation and anion from the eluent are not separated topographically contrary to the principle of electrical neutrality.

Based on these mechanisms, we explain the phenomena we observed as follows.

The refractive index curve showed a negative peak between two positive peaks in the NaCl–potassium phosphate buffer system and a small negative peak followed by two positive peaks in the KCl–sodium phosphate buffer system (Fig. 3)

This occurred because in the NaCl–potassium phosphate buffer system the ion pair Na^+P^- was eluted more rapidly than K^+P^-; a low ion-concentration area (negative peak) was formed between the two ion pairs. On the other hand, in the KCl–

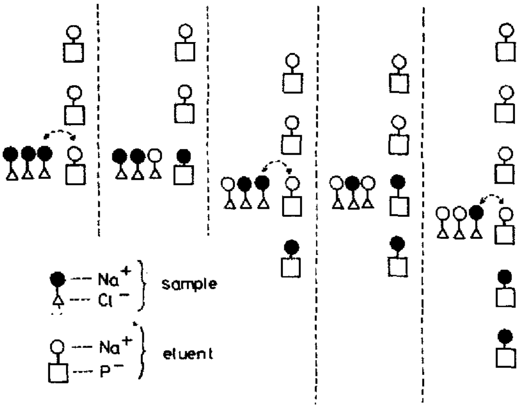

Fig. 9. Hypothetical scheme of the mechanism of the ion-exchange reaction. The time course is shown of the reaction when NaCl is eluted with sodium phosphate buffer: P^- is eluted more rapidly than Cl^-, hence a cation-exchange reaction occurs between the sample and the eluent, *i.e.*, Na^+ from the sample accompanies P^- from the eluent and is eluted more rapidly than the Cl^- from the sample which is associated with the cation from the eluent.

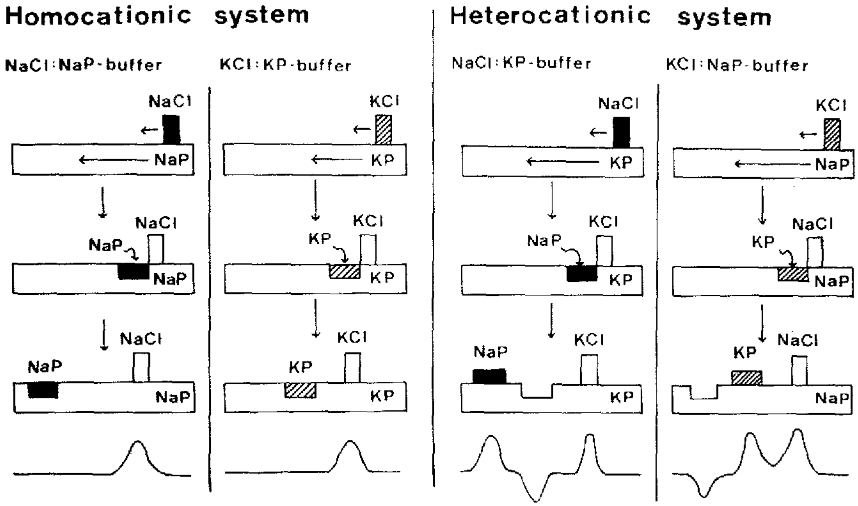

Fig. 10. Hypothetical scheme of the mechanism of formation of complex refractive index curves in homocationic and heterocationic systems.

sodium phosphate buffer system, the ion pair K^+P^- formed by the ion-exchange reaction was eluted now more slowly than the Na^+P^-. Therefore, the low ion-concentration area (negative peak) appeared in front of the ion pair K^+Cl^-. These behaviours of the ion pairs in heterocationic systems are illustrated in Fig. 10.

When an high concentration of sample salt was used in the heterocationic system, the cation from the sample spread to the later fractions (Figs. 4A and B)

The spreading can be explained as follows. The higher the concentration of the sample salt, the longer is the time needed to replace the cation from the sample with the cation from the eluent; the result is spreading. Ogata *et al.*[7] observed the spreading of magnesium ion from magnesium chloride eluted with sodium sulphate. However, they only observed the elution behaviour of Mg^{2+} and did not observe the other ions used. Therefore, they could not explain the mechanism of the spreading. This phenomenon can also be explained by the mechanism described above.

When the eluent concentration increased, the spreading of the cation from the sample decreased and the elution volume of Cl^- increased (Fig. 5C)

The spreading of the cation from the sample decreased because the time required for ion exchange is less due to the smaller difference between the salt concentration of the sample and the eluent.

The elution volume of Cl^- increased because the higher the ion concentration, the lower is the degree of hydration of both the ions and the gel matrix as assumed by Eaker and Porath[15]. This phenomenon is similar to that observed by Neddermeyer and Rogers[4] and Ueno *et al.*[16]

When extremely high concentrations of the sample salt were used in the heterocationic system, the sample's cation made a spike (Fig. 4A and B)

The spike fractions contained cation not associated with P^- entirely, but with Cl^-.

