

EFFECT OF HYDROPHOBICITY ON REVERSED MICELLAR EXTRACTION OF OLIGOPEPTIDES

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Introduction

Recently, a new extraction method using reversed micelles has been investigated by many investigators for separation of amino acids^{1,3,4,8)}, peptides^{4,6,7)} and proteins^{2,5)}. One of the advantages of this system for protein extraction is that the extraction can be carried out under mild conditions. Also, in the cases of amino acid³⁾ or peptide⁶⁾ extraction, enrichment of amino acids or peptides is possible by choosing an appropriate operating condition. For industrial application of this system, it is important to estimate both selectivity of separation and enrichment of products. For this purpose, the partition coefficient is an important variable since we can evaluate selectivity and enrichment with it. In our previous paper⁶⁾, the effect of hydrophobicity and charge density on the partition coefficient of peptides was reported. We noted there that the partition coefficient of peptides generally increased with increase in hydrophobicity and charge density (electric charge divided by molecular weight). Also, we found that there is a plateau region in the relation between the partition coefficient and the charge density. However, the relation between the partition coefficient and hydrophobicity as described in the previous paper was rather qualitative. Thus, the purpose of this paper is to present a quantitative correlation between the partition coefficient and hydrophobicity of oligopeptides.

1. Experimental Methods

1.1 Reagent

Sodium di-2-ethylhexylsulfosuccinate (AOT) used

was a product of Nakalai Tesque, Inc., Kyoto. Isooctane was analytical-grade reagent made by Wako Chemical Co., Tokyo. Peptides were the products of Sigma Chemical Co., St. Louis, MO, U.S.A. They were used without further purification.

1.2 Peptide extraction

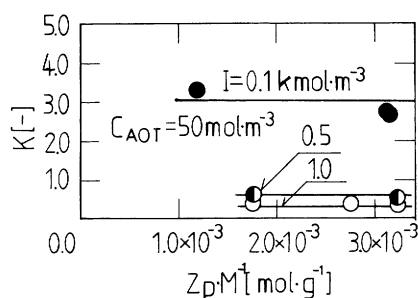
The extraction equilibrium was measured using a 100 cm³ Erlenmeyer flask. The AOT concentration in the organic phase was 50 mol/m³. An aqueous solution of 20 cm³ was mixed with an organic solution of 20 cm³. The value of w_0 was 18.5. To mix the organic and aqueous phases in the vessel, a four-blade turbine of 2 cm diameter was used at ca. 600 rpm. To settle the droplets, the solution was centrifuged. Time necessary to reach equilibrium was measured in a preliminary experiment and it was found that one hour was sufficient. Temperature was regulated at 300 K. Peptides in the organic phase were analyzed after extracting them into the aqueous phase. Analysis of peptides was carried out by the ninhydrine method.

2. Results

2.1 Effect of ionic strength in plateau region

In the previous paper⁶⁾, a plateau region, where the partition coefficient is unaffected by variation of the electric charge density, was found in the region of low ionic strength, i.e. $I=0.1$ mol/dm³. In the present study, the plateau region was observed as well for Gly-Phe-Ala in the region of higher ionic strength, i.e. $I=0.5$ and 1.0 mol/dm³, as shown in **Fig. 1**. The partition coefficient decreased with increasing ionic strength in the region $\text{pH} < \text{pI}$. In contrast to this observation, Kuboi *et al.*⁷⁾ have reported that the partition coefficient increased with increasing ionic strength at $\text{pH} = \text{pI}$. From consideration of the above two results, the electrostatic interaction seems to be

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predominant in the region $\text{pH} < \text{pI}$ rather than the hydrophobic interaction. The partition coefficient at pI was about two orders of magnitude smaller than that in the plateau region. It increased rapidly with decreasing pH of the solution toward the value at the plateau⁶⁾. That is, the value of the partition coefficient seemed to saturate quickly with decrease of pH of the solution from pI . In the experiment by Kuboi *et al.*, the hydrophobic interaction seemed predominant, since their solution was adjusted at the isoelectric point of the peptides.

2.2 Correlation between partition coefficient and hydrophobicity

| No | Peptide | No | Peptide | No | Peptide | No | Peptide |
|----|---------|----|---------|----|---------|----|---------|
| 1 | WG | 7 | YV | 13 | YGG | 19 | FFF |
| 2 | FG | 8 | WGG | 14 | GFA | 20 | LLY |
| 3 | GY | 9 | F GG | 15 | GAY | 21 | YYF |
| 4 | YG | 10 | L GG | 16 | GFF | 22 | YYY |
| 5 | FV | 11 | G SF | 17 | GLF | | |
| 6 | LV | 12 | G FS | 18 | LSF | | |

