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Peptide bond formation catalyzed by α-chymotrypsin in ionic liquids

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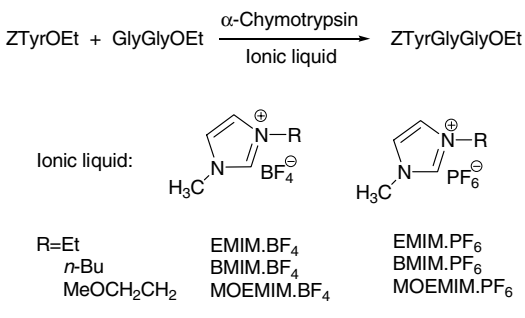
Abstract—α-Chymotrypsin catalyzed peptide bond formation was studied in ionic liquids using the synthesis of a protected fragment of Leu-enkephalin, ZTyrGlyGlyOEt, as model reaction. MOEMIM·PF_6 was found to be the most favorable solvent among the six different 1-alkyl-3-methylimidazolium hexafluorophosphates and tetrafluoroborates ionic liquids screened. With MOEMIM·PF_6 as reaction media, several di- or tripeptide derivatives were successfully prepared in 68–75% isolated yields.

Ionic liquids are salts that are generally liquid at room temperature, and have attracted much attention in recent years,[1] since they (i) have a good solubility for a wide range of organic, inorganic and organometallic materials; (ii) enjoy novel properties such as high thermal stability, almost nonexistent vapor pressure, nonflammability as well as easy recycle; (iii) possess alterable hydrophobic or hydrophilic behavior via regulating the nature of their cations and anions; (iv) serve as good media for a variety of organic syntheses. Compared with volatile solvents that have a detrimental impact on the environment, ionic liquids are considered as environmentally friendly green solvents. It is reported that different enzymes such as lipase,[2–4] protease,[5–9] peroxidase,[10] dehydrogenase, and glycosidase[11] could maintain their activity when suspended in ionic liquids, exhibiting that ionic liquids are very promising green alternatives to organic solvents for biotransformations.[12]

The enzyme catalyzed reactions, in particular, transesterification, with ionic liquids as solvent were well investigated;[1] however, peptide bond formation was seldom introduced, except, for example, thermolysin catalyzed *Z*-aspartame synthesis in BMIM·PF_6.[5] There are a few reports that α-chymotrypsin is able to catalyze ester bond formation in ionic liquids,[6–9] however, it is still unclear to utilize α-chymotrypsin as catalyst for peptide synthesis. Based on the previous study of enzyme catalyzed synthesis in non-aqueous media,[13–18] in this Letter, we report a tripeptide, ZTyrGlyGlyOEt, the protected fragment of Leu-enkephalin, synthesized by α-chymotrypsin in ionic liquids. Six different ionic liquids were employed in this study, which belong to two classes, hexafluorophosphate and tetrafluoroborate salts. Their structures and the model enzymatic reaction are shown in Scheme 1. The reaction conditions that influenced the enzymatic reaction in ionic liquids were studied, and we found that MOEMIM·PF_6 was the best solvent among the ionic liquids we used.

Six 1-alkyl-3-methylimidazolium hexafluorophosphates and tetrafluoroborates were prepared by the literature



Scheme 1. Six ionic organic liquids and the model enzymatic reaction investigated in this Letter.

Keywords: α-Chymotrypsin; Ionic liquid; Peptide synthesis.
Abbreviations: BMIM·PF_6, 1-butyl-3-methylimidazolium hexafluorophosphate; EMIM·PF_6, 1-ethyl-3-methylimidazolium hexafluorophosphate; MOEMIM·PF_6, 1-methoxyethyl-3-methylimidazolium hexafluorophosphate; BMIM·BF_4, 1-butyl-3-methylimidazolium tetrafluoroborate; EMIM·BF_4, 1-ethyl-3-methylimidazolium tetrafluoroborate; MOEMIM·BF_4, 1-methoxyethyl-3-methylimidazolium tetrafluoroborate.
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procedures.[2] All the ionic liquids were dried over P_2O_5 under vacuum at least for 24 h before use. For a general case with MOEMIM·PF_6 as solvent, the reaction was performed at room temperature and the desired tripeptide was produced as a precipitate from the solution. After simple filtration and recrystallization, a pure ZTryGlyGlyOEt was obtained.[19]

