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The elementary catalytic system model as a building unit of large enzymatic systems

General model

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A model of an elementary catalytic system (ECS) is suggested, derived from the notions of the general theory of systems, which is capable of performing a single elementary catalytic act (ECA). For large chemical systems involving high numbers of ECAs occurring on various ECSs, the system's action becomes increasingly effective when component ECSs are coupled into complex structures. Models are suggested to describe these couples and several fundamental theorems are proved concerning the possible structures. Complex catalytic systems are shown to contain finite numbers of ECSs.

1. Introduction

Problems connected with analysis of catalytic reaction networks are studied in systems theory, control theory of metabolic networks and formal enzyme kinetics. There are numerous references on these subjects collected in monographs. This paper refers to one of the more recent books [1] as a source of the literature.

There is now compelling evidence, both empirical and theoretical, that most (if not all) intermediary metabolism takes place in organized states [2]. The interior of living eukaryotic cells is subdivided by membranes into various regions (macrocompartments) and, by more subtle means of metabolite sequestration, into dynamic microcompartments [3]. Living systems possess mechanisms for vectorial flow of matter and energy [4,5]. It seems clear now that the traditional reductionistic manner of extracting and studying individual enzymes in vitro may have little relevance to conditions extant in vivo [6].

Many models of enzyme systems have been proposed. The development of mathematical methods makes it possible to solve numerically more and more complicated kinetic models in formal enzyme kinetics [7]. Treatment of an enzyme system as a whole allows us to use methods of control theory [8]. The formal structural kinetic models [9] make it possible to express how subunit interactions and quaternary constraints control the rate of an allosteric enzymatic process.

Each existing model postulates the existence of an absolute continuous medium (involving the use of differential equations, and terms expressing 'diffusion', 'free energy', 'rate constant', etc.). This approximation agrees with modern paradigms in kinetic, thermodynamic and catalytic theories, but is not in accord with the mentioned experimental evidence furnished by modern enzymology. Using

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these models it is difficult to single out catalytic, binding and transport functions inside an enzyme system. On the other hand, rather than the binding of a substrate, the most important property of an enzyme is to catalyze the conversion of a substrate into a product [9].

Most catalytic reactions occur in large chemical systems, i.e., those containing very large numbers of molecules. Any single catalytic conversion of a substrate into a product proceeds in a very small part of the chemical system. Catalysis itself, viz., quantum-mechanical interactions between the catalytic center, the substrate and the product molecules is associated with the peculiar attributes of part of the system, which are available during a short interval of the interaction time. The local system inside the chemical system, as simply as possible, within which a single catalytic conversion of a substrate into a product may occur, will henceforth be called the elementary catalytic system (ECS).

It is just the purpose of the present paper to suggest new general models of enzyme systems composed of elementary catalytic systems. The term 'catalytic system' was first used by Rudenko [10] in his analysis of coupled catalytic and transport functions. In this paper the term has a different meaning and thus allows one to separate the catalytic function from other types.

Some fundamental theorems concerning the structures of these models are proved.

2. Elementary catalytic system

Any single catalytic act occurs in a small local space of a chemical reaction system. In large chemical systems measurable catalytic effects convey statistical information because in these systems it is impossible to separate:

(i) numerous and multiple elementary catalytic acts (ECA) proceeding on a population of catalytic centers from

(ii) the energy and mass flow inside the reaction system which occurs simultaneously with ECA.

However, catalysis itself, viz., quantum-mechanical interactions between the catalytic center (c), the substrate molecules (s) and the product molecules (p), proceeds in the local space of the reaction system. Thus, catalysis is associated with peculiar attributes of the center c and of the stoichiometric quantities of s and p. The ECS, i.e., the one in which only a single ECA occurs, may be defined in terms of the system notions used in the general systems theory.

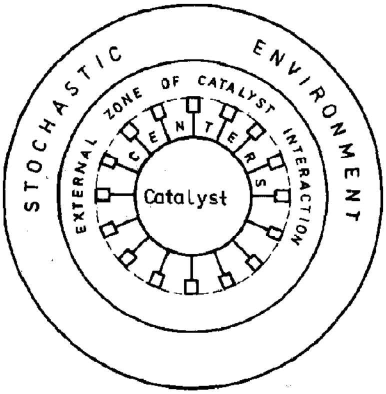

Fig. 1. A functional scheme of a catalytic reaction system.

A large chemical system in which catalytic processes occur can be represented schematically (fig. 1) by grouping the elements that perform analogous functions. Then, the ECS constitutes the minimum set of elements that perform the functions required for an ECA to occur, and is isolated from stochastic environment. The environment represents the chemical system that includes no ECS. The formal assumption of isolation of the ECS, often made in the systems theory, simplifies the modelling of a system.

The ECS (fig. 2) is composed of a set of objects c, s, p, g, k, where g is the source and k the receiver of energy quanta, and of the set of bi-ob-

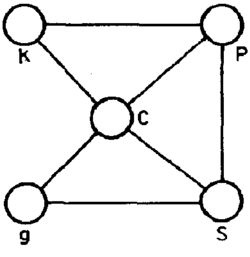

Fig. 2. A graph of the ECS structure.

ject relations. In such an ECS only those events occur which represent the very essence of catalysis. To describe objects and relations in the ECS a simple impulse-oscillation parametric model of the molecule (IOM) has been suggested. In the model any molecule is treated as a 'black box' with a finite set of outputs and inputs. For a given reaction a subset of molecular inputs/outputs which involve interactions may be isolated. Each input and output is represented by two parameters (Δt, I). The parameter Δt represents an attribute of an input (output) and signifies the duration of the active state (impulse) required for the reaction to occur; the parameter I, again an attribute of the molecule, represents the constant time interval (oscillation time) between the moments of appearance of two successive active states of the molecule. It is assumed that $I \gg \Delta t$. The IOM assumes molecules 1 and 2, endowed with respective parameters I_1 and I_2 and situated at an optimum distance and in a favorable mutual orientation, to interact if and only if the moment $\mathrm{SYN}_{1,2}$ of synchronization of appropriate inputs in an active state can be attained. The moment $\mathrm{SYN}_{1,2}$ is defined as

$$\mathrm{SYN}_{1,2} = a_1 + n_1 I_1 = a_2 + n_2 I_2 \qquad (1)$$

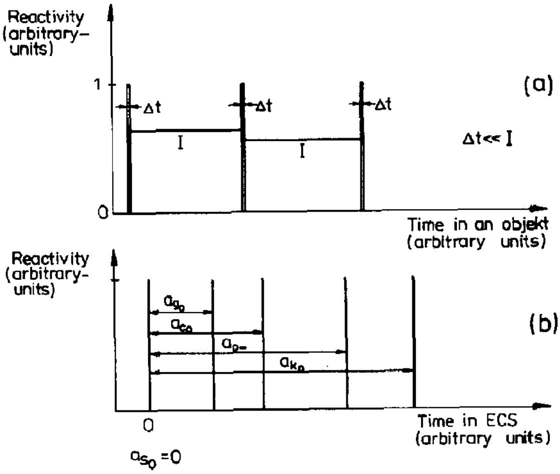

Fig. 3. A scheme of the relation between (a) the time inside the object and (b) the time in the system according to the IOM model.

