Chapter 17

Bovine Heart Cytochrome c Oxidase

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1. INTRODUCTION

Mitochondrial cytochrome c oxidase reduces molecular oxygen (O₂, hereafter) to water, and this process is coupled to the pumping of protons through the mitochondrial inner membrane from matrix space to intermembrane space (Ferguson-Miller and Babcock, 1996). The elucidation of the reaction mechanism of this enzyme continues to be one of the most important and intriguing subjects in biological science. Elucidation of the reaction mechanism of an enzyme means complete description of changes in the three-dimensional structure of the enzyme during the enzymic turnover triggered by its substrates. Thus, solving of the three dimensional structures in various intermediate states during enzymic turnover is crucial for a complete understanding of the reaction mechanism. A powerful method for determination of the three-dimensional structure of a protein is X-ray crystallography. However, using this method for the identification of the structures of short-lived transient intermediates at the active site can be quite difficult. Furthermore, the resolution of the X-rays scattered from crystals of enzymes is, in most cases, not high enough for a complete
evaluation of the chemical reactivity at the catalytic site. Thus, implementations of additional spectroscopic methods are indispensable for a complete description of chemical events that take place at the catalytic site of an enzyme. However, any spectroscopic method is sensitive only to the chromophores and it provides essentially one-dimensional information. Thus, if an X-ray structure for a particular enzyme is not available, chemical events at the catalytic site cannot be completely elucidated. Thus crystallographic and spectroscopic methods are quite complementary and both types of methods are required for a complete examination of any enzyme reaction mechanism.

Several requirements need to be considered in order to obtain the X-ray structure of an enzyme at high resolution. First of all, the enzyme must be purified from cells for crystallization, and the chemical composition of the isolated enzyme needs to be determined. These initial steps in elucidation of the reaction mechanisms of large multicomponent membrane proteins such as cytochrome c oxidase are usually quite time-consuming. In this article, the first and fundamental step required for structure-function investigations of cytochrome c oxidase, determination of the composition of the purified enzyme and its crystallization, will be reviewed. Then, mechanisms of O₂ reduction and proton pumping will be discussed with emphasis on the importance of the X-ray crystallographic findings for increasing our understanding of the reaction mechanism.

2. COMPOSITION OF BOVINE HEART CYTOCHROME C OXIDASE

2.1. Purification

Usually it is very difficult to isolate membrane proteins since typical membrane proteins have large hydrophobic surfaces surrounding a central core region. These hydrophobic surfaces interact with the phospholipid bilayer of the biological membrane. On the other hand, the two surfaces at the outer ends of membrane proteins are exposed to the aqueous phases on both sides of the biological membrane. This common arrangement of hydrophobic and hydrophilic surfaces on membrane proteins indicates that in their isolated form membrane proteins are unstable in an isolated aqueous medium or in an isolated hydrophobic medium. Actually, an example of a membrane protein that has been isolated in an organic solvent cannot be found. Thus, the best method for isolation of membrane proteins is to extract the protein from the phospholipid bilayer by exchanging the membrane phospholipids bound to the hydrophobic surface of the