Biological modelling / Biomodélisation

Introduction to the concept of functioning-dependent structures in living cells

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Abstract

The assembly of proteins into larger structures may confer advantages such as increased resistance to hydrolytic enzymes, metabolite channelling, and reduction of the number of proteins or other active molecules required for cell functioning. We propose the term functioning-dependent structures (FDSs) for those associations of proteins that are created and maintained by their action in accomplishing a function, as reported in many experiments. Here we model the simplest possible cases of two-partner FDSs in which the associations either catalyse or inhibit reactions. We show that FDSs may display regulatory properties (e.g., a sigmoidal response or a linear kinetic behaviour over a large range of substrate concentrations) even when the individual proteins are enzymes of the Michaelis–Menten type. The possible involvement of more complicated FDSs or of FDS networks in real living systems is discussed. From the thermodynamic point of view, FDS formation and decay are responsible for an extra production of entropy, which may be considered characteristic of living systems.

Résumé

Introduction au concept de structures dépendant de leur fonctionnement dans les cellules vivantes. L’association de protéines en complexes plurimoléculaires peut leur conférer des avantages tels qu’une augmentation de la résistance aux enzymes d’hydrolyse, la canalisation des métabolites et une réduction du nombre de protéines ou autres molécules actives nécessaires au fonctionnement cellulaire. Nous proposons d’appeler structures dépendant de leur fonctionnement (FDS) les associations de protéines qui sont créées et maintenues par le fait qu’elles sont en train d’accomplir leur fonction. De telles situations ont été décrites à diverses reprises dans la littérature. Ici, nous avons modélisé les cas les plus simples possibles, c’est-à-dire les FDS à deux partenaires, catalytiques ou inhibitrices. Nous montrons que les FDS peuvent présenter des propriétés régulatrices (comportements cinétiques sigmoides, ou linéaires sur une vaste plage de concentrations), même lorsque les protéines constitutives de ces FDS sont de type Michaélien. L’éventuelle intervention de FDS à plus de deux partenaires ou même des réseaux de FDS...
Abbreviation

FDS, functioning-dependent structure

1. Introduction

Proteins involved in a cooperative cellular task, such as a metabolic or signalling pathway, are not always randomly distributed but may exist in the form of multimolecular complexes (reviewed in Mathews [1]), which have been termed metabolons in the case of metabolic pathways [1–3], transducins in the case of signal transduction [4], or, more generally, hyperstructures (possibly including not only protein subunits, but also other components such as nucleic acids or lipids and implicating equilibrium as well as non-equilibrium molecular associations) [5].

As a particular case of the above, the complexes might assemble only in an activity-dependent manner, that is, proteins do not associate spontaneously but only when they are actually engaged in the process of transport and/or transformation of a substrate or transduction of a signal [1,5–10]. Demonstrative examples of such behaviour are (i) the control of several steps of the glycolytic pathway by metabolite-modulated dynamic enzyme associations [6] and (ii) the ATP- and pH-dependent association/dissociation of the V1 and V0 domains of the yeast vacuolar H+ -ATPases [11]. We propose to term functioning-dependent structure (FDS) a dynamic assembly that forms and maintains itself by the very fact that it is accomplishing a task and that disassembles when no longer functioning. Some advantages conferred by molecules being in such assemblies as opposed to being free are obvious (increased resistance to hydrolytic enzymes, substrate channelling, reduction of the number of proteins or other active molecules required for cellular processes). Metabolite-induced metabolons [10] exemplify such FDSs. In this paper we model the particularly simple example of an enzymatic two-partner FDS with a view to unravelling the basic kinetic properties of FDSs under steady-state conditions. Then we discuss briefly the possibility of developing FDS models of increasing complexity in order to represent subcellular structures more realistically.

2. The two-enzyme models

Consider a reaction medium containing two different enzymes, E and F, with E catalysing the transformation of S to P and F catalysing the transformation of P to Q, i.e.

\[ S \underset{k_{f}}{\overset{k_{r}}{\rightleftharpoons}} P \underset{k_{f}}{\overset{k_{r}}{\rightleftharpoons}} Q \]

(1)

In the conventional case (Fig. 1), the enzymes E and F work independently of each other according to the series of steps characterised by their rate constants. In this case, the intermediate substance P must go from the enzyme molecule E, which has released it, to an enzyme molecule F where it is transformed into the final product Q, via an enzyme complex FP, and which is then released into the reaction medium. The parameters \( k_{f} \) and \( k_{r} \) are the forward and reverse rate constants of each reaction, \( j \).

