the overall synthesis of carbamoyl phosphate.

The use of ^{31}P NMR appears to be the method of choice for the measurement of positional isotope exchange reactions in phosphate esters. The amount of material used in these studies is about the same as that used with the mass spectral method, and there is no need for extensive degradation of the products. The major advantage of the NMR method is that the reaction can be followed continuously if adequate sensitivity and resolution are obtained.

Acknowledgments

We thank Dr. P. M. Anderson for the generous gift of carbamoyl-phosphate synthetase (GM-22434).

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Structure of the Cytochrome *c* Oxidase Complex: Labeling by Hydrophilic and Hydrophobic Protein Modifying Reagents†

L. Prochaska, R. Bisson, and R. A. Capaldi*

ABSTRACT: Beef heart cytochrome *c* oxidase has been reacted with [^{35}S]diazobenzenesulfonate ([^{35}S]DABS), [^{35}S]-*N*-(4-azido-2-nitrophenyl)-2-aminoethylsulfonate ([^{35}S]NAP-taurine), and two different radioactive arylazidophospholipids. The labeling of the seven different subunits of the enzyme with these protein modifying reagents has been examined. DABS, a water-soluble, lipid-insoluble reagent, reacted with subunits II, III, IV, V, and VII but labeled I or VI only poorly. The arylazidophospholipids, probes for the bilayer-intercalated portion of cytochrome *c* oxidase, labeled I, III, and VII heavily and II and IV lightly but did not react with V or VI. NAP-taurine labeled all of the subunits of cytochrome *c* oxidase. Evidence is presented that this latter reagent reacts with the enzyme from outside the bilayer, and the pattern of labeling with the different hydrophilic and hydrophobic labeling reagents is used to derive a model for the arrangement of subunits in cytochrome *c* oxidase.

Cytochrome *c* oxidase is the terminal member of the electron transport chain, an integral part of coupling site III, and an intrinsic component of the mitochondrial inner membrane. The protein complex contains two heme moieties (*a* and a_3) and two copper atoms as electron acceptors along with seven (or possibly more) polypeptides in a complex of molecular weight around 140 000 [for reviews, see Erecinska & Wilson (1978) and Capaldi (1979)]. Recently, considerable progress has been made in determining the structure of this complex. The gross shape and approximate size of cytochrome *c* oxidase from beef heart mitochondria has been obtained by electron microscopy and image reconstruction studies (Henderson et al., 1977; Fuller et al., 1979). The protein is seen as Y shaped and made up of three domains, two of which (the M_1 and M_2 domains) span the lipid bilayer; the third (or C domain) is outside the bilayer (Fuller et al., 1979). Cytochrome *c* binding (S. D. Fuller and R. A. Capaldi, unpublished results) and antibody binding experiments (Frey et al., 1978) indicate that the C domain is located on the cytoplasmic side of the mitochondrial inner membrane (hence the nomenclature); the two M domains extend a small way into the matrix space. The arrangement of the subunits in cytochrome *c* oxidase has been examined by reacting the enzyme with [^{35}S]DABS (Eytan & Schatz, 1975; Eytan et al., 1975; Eytan & Broza, 1978; Ludwig et al., 1979), by lactoperoxidase-catalyzed iodination of the complex (Eytan & Schatz, 1975), by antibody binding experiments (Chan & Tracy, 1978), and by using iodoaryl azides (Cerletti & Schatz, 1979) and arylazidophospholipids (Bisson et al., 1979a,b). The orientation of the enzyme in the mitochondrial inner membrane has also been explored by labeling with [^{35}S]DABS (Eytan et al., 1975; Ludwig et al., 1979) and by antibody binding (Chan & Tracy, 1978).

The concensus from these studies is that subunits II and III are on the cytoplasmic side of the inner membrane and thus a part of the C domain of the cytochrome *c* oxidase complex. Subunit IV is generally accepted to be on the matrix side of the membrane, while subunit I is considered to be predomi-

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nantly buried within the bilayer-intercalated portion of the protein. There are as yet conflicting data on the location of other subunits in the complex.

