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Enzymatic Synthesis of Oligopeptides in Mixed Reverse Micelles

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Abstract: Some oligopeptide derivatives were successfully synthesized in 56-88% yield by α-chymotrypsin in AOT-Brij30/*n*-heptane mixed reverse micelles. The reaction conditions such as W_0 value, reaction time and the concentration of enzyme in the water pool were studied with ZTyrGlyGlyOEt as model peptide. © 1999 Elsevier Science Ltd. All rights reserved.

Reverse micelles can be used as microreactors for enzymatic peptide synthesis and this has attracted attention in recent years.[1] The enzyme can be solubilized in the polar core of the reverse micelles formed by the surfactant molecules and its catalytic activity is maintained. In the past, the enzyme properties in reverse micelles, particularly the kinetic parameters, were widely investigated.[2-3] Only a few papers reported that peptides were synthesized by enzymes in reverse micelles.[4-6] In our previous work,[7-9] we studied and synthesized a series of peptide derivatives including *N*-protected Leu-enkephalin using proteases in organic solvents. In this communication, we focus on enzymatic peptide synthesis in reverse micelles, employing a mixed reverse micellar system successfully to obtain some oligopeptide derivatives in good isolated yields. The influence of reaction conditions was also studied.

A tripeptide ZTyrGlyGlyOEt, a protected fragment of Leu-enkephalin, was used as model peptide and synthesized by α-chymotrypsin in reverse micelles. We found that AOT-Brij30/*n*-heptane[10] mixed reverse micelles were more suitable for the enzymatic peptide synthesis since the isolated yield (77%) was higher than that (73%) in AOT/*n*-heptane system. In this work, the model peptide was synthesized in 0.09M AOT-0.01M Brij30/*n*-heptane mixed reverse micelles.

An important parameter W_0, defined as the molar ratio of water to surfactant, was studied because the catalytic activity of the enzyme was dependent on its value. Fig. 1 illustrates the influence of W_0 on the isolated yield of ZTyrGlyGlyOEt. When the water content was low (W_0=1), the product was obtained in poor yield. With the amount of water in the system increasing (W_0=2-6), the isolated yield increased dramatically and then held steady. The yield can increase to 86% (W_0=6), but as the water content increased beyond this

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(W_0=8-25) the yield gradually declined. The dimension of reverse micelles was small when the W_0 value was quite low. α-Chymotrypsin was not able to dissolve in the water pool and was exposed to the organic phase, consequently, the catalytic activity of the enzyme could not be shown. At the optimum W_0, α-chymotrypsin was able to dissolve in the polar core and there was enough essential water on the enzyme surface. The inner diameter of the empty micelle almost corresponds to the size of the α-chymotrypsin molecule. In this case, the enzyme could retain its active conformation and display high activity in peptide bond formation.[11] When the W_0 value was high, the flexibility of enzyme molecules increased causing the loss of enzymatic activity to a certain degree. Accordingly, the yield of the peptide decreased.

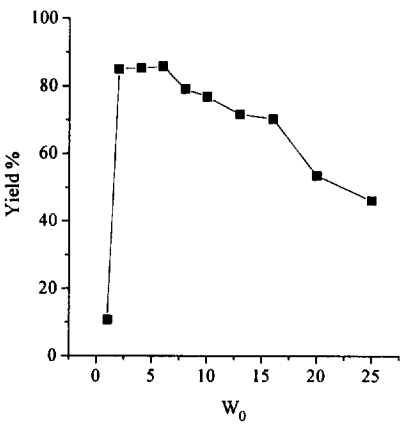

Fig. 1. The effect of W_0 on the yield of ZTyrGlyGlyOEt. Reaction conditions: 400μM α-chymotrypsin in water pool, substrate concentration being 50mM and stirring for 24 hours.

Other experimental factors such as reaction time were also investigated. It was observed that the product was precipitated from the reaction solution in a few minutes, indicating that the reaction was fast. After 24 hours the target tripeptide was obtained in maximum yield (86%). When the reaction time was prolonged to 72 hours the yield decreased to 74%.

The concentration of α-chymotrypsin in the water pool was varied from 4mM to 4μM and the corresponding isolated yield was shown in Table 1. The results showed that when the enzyme concentration was high, the model peptide was synthesized in good yield (92%). Even if the absolute amount of α-chymotrypsin was very low (21.6μg/mmol substrate) in the reaction, the tripeptide was still obtained in modest yield (41%). This will be useful in practical peptide synthesis.

Table 1. The effect of α-chymotrypsin concentration in the polar core on the yield of ZTyrGlyGlyOEt

Enzyme concentration in polar core	Absolute amount of enzyme	Yield %
4mM(100mg/ml)	21.6mg/mmol substrate	92
2mM(50mg/ml)	10.8mg/mmol substrate	91
400μM (10mg/ml)	2.16mg/mmol substrate	86
40μM (1mg/ml)	0.216 mg/mmol substrate	71
4μM (0.1mg/ml)	21.6μg/mmol substrate	41

Reaction conditions: W_0=6, substrate concentration being 50mM and stirring for 24 hours.

Under these reaction conditions (see Table 2), other different peptide derivatives were also prepared with 0.09M AOT-0.01M Brij30/*n*-heptane reverse micelles in 56-88% yield.[12] The results indicated this reaction system was quite favorable for the enzymatic peptide synthesis. From Table 2, it is known that, among the several common protecting groups used in the amino component, phenylhydrazino is more suitable for the α-chymotrypsin-catalyzed reaction than methyl and ethyl. In addition, the data also indicate that glycine is a more favorable substrate than alanine in P_1' position for α-chymotrypsin. It is hoped that the results presented in this work will be helpful for the detailed study of enzymatic peptide synthesis in mixed reverse micelles.

Table 2. The peptide derivatives synthesized by α-chymotrypsin in mixed reverse micelles

Acyl donor	Nucleophile	Product	m.p. (°C)	Yield %
ZTyrOEt	GlyGlyOEt	ZTyrGlyGlyOEt	166-168	86
BocTyrOEt	GlyGlyOEt	BocTyrGlyGlyOEt*	190-192	88
BocTyrOEt	GlyNHNHPh	BocTyrGlyNHNHPh	198-200	88
ZTyrOEt	GlyNHNHPh	ZTyrGlyNHNHPh	194-196	86
ZTyrOEt	GlyOMe	ZTyrGlyOMe	139-141	59
ZTyrOEt	GlyOEt	ZTyrGlyOEt	173-175	66
ZTyrOEt	AlaOEt	ZTyrAlaOEt*	150-152	56

Reaction conditions: 400μM α-chymotrypsin in water pool, W_0=6, substrate concentration being 50mM and stirring for 24 hours.

* New compounds were confirmed by MS, elemental analysis and specific rotation.

The physical constants of the other compounds were identical to the literatures.

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12. The general procedure for the enzymatic peptide synthesis in reverse micelles (ZTyrGlyGlyOEt as an example). 0.25mmol (50mg) HCl • GlyGlyOEt was suspended in 5ml 0.09M AOT-0.01M Brij30/*n*-heptane reverse micellar system. 70μl Et_3N and 54μl water solution of α-chymotrypsin (10mg/ml) were added. After the substrate was completely dissolved by shaking on a vibromixer, 0.25mmol (85mg) ZTyrOEt was immediately added. The observed pH value of the solution was about 10.0 which was determined by dropping a small amount of the mixture on a damp pH paper. The mixture was stirred at room temperature for 24 hours. At the end of the reaction, the precipitate was filtered and washed with 1M hydrochloric acid (4ml × 2) and water (4ml × 2). The product was obtained in 86% yield.