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Elizaveta I Bon *

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Review Article

Ultrastructure of the mitochondrial membrane and respiratory ensembles

Bon E.I., Maksimovich N.Ye., Ostrovskaya O.B and Vishnevskaya E.I.

Grodno State Medical University, Gorkogo St, Grodno, Republic of Belarus

***Corresponding Author:** Elizaveta I Bon, Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria.

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Abstract:

Electron microscopy is the leading method for studying the ultrastructure of cells. The mitochondrial membrane system serves not only as a structural case, but also contains highly organized ensembles of respiratory enzymes that participate in ATP synthesis, in the osmotic work of active transport and in the mechanical coupling underlying changes in the shape and volume of mitochondria. Now we will consider the ultrastructure of the mitochondrial membrane according to chemical analysis and electron microscopy and other physical studies, as well as the molecular and enzymatic architecture of respiratory ensembles, which are believed to be structural units of the mitochondrial membrane.

Keywords: ultrastructure; mitochondria; membrane; respiratory ensembles

Introduction

The mitochondrial membrane system serves not only as a structural case, but also contains highly organized ensembles of respiratory enzymes that participate in ATP synthesis, in the osmotic work of active transport and in the mechanical coupling underlying changes in the shape and volume of mitochondria. Now we will consider the ultrastructure of the mitochondrial membrane according to chemical analysis and electron microscopy and other physical studies, as well as the molecular and enzymatic architecture of respiratory ensembles, which are believed to be structural units of the mitochondrial membrane. [1]

The chemical composition of the mitochondrial membrane

A number of preparations of mitochondrial membranes have been subjected to detailed analysis; these include Keilin—Hartree particles, electron-carrying green particles and "holate" Ball and Cooper particles. All these drugs are derived from the mitochondria of the heart. In addition, the composition of digitonin fragments of rat liver mitochondria obtained [2] was studied. As already noted, such membrane preparations are largely similar in their enzymatic composition. They usually contain 35-40% lipids, mainly phosphatides, and 60-65% protein. The slight differences between the drugs, which are sometimes observed, seem to be due to different production conditions using various physical and chemical methods of destroying the structure of mitochondria.[3]

The composition of lipids. The composition of mitochondrial lipids from rat liver and bovine heart has been studied in several laboratory studies. In general, almost all mitochondrial lipids, especially in preparations of bovine heart mitochondria, are found in the membrane fraction. Over 90% of membrane lipids are phospholipids; the rest are mainly composed of triglycerides, diglycerides [4]and cholesterol. Rat liver mitochondria contain significant amounts of phosphatidylethanolamine, phosphatidylcholine [5], inositphosphatides, cardiolipin and phosphatidylserine; the content of plasmalogen and sphingomyelin is low, sometimes they are completely absent. The mitochondria of the bovine heart, on the contrary, are very rich in plasmalogens; they contain derivatives of both ethanolamine and choline.

Mitochondria of both types contain a lot of cardiolipin; almost all of the cardiolipin of intact cells is found in mitochondria [6]; in microsomes, on the contrary, the content of cardiolipin is very low, and sometimes it is not detected at all. Phosphatidine acid in most preparations is contained in vanishingly small amounts. Lysophosphatides are found in extracts of intact mitochondria and digitopine fragments obtained using a chloroform—methanol mixture, but it is quite possible that they are formed as a result of hydrolysis of the initial lipids during mitochondrial extraction. Indeed, lysophosphatides are known to cause mitochondrial swelling and the uncoupling of oxidative phosphorylation. [7]

The composition of fatty acids in lipids of mitochondria and microsomes of rat liver was also studied. Lipids of liver mitochondria contain palmitic (17.8%), stearic (14.9%), oleic (11.9%), linoleic (23.4%) and arachidonic (17.0%) acids, which are the main components. In general, compared with unfractionated liver tissue, mitochondria contain less palmitic and oleic acid, but are characterized by a significantly higher relative content of linoleic, arachidonic and docosahexaenoic acids. [8]

The fatty acids of cardiolipin are almost entirely represented by linoleic acid. However, the mitochondria of other tissues and other animal species do not always contain such large amounts of linoleic acid.

Undoubtedly, the bulk of the membrane lipids performs an exclusively structural function, forming a two-layered lipid core of the membrane. However, it has been shown that some membrane lipids perform one or another specific function in the transfer of electrons, the movement of ions and in the processes of swelling and contraction. For example, phosphatidic acid [9] and (or) cardiolipin, as well as phosphatidylinosite, are involved in the mechanism of mitochondrial contraction, as already shown in Chapter IX. Lecithin, apparently, performs a specific function, participating in the action of cytochrome oxidase and dehydrogenase of p-hydroxybutyric acid, as shown by the work of Green's laboratory. Of some interest is the possible presence of small amounts of lipoaminoacids and lipopeptides in mitochondria, since such lipids can participate in the biosynthesis of membrane proteins. However, they have not yet been discovered. [10]

