

BOOK REVIEWS

Thermostability of Enzymes. Gupta, M. N. ed. Narosa Publishing House, New Delhi. 1993. 213 + 5 pp.

This book is a positive effort to compile various aspects of enzyme stability. It consists of 11 chapters written by several authors and discusses a wide range of topics from protein structure, stability, isolation and purification of proteins to immobilization techniques for altering thermal stability of enzymes and protein engineering for thermal stabilization. The first seven chapters contain an overview and deal with an understanding of thermostability, while the last four describe different techniques of thermostabilization. The last chapter discusses the inter dependence of the twin areas of thermostability and thermostabilization.

The stability of native protein structure is governed by a complex interplay of forces—electrostatic and hydrogen bonds and covalent crosslinks, rendering the predictions of specific mutations difficult. Denaturation is a process of transformation of protein to a non-active form and in the case of an enzyme to an inactive form. Denaturation generally involves either variation to temperature or addition of chaotropic agents as a means of inducing unfolding. The unfolded state of a protein contributes appreciably to the overall entropy of a protein.

The common procedures employed to enhance thermal stability of proteins include (i) introduction of disulphide bonds (ii) chemical modifications at reactive side chain (iii) alteration of packing arrangements (iv) engineering of helix-dipole interactions (v) introduction of new hydrogen bonds and (vi) immobilization.

Thermostable enzymes are isolated using the same methodology as applied for purification of proteins. Thermal denaturation of heat-sensitive proteins may also be exploited to purify the thermostable ones.

Organisms that live and thrive under conditions of extreme temperatures (40–45°C) are known as thermophiles. Thermophiles are important sources of thermostable enzymes and have probably evolved a number of mechanisms in order to adapt to life at a high temperature. The two theories involved in thermophile mechanisms are (i) static, according to which all cellular components of thermophilic microorganisms have an intrinsic stability higher than their mesophilic

counterparts as the genetic information in a thermophile is different from that of a mesophile and (ii) dynamic, according to which active metabolism and rapid resynthesis of key enzymes are essential for maintenance of thermophilic growth.

Thermoresistance of enzymes is of great interest as application of thermostable enzymes has many advantages. The most important ones are (i) acceleration of rates of reactions catalysed by enzymes (ii) shift of thermodynamic equilibrium (iii) increase of operational stability (iv) high stability towards denaturing conditions and (v) ease of operation at elevated temperatures.

Immobilization increases protein stability against denaturing actions such as temperature, organic solvents, surfactants etc. and stabilizing effect depends on the number of bonds between an enzyme and support. However, if activation is caused by reasons other than unfolding, stabilization by immobilization is unsuccessful.

Ajit Sadana provides an insight into enzyme deactivation, an important factor in the practical application of enzymes. Models for enzyme deactivation are presented to help understand enzyme deactivation mechanisms.

Protein stability can be determined from denaturation curve followed by changes in conformational properties of proteins. Proteins can be denatured by various chemical and physical treatments. Some of the main conclusions are (i) urea, guanidine hydrochloride and guanidine thiocyanate give the most extensively denatured states at 25°C and (ii) extreme pH, heat organic solvents and solutes and inorganic salts give partially denatured state.

It is well known that heating of proteins to moderately high temperatures for shorter durations causes thermoinactivation. Higher temperature and longer durations lead to irreversible thermoinactivation. One of the primary reasons for thermoinactivation is the instability of disulphide bridges. A single disulphide bond contributes 2–5 kcal/mol towards stability. 'Scrambling' results due to inactivation of disulphide bridges. Cysteine bridges are also susceptible to decomposition.

Enzymes possess remarkable properties and therefore are useful in food, chemical and pharmaceutical industries. However, their full potential has not been exploited due to their instability. Efforts have been made to increase stability by use of solu-

ble additives.

Soluble additives interact non-covalently and reversibly with enzyme to form complexes, they may interact with solvent medium or with other substances present in solution which tend to inactivate the enzymes. Soluble additives commonly used to stabilize enzymes are (i) organic molecules such as acetone, ethanol, butanol etc. (ii) polyhydric alcohols (iii) substrate and substrate-like ligands.

Chemical alteration of protein structure with or without changes in biological function is termed as chemical modification. This aspect has been covered by Renu Tyagi and M. N. Gupta. Thermostability can be enhanced by (i) chemical modification and (ii) chemical crosslinking.

Immobilization is known to be used for stabilization of enzymes by suppression of unfolding of the protein molecule or by suppression of certain secondary processes. Cabral and Kennedy have reviewed the methodology involved in the preparation of immobilized enzymes and immobilization methods on the thermostability of enzymes. Common immobilized techniques generally used for stability of enzymes include (i) adsorption (ii) affinity immobilization (iii) ionic binding (iv) covalent binding and (v) entrapment.

Stabilization of proteins is essential for studies in biotechnology. Protein engineering is a promising strategy for stabilizing proteins. Rapid advances in recombinant DNA techniques have made it possible to replace at will one or more amino acid residues in a protein, by substituting one or more nucleotides in gene coding for the protein. The contributions of individual amino acids towards protein stability can then be exploited. Eventually it may be possible to construct more stable proteins than parent molecules by site-directed mutagenesis.

The book is a good attempt by both the editor and the publishers and will be a useful guide to students, teachers and researchers involved in different aspects of enzymes. It is moderately priced both for libraries and individual buyers.

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