Fig. 1. The conventional case in which two free enzymes, E and F, catalyse two sequential reactions (see (1)). The first enzyme, E, and the initial substrate, S, form a substrate-enzyme complex, ES, which releases the product P in the reaction medium, thus regenerating the free enzyme E. Then P diffuses at random until it reaches an enzyme F where it is transformed into the final product Q via an enzyme complex FP, and which is then released into the reaction medium. The parameters \( k_{f} \) and \( k_{r} \) are the forward and reverse rate constants of each reaction, \( j \).
Fig. 2. A ‘catalytic’ two-enzyme model of FDS. In this model, the free enzyme F is not capable of binding and reacting with its substrate, P, and the free enzymes, E and F, are not capable of assembling with each other. However, the binding of substrate S by enzyme E is responsible for a structural transition that confers on E an ability to bind to enzyme F thus forming the complex ESF. Then this structure catalyses the transformation of S into P (without releasing P into the reaction medium), the channelling of P to F and the fixation of another S molecule, thus forming the complex ESFP. Within this latter complex, F transforms P into Q and releases Q into the reaction medium, thus regenerating ESF. In brief, ESF catalyses the overall transformation of one S to one Q per cycle. The first step (with the rate constants $k_1$ and $k_2$) is identical to that in the conventional case in which the two enzymes are not assembled into an FDS (Fig. 1). The parameters $h_j$ are the rate constants of each reaction, $j$, involved in the FDS formation and functioning.

schematised in the usual concise way since there are certainly many more intermediate complexes involved in reality than indicated in the figures.

A two-partner model of FDS may be constructed as depicted in Fig. 2. There are two steps in the functioning of this FDS model: (i) the creation of the enzyme–enzyme bond as a consequence of the fact that enzyme E has bound its substrate S and (ii) the engagement of the bi-enzymatic FDS thus obtained in the catalysis of the overall reaction of the initial substrate, S, to the final product, Q. If substrate S were to be entirely consumed in the reaction medium, then obviously the process of FDS formation would reverse and cause this structure to break down and release the free enzymes E and F. When the concentration of S is not zero, the relative concentrations of free and assembled enzymes depend on the values of the rate constants. Such a type of FDS as described in Fig. 2 is termed catalytic, in the sense that the formation of the enzyme assembly facilitates the progress of the overall reaction from S to Q. Note that in this and the following figures, ESF is a formal description of the functioning-dependent structure, meaning that the bi-enzymatic complex EF is also bound to a substrate molecule: it does not mean that the substrate is a component of the bond of E with F.

An inhibitory FDS (Fig. 3) may also be envisaged. In such an FDS, enzymes again assemble as in Fig. 2, but only the free enzyme F can catalyse the reaction of P to Q and the complex ESF has no catalytic effect.

3. Steady-state kinetics

3.1. Statement of the problem

In the following, the concentration of any substance, X, will be symbolised [X]. To compare the steady-state kinetic behaviour of the catalytic and inhibitory FDSs (Figs. 2 and 3, respectively) with that of the similar, ‘non-assembled’ enzymes that do not form an FDS (Fig. 1), we use the simplifying assumptions that (a1) the reaction medium is homogeneous, (a2) the channelling of P from E to F within the FDS is perfect (i.e., there is no liberation of P into the reaction medium), (a3) all the reactions of formation or decay of complexes other than those indicated in the figures, for instance

$$ES + FP \rightleftharpoons ESFP$$

(2)
are negligible, \( (a_4) \) the free enzymes \( E \) and \( F \) are of the Michaelis–Menten type (i.e. apart from their possible changes in their structure due to their assembling into an \( FDS \), there are no allosteric transitions due to the fixation/release of regulatory ligands), \( (a_5) \) all the stoichiometric coefficients involved in the reactions are taken to be equal to 1 and \( (a_6) \) under steady-state conditions, \( [S] \) is maintained at a constant value, \( [S]_0 \), and \( [Q] \) at a zero value.

