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Hypothesis

Hormonal regulation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase: kinetic models

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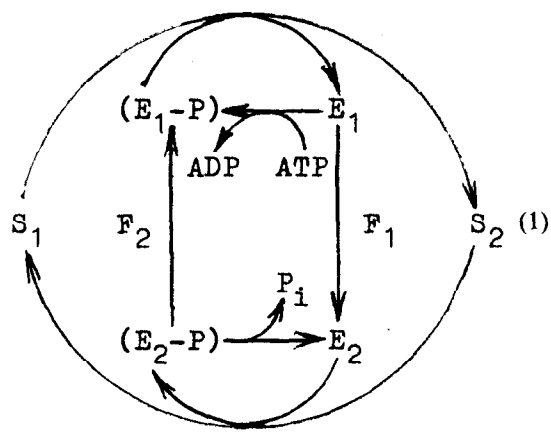
A graphical method to reveal the so-called 'critical fragments' in schemes of biochemical systems is considered. These fragments produce multiple steady states or self-oscillations in systems. As an example, the bifunctional enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, regulated by glucagon through enzyme phosphorylation, is discussed. It is shown that this enzyme may act as a metabolic switching mechanism in discontinuous or oscillatory regimes, depending on the specific structure of its kinetic scheme. The boundaries of concentrational and parameter domains for these critical phenomena are also predicted.

Bifunctional enzyme; Graph-theoretical method; Glucagon switching signal; Critical phenomenon

1. INTRODUCTION

The recently discovered bifunctional enzyme (E_1/E_2) catalyses the substrate cycle $S_1 \underset{E_2}{\overset{E_1}{\rightleftharpoons}} S_2$ [1]. Protein kinase, F_1, activates E_2 and simultaneously inhibits E_1. Phosphatase, F_2, induces the opposite effect [1]. Both E_1 and E_2 involve a phosphoryl-enzyme intermediate, (E_1-P) and (E_2-P) [2]. The modification (phosphorylation) of the enzyme was shown to reduce the amount of (E_2-P) and increase that of its counterpart E_2 [2]. Therefore, the following reaction mechanism may be postulated:



(1)

Moreover, the activity of F_1 is controlled (inhibited) by substrate S_2 [3] and, presumably, by substrate S_1. The glucagon regulatory signal ac-

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Abbreviations: E_1, 6-phosphofructo-2-kinase; E_2, fructose-2,6-bisphosphatase; S_1, fructose 6-phosphate; S_2, fructose 2,6-bisphosphate; F_1, cAMP-dependent protein kinase; F_2, phosphoprotein phosphatase

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tivates F_1, dramatically changing two reciprocal enzyme activities and thereby switching hepatic glycolysis to gluconeogenesis [1].

External fluxes, $\rightarrow S_1 \rightarrow$, $\rightarrow S_2 \rightarrow$, may be included in mechanism 1, but are not essential for our model (contrary to a similar model, based on empirical equations [4]).

Two 'critical' kinetic regimes of the system (mechanism 1) may be predicted: discontinuous (hysteretic) switching, similar to the one described recently [5], and self-oscillations. Both regimes may be important for switching of opposing metabolic fluxes [4].

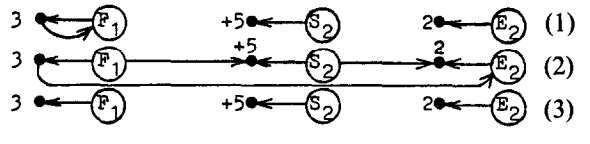
It is shown in this paper that the critical kinetics may be identified from the specific structure of kinetic schemes.

2. CRITICAL FRAGMENTS IN KINETIC SCHEMES

We use a graphical method [6–10] to reveal 'critical fragments' in kinetic schemes ('graphs'). Circles and points in the graphs (see figs 1 and 3) refer respectively to different reactants and reactions. A critical fragment is a combination of the so-called 'even cycles', leading through common reactants and reactions (see, e.g. [6]). An even cycle is formed by an even number (0, 2, ...) of 'negative paths', ○→●←○, and/or any number of 'positive paths', ○→●→○ [7]. Critical fragments give rise to 'destabilizing' negative terms in the characteristic polynomial for a schematically presented system [8].

