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# Activity of α-Chymotrypsin Enhanced in the Presence of Iron Oxide Nanoparticles in Organic Solvent: Application to Peptide Synthesis

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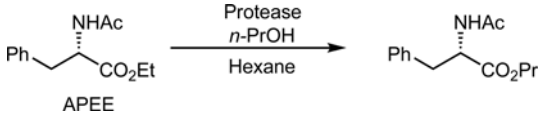
Enzymes are a useful class of catalysts for the preparation of enantiomeric compounds.[1] The applications of enzymes in synthetic transformations, however, are limited by their reduced activities in organic solvent. Particularly, proteases such as subtilisin and α-chymotrypsin display several orders of magnitude lower activities in organic solvent than their aqueous counterparts.[2] A useful method for enhancing the nonaqueous activities of proteases is to use them after lyophilization in the presence of excipients such as lyoprotectants and inorganic salts.[3] For example, subtilisin displayed a 3750-fold enhanced activity in hexane if it was lyophilized in the presence of 98%(w/w) KCl salts.[4] In case of α-chymotrypsin (α-CT), its lyophilization in the presence of KCl salts induced a 50-fold activation in hexane.[4] However, no activation took place if the salts were added to enzymes suspended in organic solvent. Lately, we reported that iron oxide nanoparticles (IONs) enhanced the activity of subtilisin by a factor of $10^3$ even though they were simply added with subtilisin in organic solvent before use.[5] We now wish to report that α-chymotrypsin (α-CT) was also activated by added IONs in organic solvent and IONs-activated α-CT catalyzed the synthesis of peptides efficiently.

In typical procedures, α-CT from its commercial sources was lyophilized in the presence of 1% phosphate buffer (pH 7.8). The lyophilized enzyme powder and IONs (average size 12 nm)[6] were added to a vial in a sequence, followed by the addition of solution containing *N*-acetyl-L-phenylalanine ethyl ester (APEE, 10 mM) and *n*-propanol (0.85 M) in hexane. The enzymatic activity (U) was then measured as the initial rate (μmoles of product per mg of enzyme per hour) at 25 °C (Scheme 1). Separately, it was confirmed that the transesterifcation of APEE in the presence of IONs did not proceed without α-CT.

The lyophilized α-CT was poorly active (78 mU) in hexane. Its activity, however, increased rapidly with increasing the amount of IONs added (Figure 1) and reached 8.0 U (100-fold activation) in case 99 mass equivalents of IONs were added (Figure 1). The values of kinetic parameters in Table 1 indicate that the enhanced activity of α-CT is largely the result from an increase in $V_{max}$.




**Scheme 1.** α-Chymotrypsin-catalyzed transesterification of *N*-acetyl-L-phenylalanine ethyl ester (APEE).

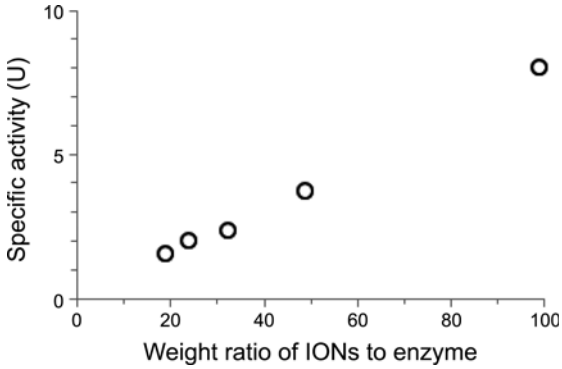



**Figure 1.** The activity of protease in the presence of IONs against the weight ratio of IONs to α-CT. U = μmoles of product per mg of enzyme per hour.

**Table 1.** Kinetic parameters of α-chymotrypsin (α-CT)[a]

| Enzyme | $V_{max}$ (U)[b] | $K_m$ (mM) | $V_{max}/K_m$ | activation |
|---|---|---|---|---|
| native α-CT | $1.8 \times 10^{-1}$ | 11 | $1.6 \times 10^{-2}$ | |
| 1% α-CT-IONs | 12 | 14 | $8.6 \times 10^{-1}$ | 54 |

[a]The kinetic parameters were obtained from the Lineweaver-Burk plot of rates (10, 5, 2.5, 1.25, 1 mM). [b]U = μmoles of product per mg of protein per hour.