In the conditions modelled here, the forward rate constants \((k_{f1} \mathrm{and \ h_{f1}})\) are expressed in \( \text{mol}^{-1} \text{s}^{-1} \text{m}^3 \) while the reverse rate constants \((k_{r1} \mathrm{and \ h_{r1}})\) are expressed in \( \text{s}^{-1} \). Moreover, for easier analysis, we treat the problem using dimensionless variables and parameters, i.e. dimensionless rate constants \( \alpha_j \) and \( \beta_j \) (corresponding to \( k_j \) and \( h_j \), respectively), the dimensionless time, \( \tau \), and dimensionless concentrations (written using lower-case letters). The definitions of these dimensionless quantities are given in Appendix A.

The equilibrium constant, \( K \), of the overall reaction of \( S \) to \( Q \) is independent of the way in which this reaction is catalysed (that is, via non-assembled enzymes or via a catalytic or inhibitory \( FDS \)). This imposes constraints on the rate constants, the consequence of which is that two of the rate constants (e.g., \( \alpha_{4f} \) and \( \beta_{4f} \)) cannot be chosen arbitrarily in the modelling process, but have to be calculated as functions of the equilibrium constant and the other rate constants. The expressions of the equilibrium constant, \( K \), and of \( \alpha_{4f} \) and \( \beta_{4f} \) are given in Appendix B. Moreover, when not at equilibrium, the overall reaction will tend to transform \( S \) into \( Q \) when \( [S]/[Q] > 1/K \) while it will tend to transform \( Q \) into \( S \) when \( [S]/[Q] < 1/K \). With \( [Q] = 0 \) according to assumption \((a_6)\), the reaction will always proceed from \( S \) to \( Q \).

### 3.2. Steady-state kinetic behaviour of the various two-partner systems

The derivation of the expression of the steady-state rate of functioning, \( u \), of the overall reaction of \( S \) to \( Q \) as a function of the concentration of substrate, \( s_0 \), in the case of non-assembled enzymes is given in Appendix C. Note that, in this and the following appendices, we have written sets of independent equations, eliminating some time derivatives (e.g., \( dE/d\tau \) and \( df/d\tau \) in Appendix C, \( de_f/d\tau \) in Appendix D, and \( de_s/d\tau \) and \( df/d\tau \) in Appendix E) as a consequence of the mass-conservation relations. Fig. 4 gives an example of the results that have been computed with a particular choice of the parameters (equilibrium and rate constants). With the many different values of the parameters we have tested, we have always obtained the same type of banal behaviour, in which \( u \) increases monotonically as a function of \( s_0 \) until reaching a saturation plateau.

The expression of the steady-state rate of functioning of a catalytic \( FDS \), \( v \), is derived in Appendix D. When computing the dependence of \( v \) on the concentration of initial substrate, \( s_0 \), there are choices of parameters with which we obtain the same banal type of behaviour (monotonically increasing curve up to a saturation plateau), as has been observed in the case of non-assembled enzymes. However, with
4. Discussion and conclusion

Apart from the obvious advantages of enzymes assembling into hyperstructures (see Introduction), it has been shown here that enzymes of the simple Michaelis–Menten type may display a richer (e.g., sigmoidal or linear) kinetic behaviour when they are engaged in functioning-dependent structures than when they remain non-assembled. Hence, under the highly structured conditions likely to exist in vivo, not only allosteric proteins, but also any sort of enzyme may exhibit regulatory properties provided it can form part of an FDS. It is also noteworthy that certain of the properties of the FDSs such as linear and sigmoid responses resemble the regulatory linear responses and step functions built into artificial electronic devices.

The possible occurrence of sigmoidal responses with FDSs is also reminiscent of apparent allosteric effects emerging in membrane-constrained co- or counter-transport proteins when the usual assumptions of very fast binding and release are relaxed [12].