The characteristic content and quantitative ratio of lipids in the mitochondrial membrane are probably due to the need to maintain a thermodynamically stable double layer of lipids forming the backbone of the membrane, which serves as a support for respiratory ensembles. Apparently, the fact that almost all lipids of the mitochondrial membrane are extracted with a mixture of chloroform [11] and methanol is of great importance. This indicates the presence of only a small number of covalent bonds between lipids and protein elements, or even their complete absence. This fact indicates a high degree of stabilization of lipids and proteins in membrane structures. The work of Green's laboratory shows that the added labeled phospholipids of the medium can significantly. the degree of exchange with phospholipids of the mitochondrial membrane, but further studies are needed to clarify this effect and its specificity, since lipids of mitochondria and hyaloplasm differ from each other in composition and rate of renewal. [12]

Squirrels

It is well known that proteins and enzymes of the mitochondrial membrane are very difficult to solubilize in their native form and are difficult to get rid of lipids. This significantly delayed the study of electron transfer enzymes and oxidative phosphorylation. There is some evidence that it is the lipids of the membrane that give proteins insolubility and cause their strong fixation. Thus, the extraction of membrane fragments with acetone or detergents such as cholate [13] often causes a weakening of such fixation or solubilization of specific membrane enzymes.

The method of solubilization and fractionation of membrane proteins with cholate was carefully developed in Green's laboratory, which made it possible to isolate cytochromes a, B and c, as well as the so-called structural membrane protein. When treated with cholate, there is no true extraction of proteins in a stable water-soluble mono-dispersed form, since after removal of the cholate, the proteins soon become insoluble again, as is typical for polymers.[14]

Criddle postulated that hydrophobic bonds determine the ability of membrane proteins and enzymes to polymerize and form insoluble polymolecular complexes with each other and with some lipids. Crane showed that cytochrome c combines with phosphatidylethanolamine to form a stable complex soluble in ether. This complex is called lipocytochrome C [15]. In it, the 2-amino groups of cytochrome c lysine residues form electrostatic bonds with lipid molecules. It is possible that this interaction of lipid—protein together with hydrophobic bonds provides stabilization of the membrane structure.

A structural protein. Criddle highlighted from bovine heart mitochondria treated with a mixture of cholate, deoxycholate and sodium dodecyl sulfate, a monomeric form, which they called the structural protein of the mitochondrial membrane [16]. This drug did not contain cytochromes and flavoproteins. Since this protein makes up 55% of the total membrane protein mass, they assumed that it is the main structural protein of the membrane; therefore, the mitochondrial membrane, like the protein envelope of a virus, consists of repeating identical protein molecules.

At a neutral pH, the structural protein is in polymer form and insoluble in water, but with the help of anionic detergents and alkalis, it can be converted into a soluble form. Monomeric forms predominate in such solutions, but when neutral pH is restored or detergents are removed, insoluble polymers and aggregates are formed. The monomeric form has a molecular weight of about 22,000, but the tendency to polymerization violates the accuracy of sedimentation and electrophoretic studies [17]. The study of the amino acid composition showed that about 41% of the residues are represented by non-polar amino acids; the total content of aspartic and glutamic acids is 16%. These data show that the structural protein is only slightly more hydrophobic than most other proteins, but perhaps the sequence of amino acids and the features of the tertiary structure ensure the predominance of nonpolar groups in certain areas of the surface.

In viral proteins consisting of repeating identical monomers, the Y-terminal and C-terminal amino acid residues of the units are identical. Such an analysis of the structural protein of mitochondria is complicated by the incompleteness of its reaction with specific substances used for the analysis of end groups. Carboxypeptidase has been shown to cleave off leucine and smaller amounts of tyrosine [18]; attempts to identify the I-terminal amino groups have not been successful.

It turned out that the structural protein combines with pure cytochromes a, B and c to form water-soluble complexes in a molar ratio of 1: 1, and the conditions of this interaction are different for each case. The combination of a structural protein with cytochrome B causes a significant change in the redox potential of the latter. It is assumed that predominantly hydrophobic bonds are formed in such complexes. Further, it turned out that the structural protein combines with phospholipids. Thus, the structural protein is capable of interacting with two other basic molecular elements of the membrane — with electron carriers and with phospholipids.

The research of Criddle and his collaborators has not yet resolved all the questions regarding the homogeneity and identity of the structural protein, however, this is currently impossible. Nevertheless, the data they have obtained seems to be very significant and promising. The tendency of cytochromes [19], flavoproteins and structural protein to exist in monomeric and polymeric forms indicates a pronounced tendency of these molecules to form very stable macromolecular ensembles with a lamellar structure.

Other protein components of the membrane.

We have already mitochondrial actomyosin was mentioned, which can be extracted from intact mitochondria of 0.6 M KS1 and precipitated by dilution to 0.15 LT KS1 [20]. Data on the size, shape and homogeneity of this protein or protein complex are still insufficient, but of great importance is the fact that its content in mitochondria is quite high and that it, at least according to According to preliminary data, it has many properties of myofibrillar actomyosin. This protein also acts as a mitochondrial contraction factor. It is probably one of the components of the mitochondrial membrane.

