Amino Acid Sequence of the Putative Protonophore of the Energy-Transducing ATPase Complex

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Introduction

The energy transducing ATPase of mitochondria, chloroplasts, and bacteria is one of the most complex enzymes. It consists of at least ten different subunit polypeptides ranging in molecular weight from 60,000 to 8000 daltons. We have concentrated our efforts during the past few years on the smallest subunit of 8000 daltons, especially on its chemical characterization. This extremely hydrophobic polypeptide occurs in the complex as an oligomer, probably as a hexamer (Sebald et al., 1978) and is thus a major subunit, comprising about 10% of the total enzyme protein. Together with at least two further hydrophobic polypeptides it constitutes the membrane factor Fo (Sone et al., 1975; Sebald, 1977), which has been shown to exhibit the properties of a proton channel (Hinkle and Horstman; 1971; Okamoto et al., 1977).

This smallest ATPase subunit was detected about ten years ago by Beechey et al. (1967) by means of the ATPase inhibitor dicyclohexylcarbodiimide (DCCD). This hydrophobic carbodiimide was found to inhibit specifically and irreversibly the ATPase complex of beef heart mitochondria. By means of the radioactive compound, a DCCD-binding protein of low molecular weight was identified (Cattell et al., 1971). This protein was called a proteolipid due to its solubility in certain organic solvents. But it was never purified to homogeneity.

During these early studies, it was discussed that the DCCD-binding proteolipid plays an essential role in oxidative phosphorylation. Nevertheless, this protein lived a shadowy existence for several years, due mainly to the following two facts: (1) To organic chemists carbodiimides are known as highly reactive compounds (Khorana, 1953). Thus the specific inhibition of the ATPase complex as well as the specific binding to one protein only remained a puzzling result. (2) Due to the difficulties encountered in the purification and chemical analysis of hydrophobic proteins, the true nature of this proteolipid could not be established. The know-how for handling such proteins has been developed only in the last few years.

In the meantime, the DCCD-binding protein became one of the best characterized subunits of the ATPase complex. This applies to its function and especially to its structure. Recently, experiments have been reported which suggest that the isolated subunit when reconstituted into a lipid membrane exhibits protonophoric activity sensitive to DCCD or oligomycin (Nelson et al., 1977; Criddle et al., 1977). Thus the DCCD-binding protein may constitute the proton channel of the ATPase complex. We have studied two aspects of the amino acid sequence of this protein: (1) The DCCD-binding protein from mitochondria of different organisms as well as from bacteria and chloroplasts was analyzed in order to determine the general and typical features of the amino acid sequence and in order to see which residues have been conserved during evolution and therefore may be important for the function and structure of the putative protonophore. (2) The action of inhibitors was analyzed. The
Isolation and Amino Acid Composition of the DCCD-Binding Protein

The DCCD-binding protein was extracted from whole membrane by neutral chloroform/methanol. The protein from *Neurospora* and yeast mitochondria is thereby obtained in a pure form (Sebald and Jackl, 1975; Sebald et al., 1978a). Figure 1 shows an SDS-gel electrophoretic pattern of such preparations. The DCCD-binding proteins from *E. coli*, spinach chloroplasts (Wachter and Sebald, 1978b), and beef heart mitochondria (Graf and Sebald, 1978) are still heavily contaminated after extraction with chloroform/methanol. They can be finally purified by chromatography on DEAE- or CM-cellulose. This method was first described by Fillingame (1976) and by Altendorf (1977) for the isolation of the *E. coli* protein.

The amino acid composition of the DCCD-binding protein from five different sources is shown in Table 1. The proteins have a slightly different size — containing between 76 and 81 amino acids. All of them exhibit low polarity, especially the *E. coli* protein, which contains only 16% hydrophilic side chains. Generally, no tryptophan and histidine and only a few lysines and arginines are present. The large amount of the small amino acids glycine and alanine, which comprise about 25% of the total residues, is striking. All amino acid compositions, besides that of the chloroplast protein, were obtained by sequence analysis (Wachter and Sebald, 1978a; Wachter et al., 1978a; Wachter et al., 1978b). The sequences of the two mitochondrial proteins from *Neurospora* and yeast, as well as that of the *E. coli* protein, which was studied in cooperation with K. Altendorf, shall be described below.