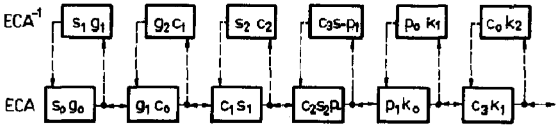

Fig. 4. A diagrammatic representation of events constituting an ECA.

where a_1 and a_2 are the moments of appearance of the first active states in the system composed of two molecules (1 and 2); n_1 and n_2 are natural numbers. In the ECS parameters a and I are assumed to be natural number counts of arbitrary time units (discrete time scale).

Relations between the time in the object and the time in the system are shown schematically in fig. 3. A detailed analysis of the general ECS model showed that an ECA may be looked upon as a sequence of bi-object events (fig. 4). The condition for each event to occur is expressed by eq. 1.

At the moment SYN_{xy} there arises a relation between the objects x and y and, as a result, the states of these objects become changed. The new states x_{i+1} ($x = s$, c, p, g, k) differ in internal energy E and in parameter I from the preceding states x_i ($i = 0$–3) (cf. fig. 4).

Numerical solutions of the system of diophantic equations, eq. 1, and a general optimum analytical solution will be outlined in a separate paper.

3. Interactions between ECS and environment

To make many successive ECAs occur, it is necessary to carry s and g into, and p and k away from, the ECS (fig. 5). While an ECA occurs, the object c is following a closed trajectory in the state space (regeneration of c); thus, the ECS model may be simplified and considered (fig. 6) as a transformer of the chemical s and the energy g input signals into the respective output signals p and k. If the transformer is placed in the stochastic environment (fig. 7) containing objects s, p, g, and k in various states belonging to the set of states required for an ECA to occur (fig. 4), it is possible to evaluate the maximum classical probability (MPr) for an ECA to be realized by an

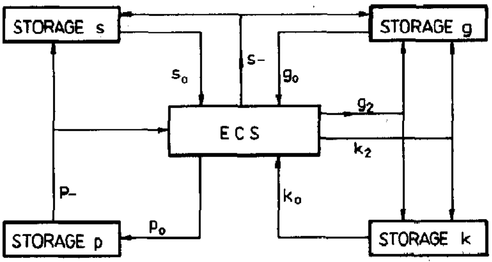

Fig. 5. A model of ECS-environment interaction.

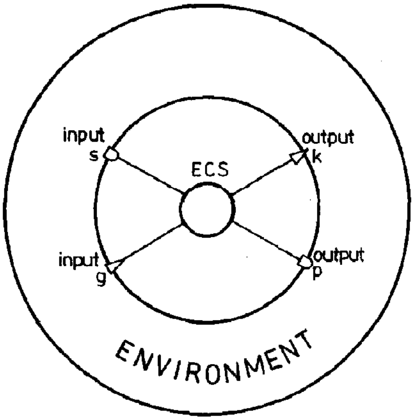

Fig. 6. A simplified model of interaction between an ECS and its environment.

ECS. In the probability theory, classical probability may be evaluated if the space of elementary events is a finite set containing exclusive possible elementary events.

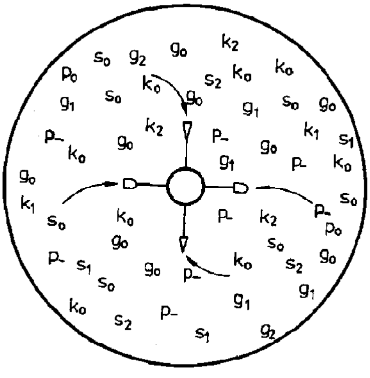

Fig. 7. A scheme of cooperation of the model of fig. 6 with a stochastic environment.

The condition for an ECA to occur is to have objects s, p, g, k in suitable states to appear in a well-defined sequence at appropriate ECS inputs and outputs (I/O). For the evaluation of MPr, the objects are assumed to appear at appropriate moments a. If this assumption is not true, the resulting probability MPr will be lower because some events with appearance times different from the appropriate a are ineffective because the condition, eq. 1, of SYN$_{xy}$ is not satisfied. This is equivalent to assuming the probabilities of all bi-object events inside the ECS to be equal to unity. Then, the MPr is a function of lower-than-unity probabilities, i.e., of only those events that depend on the interaction of the appropriate inputs to the ECS with its stochastic environment. The subset of events (fig. 4) is: $(s_0 g_0)$, $(c_2 s_2 p_-)$, $(p_1 k_0)$.

From the conditions of the maximum classical probability MPr and of the nonordered (stochastic) environment it follows that:

(i) individual events are independent

(ii) any object s, p, g, k is equally likely to appear at any ECS input/output

(iii) any object state is equally probable

(iv) any object s, p, g, k state must belong to the set of object states required for ECA to occur (fig. 4).

Points i–iv are special, rather than general, assumptions; they allow one to evaluate and to compare the MPrs of various models to be considered. The MPr will decrease if any one of the above assumptions turns out to be incorrect. The set of object states required for ECA to occur (fig. 4) is the minimum set. Any other set of object states required for an ECA to occur must contain more states; then, if assumption iv is not true, the classical probability of appearance of the appropriate state is smaller. Should some states be more probable (e.g., on account of some dependent events; assumptions iii and i are not true) than the others, then they would appear more frequently. Then, however, the assumption of a nonordered environment implies that the states will not be suitable for the well-defined sequence of ECA events and the MPr of ECA will decrease. For the same reason, MPr decreases if assumption ii is not true.

To estimate MPr it is necessary to evaluate the probabilities of events (s_0g_0), $(c_2s_2p_-)$ and (p_1k_0). From the probability theory and from the above assumptions it follows that:

(a) $P(s_0g_0)$
$= P(s_0) \cdot P(w) \cdot P(g_0) \cdot P(w) \cdot P(c_0)$

where:

$P(w) = \frac{1}{4}$ is the probability of finding by an object a correct ECS input/output;

$P(s_0) = \frac{1}{4} \cdot \frac{1}{4} = \frac{1}{16}$ is the probability of appearance of object s in a state s_0, this involving two individual independent events: choice of one object from four objects (s, p, g, k) and choice of one state from four states (s_0, s_1, s_2, s_-) (fig. 4);

$P(g_0) = \frac{1}{4} \cdot \frac{1}{3} = \frac{1}{12}$ is the probability of appearance of object g in a state g_0;

$P(c_0) = \frac{1}{4}$ is the probability of appearance of state c_0 of object c inside the ECS (c_0, c_1, c_2, c_3).

(b) $P(c_2s_2p_-) = P(p_-) \cdot P(w) \cdot P(c_2)$

is the probability of appearance of object p in a state p_- at the same moment when object c inside the ECS is in the c_2 state, where $P(p_-) = \frac{1}{4} \cdot \frac{1}{3} = \frac{1}{12}$ and $P(c_2) = \frac{1}{4}$. The state p_- indicates the lack of object p in the ECS.