Another protein component of the mitochondrial membrane is the phosphoprotein [21] described earlier. To date, it has not been isolated in its native form, but the work of Kennedy, Boyer and Wadkins shows that it contains phosphorylated residues of histidine and serine. Since the phosphorus content decreases during mitochondrial swelling, increases with ATP-induced contraction, and since phosphoproteins apparently participate in the mechanisms of ion transport in mitochondria, it is possible that the specific function of the mitochondrial membrane phosphoprotein is related to its properties of polyelectrolyte, capable of enzymatic attachment and release of charged groups. The existing data do

not exclude the possible identity of the structural protein and the mitochondrial phosphoprotein; it is known that the structural protein contains histidine and serine residues [22].

Finally, it should be noted that in mitochondria, as in other membrane systems, sialic acid was found, apparently bound to a protein. Its significance has not yet been clarified, but Thomson and McLees showed that groups carrying a negative charge and determining the electrophoretic properties of the mitochondrial membrane are characterized by a low value of pK in those areas where sialic acid is detected. Thomson and McLees also showed that the charge density of the membrane does not change with swelling, therefore, the membrane does not stretch, but straightens with an increase in the total surface.

Electron carriers and breathing ensembles.

The chemical, physical and catalytic properties of flavoproteins and cytochromes strongly bound to the mitochondrial membrane were described in detail above [23], in particular, their tendency to form insoluble polymers or complexes with each other and with a structural protein was mentioned. Simple molar ratios between electron carriers indicate that they exist in the form of specifically organized repeating "ensembles" with the properties of biochemical and molecular "machines". It is quite possible that respiratory ensembles are located in the membrane structure more or less regularly, since with the gradual destruction of membranes (under the influence of ultrasound) fragments are formed in which the ensembles themselves, the number of electron carriers per 1 mg of protein, and the lipid content remain almost constant [24].

It is now quite clear that electron transporters with catalytic activity and conjugating enzymes should make up a significant proportion of the total membrane protein mass. Spectroscopic and chemical analysis conducted in the laboratories of Ball, Green, Chance and Leninger showed that over 25% of the total membrane protein consists of these enzymes, which are part of respiratory ensembles. This fact convincingly shows that the mitochondrial membrane is not an inert shell, but an extremely complex structure consisting of repetitive proliferative systems.

This pattern also indicates that the physical and chemical properties of the membrane, such as permeability and conformation, are determined by complex enzymatic reactions [25] and equilibria occurring in the membrane. This assumption arose on the basis of the above data on the movement of water and ions in mitochondria. Therefore, the specific location and spatial orientation of individual enzyme molecules in this continuous highly ordered structure is of paramount importance for the molecular architecture and function of the membrane.

Membrane ultrastructure

Even before the advent of the electron microscope [26], convincing data were obtained indicating that most biological membranes are largely similar in their chemical and physical properties and that the backbone of these membranes is represented by a double lipid layer. These data, summarized by Dawson and Danielli [27], were confirmed by subsequent experiments. The modern concepts of membrane ultrastructure are based on the following considerations. Most biological membranes contain 30-40% lipids (mainly phospholipids) and 60-70% protein; These stoichiometric ratios are consistent with the assumption of a structure consisting of a double lipid layer approximately 60 A thick, which is covered on each side with at least one layer of protein molecules. It is assumed that in the double lipid layer, the hydrocarbon chains are oriented inward and form a continuous phase, and the polar groups of phospholipids are located on the outer surfaces. Calculations have shown that the phospholipid content [28] in the erythrocyte membrane is sufficient to form a double layer of lipid molecules over the entire cell surface.

Another significant point is that the permeability coefficient of natural membranes (i.e., the amount of substance penetrating through the membrane per unit surface) for various nonelectrolytes is proportional to the olive oil—water distribution coefficient for these substances. Indeed, lipid-soluble substances are characterized by the fastest penetration, which is consistent with the presence of a continuous lipid phase in the membrane. Tedeschi and Harris have convincingly shown that the permeability of the mitochondrial membrane also follows these patterns. Biological membranes are characterized by very high electrical resistance and capacitance; this implies the presence of structures containing a continuous dielectric layer, which can be created by a continuous lipid phase [29]. Pauli has recently established that the mitochondrial membrane is also characterized by high electrical resistance. At the same time, the results of measuring the surface tension of biological membranes are consistent with the assumption of the presence of a protein layer.

It is also worth mentioning the experiments of Schmidt, Finean, Robertson and others on myelin sheaths. These shells consist of membranes of Schwann cells surrounding a nerve fiber concentrically; as a result, a unique conformation of the membrane with repeating layers appears, which is available for study by X-ray diffraction methods, as well as using polarization and electron microscopes. Relevant studies have convincingly shown the presence of a double lipid layer with a thickness of about 55 A, forming the backbone of the myelin sheath, and two layers of protein on both sides of this backbone.