The likely relevance of the concept of functioning-dependent structure to enzyme behaviour means, we suggest, that the classical structure → function relationship in biochemistry should be complemented by a reciprocal function → structure relationship. In other words, subcellular processes exist in which transient functioning structures are created and maintained by the very fact that they are accomplishing a function (see, e.g., [6,11]). This two-way relationship may prove to occur relatively frequently in living systems, while it is not generally encountered in non-living, physical, or chemical processes. Moreover, the assembly and decay of functioning-dependent structures in a living system will be responsible for an extra production of entropy, in addition to that arising from the normal reactions and transport processes in cells. This extra production of entropy by FDSs thus may be of particular relevance to living systems.

Here we have considered only very simple, two-partner FDS models, the steady-state kinetics of which has been studied by use of relatively straightforward calculation methods. In real living systems, however, much more complicated transient associations of proteins may occur, involving multi-partner associations and possibly forming dynamic networks of FDSs. We speculate that the regulatory properties of such complex FDSs will prove to be even more numerous and...
clear-cut than in the simple cases examined here. At present, the difficulty of modelling such complex systems is considerable but may become feasible with the use of appropriate mathematical and computer techniques. Despite this difficulty, the simple approach adopted in this paper shows that the FDS concept has interesting implications and that more complex and realistic FDS models should be envisaged.

Appendix A. Definition of dimensionless quantities

Dimensionless quantities have been defined by normalising all concentrations to the sum of the total concentrations of $E$ and $F$, $[E] + [F]$, and all time values to $1/k_1$. As a consequence, the molar fractions of enzymes $E$ and $F$ are:

$$x_E = [E]/([E] + [F]), \quad x_F = [F]/([E] + [F])$$

(A.1)

with $x_E + x_F = 1$

the dimensionless concentrations of all the substances involved are:

$$s = [S]/([E] + [F]), \quad p = [P]/([E] + [F]),$$

$$q = [Q]/([E] + [F]), \quad e = [E]/([E] + [F]), \quad f = [F]/([E] + [F])$$

(A.2)

$$e_s = [ES]/([E] + [F]), \quad f_p = [FP]/([E] + [F]),$$

(A.3)

$$e_{sF} = [ESF]/([E] + [F]), \quad e_{FP} = [ESFP]/([E] + [F])$$

(A.4)

the dimensionless time, $\tau$, is:

$$\tau = t/k_1$$

(A.5)

the dimensionless reverse rate constants are:

$$\alpha_{j1} = k_{j1}/k_1, \quad \beta_{j1} = h_{j1}/k_1$$

(A.6)

(A.7)

with, obviously:

$$\alpha_{11} = k_{11}/k_1 = 1$$

(A.8)

and the dimensionless forward rate constants are:

$$\alpha_{j1} = (k_{j1}/k_1)([E] + [F]), \quad \beta_{j1} = (h_{j1}/k_1)([E] + [F])$$

(A.9)

Appendix B. Equilibrium constant and non-independent rate constants

The (dimensionless) equilibrium constant of the overall reaction (Eq. (1)):

$$K = [Q]_{eq}/[S]_{eq} = q_{eq}/s_{eq}$$

(B.1)

in which $[S]_{eq}$ and $[Q]_{eq}$ are the equilibrium concentrations of $S$ and $Q$ (and $s_{eq}$ and $q_{eq}$ the corresponding dimensionless quantities), may be calculated both in the case of enzymes not assembled in a FDS and in the case when a catalytic FDS occurs. This is written:

$$q_{eq}/s_{eq} = (\alpha_{1f} \alpha_{2f} \alpha_{3f} \alpha_{4f})/(\alpha_{1f} \alpha_{2f} \alpha_{3f} \alpha_{4f}) = K$$

(B.2)

and

$$q_{eq}/s_{eq} = (\beta_{3f} \beta_{4f})/(\beta_{3f} \beta_{4f}) = K$$

(B.3)

respectively. As a consequence, not all the rate constants are independent from one another, but two of them are functions of the other rate constants and the equilibrium constant, e.g.:

$$\alpha_{4f} = (\alpha_{1f} \alpha_{2f} \alpha_{3f} \alpha_{4f})/(K \alpha_{1f} \alpha_{2f} \alpha_{3f})$$

(B.4)

and

$$\beta_{4f} = (\beta_{3f} \beta_{4f})/(K \beta_{3f})$$

(B.5)

Appendix C. Steady-state reaction rate, $u$, in the case of enzymes not assembled in a FDS