(c) $P(p_1k_0) = P(k_0) \cdot P(w) \cdot P(c_3)$

where:

$P(k_0) = \frac{1}{4} \cdot \frac{1}{3} = \frac{1}{12}; \quad P(c_3) = \frac{1}{4}.$

Then

$$\begin{aligned}\mathrm{MPr(ECA)} &= P(s_0g_0) \cdot P(c_2s_2p_-) \cdot P(p_1k_0) \\ &= P(s_0) \cdot P(w) \cdot P(g_0) \cdot P(w) \cdot P(c_0) \\ &\quad \cdot P(p_-) \cdot P(w) \cdot P(c_2) \cdot P(k_0) \cdot P(w) \\ &\quad \cdot P(c_3) \\ &= \tfrac{1}{16} \cdot \tfrac{1}{4} \cdot \tfrac{1}{12} \cdot \tfrac{1}{4} \cdot \tfrac{1}{4} \cdot \tfrac{1}{12} \cdot \tfrac{1}{4} \cdot \tfrac{1}{4} \cdot \tfrac{1}{12} \cdot \tfrac{1}{4} \cdot \tfrac{1}{4} \\ &\approx \tfrac{1}{4} \times 10^{-8}.\end{aligned}$$

This MPr estimate shows the interaction of ECS with the stochastic environment to be possible (MPr $\neq 0$) but relatively ineffective, irrespective of the rate of occurrence of individual events.

An optimum organization of the ECS environment, so far modeled generally with the aid of STORAGE x objects $(x = s, p, g, k)$ (fig. 5), will consist of a sequence of ECA events synchronized and syn-fixed in delivery or reception of suitable objects in appropriate states at correct ECS I/O. One way to model STORAGE x is to use another ECS as the source or the receiver of the signals required in a given ECS. To describe the flow of energy from an object i to j, it is necessary to assume a flow model. In catalytic systems involving numerous ECSs, one ECS may serve as the source of energy which flows directionally to another ECS. All ECS objects are described by the IOM model. The ECS object parameters Δt and I convey information on the composition, structure and dynamic behavior of the object. Since they constitute at the same time the attributes of the object, they are independent from the properties of the microenvironment of the object. Therefore, the only energy that the flow model involves is the internal energy of the object. This represents the sum of the molecular quantum states: electronic, vibrational and rotational. The translational energy of the object is assumed to be either zero or to be rapidly redistributed between all the vibrational and rotational modes of the object just before an interaction, i.e., flow of energy from one object to another, takes place. This assumption simplifies the description of the flow model. A very small local system of two interacting ECSs is very much like the system of a single active complex in the absolute rate theory. However, in that theory the problem of energy flow within a single complex has not been resolved and, instead, a thermodynamic model of the energy flow within a large chemical system has been commonly used. This model is not useful for modeling ECSs. The approaches used in quantum chemistry, spectroscopy and photochemistry are more useful. In the theory of photochemistry it is well known [11] that the internal energy level of an energy acceptor must be lower than that of the energy donor.

In a model of the directional flow of energy, the sign of the energy gradient must be identical at the start and at the end of flow. The energy conservation law must be obeyed.

Consequently, for catalytic systems involving numerous ECSs the following model was adopted to describe the flow of energy from an object i (energy donor) to an object j (energy acceptor):

$$E_{iz} + E_{jy} = E_{i(z+1)} + E_{j(y+1)} \tag{2}$$

$$E_{iz} > E_{jy} \tag{3}$$

$$E_{(z+1)} \geqslant E_{j(y+1)} \tag{4}$$

where E_{iz} is the internal energy of object i in state z; and $z+1$ and $y+1$ indicate the states past the energy flow.

4. Catalytic systems composed of two ECSs

A system of two energy-coupled ECSs (fig. 8) will act provided the following time relationships

$$\begin{aligned} &{}^1I_{k_2} = {}^2I_{g_0}; \quad {}^1_m a_{k_2} = {}^2_m a_{g_0} \\ &{}^1I_{k_0} = {}^2I_{g_2}; \quad {}^1_{m+1} a_{k_0} = {}^2_m a_{g_2} \end{aligned} \tag{5}$$

and the energy relationships

$$\begin{aligned} &{}^2E_{g_0} = {}^1E_{k_2} \\ &{}^2E_{g_2} = {}^1E_{k_0} \end{aligned} \tag{6}$$

are fulfilled; the left superscripts and subscripts are ECS numerals in the system and ECA numerals in the ECS, respectively. By the energy flow model, eqs. 2–4, it follows that

$${}^1E_{c_0} > {}^1E_{k_2} \tag{7}$$

$${}^2E_{g_0} \geqslant {}^2E_{c_0} \tag{8}$$

Addition of eq. 7 to eq. 8 gives, in view of eq. 6,

$${}^1E_{c_0} > {}^2E_{c_0} \tag{9}$$

Thus, with ^{1}ECS and ^{2}ECS energy-coupled, the ^{2}ECS that serves as the receiver of energy from ^{1}ECS must have an object c endowed with internal energy ${}^2E_{c_0}$ lower than ${}^1E_{c_0}$ of object c in ^{1}ECS.

To see the benefits arising from energy coupling of two ECSs, consider two identical stochastic systems each containing a set of objects ${}^1s$, ${}^2s$, ${}^1p$, ${}^2p$, ${}^1g$, ${}^2k$ having the states taken from the set of states indispensable for an ECA to occur: one system contains ^{1}ECS, ^{2}ECS and objects ${}^1k$ and ${}^2g$, the other containing only the energy-coupled ^{1}ECS and ^{2}ECS.

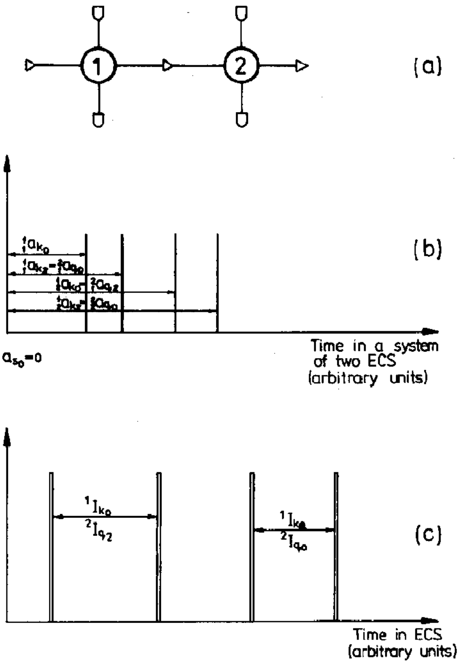

Fig. 8. Two energy-coupled ECSs. (a) (▷— and ⊃—) Objects q and s, respectively (—▷ and —⊃) objects k and p; (—▷—) energy couple, an object used as object k in ^{1}ECS and simultaneously as object g in ^{2}ECS; (b) scheme of relations (eq. 5) between times of appearance a; (c) scheme of relations (eq. 5) between parameters I.

The former system involves eight objects and eight inputs/outputs. The number of object states is the same as before (fig. 4). The corresponding probabilities are:

$$\begin{aligned} &P(s_0) = \tfrac{1}{8}\cdot\tfrac{1}{4} = \tfrac{1}{32}; && P(g_0) = \tfrac{1}{8}\cdot\tfrac{1}{3} = \tfrac{1}{24}; \\ &P(p_-) = \tfrac{1}{8}\cdot\tfrac{1}{3} = \tfrac{1}{24}; && P(c_x) = \tfrac{1}{4}; \\ &P(w) = \tfrac{1}{8}; && P(k_0) = \tfrac{1}{8}\cdot\tfrac{1}{3} = \tfrac{1}{24}; \end{aligned}$$

These values inserted into the formula (section 3) give MPr(ECA) $\approx 10^{-11}$.

