THE STRUCTURAL PROTEIN AND MITOCHONDRIAL ORGANIZATION

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In the accompanying two notes [Green et al. (1961); Criddle et al. (1961)] and in other communications from this laboratory [Green et al. (1959); Bomstein et al. (1960a, 1960b); Criddle and Bock (1958)] various phenomena have been reported which can now be systematized as expressions predominantly of the hydrophobic bond. These are as follows: (1) the tendency of structural protein (S.P.) and cytochromes a, b and c_1 to form polymers at neutral pH when isolated and freed from other mitochondrial components; (2) the monomerization of these polymers by lipophilic reagents; (3) the depolymerization of cytochromes a, b and c_1 by S.P.; (4) the depolymerization of S.P. by the cytochromes; and (5) the binding of lipid to S.P. and to the cytochromes. All these interactions in aqueous media appear to involve mainly the cohesion of a non-polar region in one molecule to a similar non-polar region of complementary configuration in a second molecule. The larger non-polar side chains of amino acids (leu, ileu, try, tyr, etc.) and of fatty acids are presumed to be the elements in proteins and lipids respectively which confer regional non-polarity on the molecule.

Hydrophobic bond

The strength of hydrophobic bonds between protein molecules or between proteins and lipids is still subject to much discussion and investigation.

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Studies do indicate, however, that the energy involved for non-polar groups associating together in aqueous solution can be considerable. The overall stability of these bonds is generally agreed to be due to the decrease of "ice-likeness" of water when such bonds are formed in aqueous solution. Kautzman (1959) and Leach (1959) have pointed out that the process of dissolving a hydrocarbon in water is accompanied by a small enthalpy change (0 to -1 k cal/mole), a large negative partial molar entropy (about -20 e.μ.) and consequently a positive value for the ΔF° of solution. The reason for this large decrease in entropy is presumed to be the ordering of water molecules in the immediate vicinity of the non-polar residues of the hydrocarbon chains. Such ordering results in an increase in ice-like structure in the water with a corresponding decrease in the entropy. The change in free energy for the transfer of a non-polar side chain of an amino acid from aqueous solution to a non-polar region (for example on a neighboring protein molecule) may well be of the order of -4 to -5 k cal/mole. It can be seen that when a large group of such side chains is involved in a protein-protein interaction of a hydrophobic nature, the overall stabilization may be considerable.

All protein molecules have sufficiently large numbers of amino acids with non-polar side chains that extensive hydrophobic regions could result from the interaction of these groups. Such regions would have the effects of both stabilizing the tertiary structure of the individual protein molecules and providing sites for the formation of interprotein complexes. S.P. which has a higher than average non-polar amino acid side chain content, and also the three cytochromes appear to have the configurational attributes which allow the formation of stable, specific hydrophobic bonds either with other molecules of the same species (polymerization) or with molecules of different species (complex formation).

Fragmentation of Mitochondria

The importance of lipid-protein complexes has long been recognized by those attempting to fractionate and purify mitochondrial components. When

separations of components were achieved by the use of the so-called "lipid reagents" it was assumed that the reagent attacked the bonds of lipid to protein. The range of action of bile acids and other "lipid reagents" has to be extended to include the additional effect of these reagents on inter-protein binding through hydrophobic areas.

The reagents which have been used to fragment the mitochondrion into daughter particles and to isolate the various cytochromes (bile acids, detergents, etc.) are capable of displacing the two molecules involved in the hydrophobic bond as follows:

$$A \text{---} B + \text{detergent} \rightleftharpoons A \text{---} \text{detergent} + B \text{---} \text{detergent}$$

The monomerization of particulate cytochrome b by cetyldiethylmethyl ammonium bromide [Bomstein et al. (1960a)], the monomerization of hexameric cytochrome c_1 by sodium dodecylsulfate [Criddle and Bock (1959)], the release of lipid from its attachment to S.P. or cytochrome by bile acids in presence of salt and butanol, and the release of cytochromes from their attachment to S.P. are manifestations of such replacement reactions. As only those reagents which rupture hydrophobic bonds are effective in the breakdown of mitochondrial complexes it seems probable that the electron transport chain is stabilized chiefly by specific interactions between non-polar regions in the component protein molecules.

Role of Structural Protein in the Mitochondrion

If we assign to S.P. a role in mitochondrial structure analogous to that of S.P. in other systems such as viruses or ribosomes, we could say that it should serve a dual function: (1) binding to other like molecules to form a polymeric array; and (2) binding to the functional components of the electron transport chain to increase the order and stability of the unit. In this case S.P. would have to be able to bind specifically certain protein components of the electron transport chain and mitochondrial lipid, as well as to form polymers with other molecules of its own kind. For such

a role it would be mandatory for S.P. to be present in abundance in the mitochondrion.

The two companion communications have shown that this protein satisfies all of the above criteria for a structural protein. It accounts for 50-75% of the total particulate protein of the mitochondrion. Specific interactions between S.P. and the cytochromes (a, b and c_1) and between S.P. and mitochondrial lipids have been demonstrated. The available evidence is, therefore, highly suggestive that S.P. indeed plays the role of a binding agent which holds the system together by means of hydrophobic interactions.

Structural Protein and Membrane Systems

Electron microscopy has revealed the remarkable similarity in membrane structure (characteristic three-banded structure) of energy-transforming devices such as the retinal elements of the rods and cones, the cell membrane, the chloroplast, the kidney tubule and the mitochondrion. Since the functional components in these devices are vastly different it has been difficult to understand why the membrane structures should be so similar. The element of universality in all membrane systems may be the structural protein - lipid molecular network which could serve as the framework for membrane systems generally.

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