Electron microscopy of mitochondrial membranes using the 'positive contrast' method

For more than ten years, osmium tetrachloride and potassium permanganate have been widely used as fixators in electron microscopic examination of the cell ultrastructure, in particular membranes and granules. These agents have a high density relative to the electron beam and are specifically deposited in cell structures, creating a so-called "positive contrast"[30]; the surrounding substance does not capture the fixator and remains transparent to electrons. This positive contrast is probably due to the chemical or physical affinity of the fixative to the specific chemical or physical structures of the object. Each of these fixators creates a positive contrast, but they act somewhat differently.

There is still no complete consensus on the interpretation of electronic microphotographs obtained using this method.

Shestrand [31] believes that under normal conditions, there is a kind of fusion of two membrane elements. When mitochondria swell, stratification occurs along the middle of the three lines detected by permanganate. Robertson and other researchers, on the contrary, believe that fusion in the region of this line is rarely observed and that there is a space between the elements of a complex membrane that can "shrink" when mitochondria are placed into a hypertonic sucrose solution [32]. The work of Robertson, Finean and Steckenius allows us to conclude that osmium and permanganate are deposited in a specific way in the mitochondrial membrane and reveal the relative location of its elements.

The question of the true location of protein and lipid molecules in the membrane has not been completely resolved. If Robertson suggested that all biological membrane systems consist of so-called elementary membranes characterized by a certain and constant thickness; in each of these elementary units, two thin dense lines separated by a light gap, 25 A thick, are revealed during permanganate treatment [33]. As the Phinean diagram shows, the total thickness of each pair of lines is 75 A. Robertson believes that each elementary membrane contains a double layer of lipid molecules with a thickness of 50 A, covered on each side with a monolayer of unfolded protein molecules with a thickness of 10-15 A, which in total amounts to about 75 A. Robertson believes that such elementary membranes can exist in isolation, for example in a cell

membrane, or they can connect in pairs, forming complex membranes for example, mitochondrial membranes and crystals.

This concept has played a positive role in the analysis and systematic study of complex membrane structures, however, recent work by Shestrand and his school has allowed us to establish the existence of significant differences between biological membranes of different types; these differences are revealed on osmium-treated preparations using high-resolution electron micrography. Electron micrographs of mitochondrial crysts [34] made by Shestrand show that when treated with osmium, significantly thicker dense lines are revealed in each of the paired membranes than in the plasma membrane, and a thin granular structure of these lines is detected.

This is to be expected, since the mitochondrial crystals contain highly organized respiratory ensembles that are not present in the plasma membrane. It is likely that the plasma membrane contains some other types of organized enzyme systems that are less studied. Robertson showed that elementary membranes can differ in thickness in different cell membranes, and suggested that these differences are due to the uniqueness of lipids and proteins in a membrane of this type [35].

Negative contrast

Recently, when studying membrane structures, and in particular mitochondrial membranes, the method of negative contrast has been increasingly resorted to. In negative contrast, a substance with a high electron density, chemically inert, is used, which does not connect to any components of membrane structures, but only surrounds the structure and reveals its outlines. Phosphowolframate and uranium acetate are usually used for this purpose. Negative contrast was particularly useful in identifying the structure of isolated viral particles. [36]

Fernandez-Moran recently applied the negative contrast technique in combination with high resolution to study the ultrastructure of the mitochondrial membranes of the bovine heart. On the micrographs of mitochondrial membranes he obtained, he found spherical or ellipsoidal particles about 70-90 A in diameter, which he called "elementary particles". These particles are connected by a narrow "leg" to the transparent backbone of the membrane [37]. Most of the Preparations show that both surfaces of this skeleton are dotted with elementary particles. In the first works, Fernandez-Moran noted that these particles are found both on outer membranes and on crystals, but in later works he focused on the latter. Fernandez-Moran also reported the presence of elementary particles in the membranes of erythrocytes and in the myelin sheath [38].

The Fernandez-Moran data were then confirmed in a number of laboratories. Stackenius observed such particles in the mitochondria of the neurospore, Parsons Greenwalt — in the mitochondria of the rat liver, A. D. Smith — in the mitochondrial crystals of insect flying muscles [39]. Stackenius and Parsons failed to establish a definite pattern in the distribution of particles, while Smith showed that elementary particles are collected on the surface of mitochondria in the form of characteristic repeating clusters or rosettes.

Smith also noted that negative contrast can reveal the characteristic thin membrane structure. He suggested that an elementary particle and its leg represent half of a symmetrical structure shaped like a dumbbell, while the other half is "walled up" in a membrane. Smith determined that there are 400 such particles per 1 mk2 of the membrane surface. The method of negative contrast also revealed other aspects of the structure of mitochondria. Horn and Whittaker showed that with negative contrast, the crystals of the mitochondria of the brain are revealed in the form of worm-like structures, which are indentations of the inner surface of the mitochondrial membrane; in some photographs, the lumen of the crystals is visible. Parsons and Greenwalt observed long, narrow ribbon-like structures covered with elementary particles in lysed mitochondria [40]. Parsons suggested that these are crystals, but Greenwalt and other researchers believe that these structures are too long, narrow and numerous to be mistaken for crystals. Recently, Shestrand suggested that the elementary particles detected by negative contrast are an artifact. However, he still supported the view that the membrane surface is covered with particles. Using the freeze—drying method [41] to study cytoplasmic membranes, Shestrand found naturally arranged particles with an average diameter of 40-45 A. He suggested that these particles represent the globular micelle of lipids.

