Extraction of Quaternary Ammonium Ions by Lipophilic Peptide Derivatives

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Abstract
Lipophilic peptide derivatives carrying p-nitrophenol group as a reporting site were prepared, and extraction equilibria of quaternary ammonium ions by them were studied. The Leu-Leu-Leu derivative most effectively extracted quaternary ammonium ions from water into chloroform. The substitution of Leu by β-Ala led to marked reduction in the extractability except for the sequence of Leu-Leu-β-Ala, and the reduction of the extractability was dependent on the position of the substituted β-Ala, indicating the significant role of the amino acid sequences in the recognition of quaternary ammonium ions.

1 Introduction
Receptors for various target molecules have been prepared by three-dimensionally controlled molecular design so far.[1-3] Recently, we have reported the host compounds to develop a new extracting reagent, which is a conjugate of two kinds of sites; one is a molecular recognition site consisting of peptides and the other a reporting site to give optical signal as a result of selective binding with a target species.[4,5] Based on this concept, lipophilic peptides shown in Fig. 1 were prepared in this work. They have a peptide moiety as the molecular recognition site, p-nitrophenol moiety as the signal transduction site, and a long alkyl chain to enhance extractability of a target species.

Lipophilic peptide derivatives 2, 3, and 4 have sequences of mono-, di-, and tri-leusines, respectively. Compounds 5, 6, and 7 have peptide moieties consisting of two leusines and one β-alanine. The lipophilic amide 1 was prepared as a control. We have already reported the selectivity of 4 and 5 toward various amines in homogeneous chloroform solution,[4, 5] and identified that the peptide 4 is much more powerful in the binding of amines than the peptide 5 as well as the amide 1.[4,5] As a further extension of previous works,[4,5] the present work was planned to clarify effects of number of leusine residues in the

Fig. 1 Structures of lipophilic peptides
recognition site (peptides 1-4) and the position of a β-alanine residue in the recognition sites consisting of two leusines and one β-alanine (peptides 5-7) on extraction of tetrabutylammonium between water and chloroform. Furthermore, the extraction of other three kinds of ammonium compounds was also studied by using the peptides 1-7.

2 Experimental

Lipophilic extractants 1-7 were synthesized as reported.[4-6] Extraction equilibria of four ammonium compounds with the extractants 1-7 were analyzed spectrophotometrically. Tested ammonium compounds were tetrabutylammonium, tetramethylammonium, choline, and acetylcholine. A chloroform solution of each extractant (1-7, 8.3 × 10^{-5} mol dm^{-3}, 20 cm^3) was contacted with an aqueous solution (20 cm^3) containing a target quaternary ammonium chloride salt (0.01 mol dm^{-3}) and hydrochloric acid (5 × 10^{-3} mol dm^{-3}) under stirring. Then, a sodium hydroxide solution (2.0 mol dm^{-3}) was added dropwise into the aqueous phase. The pH of the aqueous phase and the spectrum of the organic phase were monitored after each addition of the sodium hydroxide solution. The spectral change caused by dissociation of p-nitrophenol moiety was observed through the exchange of phenolic proton of p-nitrophenol moiety with the target quaternary ammonium ion.

3 Results and Discussion

The peptide extractants 1-7 are monoprotic acids (HL) and they can extract a cation (Q⁺) from aqueous solutions into organic phase as shown in Scheme 1. Extraction constant K_{ex} is defined by eq. (1), where subscripts a and o refer to water and organic phases, respectively.

\[
K_{ex} = \frac{[QL]_a[H^+]_a}{[HL]_a[Q^+]_a}
\]  

Here, [QL], [HL], and [Q⁺] are concentrations of ion pair, free lipophilic peptide, and quaternary ammonium ion, respectively. When a 1:1 ion pair is extracted into the organic phase, eq. (2) can be derived.

\[
\Delta A = \frac{K_{ex}ε_{QL}[HL]_a[Q^+]_a}{[H^+]_a + K_{ex}[Q^+]_a}
\]

where the subscript t refers to total concentration of each species, and where \( \Delta A \) and \( ε_{ql} \) are apparent absorbance change and molar absorptivity of QL, respectively.