Recently, the reagent [^{35}S]-*N*-(4-azido-2-nitrophenyl)-2-aminoethylsulfonate ([^{35}S]NAP-taurine) has been introduced as a small, water-soluble, lipid-insoluble protein modifying reagent for use in studies of membrane topology (Staros & Richards, 1974; Staros et al., 1975). In the experiments described here, we have examined the labeling of both detergent-dispersed and membranous cytochrome *c* oxidase by this reagent. The reactivity of NAP-taurine with cytochrome *c* oxidase is compared directly to that of DABS and with the reactivity of the enzyme to arylazidophospholipids, reagents designed to label bilayer-intercalated portions of an intrinsic membrane protein (Bisson et al., 1979a,b). Our studies provide new insights into the arrangement of the subunits in the cytochrome *c* oxidase complex.

Experimental Procedures

Enzyme Preparations. Beef heart cytochrome *c* oxidase was prepared according to the method of Capaldi & Hayashi (1972). Electron transport activity was measured polarographically by the procedure of Vik & Capaldi (1977). Oligomycin-sensitive ATPase (OSATPase) was isolated and assayed for enzymatic activity according to Serrano et al. (1976). Antibody against cytochrome *c* oxidase was prepared according to Ludwig et al. (1979).

Preparation of Protein Modifying Reagents. [^{35}S]DABS (5–9 Ci/mmol) was prepared from [^{35}S]sulfanilic acid (Amersham Searle) according to Tinberg et al. (1974). [^{35}S]-NAP-taurine (39 mCi/mmol) was synthesized from [^{35}S]-taurine by the method of Staros et al. (1975). The final product was 70% pure as judged by thin-layer chromatography on silica gel developed with chloroform–methanol. The major impurity (25%) was unreacted [^{35}S]taurine, as detected by 0.25% ninhydrin spray in butanol. There were also trace amounts of photolytic degradation products.

The preparation of 1-myristoyl-2-amino-[(*N*-2-nitro-4-azidophenyl)lauroyl]-*sn*-glycero-3-[^{14}C]phosphocholine (I) and of 1-palmitoyl-2-(2-azido-4-nitrobenzoyl)-*sn*-glycero-3-[^{3}H]-phosphocholine (II) has been described by Bisson & Montecucco (1980). The specific radioactivity of the two arylazidophospholipids was 177 Ci/mol and 3.9 Ci/mmol, respectively.

Labeling of Detergent-Dispersed Enzyme. Cytochrome *c* oxidase (0.6 mg/mL) was reacted with [^{35}S]DABS (20 μM) at room temperature for 10 min in a buffer containing either 0.1% Tween 80 or 0.2% Triton X-100 in 100 mM NaCl and 50 mM sodium phosphate (pH 7.5). The reaction was stopped by the addition of 10 mM histidine, and the mixture was centrifuged through 10% sucrose in 10 mM sodium phosphate for 12–16 h at 200000*g* to separate the protein from noncovalently bound protein modifying reagent. The isolated enzyme (0.6 mg/mL) was also reacted with [^{35}S]NAP-taurine in a buffer containing either 0.1% Tween 80 or 0.2% Triton X-100 with 100 mM NaCl and 50 mM sodium phosphate (pH 7.5). Experiments were conducted over a range of concentration of NAP-taurine with 1.3 mM being used in experiments shown in Figure 1. The reaction was initiated by illuminating the sample with high-intensity white light [$(0.5–2.0) \times 10^6$ ergs/(cm^2 s)] that was filtered through a $CuSO_4$ solution to remove infrared light. Illumination was continued for 10 min at 20 °C in a water-jacketed cell. Cytochrome *c* oxidase was separated from noncovalently bound NAP-taurine by centrifugation through 10% sucrose in 10 mM sodium phosphate (pH 7.4) as in the DABS labeling experiments. OSATPase was labeled with both [^{35}S]DABS and [^{35}S]NAP-taurine in a buffer containing 100 mM NaCl and 50 mM sodium phosphate (pH 7.5) by the same procedures described for the experiments with cytochrome *c* oxidase.

