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Brief 69-10207

AEC-NASA TECH BRIEF

Purification and Characterization of Two Fully Deuterated Enzymes

Comparative data have been presented (*1*) on the kinetic and thermal stabilities of pure preparations of two ordinary enzymes (an alkaline phosphatase and a plant ribonuclease) and their fully deuterated counterparts. Side-chain deuteration appears to have little or no effect upon the kinetic properties of the enzymes, although the thermal stability of the deuterated phosphatase appears somewhat modified. Generally the kinetic measurements and thermal studies indicate very little difference in structure and function between the fully deuterated and the ordinary enzymes.

Earlier studies were concerned with the action of ordinary enzymes on deuterated substrates, or on hydrogen substrates in a D_2O medium. With the successful cultivation of fully deuterated organisms, however, their preparation and study become feasible. A fully deuterated enzyme contains deuterium in place of hydrogen at unexchangeable positions in the side chains of constituent amino acids of the enzyme.

In this study, only highly purified enzymes were used because, in the absence of accurate information about enzyme concentration, no valid conclusions can be reached about the magnitude of deuterium-isotope effects on the kinetic properties of a fully deuterated enzyme. No conformational changes resulting from the presence of fixed deuterium are felt at the active site of alkaline phosphatase; thus the kinetic properties of the deuterated enzyme are essentially the same as for the ordinary enzyme. The active site of the deuterated enzyme appears not be be altered by side-chain deuteration.

The effects of temperature on the enzymes proved to be consistent with earlier results. In the case of a phycocyanin undergoing denaturation at 49°C, side-chain deuteration lowers the denaturation temperature by about 7°C. Phycocyanin from a thermophilic alga denatures at 60°C, and deuteration lowers this temperature by 2°C. Alkaline phosphatase is inactivated at 85° to 90°C, and the ribonuclease activity is stable to about 100°C. No isotopic effect on thermal denaturation is observed; thus it appears that, at higher temperatures, side-chain deuteration has no significant effect on the thermal stability of proteins.

Reference:

1. S. Rokop, S. Parmerter, H. L. Crespi, J. J. Katz, *J. Biol. Chem.*, in press.

Notes:

1. This report may interest biological-research laboratories, fermentation companies, and hospital researchers.
2. Inquiries may be directed to:

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Patent status:

Inquiries concerning rights for commercial use of this innovation may be made to:

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Category 04