The latter system of energy-coupled ^{1}ECS and ^{2}ECS involved six objects and six inputs/outputs; therefore, the probabilities are:

$$P(s_0) = \tfrac{1}{6} \cdot \tfrac{1}{4} = \tfrac{1}{24}; \quad P(g_0) = \tfrac{1}{6} \cdot \tfrac{1}{3} = \tfrac{1}{18};$$
$$P(p_-) = \tfrac{1}{6} \cdot \tfrac{1}{3} = \tfrac{1}{18}; \quad P(c_x) = \tfrac{1}{4};$$
$$P(w) = \tfrac{1}{6}; \quad P(k_0) = \tfrac{1}{6} \cdot \tfrac{1}{3} = \tfrac{1}{18};$$

For ^{1}ECS and ^{2}ECS (fig. 8) we have:

$$\mathrm{MPr}({}^1\mathrm{ECA}) = P(s_0 g_0) \cdot P(c_2 s_2 p_-)$$

$$\mathrm{MPr}({}^2\mathrm{ECA}) = P(s_0) \cdot P(w) \cdot P(c_0) \cdot P(c_2 s_2 p_-) \cdot P(k_0 p_1)$$

and hence

$$\mathrm{MPr}(\mathrm{ECA}) \approx 10^{-8}$$

$$(\mathrm{MPr}({}^1\mathrm{ECA}) \approx 4 \times 10^{-8};$$
$$\mathrm{MPr}({}^2\mathrm{ECA}) \approx 0.9 \times 10^{-8})$$

Evidently, energy coupling has resulted in the probability MPr being raised by a factor of several hundred.

The system of the chemically coupled ECSs (fig. 9) will act provided the following time relationships

$$\begin{aligned} &{}^1I_{p_0} = {}^2I_{s_0}; \quad {}^1_n a_{p_0} = {}^2_n a_{s_0} \\ &{}^1I_{p_-} = {}^2I_{s_-}; \quad {}^{\,\,1}_{n+1} a_{p_-} = {}^2_n a_{s_-} \end{aligned} \tag{10}$$

and energy relationships

$${}^1E_{p_0} = {}^2E_{s_0} \tag{11}$$

$${}^1E_{p_-} = {}^2E_{s_-} \tag{12}$$

are satisfied. States p_- and s_-, introduced apparently formally into the general ECA scheme, serve to convey information of the lack of object p and s in the system; without this information individual ECSs could not operate correctly in a complex ECS.

The probabilities MPr, evaluated as before, show chemical coupling to make ECA more likely to occur by a factor of several hundred.

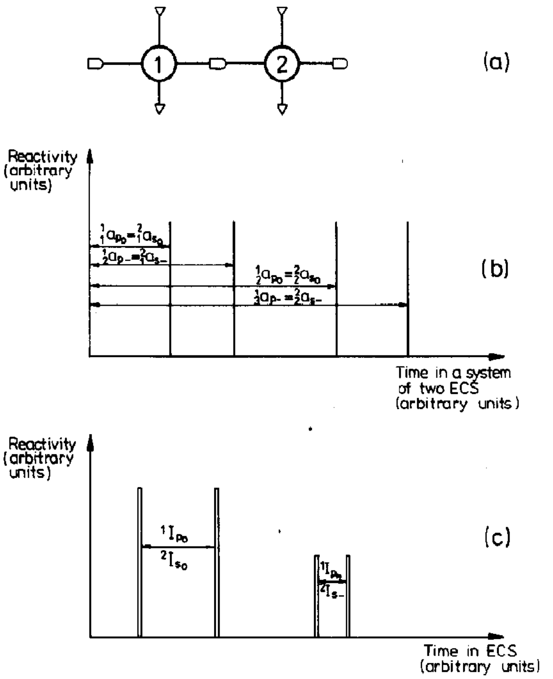

Fig. 9. Two chemically coupled ECSs. (a) (-▭) Chemical couple, an object used as object p in ^{1}ECS and simultaneously as object s in ^{2}ECS; (b) scheme of relations (eq. 10) between times of appearance a; (c) scheme of relations (eq. 10) between parameters I.

If ${}^1E_{p_0} > {}^1E_{s_0}$, the energy relationships in the chemical couple imply

$${}^2E_{s_0} > {}^1E_{s_0} \tag{13}$$

and, for the inverse case,

$${}^2E_{s_0} < {}^1E_{s_0} \tag{14}$$

The catalytic systems considered above, composed of two single-coupled ECS, i.e., as simple as can be, may be treated as fragments of multicatalytic systems (MCS).

5. Structures of multicatalytic systems (MCSs)

If chemical and energy couples are treated as branches and if systems of single ECSs with no objects participating in the couple are treated as vertices of a graph, then the structure of any set of

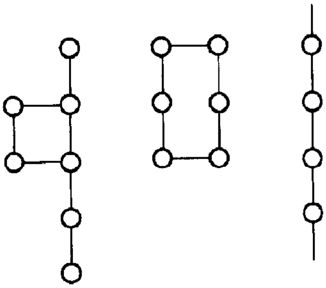

Fig. 10. Illustrative graphs of MCS structure.

coupled ECSs may be described as a structural graph. If the graph contains no cyclic paths, the MCS will be termed the chain type. If the set of all branches in the graph forms a Euler cycle, the MCS will be termed the cyclic type. If the graph contains a subset of branches producing a cyclic path, the MCS will be termed the mixed type. Examples of these structures are shown in fig. 10.

Chain and cyclic MCSs, can produce structures containing either chemical couples or energy couples or both. Mixed-type MCSs must always include both chemical and energy couples.

5.1. Chain MCS

Any energy-coupled chain (fig. 11) is composed of n coupled ECSs and $(n-1)$ energy couples which act provided that for any m $(0 < m \leqslant n-1)$ the following relationships hold:

$$^{m}E_{g_0} = {}^{m+1}E_{k_2} \tag{15}$$

$$^{m}E_{g_2} = {}^{m+1}E_{k_0} \tag{16}$$

Theorem 1

Any energy-coupled chain-type MCS contains a finite number of ECS.

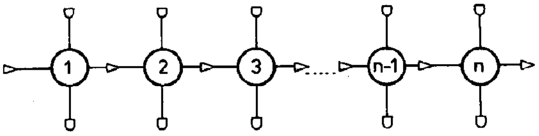

Fig. 11. Energy-coupled chain MCS.

Proof

By eq. 9, it follows that

$$\begin{aligned} &^{1}E_{c_0} > {}^{2}E_{c_0} \\ &^{2}E_{c_0} > {}^{3}E_{c_0} \\ &^{3}E_{c_0} > {}^{4}E_{c_0} \\ &\dots\dots\dots \\ &^{n-1}E_{c_0} > {}^{n}E_{c_0} \end{aligned} \tag{17}$$

and hence

$$^{1}E_{c_0} > {}^{2}E_{c_0} > {}^{3}E_{c_0} > {}^{4}E_{c_0} > \dots > {}^{n-1}E_{c_0} > {}^{n}E_{c_0} \tag{18}$$

For any object i in energy state m the following trivial relations are true:

$$E_{i_0} > 0 \tag{19}$$

$$0 < E_{i_{\max}} < \infty \tag{20}$$

$$E_{i_0} \leqslant E_{i_m} \leqslant E_{i_{\max}} \tag{21}$$

where E_{i_0} and $E_{i_{\max}}$ denote, respectively, the ground-state internal energy of object i and the maximum internal energy state available for i with its structure graph and chemical composition remaining unaffected.

