Principles of symmetrical organization for the pyruvate dehydrogenase complex

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Abstract The experimentally observed phenomenon of non-equimolarity for enzyme components, assembled into multienzyme complexes of the 2-oxo acid dehydrogenases family, is structurally interpreted to predict the only possible stable symmetrical distribution of peripheral components on the complex core. To obey the equivalent neighboring, that is necessary for unique self-assembled structures, we should deduce discrete conformational states for core subunits, those with different affinity for peripheral components. Two kinetically different types of substrate-intermediate pathways through the lipoyl network of the mammalian pyruvate dehydrogenase complex follow from this structural theory. The theory predicts unusual kinetic behavior for the multienzyme complex.

Key words: Enzyme structural symmetry; Kinetic mechanism; 2-Oxo acid dehydrogenase

1. Introduction

Multisubunit enzymes are known to self-assemble from a certain number of individual subunits into unique structures. These structures have been shown [1,2] to obey the principle of equivalent neighboring: identical subunits in the assembly should have identical contacts with corresponding identical neighbors. This causes enzyme structures, involving identical subunits, to have at least one symmetry axis [1]. Multienzyme complexes of the 2-oxo acid dehydrogenase family are a good example. These multienzyme complexes comprise multiple copies of three or more enzyme components. Their three catalytic components, E1, E2, E3, catalyze consecutive reactions of a pivotal importance in metabolism [3–5]. Peripheral components, E1 and E3, are organized on the core component E2 to form a specifically functioning assembly. The symmetry of the cores for the 2-oxo acid dehydrogenase complexes is octahedral or icosahedral [3–5] according to the number, 24 or 60, of their identical E2 subunits.

The number of the peripheral E1 and E3 subunits, bound on the core, should be equal to that of the core or greater by a factor of integer to satisfy the principle of equivalent neighboring [1,6]. However, in reality, this is not the case: the polypeptide chain E1:E2:E3 ratios, observed in the assembled multienzyme structures, are non-equimolar [7]. This seems to violate the self-assembly principles for the unique multienzyme structures [7]. Considering this, the arrangement of the peripheral components on the core is still not understood. The electron microscopy and X-ray data available do not resolve this problem. We give here an approach to resolve it. To locate the peripheral components on the core in a unique symmetrical structure self-assembled, we share the core E2 subunits among a small number of conformations in accordance with the ratio of E1:E2:E3 known and with equivalent neighboring for each of the components. On this structural model, the unusual kinetic properties of the mammalian pyruvate dehydrogenase complex are discussed.

2. Theory

Spatial symmetries for multisubunit protein structures of finite size are covered by point group symmetries, involving only rotation axes other than mirror planes or centers of inversion [1,8]. Point group symmetries that fall into this category are the following: cyclical, dihedral, tetrahedral, octahedral and icosahedral.

A rotation symmetry axis disappears if, for example, identical protein subunits arranged around the axis take different conformations or substitute for other polypeptide chains. In this case the symmetry of the structure is lowered. Our detailed analysis of the above-listed point groups, having regard to Goldstein et al. [9], shows the structures with lowered symmetry are described by the subgroups of the symmetry group for the starting structure if the principle of equivalent neighboring is obeyed. For choosing a particular subgroup from the set available, we referred to the Lagrange theorem [10], according to that the order (the number of elements) of a subgroup of the finite group is a divisor of the group order.

Selection of the unique lowered symmetry fitting the experimental stoichiometric data for the components of the multienzyme complex enables us to find the location of the peripheral components on the core and, therefore, postulate the mechanism of functioning and regulation for the 2-oxo acid dehydrogenase complexes. The mechanism so deduced can explain unusual kinetic behavior for these multienzyme complexes on the rigorous structural principles.

3. Results and discussion

To explain the structural organization observed for the multienzyme complexes we presume the following:

(1) the identical core subunits assembled are shared among a certain number of discrete conformations, those characterized by the different affinity for the peripheral components. This sharing is defined by the principle of equivalent neighboring;