We enumerate 'sub-graphs' in a fragment to predict its critical properties. We term all the fragment reactants together with its uncrossing 'cycles' and/or its uncrossing 'half-paths', ○→●, as a sub-graph [7]. The number of sub-graphs, containing an odd number of even cycles, must be greater than the number of other sub-graphs in the critical fragment [8]. This rule determines the structure of critical fragments for reaction steps with stoichiometric coefficients equal to unity.

The critical fragment, shown in fig.1 by thick lines, contains the following three sub-graphs:



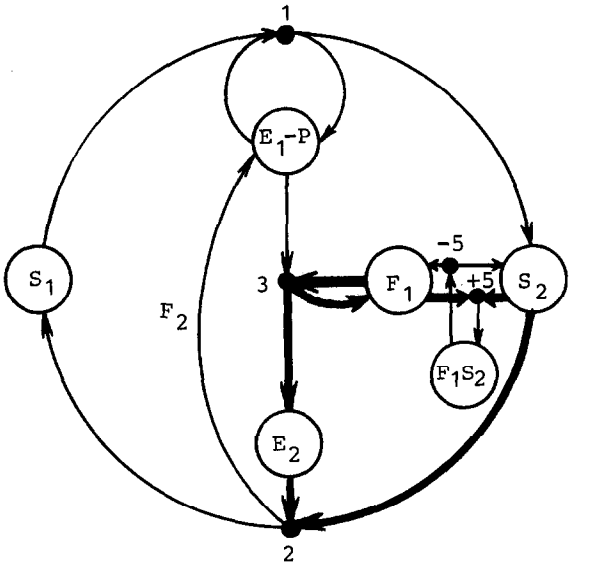


Fig.1. Graph producing multiple steady states. Thick lines show the 'critical fragment' which contains 3 reactions (3, +5, 2) and 3 reactants (E_2, F_1, S_2). The number of the fragment reactants is equal to the number of independent ones, since there are 3 constraints, i.e. $\Sigma[E] = [E]_0$, $\Sigma[F] = [F]_0$, $\Sigma[S] = [S]_0$; therefore it is a fragment of 'highest order'.

The first two each contain an odd number (one) of even cycles.

Multiple steady states may be obtained only from a critical fragment of 'highest order' (i.e. with all independent reactants). Self-oscillations may be obtained only from a fragment of 'lower order'. The presence of critical fragments gives only the necessary condition for critical phenomena. This condition becomes also sufficient, if none of the reactants of the critical fragment enter irreversibly the reactions of other fragments.

3. MULTIPLE STEADY STATES

Step $E_1 \xrightarrow[\text{ATP}]{\text{ADP}} (E_1\text{-P})$ may be rapid at high ATP levels. Therefore, the level of E_1 may be negligibly low. Consequently, the following modelling reactions may be considered:

$$(E_1\text{-P}) + S_1 \longrightarrow (E_1\text{-P}) + S_2$$

(forward reaction), (1)

$$E_2 + S_2 \longrightarrow (E_2\text{-P}) + S_1 \text{ and } (E_2\text{-P}) \longrightarrow E_2$$

(reverse reaction), (2)

$(E_1\text{-}P) + F_1 \longrightarrow F_1 + E_2$ (phosphorylation), (3)

$(E_2\text{-}P) + F_2 \longrightarrow F_2 + (E_1\text{-}P)$

(dephosphorylation), (4)

$F_1 + S_2 \rightleftharpoons (F_1S_2)$ (substrate inhibition). (5)

These reactions may be treated as obeying mass action kinetics. If reaction 4 is very rapid, and reaction $(E_2\text{-}P) \longrightarrow E_2$ is slow, which can apply to the isolated enzyme [2], we substitute reactions 2 and 4 for a single reaction (2): $E_2 + S_2 \longrightarrow S_1 + (E_1\text{-}P)$. The obtained graph, shown in fig.1, contains two critical fragments of highest (third) order: the one shown by thick lines, and the other that includes reactants $(E_1\text{-}P)$, F_1, S_2 and reactions 1, 3 and +5. However, only the first one produces a critical phenomenon, because only from this fragment do no additional effluxes (half-paths, $\circ\!\!\rightarrow\!\!\bullet$) originate.

