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Supramolecular organization of tricarboxylic acid cycle enzymes*

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We propose a spatial structure for the tricarboxylic acid cycle enzyme complex (tricarboxylic acid cycle metabolon). The structure is based on an analysis of data on the interaction between tricarboxylic acid cycle enzymes and the mitochondrial inner membrane, as well as on data on enzyme-enzyme interactions. The α-ketoglutarate dehydrogenase complex, adsorbed along one of the 3-fold symmetry axes of the mitochondrial inner membrane, plays a key role in formation of the metabolon. In the interaction with the membrane, two association sites of the α-ketoglutarate dehydrogenase complex participate, placed on opposite sides of the complex. The tricarboxylic acid cycle enzyme complex contains one molecule of the α-ketoglutarate dehydrogenase complex and six molecules of each of the other enzymes of the tricarboxylic acid cycle, as well as aspartate aminotransferase and nucleoside-diphosphate kinase. Succinate dehydrogenase, which is the integral protein of the mitochondrial inner membrane, is a component of the anchor site responsible for the assembly of the metabolon on the membrane. The molecular mass of the complex (without regard to succinate dehydrogenase) is 8×10^6 Da. The metabolon symmetry corresponds to the D_3 point symmetry group.

Keywords: Tricarboxylic acid cycle; Metabolon; Supramolecular organization; Protein-protein interactions.

1. Introduction

At present it is generally accepted that the enzymes catalyzing the reactions of common metabolic pathways are organized into multienzyme complexes (Welch, 1977; Friedrich, 1984; Srere, 1987) These have been referred to as metabolons (Srere, 1985b) The Physiological significance of the phenomenon lies in the kinetic advantage owing to the reduction of the transient and transit times, due to process compartmentation preventing inappropriate diffusion of intermediates into other metabolic pathways (Welch, 1977; Gaertner, 1978; Snol', 1979; Wombacher, 1983; Keleti, 1984; Friedrich, 1984, 1985), as well as in the possibility to control the organized metabolic pathway as a whole (Kurganov, 1986a–d, 1987a,b).

Kuzin (1970) and Srere (1972) supposed the existence of supramolecular organization for one of the most important metabolic pathways, namely the tricarboxylic acid (TCA) cycle. This supposition has been confirmed recently (see Srere, 1985a, 1987). Thus, extensive experimental data have been accumulated on protein-protein interactions for TCA cycle enzymes (Koch-Schmidt et al., 1977; Srere et al., 1978; Beeckmans and Kanarek, 1981; Fahien and Kmiotek, 1983; Porpaczy et al., 1983, 1987; Tyiska et al., 1987) and interactions between most of the TCA cycle enzymes and the mitochondrial inner membrane (Matlib and O'Brien, 1975; D'Souza and Srere, 1983; Moore et al., 1984; Sümegi and Srere, 1984b; Tyiska et al., 1987). It is worth noting that the protein concentration in the mitochondrial matrix where the TCA cycle enzymes are localized is 40 wt% (Srere, 1985a). Under such conditions diffusion is hindered not only for proteins, but for such

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large molecules as nucleotide coenzymes. In addition calculations presented by Srere (1985a) which show that the average distance between inner membrane surfaces for heart mitochondria are 15—30 nm, can also be taken as evidence that most of the matrix enzymes contact the inner membrane.

Recently a supramolecular aggregate containing five TCA cycle enzymes (fumarase, malate dehydrogenase, citrate synthase, aconitase and isocitrate dehydrogenase) was identified in extracts from gently disrupted *Escherichia coli* cells (Barnes and Weitzman, 1986). Preliminary data from the same authors (Barnes and Weitzman, 1986) testify that similar aggregates are present in mitochondrial lysates as well.

In the present work, based on data on enzyme-enzyme interaction, an attempt is made to design a spatial structure for the TCA cycle enzyme complex. Data on enzymes from pig and bovine heart mitochondria are used. However, we consider that the principles for metabolon assembly proposed by us should be valid in the case of TCA cycle enzyme complexes from other sources as well.

2. Principles for metabolon construction

The TCA cycle occupies a central position in cellular metabolism. The cycle is initiated by a reaction in which the acetyl group bound to CoA and being the product of carbohydrate, fat and amino acid degradation, joins to oxaloacetate to form citrate. During subsequent reactions the citrate is successively oxidized to oxaloacetate producing two molecules of carbon dioxide. Reduced coenzymes formed in the dehydrogenase reactions are reoxidized by the electron transfer chain localized in the mitochondrial inner membrane. One of the TCA cycle enzymes, succinate dehydrogenase, is also a component of electron transfer chain complex II. Other TCA cycle enzymes are located in the mitochondrial matrix.