Labeling of Membranous Cytochrome c Oxidase. Egg lecithin (Sigma) dissolved in $CHCl_3$–CH_3OH (2:1) was dried under a stream of nitrogen. The lipid was then suspended in a buffer of 10 mM sodium phosphate (pH 7.5) to a 0.4% solution by sonication using a Branson bath sonicator on the full setting. The sonication step was continued until the phospholipid solution was optically clear. Cytochrome *c* oxidase was incorporated into the egg lecithin vesicles by the method of Eytan & Broza (1978). One aliquot was labeled by [^{35}S]DABS and another aliquot by [^{35}S]NAP-taurine by using the same conditions as those for the detergent-dispersed enzyme. Vesicles containing arylazidophospholipid were prepared according to Bisson et al. (1979b). The ratios of arylazidophospholipid to egg lecithin used were 0.4 and 0.1% for azidophospholipids I and II, respectively. Reaction of cytochrome *c* oxidase and the azidophospholipids was initiated by illuminating the sample under UV light through glass [intensity $(1–3) \times 10^3$ ergs/(cm^2 s)] for 30 min on ice. After vesicles were labeled by [^{35}S]DABS, [^{35}S]NAP-taurine, or arylazidophospholipids, they were dissolved in 1% Triton X-100 and then centrifuged through sucrose (15%) for 12 h to separate the enzyme from nonbound labeling reagent and from excess phospholipid.

Gel Techniques. $NaDodSO_4$–polyacrylamide gel electrophoresis was performed by the procedure of Swank & Munkres (1971) using 15% acrylamide and *N,N'*-methylenebis(acrylamide) (1:30). Gels were stained and destained as described by Downer et al. (1976). Radioactive gels were sliced into 1-mm thick slices with a Mickle gel slicer. These were dissolved in 1 mL of 15% H_2O_2 at 80 °C overnight, 6 mL of a solution of Omnifluor (NEN) (2:66 g/L) in toluene–Triton X-100 (2:1) was added to each vial, and the radioactivity was measured in a Packard liquid scintillation counter.

Other Methods. Protein was estimated as described by Lowry et al. (1951). Light intensity was measured with a Yellow Springs Instrument Co. Model 50 radiometer.

Results

The labeling of detergent-dispersed beef heart cytochrome *c* oxidase by [^{35}S]DABS is shown in Figure 1, trace b. In agreement with previous reports (Eytan et al., 1975; Eytan & Broza, 1978; Ludwig et al., 1979), subunits II, III, IV, V, and VII were all labeled by this reagent but there was essentially no labeling of either subunit I or VI. The relative labeling of subunits by DABS, arbitrarily defined as the number of counts in each subunit divided by the molecular weight of that component, is given in Figure 2. It can be seen that the labeling of subunit II is low in relation to that of subunit III. This was not obvious in our previous experiments (Ludwig et al., 1979) because a portion of subunit III was aggregated and migrated on top of the gels. The experiment in Figure 1b was with cytochrome *c* oxidase dissolved in Triton X-100. Experiments in which the enzyme was dissolved in Tween 80 gave very similar results except that subunits IV and V were considerably more reactive to the protein modifying reagent (Figure 2).

The labeling of detergent-dispersed cytochrome *c* oxidase with NAP-taurine is shown in Figure 1a (traces a and b are actually for two aliquots of the same sample, the DABS and NAP-taurine labeling was done simultaneously, and the gels were run together). All of the subunits of cytochrome *c* oxidase as well as impurities of the enzyme preparation were labeled

