

Novel approach to enzymatic synthesis of peptides containing chromogenic and fluorogenic moieties

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Enzymatic peptide synthesis is a promising technique for production of optically pure biologically active peptide and their analogs, especially in organic media.

We developed a new approach for enzymatic peptide synthesis using as catalysts proteases which are covalently immobilized on macroporous carrier, poly(vinyl alcohol) (PVA) cryogel. Immobilized subtilisin 72, thermolysin, α -chymotrypsin, trypsin were prepared by chemical coupling of corresponding enzyme to the carrier and their synthetic efficiency in nonaqueous media was examined. The immobilized enzymes were able to catalyze peptide bond formation with high yield in DMF-MeCN mixtures with low water content.

The series of N-protected p-nitroanilides of tetrapeptides with general formula of Z-Ala-Ala-Xaa-Yaa-pNA (Xaa = Leu, Lys, Glu; Yaa = Phe, Asp) was synthesized in 70 - 98% yield using immobilized subtilisin as a biocatalyst in a DMF-MeCN (6/4) mixture. Synthesis of intramolecularly quenched fluorogenic substrate for pepsin, Abz-Ala-Ala-Phe-Phe-Ala-Ala-DeD, was carried out in DMF-MeCN mixture with equimolar amounts of amino- and acylating components and at [E]/[S] molar ratio of 1:800. The yield of the product (by HPLC) was 88% after 24 h. Immobilized thermolysin and α -chymotrypsin catalyzed the formation of chromogenic subtilisin substrate, Z-Ala-Ala-Leu-pNA in the DMF-MeCN mixture to yield 90% (1h) and 60% (24 h), respectively. Synthesis of Z-Phe-Arg-Leu-pNA from Z-Phe-Arg-OMe and Leu-pNA, with unprotected guanido group of arginine, was catalyzed by trypsin-cryoPVAG in MeCN yielding 60% of tripeptide in 24h.

Our studies have demonstrated high potential of cryoPVAG-immobilized proteinases as catalysts of synthetic reactions in organic media with low water content. The main advantage of our novel biocatalysts is that they can be used repeatedly without essential loss of activity and might be easily removed from the reaction mixture.

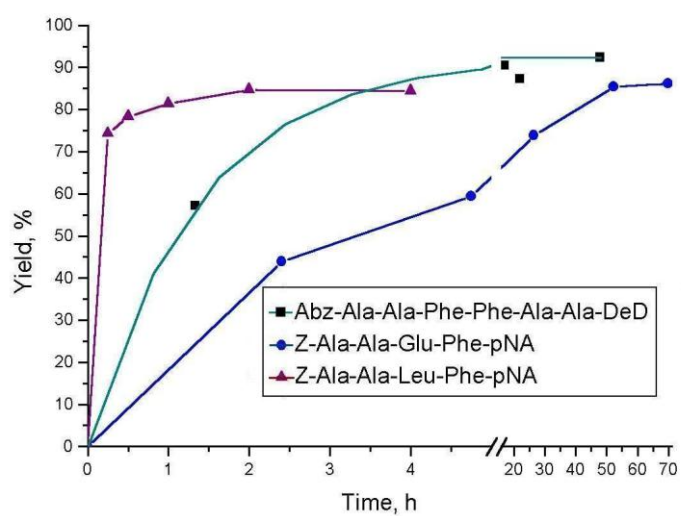


Fig. 1 - The time dependence of the peptide yield in synthesis, catalyzed by subtilisin-cryoPVAG

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