The TCA cycle is linked to many metabolic processes. The closest association is that with reactions catalyzed by aspartate aminotransferase and nucleoside-diphosphate kinase. Aspartate aminotransferase, along with malate dehydrogenase, forms the malate-aspartate shuttle, which enables mitochondria to oxidize extramitochondrial NADH. For aspartate aminotransferase the interaction with some TCA cycle enzymes has been demonstrated experimentally (Backman and Johansson, 1976; Beeckmans and Kanarek, 1981; Fahien and Kmiotek, 1983). Nucleoside-diphosphate kinase transfers the phosphate from GTP emerging from the succinic thiokinase reaction to ADP. It is a principal way of utilizing the GTP formed in the TCA cycle (Jucobus and Evans, 1977).

We consider that the TCA cycle enzyme complex should include all TCA cycle enzymes as well as aspartate aminotransferase and nucleoside-diphosphate kinase. The information on molecular mass and subunit structure of the corresponding enzymes is shown in Table 1.

A main principle of metabolon assembly is that it is formed on some biological support — biological membranes, structural muscle proteins or other cell structures (Kurganov, 1986a). The support contains a protein with an "anchor" function. Fixation on a support provides uniqueness of complex assembly as well as creating a centre of system control sensitive to new control factors, and in the first place to second messengers (Kurganov, 1986 a—c).

The important reason for the supramolecular structure uniqueness is its symmetry (Goldstein et al., 1986). The symmetry of metabolons adsorbed on membranes has to reflect the symmetry of integral membrane proteins acting as anchor sites (Kurganov, 1984). It should be noted here that the number of integral proteins, especially channel-forming proteins, are functioning in the membrane in the form of trimers with 3-fold symmetry axis perpendicular to membrane plane (Kagawa, 1978; Klingenberg, 1981). It may be assumed that such a type of integral protein symmetry is favoured by the hexago-

TABLE 1

Properties of the tricarboxylic acid cycle enzymes from mammalian heart.

No.	Enzyme	EC no.	Molecular mass (Da)	Subunit structure	Reference
1	Citrate synthase	4.1.3.7	98 000 (pig)	α_2	Srere, 1985a
2	Aconitase	4.2.1.3	66 000 (pig)	α	Srere, 1985a
3	Isocitrate dehydrogenase (NAD-specific)	1.1.1.41	160 000 (pig)	$\alpha_2\beta\omega$	Srere, 1985a
4a	α-Ketoglutarate dehydrogenase	1.2.4.2	216 000 (pig)	α_2	Oliver and Reed, 1982
4b	Transsuccinylase	2.3.1.61	41 000 (pig)	α	Oliver and Reed, 1982
4c	Lipoamide dehydrogenase	1.6.4.3	108 000 (pig)	α_2	Oliver and Reed, 1982
5	Succinic thiokinase	6.2.1.4	70 000 (pig)	$\alpha\beta$	Srere, 1985a
6	Succinate dehydrogenase	1.3.99.1	97 000 (beef)	$\alpha\beta$	Srere, 1985a
7	Fumarase	4.2.1.2	194 000 (pig)	α_4	Srere, 1985a
8	Malate dehydrogenase	1.1.1.37	70 000 (pig)	α_2	Srere, 1985a
9	Aspartate aminotransferase	2.6.1.1	93 000 (pig)	α_2	Barra et al., 1976
10	Nucleoside-diphosphate kinase	2.7.4.6	103 000 (beef)	α_6?	Colomb et al., 1969

nal packing of lipids in membrane plane (Mikelsaar, 1987). Metabolons formed on such integral proteins have to possess the 3-fold symmetry axis perpendicular to the membrane plane.

When determining the relative disposition of enzymes in the complex we were guided by the following principles.

(1) Enzymes that catalyze consecutive steps of the TCA cycle must occupy neighbouring positions in the complex, i.e. they must have corresponding recognition sites (Kurganov et al., 1985). Such a disposition of enzymes provides a possibility for chemical transformation of metabolic intermediates by conveyer principle in the metabolon microcompartment. Data are available on interaction between malate dehydrogenase and fumarase (Beeckmans and Kanarek, 1981), malate dehydrogenase and citrate synthase (Koch-Schmidt et al., 1977; Srere et al., 1978; Beeckmans and Kanarek, 1981; Fahien and Kmiotek, 1983), malate dehydrogenase and aspartate aminotransferase (Backman and Johansson, 1976; Beeckmans and Kanarek, 1981; Fahien and Kmiotek, 1983), citrate synthase and aconitase (Tyiska et al., 1987), citrate synthase and aspartate aminotransferase (Fahien and Kmiotek, 1983), isocitrate dehydrogenase and α-ketoglutarate dehydrogenase complex (Porpaczy et al., 1987) α-ketoglutarate dehydrogenase complex and succinic thiokinase (Porpaczy et al., 1983).

