

EFFECT OF AMINO ACID SEQUENCE ON REVERSED MICELLAR EXTRACTION OF DIPEPTIDES

KAZUYUKI KISHI AND SHINTARO FURUSAKI

Department of Faculty Engineering, The University of Tokyo, Tokyo 113, Japan

Key words: Extraction, Reversed Micelles, AOT, Dipeptides, Amino Acid Sequence

Introduction

A new extraction method using reversed micelles has recently been investigated for separation of amino acids (Furusaki and Kishi, 1990; Adachi et al., 1991; Leodidis and Hatton, 1990), peptides (Kishi and Furusaki, 1991, 1992), and proteins (Goklen and Hatton, 1985). In our previous paper (Kishi and Furusaki, 1992), it was found that peptides with different sequence order showed different partition coefficients. For example, the partition coefficients of Gly-Tyr and Tyr-Gly showed different K values. A study on the effect of amino acid sequence will be useful to understand the mechanism of solubilization of amino acids in the organic phase. Thus, the purpose of this communication is to study the effect of the order of amino acid sequence of dipeptides containing glycine on the partition coefficients.

1. Experiment

1.1 Reagent

Sodium di-2-ethylhexylsulfosuccinate (AOT) was a product of Nacalai Tesque, Inc., Kyoto, Japan. Isooctane was analytical-grade reagent from Wako Chemical Co., Tokyo. Peptides were the product of Sigma Chemical Co., St. Louis, MO, USA. They were used without further purification.

1.2 Peptide extraction

Extraction of peptides was carried out by using the AOT/isooctane-water system.

The extraction equilibrium was measured with a 100 cm³ Erlenmeyer flask at pH = 1.8. This pH region is in the plateau region, where pH does not affect partition (Kishi and Furusaki, 1992). The AOT concentration in the organic phase was 50 mol/m³. The value of W_O was 18.5 under this condition. A 20 cm³ aqueous solution was mixed with organic solution of the same volume for about 1 h. To mix the organic and aqueous phases in the vessel, a 2 cm four-blade turbine was used at ca. 600 rpm. After mixing, the aqueous and organic phases were separated with a centrifuge. The analytical methods of peptides were described in the previous paper (Kishi and Furusaki, 1992).

2. Results and Discussion

The partition coefficient of peptides between water and the reversed micellar interface, $K_{i/w}$, was defined according to Leodidis and Hatton (1990) as follows:

$$K_{i/w} = \frac{n_{p,o} / (n_{AOT} + n_{p,o})}{n_{p,w} / (n_w + n_{p,w})} \quad (1)$$

Table 1 shows the partition coefficients of dipeptides containing Glycine at pH = 1.8. For dipeptides containing Glycine, peptides which have Glycine at the N-terminal were found to show larger partition coefficients than the peptides which have Glycine at the C-terminal.

In our previous paper (Kishi and Furusaki, 1992), we have reported that the hydrophobic residue of amino acids in the peptides is solubilized into the interface (micellar phase) between the organic phase and the inner water phase. Phe-Gly has a positive charge at the N-terminal. The positive charge will interfere with the solubilization of the Phe residue of the peptide into the hydrophobic micellar phase because of the repulsion force between the charge and hydrophobic nature of the micellar phase. On the other hand, Gly-Phe shows no repulsion force between the Phe residue and the micellar phase because the Phe residue does not have charge at low pH. Furthermore, the electrostatic interaction between the head charge of AOT and the charge of Gly also increase the partition coefficient. **Fig. 1** shows the schematic diagram of this mechanism.

On the other hand, Chen *et al.* (1994, 1995) have reported that Phe-Gly has a larger partition coefficient than that of Gly-Phe under the condition of pH between 5 and 6. At a pH value between 5 and 6, both Phe-Gly and Gly-Phe have no or very little electric charge, since their pI values are 5.7. At pH = 5.2, we obtained similar results to their results. **Table 2** shows the variation of partition coefficients of both Phe-Gly and Gly-Phe with various pH values in aqueous phase. Since practical extraction will be carried out at low pH region, we examined the partition at pH = 1.8.

Diamond *et al.* (1989) studied distribution of oligopeptides in an aqueous two-phase system. They claimed that peptides with Gly in the N-terminal distributed more in the hydrophilic potassium phosphate (PK) phase than the polyethylene glycol (PEG) phase. According to Diamond *et al.*, this was caused by the fact that the relatively hydrophilic polar peptides (Gly in N-terminal)

* Received September 19, 1994. Correspondence concerning this article should be addressed to S. Furusaki.