From the practical viewpoint, the partition coefficient in the plateau region will be important rather than that at pI . Since hydrophobicity was expected to have an important influence on the partition coefficient, we paid attention to its effect here. For this purpose, evaluation of the relative hydrophobicity of the peptides is necessary. Hansch *et al.*⁹⁾ have chosen the logarithm of the partition coefficient for the water-1-OctOH (*n*-octanol) system, $\log P$, as a scale of hydrophobicity of amino acids. Furthermore, they have proposed the Hydrophobic Substituent Constant (*HSC*) as the scale of hydrophobicity. Rekker¹⁰⁾ has proposed the Hydrophobic Fragmental Constant (Hydrophobicity Index, *HI*), which is a modification of *HSC*. In the present study, the sum of (*HI*)_{AA} by Rekker for each amino acid residue, $\sum(HI)_{AA}$, was used as a scale of hydrophobicity of peptides (*HI*)_P. Rekker¹⁰⁾ has also classified the 21 amino acids into two groups: hydrophilic (H) and lipophilic (L). As shown below, we noticed that the number of lipophilic amino acid residues in the amino acid sequence of peptides (*N_L*) acted as a specific scale to characterize the partitioning of oligopeptides into reversed micelles.

Figure 2 shows the quantitative correlation between the partition coefficient in the plateau region and $(HI)_P$ for dipeptides and tripeptides. This figure tells us three points. Firstly, at a given N_L value the partition coefficient increases with increase in $(HI)_P$ value. Secondly, the same correlation holds for partition

tried a correlation between the partition coefficient and the mean value of hydrophobicity of the constituting lipophilic amino acid residue, $(HI)_P/N_L$. The correlation became better (Fig. 3). The partition coefficient increased with the increase in $(HI)_P/N_L$, as shown in the figure. The relation can be expressed by the following equation.

$$\ln K = 3.37 (HI)_P/N_L - 6.09 \quad (1)$$

The data for the peptides containing alanine did not fit the correlation. If the value of $(HI)_{AA}$ for alanine is evaluated a little larger, this discrepancy may happen. However, this point is left for further study.

Peptides are considered to be solubilized in the interface region rather than in isooctane since the solubility of peptides in organic solvents is very low. The apparent partition coefficient, K , is proportional to the partition coefficient of peptides between the interface region and the inner water pool, $K_{int/w}$, since the ratio of water to AOT in the organic phase was kept constant in this study. The latter coefficient will be a function of the electrostatic interaction, the hydrophobic interaction, the free energy change in relocating AOT molecules to bind peptides (steric effect), etc., as expressed by Eq. (2)⁸.

$$RT \ln K_{int/w} = \Delta G_{elec.} + \Delta G_{hydrophobic} + \Delta G_{steric} + \dots \quad (2)$$

Peptides used in this study have a maximum charge of +1 at pH sufficiently smaller than pI . For these peptides, the effect of the electric charge and the steric effect on the partition coefficient seem almost identical since the coefficient could be expressed only by a function of hydrophobicity index; that is, the terms other than $\Delta G_{hydrophobic}$ on the right side of Eq. (2) are almost constant.

The behavior in Fig. 3 seems to suggest that only one hydrophobic residue is solubilized into the interface between the oil phase and the inner water phase, regardless of dipeptides or tripeptides. The result that $\ln K$ is related to an average of the hydrophobicity may be due to a statistical distribution of probability of interactions concerning which one of the specific hydrophobicity groups of the amino acids will bind with the AOT molecule. Some kind of averaged hydrophobicity might be adequate for such a system with the distribution of interactions.

Conclusion

Extraction of oligopeptide using reversed micelles was investigated. Correlation of the partition coefficient with the hydrophobicity of the peptide was

characterized by the number of hydrophobic amino acids. The partition coefficient of the peptides increased with increasing value of hydrophobicity of the peptide.

If we divide the hydrophobicity index by the number of hydrophobic amino acids, N_L , we obtain the mean hydrophobicity index of the constituting lipophilic amino acids. By using this value, a unique correlation of the partition coefficient was obtained. The logarithm of the partitioning coefficient was linearly related to the averaged value of hydrophobicity index, $(HI)_P/N_L$.

Nomenclature

| | | |
|--------------------------|-----------------------------------------------------------------------------------|------------------------|
| C_{AOT} | = concentration of AOT in organic phase | [mol/m ³] |
| $\Delta G_{elec.}$ | = free energy change of binding due to the electrostatic force | [J/mol] |
| $\Delta G_{hydrophobic}$ | = free energy change of binding due to the hydrophobic interaction | [J/mol] |
| ΔG_{steric} | = free energy change of relocating AOT molecules caused by binding | [J/mol] |
| HI | = Hydrophobicity Index | [—] |
| HSC | = Hydrophobic Substituent Constant | [—] |
| I | = ionic strength | [kmol/m ³] |
| K | = partition coefficient | [—] |
| $K_{int/w}$ | = partition coefficient of peptides between interface and inner water phase | [—] |
| M | = molecular weight | [g/mol] |
| N_L | = number of hydrophobic amino acids in the amino acid sequence of peptide | [—] |
| pI | = isoelectric point | [—] |
| w_o | = ratio of water concentration to AOT concentration in moles in the organic phase | [—] |
| Z_P | = average electric charge | [—] |

<Subscripts>

| | |
|------|--------------|
| AA | = amino acid |
| P | = peptide |

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