(2) Enzymes which utilize and regenerate NAD must be in close contact with one another (Kurganov et al., 1985). It may be supposed that the three TCA cycle dehydrogenases using NAD — namely isocitrate dehydrogenase, lipoamide dehydrogenase and malate dehydrogenase — are placed side by side and in contact with integral membrane-bound protein, namely complex I of the electron transfer chain providing regeneration of coenzyme oxidized form. Such a disposition of NAD-dependent enzyme systems allows effective coenzyme recycling in the metabolon microcompartment.

When constructing the model we should also take into account the experimental data on interactions of enzymes which are not connected by common metabolites. Thus, the interaction between citrate synthase and fumarase was shown (Beeckmans and Kanarek, 1981). Aspartate aminotransferase under the same conditions does not interact with fumarase. The absence of contact

between citrate synthase and α-ketoglutarate dehydrogenase complex has also been demonstrated (Porpaczy et al., 1983).

3. α-Ketoglutarate dehydrogenase complex is the nucleus of the tricarboxylic acid cycle metabolon

A difficulty in construction of the TCA cycle enzyme complex is a significant difference in size between the α-ketoglutarate dehydrogenase complex (with molecular mass of 2.7×10^6 Da) and the rest of enzymes whose molecular masses do not exceed 2×10^5 Da. Although it was suggested (Srere, 1985a) that in mitochondria the α-ketoglutarate dehydrogenase complex exists as a smaller aggregate, in our opinion the α-ketoglutarate dehydrogenase complex is sufficiently large so as to be a nucleus around which all the rest of the proteins of the TCA cycle metabolon are assembled.

The core of the α-ketoglutarate the dehydrogenase complex consists of 24 subunits of trans-succinylase, creating a cubic structure with three 4-fold symmetry axes; four 3-fold symmetry axes and six 2-fold symmetry axes (the O symmetry group) (Oliver and Reed, 1982). Six dimers of α-ketoglutarate dehydrogenase and lipoamide dehydrogenase are joined to them (Oliver and Reed, 1982), which diminishes the symmetry. Similar organization is seen in the α-ketoglutarate dehydrogenase complex from *Escherichia coli* (Oliver and Reed, 1982).

The exact structure of the α-ketoglutarate dehydrogenase complex is still obscure. Wagenknecht et al. (1983) by virtue of the data on in vitro interactions for components of the α-ketoglutarate dehydrogenase complex from *Escherichia coli* came to the conclusion that the complex does not have a unique quaternary structure but represents a family of structural isomers with overall numbers up to 125,000.

However, in vivo the α-ketoglutarate dehydrogenase complex is adsorbed on the membrane, and so we may assume under native conditions the existence of an unambiguous complex structure. In the case of mammalian heart mitochondria, the problem of clarifying the type of α-ketoglutarate dehydrogenase complex fixation on the membrane is simplified because the complex size is comparable with the distance between the inner surfaces of the mitochondrial inner membrane. The edge of the cube formed by trans-succinylase has a length of 11.7 nm (Tanaka et al., 1972). Thus, the length of the cube diagnonal (20 nm) corresponds to the average distance between the inner surfaces of the inner membrane in heart mitochondria (15—30 nm according to data presented by Srere (1985a)). Thus we expect that the trans-succinylase is arranged in such a way so that the two opposite cube vertices are in touch with opposite membrane surfaces, the 3-fold symmetry axis being perpendicular to those surfaces (Fig. 1). Six lipoamide dehydrogenase molecules are placed on the cube faces, each touching one of the membrane surfaces (this is necessary for

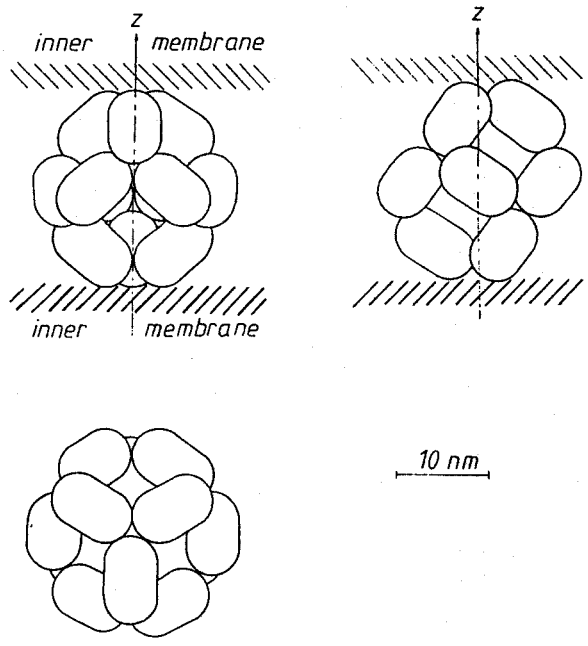

Fig. 1. Structure of α-ketoglutarate dehydrogenase complex core (three projections). Ovaloids are the dimers of transsuccinylase. *z* is 3-fold symmetry axis.