Any term in eq. 18 represents a value E_{i_0} of the ground-state internal energy of an object c. Two objects c cannot have approximately identical E_{i_0} values, i.e., differ only infinitesimally in energy. Should we assume this difference to be infinitesimally small then the fundamental assumption of qualitative spectroscopic analysis will fail: different objects will produce identical spectra. Therefore, if differences between energy states, E_{i_0} values, are finite, the number of terms in eq. 18 must also be finite.

The ratio of probability MPr of an ECA to occur on elements of a set of n energy-coupled ECSs (with terminal ECSs disregarded) to one of an ECA occurring on elements of a set of n uncoupled ECSs, may be estimated as follows:

The system of n uncoupled ECS involves $4n$ objects and $4n$ inputs/outputs. The number of

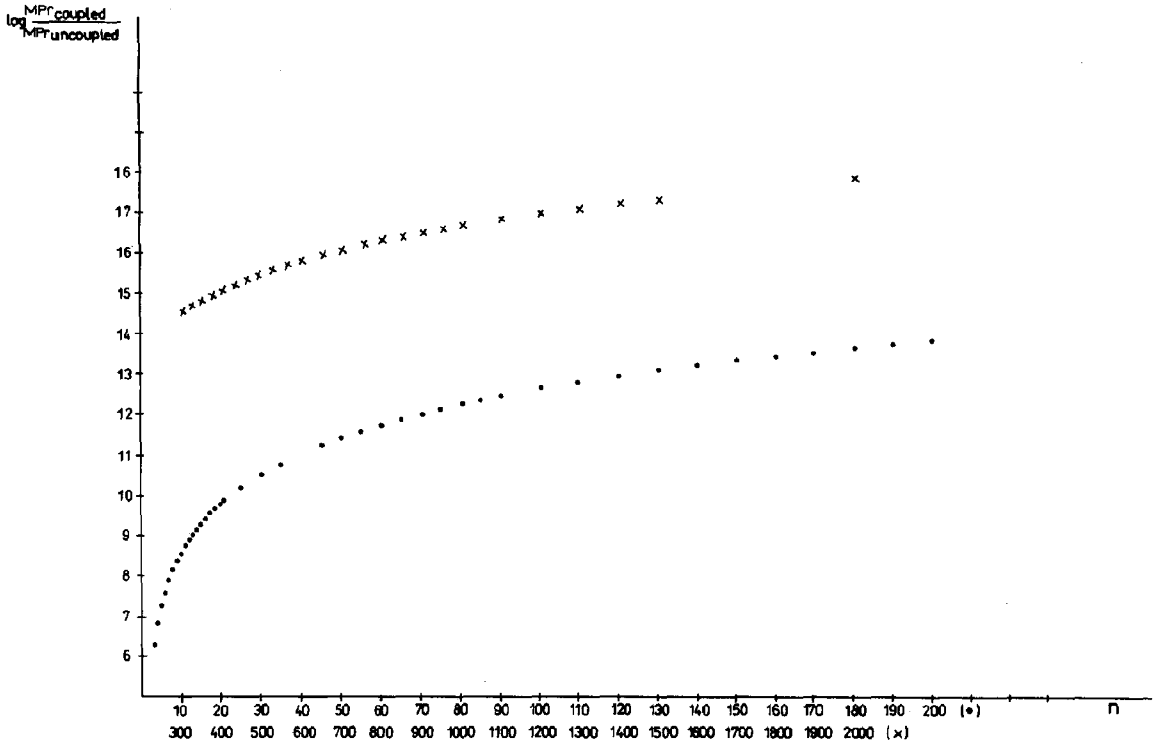

Fig. 12. Relationship between classical probability (MPr) of an ECA and the number (n) of ECSs in an energy chain.

object states is the same as before (fig. 4). The probabilities are:

$$P(s_0) = \frac{1}{4n} \cdot \frac{1}{4} = \frac{1}{16} \cdot \frac{1}{n};$$

$$P(g_0) = \frac{1}{4n} \cdot \frac{1}{3} = \frac{1}{12} \cdot \frac{1}{n};$$

$$P(p_-) = \frac{1}{4n} \cdot \frac{1}{3} = \frac{1}{12} \cdot \frac{1}{n}; \quad P(c_x) = \frac{1}{4};$$

$$P(w) = \frac{1}{4n}; \quad P(k_0) = \frac{1}{4n} \cdot \frac{1}{3} = \frac{1}{12} \cdot \frac{1}{n};$$

With these values, the formula for MPr(ECA) (section 3) gives

$$\mathrm{MPr(ECA)}_{\text{uncoupled}} \approx 2 \times 10^{-9} \times n^{-8}$$

Two systems of energy-coupled n ECSs are possible: one with $4n$ objects and $(2n + 2)$ inputs/outputs, and the other with $(2n + 2)$ objects and $(2n + 2)$ inputs/outputs. In the former, the probabilities are as above except that $P(w) = 1/(2n + 2)$. Then

$$\mathrm{MPr(ECA)}_{\text{coupled}} = P(s_0) \cdot P(w) \cdot P(c_0) \cdot P(p_-) \cdot P(c_2) \cdot P(w)$$

and

$$\mathrm{MPr(ECA)}_{\text{coupled}} \approx 3 \times 10^{-4} \times [n(2n + 2)]^{-2}$$

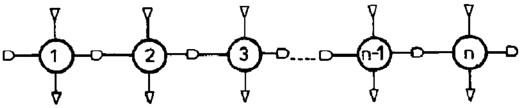

Fig. 13. Chemically coupled chain MCS.

In the latter, the probabilities are:

$$P(s_0) = \frac{1}{2n+2} \cdot \frac{1}{4}; \quad P(w) = \frac{1}{2n+2};$$

$$P(c_x) = \tfrac{1}{4};$$

$$P(p_-) = \frac{1}{2n+2} \cdot \frac{1}{3}$$

and eventually

$$\mathrm{MPr(ECA)}_{\mathrm{coupled}} \approx 5 \times 10^{-3} \times (2n+2)^{-4}.$$

Thus, the $\mathrm{MPr(ECA)}_{\mathrm{coupled}}/\,\mathrm{MPr(ECA)}_{\mathrm{uncoupled}}$ ratios become

$$\begin{aligned} &2 \times 10^{5} \times n^{6} \times (2n+2)^{-2} \text{ for the first and} \\ &2 \times 10^{6} \times n^{8} \times (2n+2)^{-4} \text{ for the second system.} \end{aligned} \tag{22}$$

If n is very large, the ratios vary approximately with n^4 (fig. 12). The above formulas are true for $n \geqslant 3$.

In full analogy to the energy-coupled chain, any chemically coupled chain (fig. 13) is composed of n ECSs and $(n-1)$ couples which, if $0 < m \leqslant (n-1)$, act provided the relationships

$${}^{m+1}E_{p_0} = {}^{m}E_{s_0} \tag{23}$$

$${}^{m+1}E_{p_-} = {}^{m}E_{s_-} \tag{24}$$

are fulfilled.

If for each ECS of a chemically coupled chain the difference $(E_{s_0} - E_{p_0})$ is always positive or always negative, the chain will be termed the homogeneous chemical chain; otherwise the chain will be called the nonhomogeneous or alternating chain.