With non-assembled enzymes (Fig. 1), the mass-conservation equations are:

$$x_E = e + e_s, \quad x_F = f + f_p$$

(C.1)

a set of independent equations governing the system under steady-state conditions is:

$$dp/d\tau = -\alpha_{2f} pe + \alpha_{3f} e_s - \alpha_{3f} pf + \alpha_{3f} f_p = 0$$

(C.2)

$$de_{s}/d\tau = \alpha_{1f} se - \alpha_{1f} e_s + \alpha_{2f} pe - \alpha_{2f} e_s = 0$$

(C.3)

$$df_p/d\tau = \alpha_{3f} pf - \alpha_{3f} f_p + \alpha_{4f} qf - \alpha_{4f} f_p = 0$$

(C.4)

with, according to assumption $a_6$:

$$s = s_0, \quad q = 0$$

(C.5)

and the initial conditions are:

$$p(0) = 0, \quad e_{s}(0) = 0, \quad f_p(0) = 0$$

(C.6)
The expressions of the variables \(e, f, e_s, f_p,\) and \(p\) are easily found to be:

\[
e_s = x_E (\alpha_1 s_0 + \alpha_2 p)/(\alpha_1 s_0 + \alpha_1 e + \alpha_2 p + \alpha_3 e)
\]
\[
f_p = x_F (\alpha_3 p)/(\alpha_3 p + \alpha_3 e + \alpha_4 e)
\]
\[
e = x_E - e_s
\]
\[
f = x_F - f_p
\]
\[
p = (-D s_0 - G - (D s_0 + G)^2 - 4A H s_0)^{1/2}/2A
\]

with \(A, D, G\) and \(H\) being expressed as:

\[
A = -\alpha_2 \alpha_3 (\alpha_1 e + e_0)
\]
\[
D = \alpha_1 \alpha_3 (\alpha_2 e - e_0)
\]
\[
G = -\alpha_1 \alpha_2 (\alpha_3 e - \alpha_3 \alpha_4 (\alpha_1 + \alpha_2) e_0)
\]
\[
H = \alpha_1 \alpha_2 (\alpha_3 e + \alpha_4 e_0)
\]

and the reaction rate, \(u\) (which corresponds to both the consumption of \(S\) and the production of \(Q\)), is written:

\[
u = \alpha_1 s_0 e - \alpha_1 e_s = \alpha_4 r f_p
\]

Appendix D. Steady-state reaction rate, \(v\), in the case of a catalytic FDS

The mass-conservation equations of the catalytic FDS (Fig. 2) are:

\[
x_E = e + e_s + e_f + e_f s_p
\]
\[
x_F = f + f_p + e_s
\]

(D.1)

Then three independent steady-state equations are derived in a manner similar to that in the case with non-assembled enzymes, e.g.:

\[
de/dt = -\alpha_1 s e + \alpha_1 e_s = 0
\]
\[
d_e s/dt = \alpha_1 s e - \alpha_1 e_s + \beta_2 e f_s - \beta_2 f e_s = 0
\]
\[
d_e f s_p/dt = \beta_3 s e f_s - \beta_3 e f_s p + \beta_4 q e f_s - \beta_4 e f_s p = 0
\]

(D.2)

(D.3)

(D.4)

with the conditions:

\[
s = s_0, \quad q = 0, \quad p(0) = 0, \quad e_s(0) = 0, \quad e_f s(0) = 0, \quad e_f s_p(0) = 0
\]

(D.5)

Defining \(P1, P2, P3, A, B\) and \(C\) as:

\[
P1 = (\alpha_1 \beta_2 (s_0))/(\alpha_1 \beta_2)
\]
\[
P2 = 1 + ((\alpha_1 s_0)/(\alpha_1 e))
\]
\[
P3 = 1 + ((\beta_3 s_0)/(\beta_3 s + \beta_4))
\]
\[
A = P1 P2 P3
\]
\[
B = P2 - (P1 P3(x_E - x_F))
\]
\[
C = -x_E
\]

(D.6)

(D.7)

(D.8)

(D.9)

(D.10)

(D.11)

(D.12)

(D.13)