exchange of NAD and NADH with complex I of the electron transfer chain). Six α-ketoglutarate dehydrogenase molecules are attached to those cube edges having no contact with membrane. Therefore the α-ketoglutarate dehydrogenase complex (Fig. 2), apart from its 3-fold symmetry axis, possesses three 2-fold symmetry axes as well (D_3 point symmetry group). Around this structure other enzymes will arrange themselves forming the TCA cycle metabolon.

4. Hypothetical structure of the tricarboxylic acid cycle enzyme complex

Using the principles mentioned above we have constructed a structural model for the TCA cycle enzyme complex. It is assumed that the symmetry of the TCA cycle enzyme complex as well as the symmetry of the adsorbed α-ketoglutarate dehydrogenase complex belongs to the D_3 point group. The number of asymmetric molecules of every kind corresponds to a group order (Goldstein et al., 1986) which is equal to 6 in the case of D_3 group. Hence, the complex must include six molecules of each enzymes.

The structure of the TCA cycle enzyme complex may be represented as a hexamer consisting of six identical asymmetric subunits (Fig. 3). Every asymmetric subunit (Fig. 4) contains a tetramer of transsuccinylase, one molecule of every other enzyme and an anchor protein (Ω).

A general view of the TCA cycle enzyme complex is shown in Fig. 5. Enzyme sizes on the chart accord with their molecular masses. The complex is "squeezed" between opposite surfaces of the inner membrane, the membrane being in contact with all enzymes apart

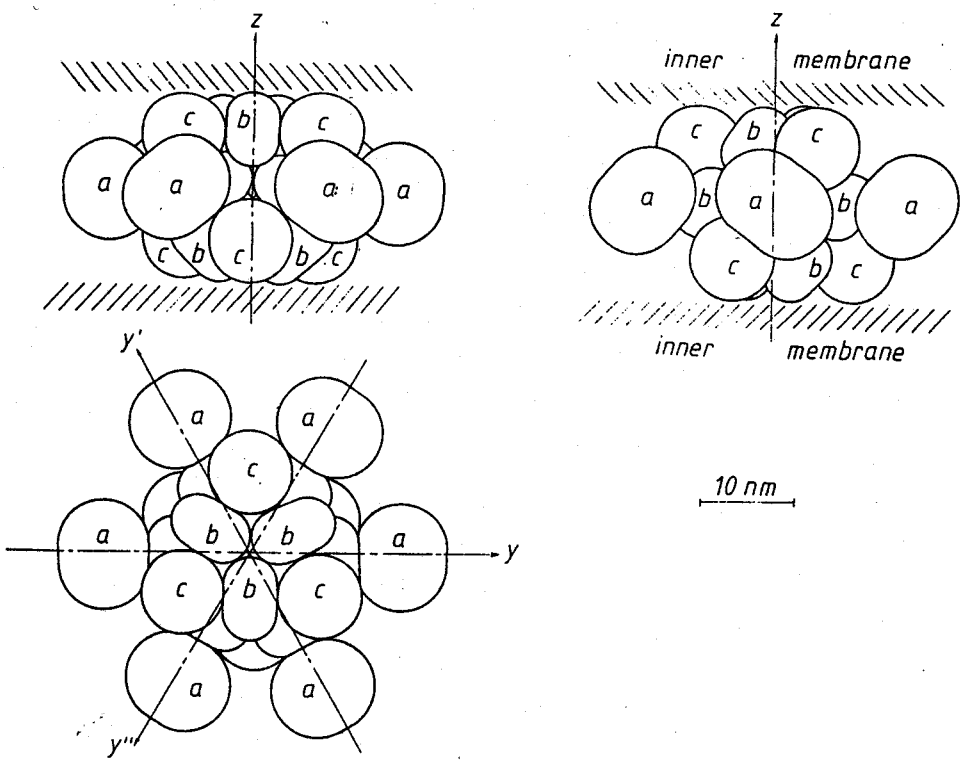

Fig. 2. α-Ketoglutarate dehydrogenase complex (three projections). Designations: *a* is α-ketoglutarate dehydrogenase, *b* is dimer of transsuccinylase, *c* is lipoamide dehydrogenase, *z* is 3-fold symmetry axis, *y*, *y'* and *y''* are 2-fold symmetry axes.