The structure of the double lipid layer

It is likely that in the not too distant future we will learn how to reconstruct membrane systems with an ordered arrangement of enzymes and enzyme systems; this is confirmed by recent successes in creating stable two-layer lipid structures and in understanding the physical principles of their formation and stabilization. Radin, Muller and their collaborators have recently shown that stable two-layered lipid structures, resembling in their physical properties the lipid layers of natural membranes, can be reconstructed from crude extracts of brain lipids. A drop of brain lipid solution in a chloroform—methanol mixture [42] was placed in a narrow slit in a plastic partition between chambers containing a solution of KS1. As the solvents diffuse from the droplet into the aqueous phase, the oriented phospholipid molecules at the boundary of the two solvents in the droplet approach each other and eventually combine into a fairly stable double layer located across the gap.

The thickness of such a double layer corresponds to the thickness of the double lipid layer of natural membranes; it has high electrical resistance and other properties characteristic of these membranes.

Thompson and Huang improved the Radin and Muller method and developed more precise methods for studying the optical, electrical, mechanical properties and permeability of double lipid layers. They studied the formation of double layers of highly purified lipids of known composition in order to determine the basic prerequisites for the formation of a self-stabilizing two-layer system at the molecular level. Thompson and his collaborators found that such stable double layers are not formed from any phospholipid or neutral lipid; To obtain a stable system, a certain combination of specific lipids is necessary [43].

The simplest stable system is formed by the interaction of egg lecithin and "-tetradecane, which can be replaced by methyloleate, or cholesterol with "-decane. Contrary to expectation, it turned out that such a simple system is very similar in properties to natural membranes.

Thompson and his collaborators believe that the structure and stability of the double lipid layers may eventually be determined by the same factors that determine the formation of a stable secondary, tertiary and quaternary protein structure. Their stability is determined by the nature and ratio of their lipid molecules, their electrical charges and spatial packaging. The stabilization of double lipid layers is mainly determined by dispersion van der Waals forces acting between methylene groups [44] of adjacent hydrocarbon chains. In each individual case, these forces are very weak, but when repeated repeatedly in three directions along tightly packed hydrocarbon chains, they become irresistibly strong.

Thompson emphasized that different types of lipids can have the same importance for the structure of membranes as amino acids for the structure of protein, it's secondary, tertiary and quaternary structure of membranes is determined by the structure of its constituent lipid molecules, their spatial packaging, their quantitative ratio and flat shape. Indeed, such double layers of lipids represent one of those two or three thermodynamically stable conformations of phospholipid—water systems [45] described by Lusatti and Hasson. In addition to the lamellar arrangement, rectangular and inverted micellar shapes are also possible. The latter do not create a continuous hydrocarbon phase and are unlikely to occur in natural membranes.

Ultrastructure of the respiratory ensemble

We have already seen that respiratory ensembles consisting of a complete set of electron carriers and conjugating enzymes are located in the mitochondrial membrane and are separated by equal intervals. These ensembles make up 25% (or more) of the total membrane protein mass, which is released by destroying membranes by physical or chemical methods. Based on the known carrier content, we can calculate how many ensembles there are per mitochondria [46]. Estabrook and Golovinsky determined that one liver mitochondria contains about 17,000 cytochrome a molecules, and one heart mitochondria contains 50,000. If each respiratory ensemble contains one cytochrome a molecule, then there should be about 17,000 respiratory ensembles in each liver mitochondria; if the ensembles are not monomers, then their number will be correspondingly smaller. It should be remembered that cytochrome oxidase can exist as a complex containing 6 cytochrome a molecules.

Apparently, the ensembles are localized mainly in crist membranes, the surface area of which is much larger than the area of the outer membrane. Moreover, it turned out that the mitochondrial respiration rate correlates with the number of crysts and is maximal in the mitochondria of the insect flying muscle.

The components included in the minimal respiratory ensemble and their molecular weights are listed below. It is assumed that such a minimal ensemble contains one molecule of NAD * H2 dehydrogenase, succinate dehydrogenase[47] and cytochromes a, b, c and C4. It should be noted that the molecular weight of the largest component of NAD * H2 dehydrogenase is not precisely established, but the table shows data for the Synger enzyme, which, apparently, it is the most "native". Based on these data, the molecular weight of the particle representing the minimum respiratory ensemble turned out to be 1,350,000.