We speculate that two factors might contribute to the IONs-induced activation of α-CT. They include a large surface-to-volume ratio of IONs and water on the surface of IONs. IONS used in this work contained 3.1-3.5%(w/w) water which was determined by the Karl-Fisher titration method. It is expected that enzyme molecules are physically adsorbed on IONs in organic solvent and probably dispersed on the large surface of IONs by shaking during reaction, thus lowering mass transfer barrier. Previously, it was suggested that the activation of enzyme by nanoparticles (fumed silica: diameters, 5-7 nm) was due to the distribution of enzyme on the large surface.[7] We have also demonstrated this possibility in the IONs-activation of subtilisin.[5] On the other hand, water on the surface of IONs could provide aqueous microenvironments around enzymes, thus allowing for efficient

**Table 2.** Synthesis of peptides using IONs-activated α-CT.

| Acyl donor | Acyl acceptor | Time (h) | Product[a] | Yield (%)[b] |
|---|---|---|---|---|
| *N*-Ac-L-Phe-OEt | Gly-$NH_2$ | 4 | *N*-Ac-L-Phe-Gly-$NH_2$ (**1**) | > 97 |
| *N*-Ac-L-Phe-OEt | L-Ala-OMe | 6 | *N*-Ac-L-Phe-L-Ala-OMe (**2**) | > 97 |
| *N*-Ac-L-Phe-OEt | L-Ala-$NH_2$ | 7 | *N*-Ac-L-Phe-L-Ala-$NH_2$ (**3**) | > 97 |
| *N*-Ac-L-Phe-OEt | L-Val-OMe | 8 | *N*-Ac-L-Phe-L-Val-OMe (**4**) | > 97 |
| *N*-Z-L-Ala-L-Phe-OMe | Gly-$NH_2$ | 8 | *N*-Z-L-Ala-L-Phe-Gly-$NH_2$ (**5**) | > 97 |
| *N*-Z-L-Ala-L-Phe-OMe | L-Ala-$NH_2$ | 4 | *N*-Z-L-Ala-L-Phe-L-Ala-$NH_2$ (**6**) | > 97 |
| *N*-Z-L-Ala-L-Phe-OMe | L-Val-$NH_2$ | 2 | *N*-Z-L-Ala-L-Phe-L-Val-$NH_2$ (**7**) | > 97 |
| *N*-Z-L-Ala-L-Phe-OMe | L-Leu-$NH_2$ | 1 | *N*-Z-L-Ala-L-Phe-L-Leu-$NH_2$ (**8**) | > 97 |

[a]All the products were confirmed on the basis of their NMR and HRMS data as well as their melting points: **1**: mp 184-186 °C (lit.[9] mp 184 °C). **2**: mp 149-150 °C; lit.[10] mp 149-155 °C). **3**: mp 233-235 °C (lit.[11] mp 232-234 °C). **4**: mp 145-147 °C (lit.[12] mp 146-147 °C), **5**: mp 179-181 °C; HRMS (FAB+) $C_{22}H_{27}O_5N_4$ calcd 427.1987 ($M+H^+$), found 427.1984). **6**: mp 195-197 °C (lit.[13] mp 194-197 °C). **7**: mp 226-230 °C; HRMS (FAB+) $C_{25}H_{33}O_5N_4$ calcd 469.2451 ($M+H^+$), found 469.2453. **8**: 223-225 °C (lit.[14] mp 223-224 °C). [b]Based on $^1$H NMR analysis. Isolated yields were almost qantitative.

catalysis.

As a useful synthetic application of IONs-activated α-CT, we explored the synthesis of peptides in organic solvent. Each reaction for the synthesis of peptide was performed with a solution containing 1 mg of α-CT, 99 mg of IONs, 50 ìmol of acyl donor, and 1.5 equivalent of acyl acceptor in hexane at 25 ºC. All the reactions proceeded smoothly to provide the products in quantitative yields (Table 2). In a previous study,[8] α-CT-catalyzed peptide synthesis in organic solvent was performed in the presence of a little amount of water to maintain high enzymatic activity during the reaction, thus resulting in the formation of hydrolyzed byproducts. This hydrolysis problem was not observed with IONs-activated α-CT.

In summary, we have demonstrated that α-CT displays a significantly enhanced activity in the presence of IONs relative to its IONs-free counterparts in organic solvent. IONs-activated α-CT catalyzed efficiently the synthesis of peptides without the formation of hydrolyzed byproducts.

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