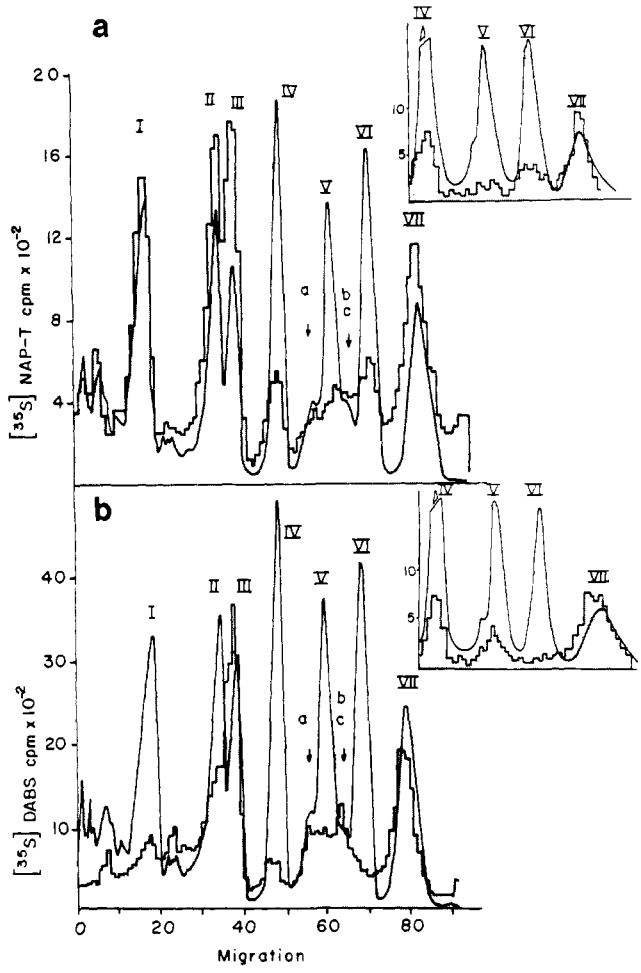

FIGURE 1: Labeling of cytochrome *c* oxidase with hydrophilic protein modifying reagents. Cytochrome *c* oxidase labeled with 1.3 mM [^{35}S]NAP-taurine (a) or 20 μM [^{35}S]DABS (b) in 50 mM sodium phosphate, 100 mM NaCl, and 0.2% Triton X-100 (pH 7.4) was collected by centrifugation and run on 15% polyacrylamide gel (1:30 cross-linked) in $NaDodSO_4$–urea as described under Experimental Procedures. 1 mol of NAP-taurine was incorporated per mol of cytochrome *c* oxidase with a 10% inhibition of electron transfer activity. 1 mol of [^{35}S]DABS was bound per 25 mol of cytochrome *c* oxidase; this effected less than 5% inhibition of activity. The inserts to a and b show the labeling of trypsin-treated enzyme in the same medium. Trypsin treatment was performed according to Ludwig et al. (1979) at a 1:20 w/w ratio of the protease to oxidase.

by this latter reagent. Reaction of trypsin-treated enzyme with NAP-taurine (this enzyme preparation is missing impurities b and c) confirmed that both subunits V and VI had been labeled (inserts to Figure 1). The relative labeling of subunits is shown in Figure 2. Experiments performed using Triton X-100 or Tween 80 as the dispersing detergent gave similar results, except for more labeling of subunit IV in Tween 80 solution.

A comparison of the labeling profiles in Figure 1 and the quantitative data in Figure 2 shows clear differences in the reaction of cytochrome *c* oxidase with NAP-taurine and DABS. Subunits I and VI were both considerably more reactive to NAP-taurine than to DABS. Subunits III, V, and VII, in contrast, were more heavily labeled by DABS than by NAP-taurine (using subunit II as a standard). The effect of chemical modification of cytochrome *c* oxidase by both DABS and NAP-taurine was monitored. Reaction of the enzyme with 50 μM DABS caused less than 5% inhibition of electron transfer activity. Reaction of cytochrome *c* oxidase with 1.3 mM NAP-taurine resulted in a 10% decrease in enzyme activity. Several different experiments were conducted in order

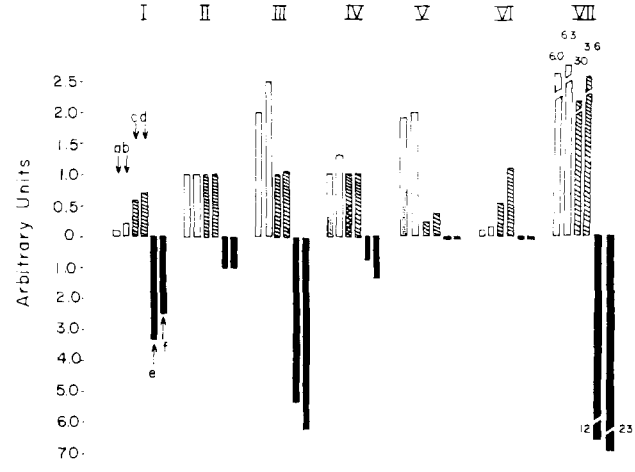

FIGURE 2: Relative labeling of the subunits of cytochrome *c* oxidase by hydrophilic and hydrophobic reagents. The figure shows bar graphs of the reactivity of each subunit to [^{35}S]DABS in detergent dispersion (a) and in vesicles (b), to NAP-taurine in detergent dispersion (c) and in vesicles (d), and to arylazidophospholipids I (e) and II (f). Values were calculated by summing the number of counts in each subunit (from gels) and dividing by the molecular weight of the component. These were then scaled to a value of 1.0 for subunit II. The high labeling of VII reflects the fact that there are multiple copies of this component(s). See Steffens & Buse (1976). The dots on the DABS labeling of subunits IV and V and the crosses on the NAP-taurine labeling of subunit IV represent reaction of the enzyme in Triton X-100.