Theorem 2

Any homogeneous chemical chain has a finite number of ECS.

Proof

By the definition of the homogeneous chemical chain, two kinds of chains are possible.

Case I

$${}^{m}E_{s_0} > {}^{m}E_{p_0} \tag{25}$$

From eqs. 25 and 23 it follows that

$$\begin{aligned} &{}^{2}E_{p_0} > {}^{1}E_{p_0} \\ &{}^{3}E_{p_0} > {}^{2}E_{p_0} \\ &{}^{4}E_{p_0} > {}^{3}E_{p_0} \\ &\cdots\cdots\cdots\cdots \\ &{}^{n}E_{p_0} > {}^{n-1}E_{p_0} \end{aligned} \tag{26}$$

and hence

$${}^{n}E_{p_0} > {}^{n-1}E_{p_0} > {}^{n-2}E_{p_0} > \ldots > {}^{3}E_{p_0} > {}^{2}E_{p_0} > {}^{1}E_{p_0} \tag{27}$$

Now the procedure used before to prove theorem 1 will show case I of theorem 2 to be true.

Case II

$${}^{m}E_{s_0} < {}^{m}E_{p_0} \tag{28}$$

Proceeding as in case I, it follows that

$${}^{1}E_{p_0} > {}^{2}E_{p_0} > {}^{3}E_{p_0} > \ldots > {}^{n-1}E_{p_0} > {}^{n}E_{p_0} \tag{29}$$

and a procedure analogous to that used before will also demonstrate case II of theorem 2 to be true.

A special category of alternating chemical chains is one of the chains in which for every other ECS the difference $(E_{s_0} - E_{p_0})$ has an identical sign.

For such regularly alternating chains, the following is true.

Theorem 3

In a regularly alternating chemical chain in which, (case a)

$${}^{2m}E_{s_0} - {}^{2m}E_{p_0} > 0 \quad \text{and} \quad {}^{2m+1}E_{s_0} - {}^{2m+1}E_{p_0} < 0$$

or

(case b)

$${}^{2m}E_{s_0} - {}^{2m}E_{p_0} < 0 \quad \text{and} \quad {}^{2m+1}E_{s_0} - {}^{2m+1}E_{p_0} > 0$$

for all ECSs included, it is true that, for case a,

$$\frac{{}^{2m}E_{s_0} + {}^{2m+2}E_{s_0}}{2} > {}^{2m+1}E_{s_0} \tag{30}$$

and

$$\frac{{}^{2m-1}E_{s_0} + {}^{2m+1}E_{s_0}}{2} < {}^{2m}E_{s_0} \tag{31}$$

and, for case b,

$$\frac{{}^{2m}E_{s_0} + {}^{2m+2}E_{s_0}}{2} < {}^{2m+1}E_{s_0}$$

and

$$\frac{{}^{2m-1}E_{s_0} + {}^{2m+1}E_{s_0}}{2} > {}^{2m}E_{s_0}$$

Proof for case a

From the above case a inequalities, it follows that

$${}^{2m}E_{s_0} > {}^{2m}E_{p_0} \quad \text{and} \quad {}^{2m+1}E_{s_0} < {}^{2m+1}E_{p_0} \tag{32}$$

By eq. 23,

$${}^{2m}E_{s_0} > {}^{2m-1}E_{s_0} \tag{33}$$

$${}^{2m}E_{s_0} > {}^{2m+1}E_{s_0} \tag{34}$$

Addition of eq. 33 to eq. 34 and subsequent rearrangement give directly relation a postulated for ${}^{2m}E_{s_0}$.

Similarly for

$${}^{2m+2}E_{s_0} > {}^{2m+2}E_{p_0} \tag{35}$$

it follows, from eq. 23, that

$${}^{2m+2}E_{s_0} > {}^{2m+1}E_{s_0} \tag{36}$$

From case a of theorem 3 it follows that

$${}^{2m+1}E_{p_0} > {}^{2m+1}E_{s_0}$$

and hence, by using eq. 23, it follows that

$${}^{2m}E_{s_0} > {}^{2m+1}E_{s_0} \tag{37}$$

Addition of eq. 36 to eq. 37 gives directly the relation a postulated for ${}^{2m+1}E_{s_0}$.

Case b is proved similarly. Regularly alternating chemically coupled chains can, in special cases, have a finite number of ECSs. These cases are indicated more specifically by theorems 4 and 5.

Theorem 4

Regularly alternating chemical chains for which, from case a of theorem 3,

$${}^{2m}E_{s_0} = q\,{}^{2m-1}E_{s_0}$$

$${}^{2m+1}E_{s_0} = h\,{}^{2m}E_{s_0} \tag{a}$$

and, from case b of theorem 3

$${}^{2m}E_{s_0} = h\,{}^{2m-1}E_{s_0}$$

$${}^{2m+1}E_{s_0} = q\,{}^{2m}E_{s_0} \tag{b}$$

q and h being real numbers constant for a given chain,

$$q > 1 \tag{38}$$

$$h < 1; \quad h \neq 1/q \tag{39}$$

$$2q > 1 + qh > 2h \tag{40}$$

form chains containing finite numbers of ECS.

Proof

It is to be demonstrated that the expressions for cases a and b of theorem 4 satisfy theorem 3 and that the series of numbers E are finite. If we show that the numbers E as defined in cases a and b form a geometric series, then the procedure applied for theorem 1 will yield a direct proof of theorem 4. As an illustration, let us prove case a only. For this purpose let us substitute the relationships for case a of theorem 4 into those of theorem 3, eqs. 30 and 31, to eliminate all the E_{s_0} terms except ${}^{2m}E_{s_0}$. Then,

$$\frac{{}^{2m}E_{s_0} + qh\,{}^{2m}E_{s_0}}{2} > h\,{}^{2m}E_{s_0} \tag{41}$$

$$\frac{\dfrac{{}^{2m}E_{s_0}}{g} + h\,{}^{2m}E_{s_0}}{2} < {}^{2m}E_{s_0} \tag{42}$$

and, after simplification

$$1 + qh > 2h \tag{43}$$

$$1 + qh < 2q \tag{44}$$

which leads to eq. 40. This means that, if eq. 40 is satisfied, then all the corresponding expressions in theorem 3 are true.

Then, in turn, from case a of theorem 4 it follows that

$$ {}^{2m+1}E_{s_0} = hq\,{}^{2m-1}E_{s_0} \tag{45} $$

which means that – for $h \neq q^{-1}$ – the odd terms produce a geometric series. Similarly, for the even terms,

$$ {}^{2m+2}E_{s_0} = q\,{}^{2m+1}E_{s_0} = qh\,{}^{2m}E_{s_0} \tag{46} $$

Therefore, the number of ECSs must be finite. Similarly, the following may be proved.

Theorem 5

Regularly alternating chemical chains for which, as for case a of theorem 3

$$ \begin{aligned} {}^{2m}E_{s_0} &= {}^{2m-1}E_{s_0} + r \\ {}^{2m+1}E_{s_0} &= {}^{2m}E_{s_0} - d \end{aligned} \tag{a} $$

and, as for case b of theorem 3,

$$ \begin{aligned} {}^{2m}E_{s_0} &= {}^{2m-1}E_{s_0} - d \quad (d > 0) \\ {}^{2m+1}E_{s_0} &= {}^{2m}E_{s_0} + r \quad (r > 0) \end{aligned} \tag{b} $$

form chains with finite numbers of ECSs.

