

It is proposed to examine the folding process of as many enzymes as can be obtained in reasonable quantity and purity from thermophilic bacteria, with an emphasis on the use of affinity chromatography. This technique which my colleagues, Cuatrecasas and Wilchek and I developed in 1968 while at the National Institutes of Health should be of great value in these experiments since it can be employed not only for studying the formation and binding properties of active centers, but also as a convenient method for purification. We have, in the past, been able to purify a number of proteins from crude extracts to homogeneity in one step because of the highly specific nature of active site binding to a unique ligand designed to catch the enzyme in question. Although a considerable number of proteins from thermophiles have been isolated and studied in terms of their activity and stability in the high temperature range, there has been very little on the chemical nature of the enzymatic binding sites in terms of the portions of the peptide chains involved and the nature of amino acid replacements in going from mesophilic to thermophilic enzyme species. If the proposed experiments work out well, we should be able to add some interesting and useful information to the nature of the side chain interactions that lead to tertiary structure stabilization at elevated temperatures. It has been pointed out that a significant understanding of protein folding and active center formation in thermophilic enzymes will probably eventually require crystallographic analysis of the three-dimensional structure. It has been shown by Thomas Powers and his colleagues, Frederic Richards and Harold Wykoff, that X-ray crystallography can be carried out using the more or less standard procedures on crystals at high temperatures. It has also been found by Zierer, et al., at the Max Planck Institute for Molecular Genetics that thermophilic proteins can be crystallized in the usual manner. A DNA binding protein from Thermus thermophilus was isolated from cells grown at 75°C and, following purification, formed good hexagonal crystals which diffracted to at least 3 angstroms. Comparison of such a structure in the presence and absence of DNA should be of considerable interest.

Affinity columns are being prepared employing the procedures of modification of silica by glycidoylation followed by periodate oxidation and attachment of the ligand of interest, generally separated from the backbone

by an aminohexyl "arm." Such columns have worked quite well in HPLC equipment although the temperatures so far examined have not exceeded the usual room temperature conditions. Testing and further development of such columns will undoubtedly require a bit of experimentation. It is hoped to have columns prepared that are suitable for several dehydrogenases and for beta galactosidase within the next few months, and the preparation of such columns will be facilitated by the kind assistance of experts at the Milligen Corporation who have offered to make available their column packing equipment.

Since crystallography does appear to be a very desirable adjunct to the studies of structure and function, we will attempt to select a particularly favorable enzyme in terms of its quantity in the bacterial cells and its ease of purification to prepare quantities sufficient for crystal preparation and diffraction pattern examination. A colleague in this department, Professor Evangelos Moudrianakis, as well as an active crystallographic group in the Biophysics Department nearby will assist us with advice and equipment when this stage is reached.

There is a possibility that the work that is carried out might be of some value in industrial application. The use of thermostable enzymes in the processes of oxidation, reduction, hydrolysis or introduction of substituents on aromatic and other backbone structures will be examined.

The overall program will clearly involve the standard techniques of protein chemistry including amino acid analysis, fluorescence and spectrum determination.