to establish whether inhibition by NAP-taurine was due to denaturation of the protein. Firstly, the labeling of the enzyme with NAP-taurine was conducted over a range of concentrations of the protein modifying reagent. The relative labeling of subunits was found to be the same at all levels of NAP-taurine tested. Secondly, the absorption spectrum of the enzyme was examined after labeling with NAP-taurine. No evidence of a structural change (around the heme) was seen in these experiments. Lastly, cytochrome *c* oxidase was reacted with nonradioactive NAP-taurine (1.3 mM) and then with [^{35}S]DABS. The labeling profile obtained was identical with the one shown in Figure 1, trace b. Taken together, the above findings are a clear indication that reaction with NAP-taurine does not alter significantly the protein structure. Therefore, the differences in the labeling pattern with NAP-taurine and DABS must reflect differences in the reactivities of the two reagents and do not result from unfolding or other alterations of cytochrome *c* oxidase by the photoactive reagent.

Membranous cytochrome *c* oxidase was prepared by sonicating the enzyme with egg lecithin, and these vesicles were labeled with DABS, NAP-taurine, and arylazidophospholipids. The reactivity of membrane-bound cytochrome *c* oxidase with either [^{35}S]DABS or [^{35}S]NAP-taurine was essentially the same as that seen for enzyme dissolved in Tween 80 (Figure 2). Subunits I and VI were again labeled by NAP-taurine but not by DABS, while subunits III, V, and VII were more reactive to DABS than to NAP-taurine (using subunit II as a standard). The labeling of cytochrome *c* oxidase by arylazidophospholipids is shown in Figure 3. These reagents have been synthesized by Bisson & Montecucco (1980) as probes of the bilayer-intercalated portions of intrinsic membrane proteins. The upper trace of Figure 3 shows the reaction of the membranous enzyme with arylazidophospholipid I which contains the reactive nitrene at the methyl terminus of the fatty acid chains. Subunits I, III, and VII were all heavily labeled by this reagent from inside the bilayer and there was also a small amount of labeling of II and IV. The peak of radioactivity in the region of the gels containing subunits V and

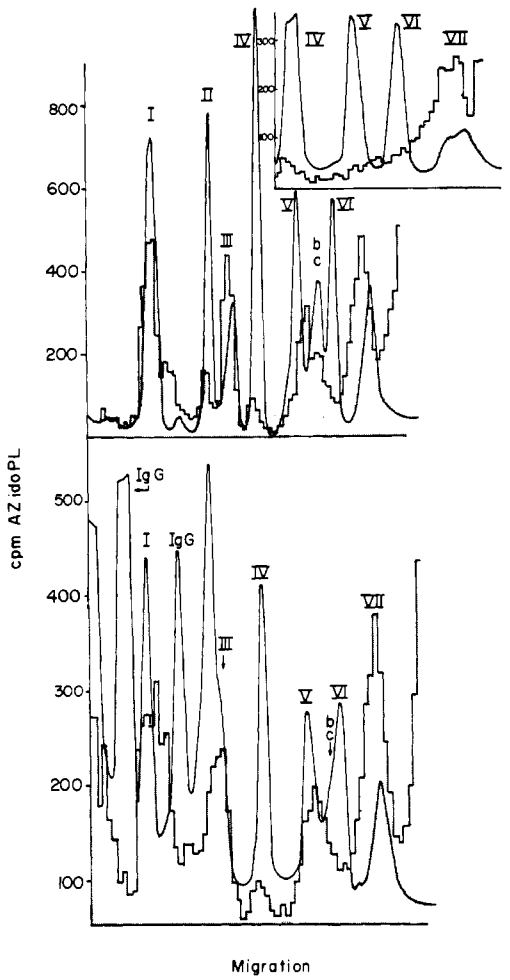

FIGURE 3: Labeling of cytochrome *c* oxidase with arylazidophospholipids. Cytochrome *c* oxidase was mixed with egg lecithin containing arylazidophospholipid I and the reaction of the hydrophobic probe with the protein monitored as described under Experimental Procedures. The upper trace shows labeling of the different subunits by the probe; the insert shows the reactivity of the smaller subunits of trypsin-treated enzyme. The lower trace shows the labeling of cytochrome *c* oxidase which had first been reacted with antibody against the enzyme. The large and small subunits of the antibody are labeled IgG.

VI is seen to run after V but before impurities b and c. This peak of radioactivity was not seen when trypsin-treated enzyme was reacted with the arylazidophospholipid (insert to Figure 3), thus establishing that the incorporation of the probe was into an impurity (probably b) and not into subunit VI as proposed by Bisson et al. (1979a).