In NaCl–potassium phosphate buffer, the concentration of Na^+ in the spread was higher in the later fractions and the sodium spike did not appear at lower concentrations (0.72 and 1.44 M) of NaCl (Fig. 4A); in KCl–sodium phosphate buffer, the concentration of K^+ in the spread was lower in the later fractions and the potassium spike appeared even with the lowest concentration (0.56 M) of KCl used (Fig. 4B)

These elution profiles indicate that NaCl ionizes efficiently to the maximum level throughout under the ionic strength, while KCl ionizes at a fixed rate, resulting in a decrease of the amount of K^+ as the unionized KCl decreases.

The sodium spike and potassium peak were in different fractions in the NaCl–potassium phosphate buffer system (Fig. 4A), and in about the same fractions in KCl–sodium phosphate buffer (Fig. 4B)

Since in the NaCl–potassium phosphate buffer system, the ion pair K^+Cl^- formed was eluted more slowly than the sample (Na^+Cl^-) they were separated. In the KCl–sodium phosphate buffer system the ion pair Na^+Cl^- was expected to be eluted slightly earlier than the sample ion pair K^+Cl^-. However, the two ion pairs were eluted together, as if they were prevented from separating.

When acidic buffer was used, the elution volume, especially of the cation from the sample, increased (Fig. 5B)

Yoza and Ohashi[17] and Ueno *et al.*[6] showed that the elution volume of metal ions eluted with an acidic eluent was different depending on the anion used in the eluent. This was assumed to be due to the different adsorbability of the anions to the gel matrix.

The phenomenon we observed, however, might be ascribed to the decreased dissociation efficiency of both the sample and eluent; this results in a decreased efficiency in replacing the cation from the sample with the cation from the eluent.

In both homocationic and heterocationic systems, the negative peak of P^- was observed in chloride fractions and positive peak of P^- in the earlier fractions (Figs. 2, 3 and 5)

This phenomenon indicates that when P^- entered into the chloride region in the column, it passed through the region quickly, making a negative peak in the region and a positive peak in the earlier fractions. A possible mechanism is that Cl^- in the Sephadex beads excludes P^- from the beads, and the phase of the beads to which P^- can penetrate became smaller. Yoza described a similar phenomenon[1] and attributed it to the Donnan exclusion effect[1,16]. We do not know yet which mechanism is correct.

Saunders and Pecsok[13] described an ion-exchange reaction, *i.e.*, when a solution containing equal concentrations of barium chloride and potassium perchlorate is eluted with distilled water from Bio-Gel P-2 (100–200 mesh), an ion-exchange reaction occurs between the two sample salts, and KCl is eluted first, followed by barium perchlorate. They explained this phenomenon by partition of the ions, because the K_D values corresponding to the two peaks correlate well with those predicted for KCl and $Ba(ClO_4)_2$.

However, the mechanism of the ion-exchange reaction we observed is based on the assumption that P^- is eluted earlier than Cl^- from Sephadex G-15. The experimental results we obtained[18] indicate that this is the case. The differential elution of

P^- and Cl^- is possibly caused by the lower adsorbability of P^- than Cl^- on the Sephadex. Sinibaldi and Lederer[8] observed the adsorption of inorganic ions other than P^- and Cl^- on Sephadex G-25 and LH-20 in some eluents. The second possibility is that since Sephadex is considered as an inert support of the aqueous portion of the eluent just as the paper in paper chromatography is, the differential elution of P^- and Cl^- is possibly due to the different partition of P^- and Cl^- to the water which is bound on the Sephadex[19]. The third possibility is that P^- and Cl^- are eluted differentially by the molecular sieving function of the gel, *i.e.*, the ionic size of the hydrated P^- is sufficiently large to be eluted earlier than the hydrated Cl^-. That the ion-exchange reaction did not occur in a Sephadex G-25 column (Fig. 8) seems to show that the third possibility is plausible. Whatever the mechanism is, the P^- from the eluent is eluted earlier than Cl^- from the sample, and a cation-exchange reaction occurred between the sample and eluent. Therefore, in the case where the cation and anion from a sample are eluted differentially, one should consider ion-exchange reaction between the sample and eluent as a possible mechanism.

CONCLUSION

When sodium chloride or potassium chloride was eluted with sodium or potassium phosphate buffer from Sephadex G-15 the cation from the sample was accompanied by the anion from the eluent (P^-) and eluted in earlier fractions, and the cation from the eluent was accompanied by the anion from the sample (Cl^-) and eluted in later fractions. This cation-exchange reaction was assumed to occur by the differential elution of P^- and Cl^-.

This mechanism would raise a general rule, *i.e.*, cation-exchange reaction occurs between the sample and the eluent when anions from the sample and the eluent are eluted differentially due either to the different adsorbabilities of the anions on the Sephadex, partition of the ions on the Sephadex or the molecular sieving function of the Sephadex.

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