When assessed as before, the formation of a chemically coupled chain brings about 150% of the benefits that can be due to the formation of an energy-coupled chain.

5.2. Cyclic MCS

Theorem 6

A cyclic energy-coupled MCS cannot exist (fig. 14).

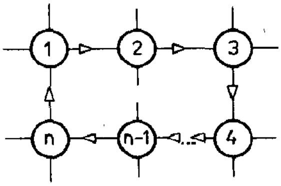

Fig. 14. Energy-coupled cyclic MCS (impossible).

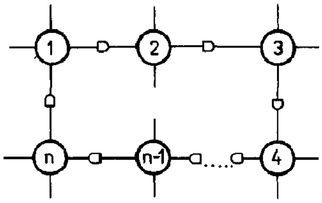

Fig. 15. Chemically coupled cyclic MCS.

Proof

By theorem 1,

$$ {}^{1}E_{c_0} > {}^{2}E_{c_0} > {}^{3}E_{c_0} > \ldots > {}^{n-1}E_{c_0} > {}^{n}E_{c_0} > {}^{n+1}E_{c_0} > \cdots $$

In a cyclic MCS containing n ECSs should hold

$$ {}^{n+1}E_{c_0} = {}^{1}E_{c_0} \tag{47} $$

which contradicts the above series of inequalities (*qed*)

Theorem 7

A cyclic chemically coupled MCS (fig. 15) is composed of a nonhomogeneous chemical chain.

Proof

By eq. 23, it follows for individual couples that

$$ \begin{aligned} {}^{1}E_{p_0} &= {}^{2}E_{s_0} \\ {}^{2}E_{p_0} &= {}^{3}E_{s_0} \\ &\cdots\cdots \\ {}^{n-1}E_{p_0} &= {}^{n}E_{s_0} \\ {}^{n}E_{p_0} &= {}^{1}E_{s_0} \end{aligned} \tag{48} $$

Addition by sides and subsequent rearrangement give

$$ \sum_{i=1}^{n} \left({}^{i}E_{s_0} - {}^{i}E_{p_0} \right) = 0 \quad (qed) \tag{49} $$

With n ECSs coupled into a cyclic system, the probability MPr of an ECA rises by a factor of 5, compared with one of the ECAs occurring in n ECSs coupled into a chemical chain.

5.3. Dendritic MCS structures

These structures (fig. 16) can be formed from n ECSs provided that

$$n = 2^{i+1} - 1 \ (i = 1, 2, 3, \ldots) \tag{50}$$

The number of dendrite branches is

$$A = 2^{i+1} - 2 \tag{51}$$

The number of MCS I/O is

$$B = 2^{i+2} \tag{52}$$

The number of I/O in the set of n uncoupled ECSs is

$$D = 2^{i+3} - 4 \tag{53}$$

with i tending to infinity, the B/D ratio tends to 0.5.

5.4. General aspects of formation of optimum MCS structures

The possibly simplest MCS structures considered here may be treated as elements of more complex structures. In a stochastic chemical system n ECSs have been shown to be more effectively operative after they have been organized into an MCS. The rise of performance of a catalytic system via formation of preferential couples is limited not only by the availability of object sets but also by the objective of the MCS action. Generally, this objective may be formulated as follows:

For a given set $\{{}^1S_0, {}^2S_0, {}^3S_0, \ldots, {}^mS_0\}$ produce, with maximum effectiveness, a set $\{{}^1P_0, {}^2P_0, {}^3P_0, \ldots, {}^mP_0\}$ in a given chemical system containing sets $\{{}^iS_0\}$, $\{{}^iP_0\}$, $\{{}^iG_0\}$, $\{{}^iK_0\}$, $\{{}^iC_0\}$ where $i \neq m$ is the power of the set.

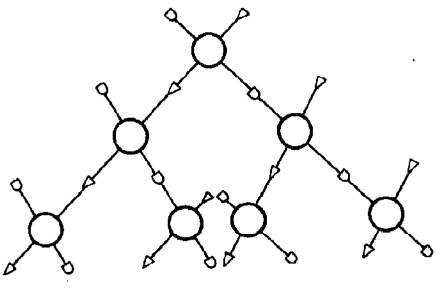

Fig. 16. Dendritic MCS structure.

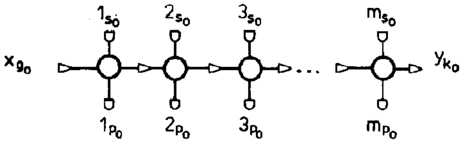

Fig. 17. Illustrative optimum structure.

In the formulation of the objective the condition has been imposed of a minimum number of chemical MCS I/O. If a system contains a pair of elements X_{g_0}, Y_{k_0} from sets $\{{}^iG_0\}$ and $\{{}^iK_0\}$, respectively, which can create for the elements of a subset of the set $\{{}^iC_0\}$ a suitable energy-coupled chain (fig. 17), then the resulting MCS assumes an optimum structure. If such a pair does not exist, or the real probability of appearance of the pair at appropriate MCS I/O is negligibly low compared with that of the appearance of other states,i.e.,

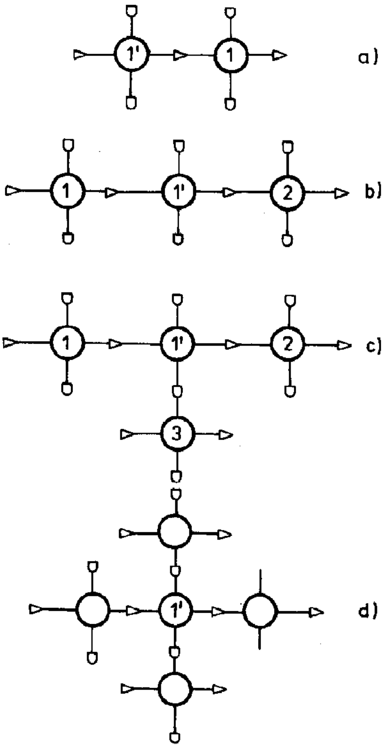

Fig. 18. Schemes of complex MCS structures utilizing ancillary ECSs (primed symbols).

elements of $\{{}^iG_0\}$ and $\{{}^iK_0\}$, then it is necessary to search for another MCS structure in which ECSs can appear able to transform the 'unsuitable' energy state available in the system into one rendering the course of a given ECA possible. Such ancillary ECSs will utilize suitable available elements of the sets $\{{}^iC_0\}$, $\{{}^iG_0\}$, $\{{}^iK_0\}$, $\{{}^iS_0\}$, $\{{}^iP_0\}$ and, in a general case, these will be the elements not belonging to a corresponding set from among the sets $\{{}^iC_0\}\ldots\{{}^iP_0\}$.

Fig. 18 shows schematically the possible instances of utilization of one ancillary ECS.

Summing up, production of complex MCS structures has been shown to result in increased probability MPr of occurrence of catalytic processes on individual ECSs in the stochastic environment. Formation of appropriate MCS structures can result in a readjustment of a chemical system to realize catalytic processes in an optimum structure via formation of ancillary objects s, p, g or k in the MCS elements.