Labeling of membranous cytochrome *c* oxidase with an arylazidophospholipid containing the reactive nitrene near the head group (probe II) gave similar results to those shown in Figure 3 except for there being less labeling of I and more labeling of VII [Figure 2 and see Bisson et al. (1979b)]. In the experiment shown in Figure 3, lower trace, antibodies against cytochrome *c* oxidase were added to the vesicles before photoactivation of the azido-containing phospholipid (probe I). It can be seen that the bound IgG was not labeled. This experiment is an important control in understanding the reaction of photoaffinity phospholipids as discussed later.

In a complementary study, oligomycin-sensitive ATPase from beef heart mitochondria has been labeled by both [^{35}S]DABS and [^{35}S]NAP-taurine. These experiments help to clarify whether NAP-taurine can react with intrinsic membrane proteins from within the bilayer and so are presented here. As shown in Figure 4, the labeling of OSATPase

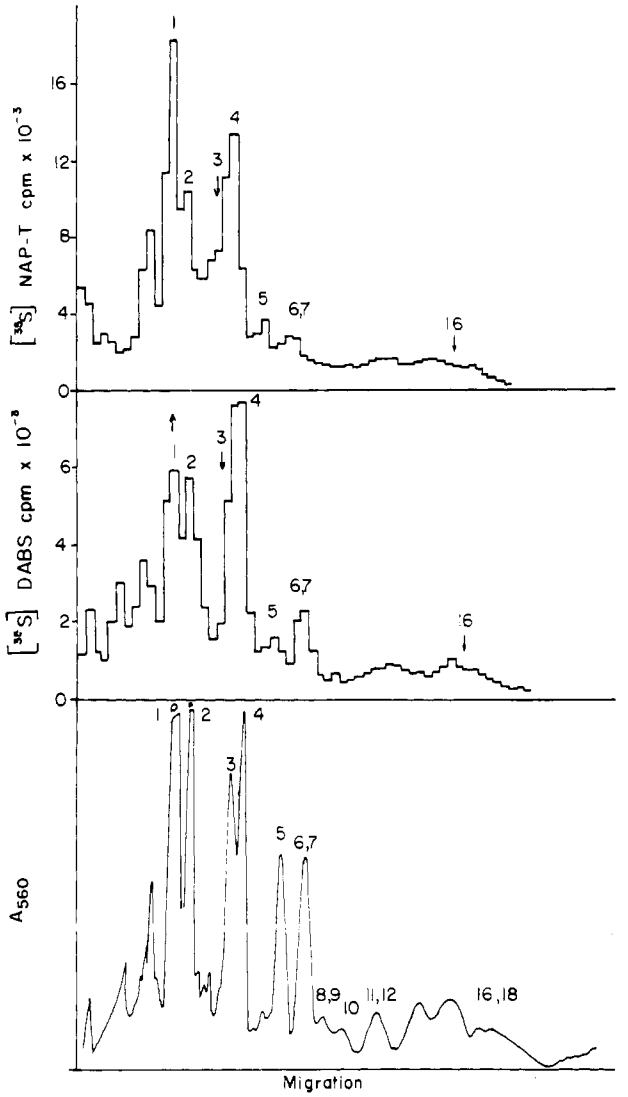

FIGURE 4: Labeling of OSATPase with hydrophilic protein modifying reagents. OSATPase was reacted with 1.3 mM [^{35}S]NAP-taurine or 20 μM [^{35}S]DABS and the protein run on 15% polyacrylamide gels in NaDodSO$_4$–urea as detailed under Experimental Procedures. The enzyme had an ATPase activity of 6.8 μmol of ATP hydrolyzed per min per mg of protein and this was 75% sensitive to oligomycin.

by [^{35}S]NAP-taurine and [^{35}S]DABS is very similar. In both cases the reagent reacted with F_1ATPase components 1, 2, 3, and 6 but not with the smaller polypeptides of the membrane sector of the complex, including the very hydrophobic proteolipid protein or DCCD binding protein (component 16). For a more complete description of the subunit structure of OSATPase, see Ludwig et al. (1980). Recently, Montecucco et al. (1979) have reported on the reaction of OSATPase with arylazidophospholipids identical with the ones used here. They found very heavy labeling of the proteolipid protein and other smaller molecular weight components of the membrane sector by these hydrophobic probes but no labeling of the F_1ATPase subunits.