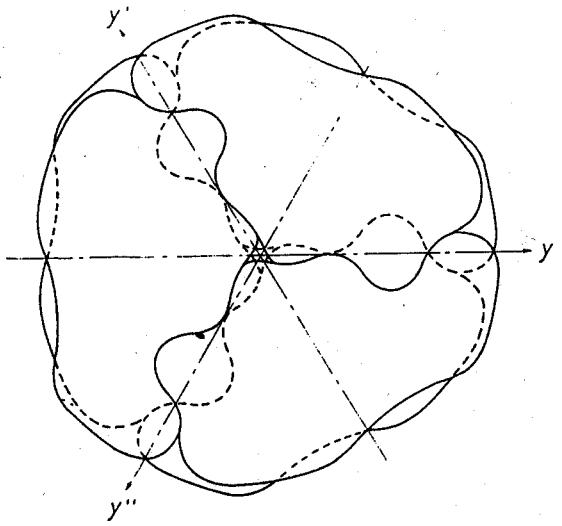

Fig. 3. The schematic representation of hexameric structure of the TCA cycle enzyme complex. The 3-fold symmetry axis is perpendicular to plane of figure. *y*, *y'* and *y''* are 2-fold symmetry axes.

from α-ketoglutarate dehydrogenase and aspartate aminotransferase. The integral proteins of the mitochondrial inner membrane, including succinate dehydrogenase, act as the anchor proteins responsible for assembly of the TCA cycle enzyme complex on the membrane. The complex height along the 3-fold symmetry axis is 20 nm and the diameter of the complex is 50 nm. Molecular mass (without succinate dehydrogenase) is 8×10^6 Da.

Under our assumptions (contacts of the metabolon with the membrane, D_3 symmetry group, central position of the α-ketoglutarate dehydrogenase complex in the metabolon, postulated enzyme-enzyme contacts) the metabolon structure is unique. It should be noted that in the completely formed metabolon each enzyme is in contact with several neighbours. Owing to this circumstance the process of metabolon assembly will be more thermodynamically advantageous in comparison with the