6. Discussion

The present general models of enzymatic systems involve an elementary catalytic system (ECS) model as a structural unit. In this ECS model the catalytic function has been separated from the transport and binding functions. This separation made it possible to analyze the very essence of catalysis and also to study secular and energetic aspects of the effects of the ECS environment properties on the course of an elementary catalytic act (ECA). To describe ECS objects resort was taken in a simple parametric impulse-oscillation model (IOM) whose secular parameters Δt (reactivity impulse) and I (object oscillation time) may be physically interpreted as the moment at which the bond length attains a maximum during the stretch and the reciprocal frequency of oscillation of the molecule as a whole, respectively. These parameters are the internal attributes of the molecule and convey information on the properties of the bond active in a given reaction as also on the properties of the entire molecule. In energy considerations this fact implied the use of the internal, rather than free, energy (entropic term is concealed in the IOM parameters).

An ECS (fig. 2) is the simplest system in which ECAs may occur. Thus, in any enzyme, the molecular moieties responsible for an enzyme's purely catalytic function appear to involve not only the center but also those structural fragments which make directional flow of energy from and to the center possible. This fully bears out Atkinson's [12] view that an enzyme's catalytic action cannot be explained in terms of merely properties of the center. Therefore, it appears useful to add a function of directional energy flow to the generally accepted functions performed by an enzyme in vivo (increased transport effectivity via microcompartment and compartment phenomena [3] or via surface diffusion [13]; binding; catalysis; allosteric control).

The prerequisite for an ECS to act is that objects in suitable energy states (characterized by I) appear at well-defined moments a at the inputs to the system and that the objects transformed within the ECS leave the ECS system through its outputs at suitable moments. A general model of purposeful cooperation of a single ECS with its environment (fig. 5) may be confronted with the system (fig. 7) in which the environment of ECS is modeled as a completely random set of objects (g, k, s, p) in various allowable states (from the set of states, fig. 4).

The general character of the present considerations has prompted us to make assumptions some of which need not be obligatorily satisfied in individual cases. Thus, for example, the assumptions that all elementary events are independent (especially when an object in a well-defined state is chosen) and that, under concrete conditions of relative concentrations, temperatures, etc., objects are equally likely to appear, should be suitably verified and precisely defined. The MPr(ECA) estimates both for the system of fig. 7 and in the subsequent considerations should be viewed as the orders of magnitude rather than exact values. In this sense, the value MPr(ECA) $\approx (1/4)\times 10^{-8}$ shows an ECS to be able to cooperate with its stochastic environment (MPr $\neq 0$) even if compared with the ideal theoretical model of fig. 5 for which MPr(ECA) $= 1$, the effectiveness of this cooperation is seen to be rather low. As the number of various ECSs immersed in the stochastic environment is increased, the effectiveness of each ECS decreases as compared with that for a single ECS (for two ECS($n = 2$) MPr(ECA) $\approx 10^{-11}$; for $n \geqslant 3$ MPr(ECA) $\approx 2 \times 10^{-9} \times n^{-8}$; e.g., for $n = 3$, 5, 10 and 100, MPr(ECA) $\approx 3 \times 10^{-13}$; 5×10^{-15}; 2×10^{-17} and 2×10^{-25}, respectively). Even with these estimates it is evident that, as the number of various ECSs immersed in the stochastic environment is increased, the individual free ECS becomes less and less likely to act. Unless internally well organized, a populous set of various enzymes in a single cell would be absolutely unable to act.

One way to model the purposeful behavior of the microenvironment of a given ECS is to employ another ECS as a source or as a receiver of either the energy or substrate (product) for the given ECS. In this paper, such a system of two ECSs has been called the energetically or chemically coupled system. In modern enzymology the term 'coupled' has been used in a broader sense (e.g., energy coupling via ATP-ADP [14]). In the present study, coupling takes place when time and energy conditions are satisfied (eqs. 5, 6 and 10–12) and when, by the IOM model assumed, space and thermodynamic matching occurs, which as a whole means that two ECS are synchronized and synfixed. Thus, in the sense adopted here, two different enzymes immobilized on a carrier even in very close vicinity are not coupled. Energy coupling affects direct flow of energy from one ECS to another; hence, the ATP-ADP system with the necessary ATP synthase is not a simple energy-coupled system.

In view of the MPr(ECA) estimates a single energy coupling gives rise to enhanced effectiveness of individual ECS by at least two orders of magnitude as compared with the uncoupled system. A new look should therefore be postulated to be given to carriers employed in immobilization of enzymes to pick up carriers capable of an additional function of directional energy flow which could significantly enhance an enzyme's effectiveness. On the other hand, there is ample experimental evidence that most immobilized enzymes are more stable and effective than are free enzymes in solution, a fact explicable, among other things, in terms of a carrier's more or less effective function as energy receiver.

The effect ($\sim 10^2$) due to chemical coupling is by at least one order of magnitude higher than that established in the kinetic studies on immobilization of bienzyme systems like fumarase-malate dehydrogenase and malate dehydrogenase-citrate synthase on porous glass beads [15] and other [16]. The rise in effectiveness was 10-fold in the former and 40–50-fold in the latter studies. The discrepancy may be both due to the nonfulfillment of the general assumptions adopted in the evaluation of the MPr(ECA) estimates and due to technical difficulties encountered in producing correct coupling in vitro.

Systems involving many coupled ECSs, i.e., multicatalytic systems (MCSs) may be represented topologically as sets of vertices (ECSs) connected by bonds (energy and/or chemical couples). For single-bond vertices, bivertex systems are possible, for two-bond vertices chains and rings, and for three- and four-bond vertices steric structures are possible. Any complex MCS structure may be represented as a sum of simple structures which, in this work, comprise chains, rings and dendrites. For such structures the number of component ECSs was proved to be finite in energy-coupled chain MCSs, chemically homogeneous chain MCSs and in certain chemical classes of homogeneously alternating chain MCS. The remaining question is that of chemically nonhomogenous chain MCSs which may be treated as sums of locally homogeneous chain MCSs. Theoretical limitation of the number of ECSs in an MCS allows one to believe that in a hypothetical complex MCSs composed of an infinite number of ECS that tends toward enhanced effectiveness of individual ECS, an infinite number of various chain MCSs could be formed but a single MCS with an infinite number of ECSs in a chain would be impossible to form.

As expected, the effectiveness of ECSs increases as the MCS becomes more and more complex, compared with an analogous set of uncoupled ECSs. By way of illustration, for a chain MCSs composed of 3, 5, 10 and 100 ECSs, $\text{MPr(ECA)}_{\text{coupled}}$ values are 10^{-6}, 10^{-7}, 10^{-8} and 10^{-12} vs. 10^{-13}, 10^{-15}, 10^{-17} and 10^{-25}, respectively, for $\text{MPr(ECA)}_{\text{uncoupled}}$.

The present study intentionally avoids any descriptions based on thermodynamics which are inadequate for modeling single events in small local subsystems of a large chemical system. The proofs showing energy-coupled rings to be impossible to exist and chemical rings to be nonhomogeneous (proofs 6 and 7) demonstrate clearly that the present approach is not in conflict with the laws of thermodynamics.

The present considerations on general models should be treated as an introduction to more detailed studies on the general laws governing complex enzymatic systems. By the very definition these models allow one to analyze the very essence of catalysis and to study the effect of properties of the environment on the singled-out function of catalysis. These models represent therefore a well-founded starting point for constructing detailed complex models of concrete biochemical and biophysical processes for computer simulation of their behavior.

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