Discussion

Our understanding of the distribution of subunits on the aqueously exposed surface of cytochrome *c* oxidase is based on labeling studies using the water-soluble, lipid-insoluble, protein-modifying reagent [^{35}S]DABS (Eytan & Schatz, 1975; Eytan et al., 1975; Eytan & Broza, 1978; Ludwig et al., 1979) or from lactoperoxidase-catalyzed iodination of the complex (Eytan & Schatz, 1975) and finally from binding studies with

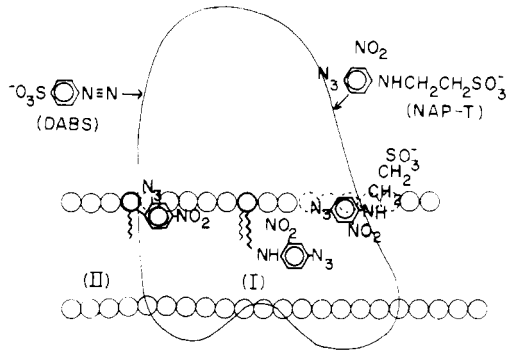

FIGURE 5: Scheme for chemical labeling of cytochrome *c* oxidase by hydrophobic and hydrophilic reagents.

antibodies made against specific subunits (Chan & Tracy, 1978). These approaches all have limitations. DABS has a limited specificity, reacting only with Lys, His, Tyr, and Cys residues, while the iodination reaction is specific for Tyr residues. Areas of the protein in the aqueous phase but not containing these nucleophilic amino acids would not be labeled by either reagent. Antibodies are bulky macromolecules and so may be sterically hindered from reacting with exposed subunits.

In an effort to avoid the above problems, we have begun a series of labeling experiments with NAP-taurine. Matheson & Scheraga (1979) have used this reagent in a study of the thermal unfolding of ribonuclease. They have shown that NAP-taurine binds only to surface-exposed amino acids and therefore does not by itself denature the protein. In the course of their work, they have examined the specificity of NAP-taurine. This photoaffinity reagent reacted with all amino acids but showed some preference for nucleophilic residues. In addition, reactivity depended on the size of the different amino acids; that is, glycine was less often modified than tryptophan or isoleucine (Matheson & Scheraga, 1979). NAP-taurine has been used by Staros et al. (1975) to label red cell membranes. The reagent was found to react with several polypeptides not labeled by DABS, and Staros et al. (1975) attributed this to differences in the specificity of the two reagents. Docktor (1979) has proposed that NAP-taurine, being amphipathic, can orient at the membrane surface with the reactive nitrene group inside the lipid bilayer. He explains the labeling of additional polypeptides in red cell membranes as being due to the reaction of NAP-taurine from inside the bilayer.

In our experiments with cytochrome *c* oxidase, NAP-taurine was found to label subunits I and VI, neither of which were labeled significantly by DABS. This additional labeling of cytochrome *c* oxidase by NAP-taurine could be with parts of the protein exposed to water but devoid of Lys, His, Tyr, or Cys residues, or, according to the argument of Docktor, it could reflect labeling from inside the bilayer. Several lines of evidence indicate that NAP-taurine is labeling cytochrome *c* oxidase predominantly from outside the bilayer (Figure 5). Subunit VI was labeled by NAP-taurine but not by arylazidophospholipids. Subunits III and VII were each more reactive to DABS than to NAP-taurine, using the labeling of subunit II as a standard. These results are the opposite of what would be expected if NAP-taurine was reacting mainly from inside the bilayer because subunits III and VII were heavily labeled by arylazidophospholipids while subunit II was only poorly labeled by the hydrophobic probes. Finally, the studies with OSATPase provide additional evidence that the reaction of NAP-taurine with membrane proteins is predominantly from the aqueous phase rather than from within the bilayer. This complex is arranged in two portions: there is the F_1ATPase portion which is outside the membrane and can be isolated as a water soluble, lipid-free protein without disrupting the lipid bilayer and there is the membrane sector made up of several very hydrophobic polypeptides such as the proteolipid protein, which are mostly buried in the lipid bilayer. [For review, see Senior (1979) and Ludwig et al. (1980).] DABS labels the F_1ATPase portion but not the smaller molecular weight and hydrophobic membrane sector polypeptides including the proteolipid protein. Arylazidophospholipids, in contrast, label the membrane sector polypeptides but not F_1ATPase subunits. The results presented here show that NAP-taurine reacts with OSATPase in much the same way as DABS does, and it does not label the proteolipid protein as would be expected if it was reacting from within the bilayer.

