

## PREFACE

This volume is dedicated to the memory of the Nobel Laureate Christian B. Anfinsen. In typical fashion he had the foresight to determine that a book dealing just with proteins from hyperthermophiles was timely, even though some of us thought that such a book might be premature. The following pages represent the product of Chris's original idea. The reader will no doubt conclude that he was right. Moreover, without Chris's urging, encouragement, and relentless enthusiasm at the initiation of this project, I doubt if a Preface would be needed at this time. Suffice it to say that this volume represents a very small monument to a man who achieved in one lifetime what few dream of in many.

A volume dedicated to the properties of enzymes and proteins derived from the so-called hyperthermophiles would not have been possible even five years ago. Hyperthermophiles are defined as microorganisms that can grow at temperatures of 90°C and above. They were discovered in shallow marine volcanic vents in the early 1980s by Karl Stetter and co-workers. Mainly through their pioneering studies, the field has greatly expanded over the past decade, and more than thirty species of hyperthermophile are known at present. However, as might be expected, because of the problems with cultivating these organisms, it was some time after they were discovered that the first proteins were purified from them. Indeed, the initial examples of hyperthermophiles were autotrophic, and their growth was obligately dependent on reducing elemental sulfur to hydrogen sulfide. Typically, they did not reach high cell densities. It was not until the mid-1980s that heterotrophs able to grow well in the absence of sulfur were isolated, such as species of *Pyrococcus* and *Thermotoga*. With these organisms, large-scale cultures became feasible, and sufficient biomass could be routinely obtained for protein purifications. Such species have been the source of most of the hyperthermophilic proteins characterized so far.

With the availability of proteins from hyperthermophiles, researchers in this field could obviously begin to address the fundamental question of how they are stabilized at temperatures near and even above the normal boiling point. With the development of this field during the 1980s, however, it became clear that the classification of the organisms themselves raised other interesting issues. Specifically, the seminal research of Carl Woese and co-workers in the late 1970s led to the descrip-

tion, on the basis of 16S rRNA analyses, of a new "domain" of life—the archaea, formerly archaeobacteria. Moreover, subsequent work by Woese indicated that the archaea domain had an ancestor in common with eukaryotes which was not shared by bacteria. Consequently, as each new hyperthermophilic species was isolated, virtually all were found to be members of the archaea. In fact, at present, only two genera of hyperthermophiles are known, *Thermotoga* and *Aquifex*, that are classified as bacteria. The ecology and taxonomy of the hyperthermophilic archaea are discussed in the first chapter by John Baross and James Holden. These researchers also present the current status of a somewhat unique topic—hyperthermophilic heat-shock proteins. Remarkably, these organisms do show both a "hot" and "cold" stress response, and the nature of the proteins involved and how they function are just beginning to be explored.

The fact that most hyperthermophilic organisms are archaea rather than bacteria raises several interesting questions regarding the nature of their biochemical pathways and the enzymes and proteins involved. Do these organisms utilize bacterial-type metabolic routes for utilizing growth substrates and for conserving energy, or are they more eukaryotic-like? The subsequent two chapters by Robert Maier and by Arnulf Kletzin and me discuss these issues with reference to the respiratory chains of sulfur-respiring organisms and to the fermentative pathways of the hyperthermophilic archaea, respectively. We also discuss the first structural information to be obtained on both a hyperthermophilic protein (rubredoxin) and a hyperthermophilic enzyme (aldehyde ferredoxin oxidoreductase). Rainer Jaenicke and co-workers provide a contrasting description of what is known about the enzymology of carbohydrate fermentation in the hyperthermophilic bacterium, *Thermotoga maritima*. The ability to metabolize complex carbohydrates and proteinaceous materials is virtually a characteristic of the hyperthermophiles, including the majority of the archaea and *T. maritima*. The enzymes involved have significant biotechnological potential, and the properties of those purified so far are discussed by Robert Kelly and co-workers. This is followed by a summary by Jocelyne DiRuggiero and Frank T. Robb of what is known about the enzymes involved in the primary metabolism of nitrogen-containing compounds.

An increasing number of hyperthermophilic organisms are being isolated from near deep sea hydrothermal vents. These occur several kilometers below sea level, and organisms living in their vicinity are exposed to two extreme conditions—high hydrostatic pressure (approximately 100 atm per 1 km depth) as well as high temperature. Although no obligately barophilic hyperthermophile has been isolated so far, there

is evidence that at least some hyperthermophilic enzymes are stabilized and/or activated by high pressure. Douglas Clark and co-workers discuss the recent data on this subject. Of course, the main "claim to fame" of hyperthermophiles in a practical sense is that they have provided heat-stable polymerases for the PCR reaction. Although the prototypical enzyme, *Taq* polymerase, was obtained from what is now considered to be a moderately thermophilic organism, the *Taq* enzyme has been superseded to some extent by a variety of polymerases from the hyperthermophiles. In addition to their commercial significance, these enzymes have also given new insights into protein structure and function, particularly with the phenomena of inteins and exteins. The current diversity of DNA polymerases is described by Francine Perler and colleagues. Of course, any analysis of such enzymes assumes that their substrate, DNA, is stable at the growth temperature of the organisms from which they were obtained. Hence, John Reeve and co-workers discuss the problems that hyperthermophiles have in maintaining DNA in a double helical form near and above 100°C and the likely role of histone-type proteins.

Key aspects of all the research discussed in this volume are the mechanisms of protein stability and the evolutionary significance of hyperthermophilic enzymes and proteins. As will become apparent, meaningful insights into the stability issue have yet to be elucidated. Although hyperthermophilic proteins are for the most part exceedingly thermostable *in vitro*, this property is apparently conferred by the same intraprotein interactions that stabilize mesophilic proteins. The problem is that the differences between the hyperthermophilic and mesophilic versions are not dramatic, or even obvious, and the "holy grail" of elucidating a general mechanism by which a given mesophilic protein can be converted into a hyperthermophilic one is not likely to be found in the near future. In contrast, the phylogenetic aspects of hyperthermophilic proteins are providing new insights into likely pathways of enzyme evolution, although the results are not always congruent with phylogenetic relationships determined by nucleic acid analyses. Future studies of proteins from both known hyperthermophiles and those yet to be discovered will no doubt provide deeper insights into both the mechanisms of protein stability and enzyme evolution.

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