(2) in accordance with this principle the complete structure

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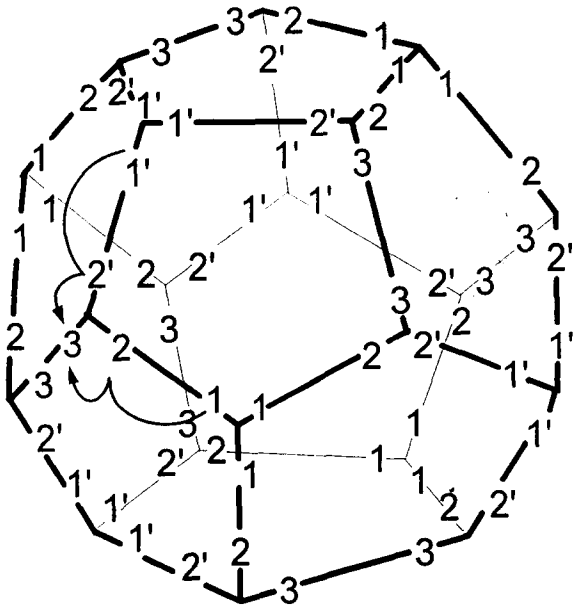

Fig. 1. Schematic presentation of the dodecahedral self-assembled structure with tetrahedral symmetry. Core subunits in five conformations are labelled 1, 2, 3, 2′, 1′. The equivalent neighboring is clearly seen for each a subunit labelled. Four homogeneous (1, 1, 1)-trimers occupy the tetrahedron vertices. Vertices of the conjugate tetrahedron are occupied by (1′, 1′, 1′)-trimers. Arrows show an example of two types for substrate intermediate pathways.

of the multienzyme complex is symmetric, and the symmetry is a subsymmetry of the core structure, because of the lower number of the peripheral subunits bound.

Under these assumptions, the problem of the quaternary structure for multienzyme complexes is the determination of the number of discrete conformations for their core subunits.

As an example, we consider here the most complicated of the 2-oxo acid dehydrogenase complexes, the mammalian pyruvate dehydrogenase complex, its core consisting of 60 E2 subunits, with icosahedral symmetry. Numerous data have been obtained in different laboratories, concerning the location of the peripheral components on the core subunits [3–5,11,12]. The peripheral components are known to bind at only a part of the potential core binding sites [13,14]. It means that initially identical potential binding sites, present on each of 60 core subunits, should become heterogeneous with binding the peripheral components. The stoichiometric data for the dodecahedral pyruvate dehydrogenase complexes show 20–30 E1-dimers [3] and 6 [3] or 12 [12] E3-dimers to bind at the core. It follows from our analysis that only the tetrahedral subgroup among all of the icosahedral subgroups is consistent with these stoichiometric data. The sharing of 60 core subunits among 5 conformations with different affinity for the peripheral components follows from this analysis.

Fig. 1 shows schematically the dodecahedral core with tetrahedral symmetry, obtained by dividing 60 core subunits into five conformational classes. It is easily seen that conformationally distinct subunits obey each the equivalent neighboring in this case. The most possible distribution of the peripheral components is shown in Fig. 1 by indices 1, 1′, 2, 2′, 3, where 1 and 1′ correspond to core conformations with the affinity for E1, 2 and 2′ are conformations with the preferential E2-activity and 3 is a conformation with the affinity for E3, mediated through protein X. The structure in Fig. 1 includes two conjugate tetrahedrons, employing the homogeneous trimers (1, 1, 1) and (1′, 1′, 1′) at their vertices. We can conclude these core trimers to have affinity to bind 24 E1-dimers. Twelve core subunits shown in Fig. 1 as 3 are located around two-fold axes, grouped into homodimers (3, 3). We suppose these dimers to have preferential affinity for E3-binding protein (protein X). The number of E3-binding protein molecules per molecule of pyruvate dehydrogenase complex has been estimated to be 6 [3] or 12 [12]. Supposing the number of protein X molecules to be 12, we can locate them each on the core subunit indicated 3 in Fig. 1. However, accepting the data from other laboratories [3], the only possible explanation for 6 protein X molecules bound to the core should be their location on the (3, 3)-core dimers with steadily changed orientation between these two core subunits to serve as 'dynamical dimers', because the disposition of protein X in this case appears not to be symmetrical [14]. These data can lead us to an unobtrusive conclusion of protein X to be a trigger between two kinetically different types of the conjugate intermediate transfer pathways distributed on the core surface, shown in Fig. 1 as $1\rightarrow2\rightarrow3$ and $1'\rightarrow2'\rightarrow3$. Such a trigger function for protein X with bound E3 may be realized by its swinging arm, contacting periodically with one of its two core neighbors.