![Scheme 1 Proposed extraction mechanism of quaternary ammonium (Q⁺) by peptide extractant (HL)](image)

(350)
Figure 2 illustratively shows the typical spectral change of organic phases as a function of equilibrium pH of water phases in the extraction of tetrabutylammonium with the peptide 4; isosbestic points are observed indicating that only a single ion pair QL is formed in the organic phase. Figure 3 shows the relationship between the absorbance at 431 nm of organic phases and pH of water phases in extraction of four kinds of ammonium compounds with the peptide 4. All data points in Fig. 3 well fitted to curves calculated from eq. (2) using a non-linear least-squares program in the package of KaleidaGraph®. Table 1 summarizes refined values of $K_{eX}$ for extraction reaction of four ammonium compounds with the peptide 4 using data shown in Fig. 3. For comparison, Table 1 also gives refined $K_{eX}$ values for the extraction reaction of tetrabutylammonium with six homologs of the peptide 4.

Fig. 2 UV-visible spectra of organic phases equilibrated with aqueous phases of various pH. HL: peptide 4; Q+: tetrabutylammonium

First, we would like to discuss the effect of number of leusine residues in the recognition site. As judged from Table 1, the peptide 4 can extract tetrabutylammonium much more effectively than the extractants 1, 2, and 3. Values of $K_{eX}$ for the peptides 1, 2, and 3 are nearly equal and less than that for the peptide 4 by about two orders of magnitudes. In other words, the extractability discontinuously increases when number of leusine residues increases from 2 to 3. This is the first interesting finding of this work.

Second, we would like to refer to the effect of the position of $\beta$-alanine residue in the recognition site consisting of two leusines and one $\beta$-alanine (peptides 5-7). The increasing order of the extractability for tetrabutylammonium is 6 < 5 << 7 < 4. This indicates that substitution of one leusine in the peptide 4 by one $\beta$-alanine reduces the extractability of the resulting peptides 5-7. However, the decrease in the extractability of 7 is not so marked compared with cases of the peptides 5 and 6. Thus, this study revealed that the extractability of the peptides 5-7 depends on the position of $\beta$-alanine in peptide moieties consisting of two leusines and one $\beta$-alanine, which is the second interesting finding of this work. Here, we would like to emphasize that our two findings mentioned above can not be explained by difference in lipophilic properties of the peptides 1-7, because log $P$ values for the peptides 1-7 are almost same (data not shown).

Last, we refer to extraction of tetramethylammonium, acetylcholine, and choline with the peptides 4 and 7. As judged from Table 1, the peptide 4 gives the selectivity sequence of tetrabutylammonium >> acetylcholine > choline > tetramethylammonium, but the peptide 7 does that of tetrabutylammonium >> choline > acetylcholine > tetramethylammonium. Both peptides 4 and 7 give quite reversed selectivity sequences toward acetylcholine.
and choline. This is the third interesting finding in this work.

We were able to add three interesting findings, which cannot be easily explained by classical theories on ion pair extraction. Since behavior of peptides in the lowly polar and non-polar solvents is complicated, we are not able to give clear explanations on our three findings at the present. However, it is expected that the more suitable sequences with the high affinity and selectivity will be able to find from a large number of various peptide sequences (peptide library).

![Fig. 3 Relationship between absorbance at 431 nm and pH of aqueous phases in the extraction of cations with the peptide extractant 4.](image)

Fig. 3 Relationship between absorbance at 431 nm and pH of aqueous phases in the extraction of cations with the peptide extractant 4. O; sodium, □; tetrabutylammonium, Δ; tetramethylammonium, ⬤; acetylcholine, ×; choline

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\(^{a}) Bar indicates that the target cation was not extracted.

**Table 1** Summary of refined \( K_{ex} \) values for extraction reactions of four ammonium compounds with peptide extractants 1-7

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**References**