The water content of the reaction medium has a great influence on the enzyme activity and the product yield. A small amount of water, which is called essential water, is required to maintain the protease catalytic conformation in non-aqueous media. On the other hand, however, excess of water in the system will lead to the undesired hydrolysis of the product. Therefore, an optimum water content range is maintained so that the enzymatic product can be obtained in high yield. The data provided in Figures 1 and 2 highlight the relationship between water content and the isolated yield of the tripeptide in the enzymatic reaction. In the case of 1-alkyl-3-methylimidazolium hexafluorophosphates, the optimum water content of MOEMIM·PF_6 was 2–3% (v/v), and BMIM·PF_6 was 1%. No product was isolated when the water content was lower than 0.5%, whereas when the water content was higher than 4%, the isolated yield of the tripeptide decreased again due to the side effect of the hydrolysis. We also examined the α-chymotrypsin catalyzed reaction with EMIM·PF_6 as medium. Since EMIM·PF_6 is a solid at room temperature which has a melting point at 61 °C, the model reaction was conducted at 70 °C. Unfortunately, no coupling product was isolated. ZTyrGlyGlyOEt has a poor solubility in ordinary organic solvents except acetone and methanol. Although we detected the tripeptide formation by TLC, through a routine extraction workup process, it was hard to isolate the expected product that was mixed with the starting materials as a solid when the reaction temperature decreased.

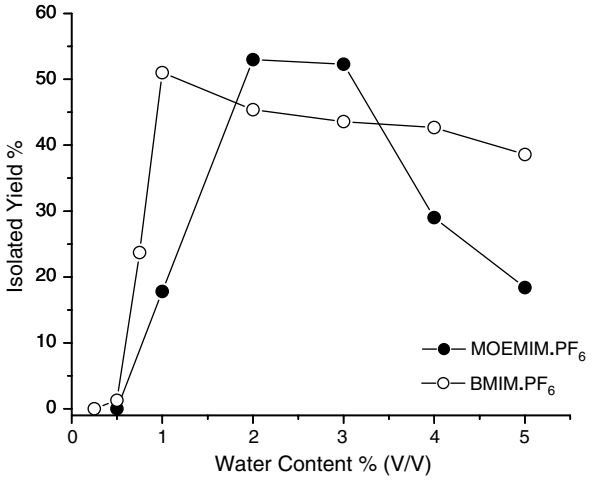

Figure 1. Effect of water content on the enzymatic reaction in 1-alkyl-3-methylimidazolium hexafluorophosphates. Reaction conditions: acyl donor and nucleophile concentrations all being 100 mM; stirring for 2 days.

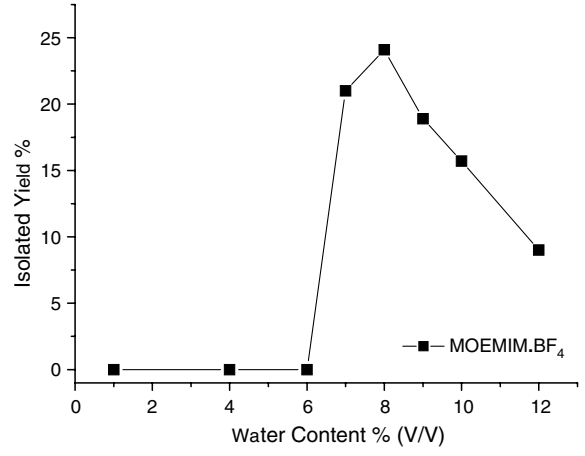

Figure 2. Effect of water content on the enzymatic reaction in 1-methoxyethyl-3-methylimidazolium tetrafluoroborate. Reaction conditions: acyl donor and nucleophile concentrations all being 100 mM; stirring for 3 days.

For 1-alkyl-3-methylimidazolium tetrafluoroborates as reaction solvent, only MOEMIM·BF_4 is appropriate for the enzymatic reaction in which the optimum water content is higher (8%). No isolated product was achieved with EMIM·BF_4 or BMIM·BF_4 as solvent as the water content varied from 2% to 10%. It is worth noting that, in some cases (water content above 6%), TLC showed that the desired product was produced, suggesting that α-chymotrypsin still exihibited its activity. However, it seems likely that the tripeptide has a large solubility in EMIM·BF_4 or BMIM·BF_4 and thereby the product cannot precipitate from the medium, and then isolate from the reaction mixture similarly as in the case of EMIM·PF_6 as reaction solvent.

1-Alkyl-3-methylimidazolium hexafluorophosphates are hydrophobic, while tetrafluoroborate salts are hydrophilic. It is observed that all hexafluorophosphate salts are not miscible in water, as a comparison, the tetrafluoroborate ionic liquids dissolve in water. This is consistent with the above results that the optimum water content of MOEMIM·BF_4 (8%) is much higher than that of MOEMIM·PF_6 and BMIM·PF_6 (1–3%). Enzyme molecules cannot adsorb the essential water to show their catalytic activity when a hydrophilic ionic liquid such as MOEMIM·BF_4 has a low water content. Also, the best isolated yield for hexafluorophosphate salts was approximately twice than that for tetrafluoroborate (see Figs. 1 and 2), which indicates that hexafluorophosphate ionic liquids are more suitable for the α-chymotrypsin catalyzed tripeptide formation.