Table 1 Effect of amino acid sequence of dipeptides containing Glycine on partition coefficient
 $C_{AOT} = 50\text{mM}$, $pH = 1.8$, $I = 0.1 \text{ kmol} / \text{m}^3$

Peptide	$K_{i/w}$	Peptide	$K_{i/w}$
Gly-Trp	5513	Leu-Gly	330
Trp-Gly	3706	Gly-Tyr	863
Gly-Phe	1825	Tyr-Gly	536
Phe-Gly	668	Gly-Val	266
Gly-Leu	675	Val-Gly	nd

nd = not detectable

Table 2 Effect of pH on partition coefficient for Gly-Phe and Phe-Gly

Peptide	pH	$K_{i/w}$
Gly-Phe	1.8	752
Phe-Gly	1.8	408
Gly-Phe	4.0	449
Phe-Gly	4.0	278
Gly-Phe	5.2	203
Phe-Gly	5.2	463

$C_{AOT} = 300\text{mM}$

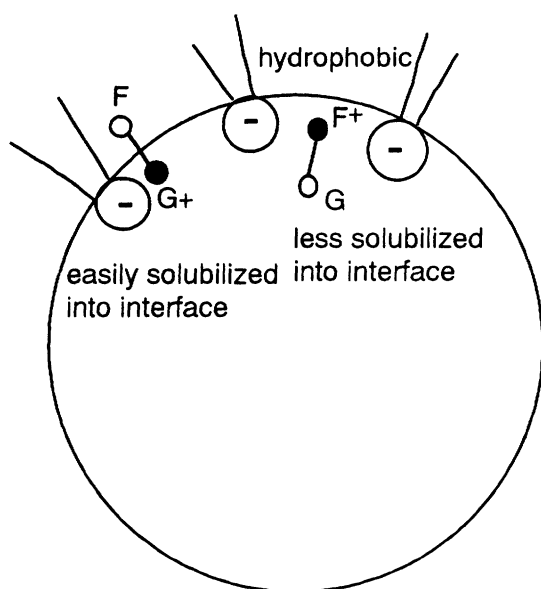


Fig. 1 Schematic diagram of mechanism of solubilization
 (●); N-terminal, (○); C-terminal

partitioned more in the hydrophilic PK phase than the relatively hydrophobic peptides (Gly in C-terminal). This result seems different from the behavior of peptides in the AOT/isooctane-water reversed micellar system, although no information on the pH value is available. We consider the contribution of the electric charge was significant in our AOT/isooctane-water system at low pH region.

Conclusion

The effect of amino acid sequence on extraction of dipeptides using reversed micelles with AOT was investigated and the following result was obtained.

For dipeptides containing Glycine, peptides with Glycine at the N-terminal had a larger partition coefficient than those with Glycine at the C-terminal. This seems to be the result of electric charge of the hydrophobic amino acid residue which exerts repulsive force against the hydrophobic group of AOT.

Literature cited

- 1) Adachi, M., M. Harada, A. Shioi and Y. Sato: *J. Phys. Chem.*, **95**, 7925 (1991)
- 2) Chen, W. Y., Y. H. Wang and L. K. Wang: *J. Chem. Eng. Japan*, **27**, 685 (1994)
- 3) Chen, W. Y.: Private Communication (1995)
- 4) Diamond, A. D., X. Lei and J. T. Hsu: *Biotech. Tech.*, **3**, 271 (1989)
- 5) Furusaki, S. and K. Kishi: *J. Chem. Eng. Japan*, **23**, 91 (1990)
- 6) Goklen, K. E. and T. A. Hatton: *Biotech. Prog.*, **1**, 69 (1985)
- 7) Kishi, K. and S. Furusaki: *Kagaku Kogaku Ronbunshu*, **17**, 614 (1991)
- 8) idem: *J. Chem. Eng. Japan*, **25**, 611 (1992)
- 9) Leodidis, E. B. and T. A. Hatton: *J. Phys. Chem.*, **94**, 6400 (1990)

Nomenclature

C_{AOT}	=	concentration of AOT in organic phase	[mol/m ³]
I	=	ionic strength	[kmol/m ³]
$K_{i/w}$	=	partition coefficient between water and the reversed micellar interfaces	[-]
n_{AOT}	=	total number of moles of AOT in organic phase	[mol]
$n_{p,o}$	=	total number of moles of peptides in organic phase	[mol]
n_w	=	total number of moles of water in water phase	[mol]
$n_{p,w}$	=	total number of moles of peptides in water phase	[mol]
W_0	=	ratio of moles of water to AOT in the organic phase	[-]