

STUDIES ON THE STABILIZATION OF ENZYMES BY COVALENT MODIFICATION WITH HYDROPHOBIC MOLECULES

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CERTIFICATE

This is to certify that the thesis entitled "Studies on the Stabilization of Enzymes by Covalent Modification with Hydrophobic Molecules" being submitted by Ms Alka Sadana, to the Indian Institute of Technology, Delhi for the award of degree of 'Doctor of Philosophy' is a record of bonafide research work carried out by her. Ms Alka Sadana has worked under my guidance and supervision, and has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard. The results contained in this dissertation have not been submitted in part or in full, to any other university or institute for the award of any degree or diploma.

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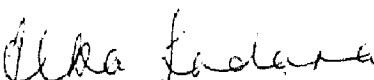
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ABSTRACT

This dissertation describes the investigations on the effect of covalently attached aromatic and steroidal molecules on the stability and catalytic activity of some enzymes in aqueous and aqueous-organic cosolvent systems. It is considered that these groups can provide large hydrophobic environment to the proteins even on low degree of modification, which may help in their stabilization and make them more compatible with the organic solvents.

The modifications of α -chymotrypsin with methyl deoxycholate and cinnamic acid derivatives show 15 and 25% substitution of the lysine groups of the enzyme respectively. The cinnamic acid-enzyme conjugate has been found to exhibit higher thermal stability than the native enzyme, retaining about 50% of its activity after 2 hours at 50°C. The steroid-enzyme conjugate, however, shows only 15% activity, the same as native enzyme after 2 hours in similar conditions. In presence of polar organic solvents, both the enzymes show higher stability than the native enzyme. Further, the steroid modified enzyme, when assayed in various aqueous-organic cosolvent systems, could withstand higher concentration of organic solvents as compared to the native enzyme. This effect is more pronounced in the systems

containing DMF, DMSO and ethanol. The cinnamic acid modified enzyme for some reasons shows reverse trend in the cosolvent systems.

The similar modifications of oxidoreductases like lactate dehydrogenase and yeast alcohol dehydrogenase with methyl deoxycholate lead to the enhancement of their activities by 20 and 15% respectively. It has also been observed that the above increase in activity, especially in the case of lactate dehydrogenase, is retained in some aqueous-organic cosolvent systems.

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