Our conclusion then is that all of the subunits of cytochrome *c* oxidase including subunits I and VI are exposed to the aqueous surface in membranous cytochrome *c* oxidase with several of these components also contributing to the bilayer-intercalated portion of the complex (based on the data with arylazidophospholipids). We now plan to use DABS, NAP-taurine, and arylazidophospholipids to map the folding of subunits within the cytochrome *c* oxidase complex. Subunits can be purified after reaction of membranous cytochrome *c* oxidase with these probes and the distribution of radioactivity within individual sequences determined. Cytochrome *c* oxidase can be isolated from labeled mitochondria or labeled submitochondrial particles and the incorporation of NAP-taurine or DABS from one side of the membrane or the other determined again with respect to the sequences of individual subunits. In this connection, the data presented here for the arylazidophospholipids are important. It has been suggested that the arylazido fatty acid in these molecules may loop back to label membrane proteins from the aqueous phase as well as from the interior of the bilayer (Bayley & Knowles, 1978).

For the purpose of testing this directly, antibody molecules were bound to membranous cytochrome *c* oxidase prior to reaction with the arylazidophospholipid probes. In this system IgG molecules were the major portion of the surface-exposed complex (cytochrome *c* oxidase plus antibody) and yet they were not labeled under conditions where there was extensive reaction of the oxidase complex itself. Other evidence that arylazidophospholipids react from within the bilayer and do not label extrinsic portions of membrane-bound proteins comes from our recent studies of cytochrome b_5 (Bisson et al., 1979c) and from the work of Montecucco et al. (1979) on oligomycin-sensitive ATPase described earlier.

The experiments reported here with trypsin-treated enzyme clarify the reactivity of different subunits with arylazidophospholipids. These hydrophobic probes react with subunits I, II, III, IV, and VII but do not react with V or VI. Subunit V is not labeled with these probes, but an impurity migrating near this component is heavily labeled. Experiments in which yeast cytochrome *c* oxidase has been labeled with hydrophobic iodoaryl azides (Cerletti & Schatz, 1979) also point to subunits I, III, and VII as providing the major part of the lipid-intercalated protein. The significance of labeling studies with hydrophobic probes is obvious when the reactivities of individual subunits are related to their amino acid sequences. The complete sequences of subunits II, IV, V, and VII (Ser) of beef heart cytochrome *c* oxidase have been obtained (Tanaka et al., 1976; Steffens & Buse, 1976; 1978; Buse et al., 1978; Sacher et al., 1979). In addition, the sequences of subunits II and III of yeast have been deduced by sequencing of mitochondrial DNA (Macino et al., 1979; Tzagoloff, personal

communication). Subunits II, III, IV, and VII each contain one or more sequences, at least 20 amino acids long, where there are no charged residues. Each of these subunits was labeled by arylazidophospholipids. Similar sequences are likely to be found in subunit I which is the most hydrophobic of all of the subunits of the enzyme. Subunit V does not show extended regions of hydrophobic amino acids; instead, charged residues are spaced all along the chain. This subunit was not labeled by arylazidophospholipids.

An attractive model for the structure of cytochrome *c* oxidase then [by analogy with purple membrane protein; see Henderson & Unwin (1975)] is one in which the hydrophobic segments of the various subunits form the membrane-intercalated portion of the protein [possibly as helices; see the X-ray diffraction studies of Blasie et al. (1977)]. The more hydrophilic parts of subunits I, II, III, IV, and VII along with all of V and VI would then form the C domain and the exposed portions of the two M domains.

The membrane-intercalated portion of cytochrome *c* oxidase has been visualized by electron microscopy and imaging of two-dimensional arrays of the enzyme embedded in glucose (Henderson et al., 1977). The M_1 and M_2 domains within the bilayer are each approximately the same size as the membrane-intercalated portion of the purple membrane protein; i.e., each could contain 6 or 8 helices for a total of 12–16/cytochrome *c* oxidase monomer. Direct evidence that the hydrophobic stretches seen in several subunits are indeed the membrane-intercalated portion of the oxidase complex requires showing that arylazidophospholipids bind to these regions while NAP-taurine and DABS are excluded from such sequences. This work is now in progress.

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