The location in the membrane, put forward on the basis of Robertson's data on the structure of the elementary membrane of the cell. It was assumed that the ensemble is an integral component of the protein monolayer of the membrane. An alternative assumption is that the ensemble is not part of the main monolayer, but is laterally attached to the membrane. This assumption is probably in better agreement with the data of Shestrade, who showed that the crist membranes are significantly thicker than the outer membrane [48].

The Leninger scheme has some advantages, since, in particular, it allows for a simple orientation of the active centers of the enzymes included in the ensemble with respect to the membrane plane, which is consistent with the vector properties of ion transfer processes and mechanochemical phenomena in the membrane.

Recently, after the discovery of the so-called elementary particles, Green, Fernandez-Moran and their collaborators suggested that these particles are respiratory ensembles in which the carriers are arranged in the form of a spherical particle connected to the membrane by means of a leg. In confirmation of this, they showed, that the respiratory ensembles isolated from the mitochondria of the heart are about the same size as elementary particles and have a spherical shape when contrasted negatively. The diameter of such isolated particles, however, is significantly larger than the particle sizes observed in intact membranes, so final conclusions about the identity of these formations are hardly possible yet.

Indeed, there are discrepancies between the size of respiratory ensembles and the weight of elementary particles. If the latter have a spherical shape and a diameter of 90 A, then their weight is significantly less than the molecular weight of the minimum respiratory ensemble (1,400,000). In addition, the calculations of Green and his collaborators used the molar ratios of carriers and the values of molecular weights, which are not completely consistent with the values obtained in other laboratories.

In such calculations, it is important to take into account that the respiratory chain does not necessarily represent a monomer, if only because the active cytochrome oxidase [49] is considered a pentamer or hexamer. But then the number of other cytochromes and flavoproteins should also be higher. Moreover, other enzymes strongly associated with respiratory particles are the same as O-p-hydroxybutyrate dehydrogenase, acyl dehydrogenase (fatty acid)-CoA and a-glycerophosphate dehydrogenase may also be part of the main respiratory ensemble; in this case, the weight of the respiratory ensemble should be higher than the weight of the elementary particle observed with negative contrast. Of great interest are Green's further experiments, which may make it possible to more accurately assess the correctness of his assumptions.

Recently, Chance has put forward a slightly different assumption about the meaning of elementary particles. He believes that each elementary particle contains one type of electron carrier molecules and probably some additional protein components, rather than representing a complete ensemble. In his opinion, adjacent elementary particles, each of which contains a specific carrier, interact with each other through oscillatory movements limited by the length and flexibility of the leg. These movements are similar to the hinge movements of the lipoic acid group in a-ketoacid dehydrogenases [50] (see Chapter III).

Vector parameters of the structure of the respiratory ensemble

An ideal model of the structure of the mitochondrial membrane and the arrangement of enzyme molecules in the respiratory ensemble should take into account not only the size and shape of the enzymes, but also two other properties of the respiratory ensemble [51]. In previous chapters, it was emphasized that the ensemble is a powerful machine that is capable not only of forming ATP, but also of moving ions and of conformational changes in membranes during swelling and contraction of mitochondria. Indeed, there is evidence that all these three types of activity are "vector" and are characterized by a certain direction. This property should have a structural basis, i.e. the asymmetric organization of individual enzymes or entire ensembles in the membrane.

The vector properties of respiratory ensembles [52] formed the basis of Mitchell's concept of the anisotropic localization of respiratory enzymes in the mitochondrial membrane and the vector action of membrane enzymes, which is opposed to the scalar (direction-independent) action of soluble enzymes. Many, if not all, enzymatic reactions are asymmetric with respect to the active center of the enzyme, but the vector characteristic cannot be expressed as movement in one direction unless some barrier restricts the rotational and vibrational diffusion of enzyme molecules [53].

Reference

1. Bon, E.I., N.Ye. Maksimovich, S.M. Zimatkin, O.B. Ostrovskaya, et. al., (2022). Changes in the Organelles of Neurons in The Parietal Cortex and Hippocampus in Incomplete Cerebral Ischemia. *International Journal of Stem cells and Medicine*. 1(2), P. 1-5.
2. E.I. Bon, N.Ye. Maksimovich, S.M. Zimatkin, O.B. Ostrovskaya, V.Yu Smirnov, et. al., (2022). Violations of the Ultrastructure of Neurons in The Cerebral Cortex of Rats with Total Cerebral Ischemia *International Journal of Stem cells and Medicine*. Vol. 1(2). P. 1-9.
3. Bennett CF, Latorre-Muro P, Puigserver P. (2022). Mechanisms of mitochondrial respiratory adaptation. *Nat Rev Mol Cell Biol*. (12):817-835.
4. LEHNINGER AL, WADKINS CL, COOPER C, DEVLIN TM, GAMBLE JL Jr. (1958). Oxidative phosphorylation. *Science*. 128(3322):450-456.
5. Chiu DC, Baskin JM. (2022). Imaging and Editing the Phospholipidome. *Acc Chem Res*. 55(21):3088-3098.