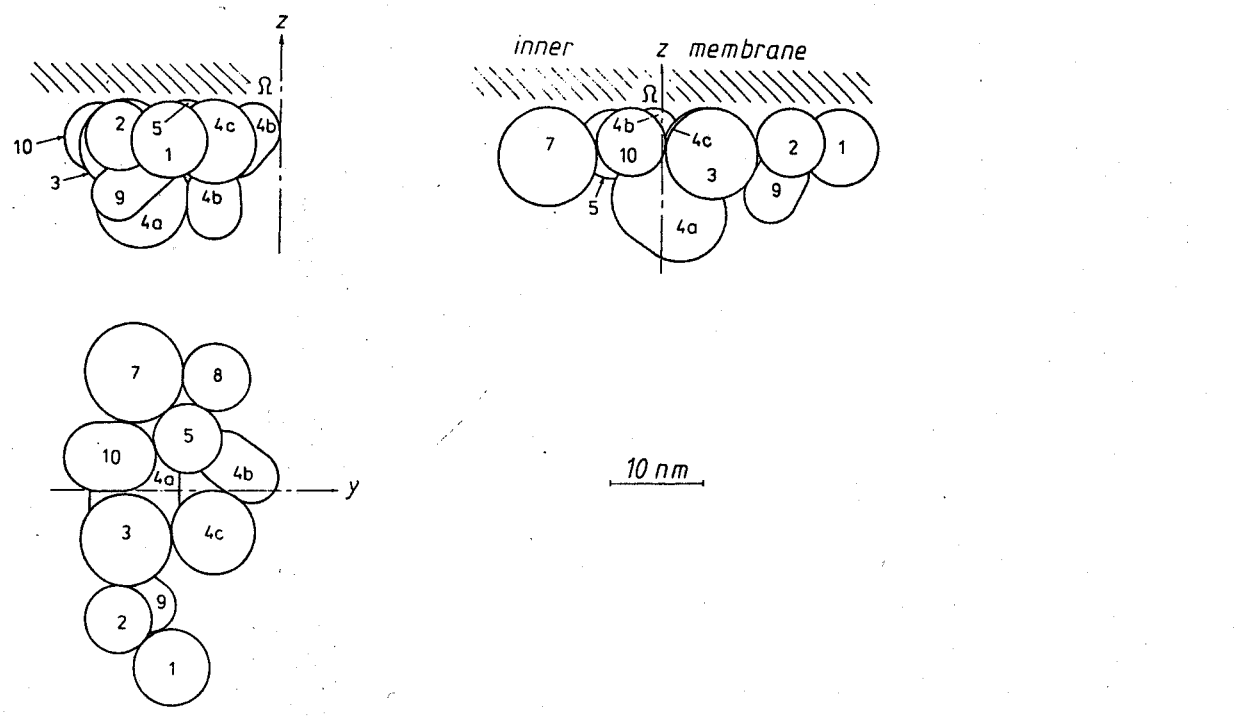

Fig. 4. Asymmetric subunit of the TCA cycle enzyme complex (three projections). The numbers refer to citrate synthase (1), aconitase (2), isocitrate dehydrogenase (3), α-ketoglutarate dehydrogenase complex (4), succinic thiokinase (5), fumarase (7), malate dehydrogenase (8), asparate aminotransferase (9) and nucleoside-diphosphate kinase (10). Ω is anchor protein. Other designations are as in Figs. 2 and 3.

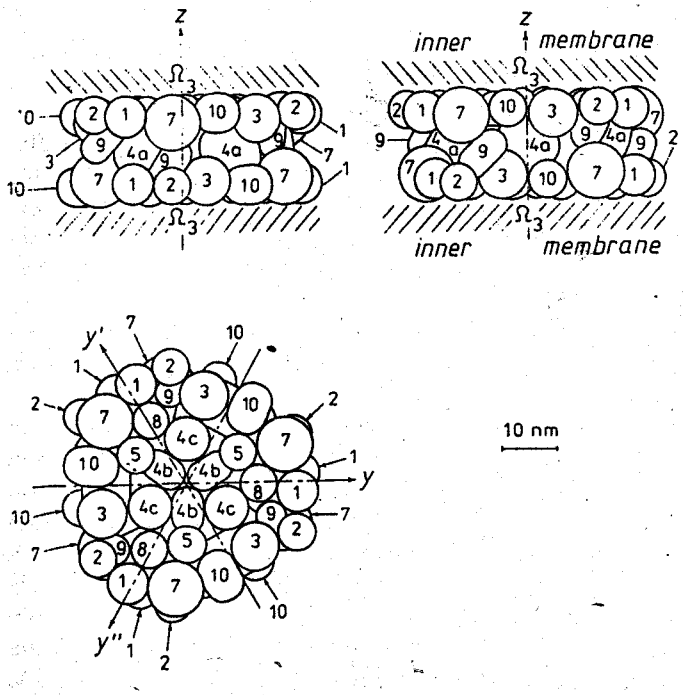

Fig. 5. Hypothetical structure of the TCA cycle enzyme complex (three projections). Ω_3 are anchor proteins including succinate dehydrogenase. Other designations are as at Fig. 4.

formation of partial complexes in which the number of contacts with neighbours will be lower. In other words, we consider metabolon assembly as a process proceeding according to the principle "all or none", i.e. as "cooperative association" (Engel and Winklmair, 1972; Klotz et al., 1975) and we assume that the formation of partial complexes may be disregarded.

The structure of our model assumes the possibility for the TCA cycle enzyme complex to interact with other enzymes and multienzyme complexes with which it is connected by common metabolities. First of all it relates to the electron transfer chain complexes in the mitochondrial inner membrane. Isocitrate dehydrogenase, lipoamide dehydrogenase and malate dehydrogenase should be in contact with complex I, and succinic thiokinase and fumarase with complex II. The interaction between malate dehydrogenase and complex I was demonstrated experimentally by Sümegi and Srere (1984b)*. In short, electron transfer chain complexes are an anchor site for assembly of the TCA cycle enzyme complex. Therefore integral and peripheral membrane-bound structures should correspond to each other in symmetry type. From this it may be assumed that the ensemble of electron transfer chain enzymes also has a 3-fold symmetry axis perpendicular to the membrane plane and coinciding with the symmetry axis of the TCA cycle enzyme complex.

*Sümegi et al. (Sümegi and Srere, 1984b; Porpaczy et al., 1987) failed to detect the physical interactions between complex I and isocitrate dehydrogenase or lipoamide dehydrogenase. However, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase complex and complex I form a ternary associate (Porpaczy et al., 1987), so we suppose that isocitrate dehydrogenase and lipoamide dehydrogenase are placed in the metabolon near complex I.

The TCA cycle enzyme complex should also interact with other matrix enzymes. In the case of heart mitochondria these are enzymes delivering acetyl-CoA to the TCA cycle, i.e. the pyruvate dehydrogenase complex and enzymes for fatty acid β-oxidation. Citrate synthase (the enzyme utilizing the acetyl-CoA) is placed in our model at the periphery of the complex. Experiments show that citrate synthase interacts with both the pyruvate dehydrogenase complex Sümegi and Alkonyi, 1983) and thiolase (Sümegi et al., 1985), which is the enzyme catalysing the final stage of fatty acid β-oxidation.