In both cases of 6 or 12 protein X molecules bound, the same structural model in Fig. 1 becomes valid. This model explains the observed stoichiometry to be stable in the assembly for all the enzymic components. Twelve sites marked 1, and 12 other sites marked 1′ we suppose to bind E1 with different affinity, as has been indicated by experimental data [15], showing two classes of E1-binding core sites, with high and low affinity.

We suppose the E1 heterodimers bound at 1 and 1′ core subunits to catalyze the acetylation of lipoyl moieties located on the next E2 subunits marked 2 and 2′, correspondingly, to transfer then intermediates to the E3 component placed at (3, 3) core dimers.

It follows from Fig. 1 the existence of two different types of the intermediate transfer, $1\rightarrow2\rightarrow3$ and $1'\rightarrow2'\rightarrow3$, which we suppose to be only kinetically different. Two types of intermediate transfer, those using common protein X and E3 bound to protein X, are possibly different due to different activities of

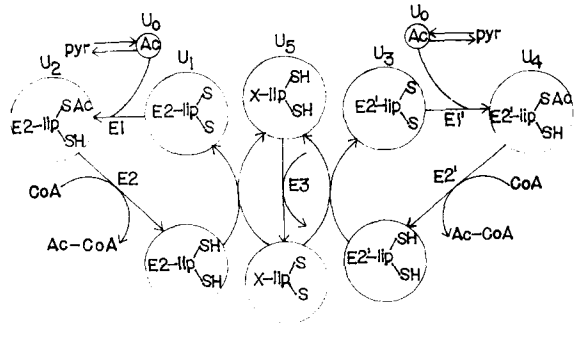

Fig. 2. The scheme for the pyruvate dehydrogenase complex-catalyzed reaction. Oxidized, reduced and acetylated lypoil moieties covalently attached to E2 and X components, as well as acetyl groups, are considered as the variables, u_0–u_5. There are two pathways (left- and right-handed) of the reaction, characterized by different kinetic parameters.

their E1 and E2. The difference in activities has been demonstrated [15] for E1 bound at high- and low-affinity core sites.

This structural model leads us to the kinetic scheme shown in Fig. 2. This kinetic scheme involves two similar reaction pathways corresponding to two intermediate transfer pathways, $1 \rightarrow 2 \rightarrow 3$, $1' \rightarrow 2' \rightarrow 3$, shown in Fig. 1. These pathways have left-handed and right-handed structural orientations to protein X. One of these pathways should be supposed to be more rapid, as it follows from the data [15]. The left and right parts of this scheme are different only in the kinetic parameter values for their steps.

This kinetic scheme shows the distribution of multiple active lipoyl moieties, attached to the core surface, among their three states: reduced, oxidized and acetylated. Their relative amounts as well as the amount of acetyl groups can be variables in kinetic equations. The catalytic interconversions are shown by oriented branches in Fig. 2. This scheme allows us to write the kinetic equations, describing the kinetic behavior of the multienzyme complex.

Kinetic scheme in Fig. 2 is similar in its topological structure to the kinetic schemes considered in our earlier works [16,17] and applied to the multienzyme complexes of the family under discussion. Now, the kinetic scheme for the mammalian pyruvate dehydrogenase complex obtains its interpretation on the self-assembly model. The kinetic schemes of such a specific structure have been shown [16,17] to generate the kinetic behavior of a critical kind at certain parameter values due to the competition of two kinetically different interconnected reaction pathways for the same substrate. Moreover, these kinetic schemes can describe the very steep change in metabolic fluxes under the relatively low chemical regulatory signal, acting through E1 phosphorylation, for example. We have predicted the bistability effect [16] and oscillations in the fluxes [17]. Such unusual kinetic behavior was observed recently for some of the multienzyme complexes [18,19].

It should be noted, that the structurally determined two types of intermediate transfer pathways, shown in Fig. 1, may serve as only preferential but not obligatory ones. The great mobility for lipoyl domains of E2 and protein X allows them to interact stochastically to some extent during the intermediate transfer. However, this does not violate the qualitative conclusions from our model, by changing only the parameter values, and conserving the prediction of the possible critical kinetic behavior.

In conclusion, our structural model for the multienzyme complexes can explain the numerous binding studies [11,14] on the basis of the principle of equivalent neighboring. The possible uncompletely assembled structures are not unique, therefore, do not follow the principle of equivalent neighboring, and are not considered here. Symmetrical organization of the multienzyme complexes seems to be reasonable for their optimal functioning and regulation.

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