6. López-Lara IM, Geiger O. (2017). Bacterial lipid diversity. Biochim Biophys Acta Mol Cell Biol Lipids. 1862(11):1287-1299.
7. Tsuchiya M, Tachibana N, Nagao K, Tamura T, Hamachi I. (2023). Organelle-selective click labeling coupled with flow cytometry allows pooled CRISPR screening of genes involved in phosphatidylcholine metabolism. *Cell Metab*. Jun 6;35(6):1072-1083.e9.
8. Fox CA, Ryan RO. (2022). Studies of the cardiolipin interactome. Prog Lipid Res. 88:101195.
9. Barroso IG, Cardoso C, Ferreira C, Terra WR. (2021). Transcriptomic and proteomic analysis of the underlying mechanisms of digestion of triacylglycerols and phosphatides and absorption and fate of fatty acids along the midgut of Musca domestica. Comp Biochem Physiol Part D Genomics Proteomics. Sep; 39:100826.
10. Welty FK. (2023). Omega-3 fatty acids and cognitive function. Curr Opin Lipidol. Feb 1;34(1):12-21.
11. Zhukovsky MA, Filograna A, Luini A, Corda D, Valente C. (2019). Phosphatidic acid in membrane rearrangements. FEBS Lett. 593(17):2428-2451.
12. Zhao H, Shao D, Jiang C, Shi J, Li Q, (2017). Biological activity of lipopeptides from Bacillus. *Appl Microbiol Biotechnol*. 101(15):5951-5960.
13. Alston TA. (2016). Noteworthy Chemistry of Chloroform. *J Anesth Hist*. Jul;2(3):85-8.
14. National Toxicology Program. Chloroform. Rep Carcinog. (2011) 12:97-100.
15. Ramzan R, Napiwotzki J, Weber P, Kadenbach B, Vogt S. (2021). Cholate Disrupts Regulatory Functions of Cytochrome c Oxidase. *Cells*. 10(7):1579.
16. Sahoo S, Ur Rehman J, Shah MR, De P, Tecilla P. (2021). Cholate Conjugated Polymeric Amphiphiles as Efficient Artificial Ionophores. *ACS Appl Polym Mater*. 3(2):588-593.
17. Koganov MM, Ksenzhek OS. (1976). Doslidzhennia rozchinnoho v izooktani kompleksu tsytokhromu c z fosfolipidamy mozku byka [Iso-octane soluble complex of cytochrome c with bovine brain phospholipids]. Ukr Biokhim Zh. 48(6):712-716. Ukrainian. PMID: 189473.
18. Kielkopf CL, Bauer W, Urbatsch IL. (2021). Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of Proteins. *Cold Spring Harb Protoc*. 2021(12).
19. Rowe SE, O'Gara JP. (2016). Electrophoretic Mobility Shift Assays. *Methods Mol Biol*. 1373:155-67.
20. Houée-Lévin C, Bobrowski K, Horakova L, Karademir B, Schöneich C, (2015). Exploring oxidative modifications of tyrosine: an update on mechanisms of formation, advances in analysis and biological consequences. Free Radic Res. 49(4):347-73.
21. Correddu D, Di Nardo G, Gilardi G. (2021). Self-Sufficient Class VII Cytochromes P450: From Full-Length Structure to Synthetic Biology Applications. *Trends Biotechnol*. 39(11):1184-1207.
22. Birker K, Ge S, Kirkland NJ, Theis JL, Marchant J, et. al., (2023). Mitochondrial MICOS complex genes, implicated in hypoplastic left heart syndrome, maintain cardiac contractility and actomyosin integrity. Elife. 12: e83385.
23. Ahmed M, Koo KM, Mainwaring PN, Carrascosa LG, Trau M. et. al., (2020). Phosphoprotein Biosensors for Monitoring Pathological Protein Structural Changes. *Trends Biotechnol*. 38(5):519-531.
24. Duan HZ, Nie ZK, Li Y, Chen YX. et. al., (2020). Unremitting progresses for phosphoprotein synthesis. Curr Opin Chem Biol. 58:96-111.
25. Edwards MJ, Richardson DJ, Paquete CM, Clarke TA. et. al., (2020). Role of multiheme cytochromes involved in extracellular anaerobic respiration in bacteria. *Protein Sci*. 29(4):830-842.
26. Yang Y, Du L, Hosokawa M, Miyashita K. et. al., (2020). Total Lipids Content, Lipid Class and Fatty Acid Composition of Ten Species of Microalgae. *J Oleo Sci*. 69(10):1181-1189.
27. Zhang Y, Narlikar GJ, Kutateladze TG. et. al., (2021). Enzymatic Reactions inside Biological Condensates. *J Mol Biol*. J433(12):166624.
28. Gilbert RJC. et. al., (2021). Electron microscopy as a critical tool in the determination of pore forming mechanisms in proteins. Methods Enzymol. 649:71-102.
29. Crans DC, Sánchez-Lombardo I, McLauchlan CC. et. al., 2019 (). Exploring Wells-Dawson Clusters Associated With the Small Ribosomal Subunit. Front Chem. 7:462.
30. Reis A, Spickett CM. (2012). Chemistry of phospholipid oxidation. Biochim Biophys Acta. 1818(10):2374-2387.
31. Woodward X, Kelly CV. (2020). Single-lipid dynamics in phase-separated supported lipid bilayers. Chem Phys Lipids. 233:104991.
32. Magnitsky S, Zhang J, Idiyatullin D, Mohan G, Garwood M, et. al., (2017). Positive contrast from cells labeled with iron oxide nanoparticles: Quantitation of imaging data. Magn Reson Med. 78(5):1900-1910.
33. Hestand MS, Ameur A. (2019). The Versatility of SMRT Sequencing. Genes (Basel). 10(1):24.
34. Rudenko SV. Characterization of morphological response of red cells in a sucrose solution. Blood Cells Mol Dis. 2009 May-Jun;42(3):252-61.
35. Laszakovits JR, Kerr A, MacKay AA. Permanganate Oxidation of Organic Contaminants and Model Compounds. Environ Sci Technol. 2022 Apr 19;56(8):4728-4748.
36. Chaban Y, Boekema EJ, Dudkina NV. Structures of mitochondrial oxidative phosphorylation supercomplexes and mechanisms for their stabilisation. Biochim Biophys Acta. 2014 Apr;1837(4):418-426.
37. Dudkina NV, Folea IM, Boekema EJ. Towards structural and functional characterization of photosynthetic and mitochondrial supercomplexes. Micron. 2015 May;72:39-51.
38. Xiao YD, Paudel R, Liu J, Ma C, Zhang ZS, Zhou SK. MRI contrast agents: Classification and application (Review). Int J Mol Med. 2016 Nov;38(5):1319-1326.
39. Hori M. High-precision molecular measurement. Science. 2020 Sep 4;369(6508):1160-1161.
40. Baba H. [Introduction to Myelin Research]. Yakugaku Zasshi. 2022;142(8):837-853. Japanese.
41. Johnston JS, Zapalac ME, Hjelmen CE. Flying High-Muscle-Specific Underreplication in *Drosophila*. Genes (Basel). 2020 Feb 26;11(3):246.
42. Venediktova N, Solomadin I, Starinets V, Mironova G. Structural and Dynamic Features of Liver Mitochondria and Mitophagy in Rats with Hyperthyroidism. Int J Mol Sci. 2022 Nov 18;23(22):14327.
43. Ward KR, Matejtschuk P. The Principles of Freeze-Drying and Application of Analytical Technologies. Methods Mol Biol. 2021;2180:99-127.
44. Klarić T, Gudelj I. HILIC-UPLC Analysis of Brain Tissue N-Glycans. Methods Mol Biol. 2017; 1503:207-216.
45. Alfredsson V, Lo Nostro P, Ninham B, Nylander T. Morphologies and Structure of Brain Lipid Membrane Dispersions. Front Cell Dev Biol. 2021 Jun 14;9:675140.
46. Gusman DJR, Cintra LTA, Novaes VCN, Matheus HR, de Araujo NJ, de Almeida JM. pH influences the biocompatibility