Appendix E. Steady-state reaction rate, \(w\), in the case of an inhibitory FDS

In the case of an inhibitory FDS (Fig. 3), the mass-conservation equations are:

\[
x_E = e + e_s + e_f s
\]
\[
x_f = f + f_p + e_s
\]

(E.1)

and a set of independent steady-state equations is written:

\[
de/dt = -\alpha_1 s e + (\alpha_1 + \alpha_2) e_s - \alpha_2 f p e = 0
\]
\[
d_e f s/dt = \beta_2 f e_s - \beta_2 e f s = 0
\]
\[
d_f p/dt = \alpha_3 p f - (\alpha_3 + \alpha_4) f_p + \alpha_4 q f = 0
\]
\[
d_p/dt = -\alpha_2 f p e + \alpha_2 e_s - \alpha_3 p f + \alpha_3 f_p = 0
\]

with

\[
s = s_0, \quad q = 0, \quad p(0) = 0, \quad e_s(0) = 0, \quad e_f s(0) = 0, \quad f_p(0) = 0
\]

(E.2)

(E.3)

(E.4)

(E.5)

(E.6)
Four of the variables \((f, e_s, f_p\) and \(e_f\)) can be expressed as functions of the other two \((p\) and \(e\)), i.e.:

\[
e_s = \frac{(\alpha_1 s_0 + \alpha_2 p)/(\alpha_1 + \alpha_2)}{e} \tag{E.7}\]

\[
f = \left(\frac{\alpha_3r + \alpha_4r}{\alpha_1(\alpha_2s_0 - \alpha_1\alpha_2p)}\right)e \tag{E.8}\]

\[
f_p = \left(\frac{\alpha_1(\alpha_2s_0 - \alpha_1\alpha_2p)}{(\alpha_4r(\alpha_1 + \alpha_2)}\right)e \tag{E.9}\]

\[
e_f = \left(\frac{\beta_2(\alpha_3 + \alpha_4r)(\alpha_1s_0 + \alpha_2p)}{(\alpha_1(\alpha_2s_0 - \alpha_1\alpha_2p))}\right)e^2 \tag{E.10}\]

Defining \(A\), \(B_1\), \(C_1\), \(B_2\) and \(C_2\) as:

\[
e_f = Ae^2, \quad e + e_s = B_1/e, \quad -x_E = C_1 \tag{E.11}\]

\[
f + f_p = B_2/e, \quad -x_F = C_2 \tag{E.12}\]

Eqs. (E.1) may be rewritten:

\[
Ae^2 + B_1/e + C_1 = 0 \tag{E.12}\]

and

\[
Ae^2 + B_2/e + C_2 = 0 \tag{E.13}\]

Since concentrations and rate constants are by nature positive quantities, \(C_1\) and \(C_2\) are negative and \(B_1\) is positive. Since the overall reaction proceeds in the direction \(S \rightarrow P \rightarrow Q\) (as a consequence of \(q\) being maintained equal to zero), the factor \((\alpha_1\alpha_2s_0 - \alpha_1\alpha_2p)\) in the expressions of \(A\) and \(B_2\) is positive and \(A\) and \(B_2\) thus are also positive. Therefore, each of Eqs. (E.12) and (E.13) has only a single solution, \(e_1\) and \(e_2\), respectively, that is biologically relevant, i.e.

\[
e_1 = \left(-B_1 + \left(B_2^2 - 4AC_1\right)^{1/2}\right)/2A \tag{E.14}\]

\[
e_2 = \left(-B_2 + \left(B_2^2 - 4AC_2\right)^{1/2}\right)/2A \tag{E.15}\]

where \(e_1\) and \(e_2\) are functions of only the variable \(p\).

There is then an easy numerical solution to the problem that is obtained by systematically varying \(p\) until the correct \(p\) value is obtained for which:

\[
e_1 = e_2 = e \tag{E.16}\]

at the desired precision. The other variables \((f, e_s, f_p\) and \(e_f\)) then are calculated from these values of \(p\) and \(e\) by using Eqs. (E.7) to (E.10) and the reaction rate, \(w\) (again corresponding to both the consumption of \(S\) and the production of \(Q\)), is written:

\[
w = \alpha_1 s_0 e - \alpha_1 e_s = \alpha_4r f_p \tag{E.17}\]

References