From the standpoint of green chemistry, it should be feasible to recycle ionic liquids for the enzymatic reaction. We found that it was easy to recycle three ionic liquids which were MOEMIM·PF_6, BMIM·PF_6 and MOEMIM·BF_4. For an instance, MOEMIM·PF_6 was recovered through the removal of the tripeptide by filtration and most starting materials via washing by deionized water. The dried and recovered MOEMIM·PF_6 was reused for the next tripeptide synthesis and an isolated yield of 59% was obtained under the same reaction conditions as before (water content of

Table 1. The peptide derivatives synthesized by α-chymotrypsin in MOEMIM·PF_6

Acyl donor	Nucleophile	Product[c]	Mp (°C)	$[\alpha]_D^{20}$	Yield (%)
ZTyrOEt	GlyGlyOEt	ZTyrGlyGlyOEt	167–168	+4.1 (*c* 2.4 HOAc)	52[a]
ZTyrOEt	GlyGlyOEt	ZTyrGlyGlyOEt	167–168	+4.1 (*c* 2.4 HOAc)	74[b]
ZTyrOEt	GlyNHNHPh	ZTyrGlyNHNHPh	196–197	−18.3 (*c* 0.5 DMF)	74[b]
BocTyrOEt	GlyGlyOEt	BocTyrGlyGlyOEt	192–194	−4.3 (*c* 0.6 DMF)	75[b]
BocTyrOEt	GlyNHNHPh	BocTyrGlyNHNHPh	201–202	−8.8 (*c* 0.3 DMF)	68[b]

Reaction conditions: 3% water content, acyl donor concentration being 100 mM and stirring for 2 days.
[a] [Acyl donor]/[nucleophile] =1:1.
[b] [Acyl donor]/[nucleophile] =1:2.
[c] All physical constants of the products were identical to the literature.[14,17]

3%). Since the recovered MOEMIM·PF_6 still contained a small amount of ZTyrOEt that cannot be removed completely by washing during the recovering procedure, the second cycle afforded a 59% yield a little higher than that of the first use (52%).

When changing the ratio of [GlyGlyOEt]/[ZTyrOEt] from 1:1 to 2:1, the yield of ZTyrGlyGlyOEt was increased from 52% to 74% (Table 1, entries 1 and 2). Under the same reaction conditions, Table 1 shows that another three di- or tripeptide derivatives were prepared with α-chymotrypsin as catalyst in 68–75% isolated yields.

In this Letter, we have successfully carried out the α-chymotrypsin catalyzed ZTyrGlyGlyOEt synthesis in 1-alkyl-3-methylimidazolium hexafluorophosphate and tetrafluoroborate ionic liquids. This is the first example to demonstrate that α-chymotrypsin can catalyze peptide bond formation in ionic liquids. The results show that MOEMIM·PF_6, BMIM·PF_6 and MOEMIM·BF_4 are three appropriate salts for the enzymatic reaction, and MOEMIM·PF_6 is the most favorable ionic liquid for the model reaction, in which the highest isolated yield was obtained. The optimum water content for MOEMIM·PF_6 and BMIM·PF_6 was about 1–3%, which was lower than 8%, the optimum water content for MOEMIM·BF_4. Moreover, MOEMIM·PF_6 was shown to be recycled easily, which illustrates that the ionic liquid can operate an environmentally friendly process as a green solvent. Besides the model peptide ZTyrGlyGlyOEt, other three di- or tripeptide derivatives were also prepared in MOEMIM·PF_6, suggesting that MOEMIM·PF_6 could be a potential ionic liquid for general α-chymotrypsin catalyzed peptide synthesis.

Additionally, in the previous study, it was found that the optimum water content of the free α-chymotrypsin catalyzed ZTyrGlyGlyOEt synthesis with dichloromethane as solvent was 0.15%.[13] The optimized water content was about 0.36–1.08% (corresponding $W_0 = 2 - 6$) when a mixed reverse micelles (0.09 M AOT–0.01 M Brij30/*n*-heptane) system was utilized for the same model reaction.[14] All of these results indicate that water content is a major factor for α-chymotrypsin catalyzed reaction in non-aqueous media. The best water content varied with the change of the reaction media. The development described herein should give us a more clearer understanding of the enzyme catalyzed reaction in non-aqueous media, especially in ionic liquids. Further exploration of the enzyme catalyzed synthesis and modification of bio-active natural compounds in ionic liquids will be reported in due course.

Acknowledgement

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19. General procedure for the enzymatic synthesis of peptide derivatives in ionic liquids, with ZTyrGlyGlyOEt as example. The enzymatic reaction was performed in a dry glass bottle with cap. To a solution of MOEMIM·PF_6 (1.5 mL) were added ZTyrOEt (0.15 mmol) and Gly-GlyOEt·HCl (0.15 mmol). After the substrates were dissolved under stirring, triethylamine (42 μL), deionized water (7.5–75 μL) and α-chymotrypsin (3.5 mg) were added. Then the mixture was stirred at room temperature for 2 days. The precipitate from the reaction solution was filtered, dissolved in acetone (1 mL) and followed by addition of water (30 mL) to give a fine needle crystal product.