of methylene blue solutions. Clin Oral Investig. 2018 Jan;22(1):361-367.
47. Nitsche JM, Kasting GB. How Predictable Are Human Stratum Corneum Lipid/Water Partition Coefficients? Assessment and Useful Correlations for Dermal Absorption. J Pharm Sci. 2018 Feb;107(2):727-738.
48. Vinothkumar KR, Zhu J, Hirst J. Architecture of mammalian respiratory complex I. Nature. 2014 Nov 6;515(7525):80-84.
49. Jodeiri Farshbaf M, Kiani-Esfahani A. Succinate dehydrogenase: Prospect for neurodegenerative diseases. Mitochondrion. 2018 Sep;42:77-83.
50. Oiki S, Iwamoto M. Lipid Bilayers Manipulated through Monolayer Technologies for Studies of Channel-Membrane Interplay. Biol Pharm Bull. 2018;41(3):303-311.
51. Rak M, Bénit P, Chrétien D, Bouchereau J, Schiff M, El-Khoury R, Tzagoloff A, Rustin P. Mitochondrial cytochrome c oxidase deficiency. Clin Sci (Lond). 2016 Mar;130(6):393-407.
52. Wessler LB, de Miranda Ramos V, Bittencourt Pasquali MA, Fonseca Moreira JC, de Oliveira J, Scaini G, Streck EL. Administration of branched-chain amino acids increases the susceptibility to lipopolysaccharide-induced inflammation in young Wistar rats. Int J Dev Neurosci. 2019 Nov;78:210-214.
53. Hajba L, Guttman A. Recent Advances in the Analysis Full/Empty Capsid Ratio and Genome Integrity of Adeno-associated Virus (AAV) Gene Delivery Vectors. Curr Mol Med. 2020;20(10):806-813.

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