The pyruvate dehydrogenase complex of bovine heart has a 3-fold symmetry axis, and its size (molecular mass of 8.5×10^6 Da) is similar to that of the proposed TCA cycle enzyme complex (Oliver and Reed, 1982). As for the enzymes of fatty acid β-oxidation, it is supposed (Sümegi and Srere, 1984a) that they are organized into the metabolon. It may be assumed that all three complexes (TCA cycle enzyme complex, pyruvate dehydrogenase complex and the complex of fatty acid β-oxidation enzymes) are organized in the heart mitochondrial matrix into a periodic structure of hexagonal lattice type.

In mitochondria of cells of other organs, the supramolecular organization of matrix enzymes may be more complex. Thus, in liver mitochondria the TCA cycle enzyme complex should also interact with pyruvate carboxylase, with glutamate dehydrogenase and other enzymes of amino acid metabolism. On the other hand, the distance between the inner surfaces of the inner membranes in liver cell mitochondria is greater than that in heart cell mitochondria (see Srere, 1985a). We may therefore suppose that the liver mitochondrial inner membrane is touched by one side only of the TCA cycle enzyme complex, the other side of the complex contacting with matrix enzymes.

It should be noted that the TCA cycle enzyme complex is a mobile structure, and is in equilibrium with free enzymes of the matrix. The mitochondrial matrix volume undergoes significant changes during transition of a mitochondrion from condensed conformation to the orthodox one (see Srere, 1985a). This process determined by the mitochondrial energy state should exert an influence on the equilibrium between TCA cycle enzyme complex and free enzymes. High protein concentrations in the matrix in the condensed conformation should encourage the formation of metabolons. When the matrix swells, there may be partially reversible decomposition of the complex into free enzymes.

It is known that adsorption of enzymes on biological membranes is sensitive to the presence of cellular metabolites (see Kurganov, 1985). Thus, it was shown (Moore et al., 1984) that the adsorption of citrate synthase on the mitochondrial inner membrane is strengthened in the presence of low concentrations of citrate and weakened in the presence of oxalo-acetate, CoA, acetyl-CoA, ATP-Mg as well as in the presence of high concentrations of citrate (above 5 μM). It may therefore be expected that the completeness of assembly of the TCA cycle enzyme complex on the membrane would depend on the concentration of certain metabolites in the mitochondrial matrix.

5. Physiological significance of metabolon formation

Metabolon assembly entails the formation of a microcompartment in which the metabolic process may proceed in isolation without releasing intermediates into the medium (Friedrich, 1984, 1985). Owing to the interaction of the enzymes in the metabolon it acts as an integrated system able to function in a cooperative way (Welch, 1977, 1984; Gaertner, 1978; Welch and Keleti, 1981; Welch and Berry, 1985). The coordinated changes of enzyme conformations in the metabolon may ensure the effective transfer of intermediates between active sites in the microcompartment; in this way the enzymes catalyzing irreversible reactions (citrate synthase, iso-

citrate dehydrogenase and α-ketoglutarate dehydrogenase) may "force" an irreversible pattern of catalytic action on the whole complex.

It should be noted that the TCA cycle enzyme complex includes a six-fold enzyme set of the corresponding metabolic pathways and so the microcompartment created by the metabolon consists of six sections. Here we can speak conventionally of two "tiers" of the microcompartment each of them consisting of three sections. Each of the sections is able to provide the whole course of metabolic cycle.

To close the TCA cycle, we need oxaloacetate to return to the first enzyme of the cycle, namely citrate synthase. However, according to Fig. 4 it is clear that the asymmetric subunit of the complex is not closed, since malate dehydrogenase has no contact with the citrate synthase in the subunit. This means that after a single pass through the metabolic cycle the oxaloacetate gets to a citrate synthase molecule of a neighbouring subunit. To have the substrate returned to the citrate synthase molecule of its initial subunit it would need to pass in a circular channel through the three sections of one "tier" of the metabolon compartment. It may be assumed that functioning of individual sections in the microcompartment (i.e. transfer of intermediates through the channel-sections) is coordinated in what may be called a "merry-go-round" action mode.

To describe the functioning of metabolons fixed on the inner surface of the mitochondrial inner membrane the autowaves theory (Ivanitskii et al.; see also Kurganov, 1986c) may prove to be useful.

