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Reversibly soluble biocatalyst: optimization of trypsin coupling to Eudragit S-100 and biocatalyst activity in soluble and precipitated forms

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Abstract

Eudragit S-100, a copolymer of methacrylic acid and methyl methacrylate is soluble at pH above 5 and insoluble at pH below 4.5. pH-dependent solubility of the polymer is used for the development of reversibly soluble biocatalyst, which combines the advantages of both soluble and immobilized biocatalysts. Activity of trypsin, covalently coupled to Eudragit S-100, was improved by protecting the active site of the enzyme with benzamidine and removing the noncovalently bound proteins with Triton X-100 in 0.15 M Tris buffer (pH 7.6). Accurate choice of coupling conditions combined with proper washing protocol produced highly active enzyme–polymer conjugate with no noncovalently bound protein. Two conjugates with 100-fold difference in the content of trypsin coupled to Eudragit S-100 were studied when the preparations were in soluble and precipitated forms. The K_m values of the soluble enzyme to the lower molecular weight substrate was less than that of the free enzyme, whereas that to the higher molecular weight substrate was closer to that of the free enzyme. Activities of the soluble and precipitated immobilized trypsin with higher molecular weight substrate were completely inhibited by soy bean trypsin inhibitor, whereas complete inhibition with soy bean trypsin inhibitor was never achieved with lower molecular weight substrate, indicating reduced access of high-molecular weight substrate/inhibitor to some of the catalytically active enzyme molecules in trypsin-Eudragit conjugate.