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Introduction

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Deciphering the molecular basis for cytochrome *c* oxidase activity and regulation has proved to be a major challenge to biochemists, biophysicists, and physiologists. Its role in controlling energy metabolism in heart and brain is a critical physiological issue; determining the environment of its unique metal centers and the kinetics of electron transfer between them continues to consume the efforts of many biophysicists and spectroscopists; and unraveling its highly complex protein structure remains a formidable task for biochemists.

One stumbling block in the analysis of this enzyme is the speed and complexity of its electron transfer reactions. Steady-state kinetic measurements show biphasic and multiphasic dependences on substrate concentration which yet defy unambigious interpretation (Cooper, 1989) and presteady-state activity measurements are hampered by the rapidity of electron flow compared to mixing methods. An important breakthrough in circumventing the latter problem has been the development of methods utilizing flash activation of carbon monoxide-inhibited oxidase to permit analysis of the initial steps of electron transfer and oxygen chemistry (Gibson and Greenwood, 1963; Chance *et al.*, 1975). This methodology has been exploited in a variety of ways with different spectroscopies including visible, resonance Raman and FTIR. With the further development of methods for laser-induced electron input from cytochrome *c*, there is promise for future expansion of time-resolved studies (Millett and Durham, 1991; Tollin and Hazzard, 1991; Pan *et al.*, 1991). Currently, intense controversy still exists over the rates and routes of electron flow between centers, the intermediates of oxygen reduction, and the mechanism by which electron transfer is coupled to proton translocation. Several of the articles in this volume present different views on the electron transfer pathways in cytochrome oxidase and possible mechanisms of coupling and control (Rousseau *et al.* (p. 165), Hill, Babcock and Varotsis (p. 71), Woodruff (p. 177), Nicholls and Butko (p. 137)). Additional perspectives on these issues are provided by recent reviews published elsewhere (Malmstrom, 1990; Chan and Li, 1991; Moody *et al.*, 1991; Babcock and Wikstrom, 1992; Larsen *et al.*, 1992).

Difficulties in understanding the kinetic behavior and the spectral characteristics of cytochrome *c* oxidase are compounded by the complexity of its protein structure (Capaldi, 1990).The mammalian enzyme is an intrinsic membrane protein with thirteen different subunits (Kadenbach *et al.*, 1983), and some tendency to assume dimeric and oligomeric states. Its activity and spectral characteristics can be profoundly influenced by the conditions of purification, the amount and nature of associated phospholipid, and the artificial hydrophobic environments provided by different detergents, as well as the immediate past history of turnover. It has been, and continues to be, a major task to define how various factors influence oxidase activity, and, moreover, to determine and maintain physiological forms of the purified enzyme. The articles of Robinson (p. 153) and Palmer (p. 145) deal with the different aspects of this issue. A tool that may be useful in probing the conformation of the protein in the vicinity of the metal centers, second-derivative visible spectroscopy, is discussed by Copeland (p. 93).

A significant advance in the study of cytochrome oxidase has been the discovery that a variety of oxidases from aerobic bacteria show remarkable

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structural, spectral and functional similarity to the mammalian enzyme, while having considerably fewer subunits (Ludwig, 1987; Saraste, 1990; Gennis, 1991). Thus, the study of bacterial oxidases circumvents some of the variables relating to purification and, more importantly, permits the application of powerful genetic tools to the investigation of structure/function relationships. The articles by Hosler *et al.* (p. 121) and Fee *et al.* (p. 103) summarize some of the recent progress in the area. Other papers that provide further perspective on this active and fruitful effort include Steinrucke *et al.* (1991), Van der Oost *et al.* (1992), Minagawa *et al.* (1992), Lemieux *et al.* (1992), and Haltia and Wikstrom (1993). This research area has generated much excitement, since a number of fundamental structural issues are already being clarified, including the nature of the metal ligands (see Hosler *et al.* p. 121) and the role of subunits (Haltia *et al.*, 1991). However, the specific structural modifications now possible with site-directed mutagenesis will give new insight into function only insofar as accurate and sensitive methods of probing the altered structure and function can be applied and meaningfully interpreted. Many unsolved kinetic, spectral, and biochemical problems will undoubtedly continue to haunt us in this endeavor (see Palmer p. 145, Caughey p. 81). Nevertheless, a number of the articles in this volume illustrate how the combination of new structural information and powerful time-resolved spectroscopies can generate novel and stimulating ideas regarding possible mechanisms of energy transduction. The future holds great promise for our ability to critically test and refine these models, leading to the likelihood of real progress in understanding the fundamental process of biological energy conservation.

One of the inspirations for the choice of contributors for this volume was a recent conference, "The First Britton Chance Research Discussion: Primary Events of Respiration," organized by Britton Chance and P. Leslie Dutton. Unfortunately, only a few of the many interesting speakers and discussants are represented here. An arbitrary decision was made to have this be a North American production and, rather than attempt to be comprehensive, the aim was to explore different views on some key issues where there have been recent experimental advances. In spite of the limited scope, it is hoped that the tone of the Chance Discussion is still partly captured: a forum where new data and new ideas were critically discussed and challenged from biochemical, biophysical, and physiological perspectives. In all these areas, Professor Britton Chance has made early, continued, and seminal contributions. In recognition and gratitude for his vital role in furthering our understanding of cytochrome *c* oxidase, I would like to dedicate this volume to him, on the occasion of his 80th birthday.

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