The enhancement of effectivity of the metabolic pathway in the microcompartment of the metabolon is not the sole advantage of the organized multienzyme structure formation. In our opinion (Kurganov, 1986a,b,d, 1987a), the physiological significance of the metabolon formation is that the cell has the possibility to control the metabolic pathway, represented by the structurally organized complex, as a whole. Formerly, the supposition was made (Kurganov, 1986c) that the metabolon must be controlled in this way using the second messengers. There is evidence that Ca^{2+} (Ezawa and Ogata, 1979; Kulinsky and Kolpakova, 1984; McCormack, 1985) and cyclic AMP (Kulinsky and Trufanova, 1975; Kulinsky et al., 1981) exert influence on the activity of certain TCA cycle enzymes. However, the activity of most TCA cycle enzymes may be determined only after mitochondria are damaged and so we cannot see the influence of such chemical signals on the TCA cycle as a whole system.

One of the TCA cycle enzymes which are sensitive to second messengers action is succinate dehydrogenase (Ezawa and Ogata, 1979; Kulinsky et al., 1981) included in the anchor site of the TCA cycle enzyme complex. Proceeding from the idea about the key role of anchor proteins in the control of metabolon functioning (Kurganov, 1986a; 1986b; 1986c), we assume that succinate dehydrogenase is playing the part of a control site in the TCA cycle enzyme complex and perhaps also in the system belonging to a superior complexity level, namely the electron transfer chain together with the TCA cycle enzyme complex.

It should be noted that most of the enzymes in the metabolon possess a subunit structure, so only a part of the active and allosteric sites of the enzymes face internal metabolon microcompartments, whereas the other part appears on the surface of the metabolon. These active and allosteric sites may be responsible for the realization of isosteric and allosteric regulation mechanisms with participation of metabolites from outside the microcompartment, as suggested by Kurganov (1986c).

6. Methods for verification of metabolon model structure

Let us discuss the question of what experiments may be useful to check the proposed structure of the TCA cycle enzyme complex.

Protein-protein contacts in our hypothetical TCA cycle enzyme complex are represented in Table 2. The most interesting is the check

TABLE 2

The predicted enzyme-enzyme contacts in the tricarboxylic acid cycle enzyme complex.

Nos.	Enzyme	1	2	3	4a	4b	4c	5	7	8	9	10
1	Citrate synthase	–	(+)	–	(–)	(–)	(–)	–	(+)	(+)	(+)	–
2	Aconitase	(+)	–	+	–	–	–	–	–	–	+	–
3	Isocitrate dehydrogenase	–	+	–	(+)	–	+	–	–	–	+	+
4a	α-Ketoglutarate dehydrogenase	(–)	–	(+)	–	(+)	(–)	–	+	+	+	+
4b	Transsuccinylase	(–)	–	–	(+)	(+)	(+)	(+)	–	–	–	–
4c	Lipoamide dehydrogenase	(–)	–	+	(–)	(+)	–	–	–	+	–	–
5	Succinic thiokinase	–	–	–	–	(+)	–	–	+	+	–	+
7	Fumarase	(+)	–	–	+	–	–	+	–	(+)	(–)	+
8	Malate dehydrogenase	(+)	–	–	+	–	+	+	(+)	–	(+)	–
9	Aspartate aminotransferase	(+)	+	+	+	–	–	–	(–)	(+)	–	–
10	Nucleoside-diphosphate kinase	(–)	(–)	+	+	–	–	+	+	–	–	–

+ signifies the presence of the contact between the enzymes. (+) signifies that the presence of the contact is proved by experiment. – signifies the absence of the contact. (–) signifies that the possibility of enzymes contacting has been studied and a negative result has been obtained.

of our predicting contacts between enzymes not bounded by common metabolites, for example between fumarase and α-ketoglutarate dehydrogenase. These contacts are necessary to stabilize the metabolon structure.

When using gentle sonic oscillation of mitochondria, Robinson and Srere (1985), obtained pellets which sedimented at 32,000 × *g* and had activity corresponding to at least five enzymes of the TCA cycle. The pellets were permeable to protein molecules and so it was deduced that in these pellets the enzymes were adsorbed to the membrane. These particles reveal a large kinetic advantage over the completely solubilized system (Robinson et al., 1987). If the TCA cycle enzymes complex in such pellets still has its native structure, then they may be a good subject to study the supramolecular organization of TCA cycle enzymes. Such pellets may also be useful to study the problem of the regulation of TCA cycle as an integrated metabolic system.

7. Conclusion

The TCA cycle takes a central place in the metabolism and is directly associated with a number of both catabolic and anabolic processes. The elucidation of the integration mechanisms of these metabolic pathways is one of the most important questions in physicochemical biology. Such a question cannot be solved without elucidation of the structural organization of the metabolic processes. Metabolons have an important place in the hierarchy of such material organization levels as well as in the hierarchy of control levels in the functioning and integration of biological systems.

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