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CHYMOTRYPSIN SUSPENDED IN ORGANIC SOLVENTS WITH SALT HYDRATES IS A GOOD CATALYST FOR PEPTIDE SYNTHESIS FROM MAINLY UNDISSOLVED REACTANTS

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Abstract

Chymotrypsin powder suspended in organic solvents in the presence of $Na_2CO_3.10H_2O$ catalyses peptide synthesis from X-Ala-Phe-OMe and Leu-NH_2 (X = Boc or Z). The reaction proceeds best in the most non-polar solvents, such as hexane, despite the fact that both reactants and products remain largely undissolved. Leu-NH_2.HCl can be used in place of the free base.

In a previous paper we reported chymotrypsin-catalyzed peptide synthesis in trichloroethylene or carbon tetrachloride with the only water source for the enzyme being the water of hydration of sodium carbonate ($Na_2CO_3.10H_2O$).[1] We have now studied the synthetic potential of this approach in more detail, using the model reactions:

Boc-Ala-Phe-OMe + Leu-NH_2 ----> Boc-Ala-Phe-Leu-NH_2 1

and Z-Ala-Phe-OMe + Leu-NH_2 ----> Z-Ala-Phe-Leu-NH_2 2

Table 1 shows how the reaction yield (peptide 1) varied in different solvents. As can be seen, the best yields were obtained in the least polar solvents. The high yields in hexane and isopropyl ether are of considerable interest, because the solubilities of both the reactants and the peptide product are very low in these solvents; they remain mostly in the form of undissolved particles. (For example, Table 1 shows solubilities we have measured for the carboxyl component Boc-Ala-Phe-OMe.) It is noteworthy also that the nucleophile in these reactions (LeuNH_2) was added in the form of its hydrochloride salt. Usually the free base would be chosen for reactions in mainly organic solvents, as the hydrochloride is almost insoluble. This and many other reactants for peptide synthesis reactions are however more readily available as hydrochlorides, so the ability

Table 1. Solubility of Boc-Ala-Phe-OMe and peptide coupling yields from it in different solvents.

Solvent	Solubility (mM)	Yield of peptide 1 (%)
Trichloroethylene	1000	<10
Ethyl acetate	400	<10
Toluene	300	35
Isopropyl ether	20	77
Hexane	1	95

0.2 mmol Boc-Ala-Phe-OMe, 0.2 mmol $LeuNH_2$.HCl, 2 ml solvent, 0.5 mmol $Na_2CO_3.10H_2O$, 0.2 μmol chymotrypsin. Reaction time: 20 h

to use these directly is of significant value. We presume that the hydrochloride reacts with a portion of the Na_2CO_3 present, to liberate the free base in the reaction mixture, and form some $NaHCO_3$.

The synthesis of peptide 2 in the same reaction system showed a large solvent dependence as well, giving the following yields: isopropyl ether (90%), trichloroethylene (89%), hexane (87%), 1,1,1-trichloroethane (84%), ethanol (14%), 3-pentanone (10%), chloroform (5%) and ethyl acetate (<10%). Again there are very good yields in solvents in which the reactants dissolve little, but in this case the reaction also proceeds well in trichloroethylene, in which Z-Ala-Phe-OMe is quite soluble.

As might be expected for a system where the reactants are largely undissolved, the rate of these reactions was not very reproducible, probably because of differences in stirring and related conditions. There was a tendency for much of the suspended material to stick to the wall of the reaction tube. (This could not be prevented by making the glass surface hydrophobic by treatment with dichlorodimethylsilane.) Hence sufficient stirring should be used.

The extreme case of the reaction in hexane was selected for further study. We used carboxyl components with two different protecting groups, and the N-component Leu-NH_2 in the form of either the free base or the hydrochloride salt (Table 2). As can be seen the reaction proceeds to good yields in all cases, though slightly better when using Leu-NH_2 as the free base.

Table 2. Chymotrypsin-catalysed synthesis of peptides in hexane.

Protecting group (X)	N-component	Composition after reaction (mole %) X-Ala-Phe-Leu-NH_2	X-Ala-Phe-OH	X-Ala-Phe-OMe
Z	Leu-NH_2	92-96	0-3	1-8
Z	Leu-NH_2.HCl	75-80	2-8	14-20
Boc	Leu-NH_2	84-94	2-6	0-14
Boc	Leu-NH_2.HCl	72-75	25	0-3

0.1 mmol each reactant in 2 ml hexane, 0.2 mmol $Na_2CO_3.10H_2O$, 0.2 μmol chymotrypsin. Reaction time 2 h.

Varying the reaction time from 2 to 8 h had no significant effect on the yields in any of these cases, indicating that 2 h was sufficient to reach essentially completion. Using an excess (0.2 mmol) of Leu-NH_2.HCl caused a slight but probably significant increase in peptide yield to 87% and 80% with Z and Boc protecting groups respectively. Increasing the volume of hexane in the reaction mixture (with the same amounts of reactants and enzyme) caused a significant reduction in yield. This seemed to be because the reactants were more attached to the walls, being less accessible to the stirrer.

We have made some preliminary studies using leucine p-nitroanilide as an alternative nucleophile, again finding good yields in hexane and isopropyl ether. We have also demonstrated the thermolysin-catalysed coupling of Boc-Ala-Phe-OH and Leu-NH_2 in the presence of $Na_2SO_4.10H_2O$ in ethyl acetate (yields over 50%) (P.Kuhl, unpublished). We have investigated whether substrate specificity is broadened under these conditions; but found no significant reaction with Z-Ala-Ser-OMe as an alternative carboxyl component for chymotrypsin-catalysed synthesis.

General discussion

Some peptides are rather insoluble in most solvents, and this has often been seen as a restriction in the selection of conditions for their coupling. The present study shows that enzymic synthesis of peptide bonds can proceed effectively even when most of the reactants are undissolved. Furthermore, the hydrochloride salt of the nucleophile (which is often more readily available in a pure form than the free base) may be used.

The reaction in these systems is really rather remarkable, since the enzyme is also undissolved, as well as most of the reactants and products. It is not clear what is the molecular basis for the rapid reaction. Even though the solubilities are low, it is possible that the solvent acts as such, transporting reactants and products between

their solid particle reservoirs and the enzyme, by means of very low dissolved concentrations. Alternatively, molecules may be transferred by some form of direct particle-particle contact, with the "solvent" acting purely as an inert suspension medium.

Experimental procedure

The starting materials were dissolved or suspended in 2 ml of the solvent, and then a solid mixture of $Na_2CO_3.10H_2O$ and 5 mg (0.2 μmol) chymotrypsin was added. The reaction mixture, in a flat bottomed vial of 14 mm internal diameter was stirred by a 12 mm teflon-coated magnetic bar, at about 1000 rpm, at room temperature (20-25 °C). After reaction the entire mixture was extracted with a suitable solvent (usually acetonitrile) and analysed by HPLC. The peptide products were identified by comparison with authentic samples.

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Reference

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