The flux through the critical fragment must be great compared to other fluxes in the hysteretic regime, therefore only rapid phosphorylation ($k_3 > k_1$) assures hysteresis (see fig.2).

If the reaction $(E_2\text{-}P) \longrightarrow E_2$ is rapid, we may simplify reactions 2 and 4:

$E_2 + S_2 \longrightarrow S_1 + E_2$ (2)

and

$E_2 + F_2 \longrightarrow F_2 + (E_1\text{-}P)$ (4).

The corresponding graph is shown in fig.3. Neither of its critical fragments produces critical phenomena, since they possess additional effluxes.

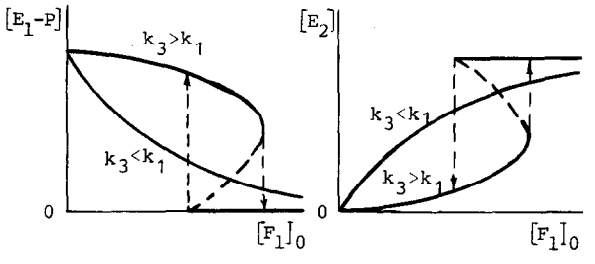


Fig.2. Dependences of two reciprocal enzymic activities (stationary concentrations $[E_1\text{-}P]$ and $[E_2]$) on protein kinase activity (initial concentration $[F_1]_0$), computed for the graph of fig.1. $[F_1]_0$ rises with glucagon addition. The phosphorylation (reaction 3) must be rapid compared to the forward reaction (1), i.e. $k_3 > k_1$, to produce hysteresis.

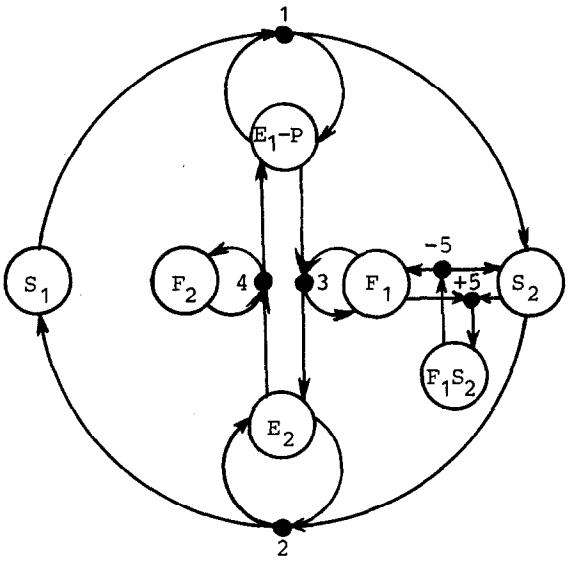


Fig.3. Graph containing two critical fragments: (a) with reactants $(E_1\text{-}P)$, F_1, S_2 and reactions 1, 3 and +5; and (b) with reactants E_2, F_1, S_2 and reactions 3, +5 and 2. The efflux, $S_2 \circ\!\!\rightarrow\!\!\bullet 2$, originates from the fragment (a), and the efflux, $E_2 \circ\!\!\rightarrow\!\!\bullet 4$, from the fragment (b); therefore they do not produce critical phenomena.

4. SUSTAINED OSCILLATIONS

Let us modify fig.1, introducing a new hypothetical inhibition,

$$S_1 + F_1 \underset{k_{-6}}{\overset{k_{+6}}{\rightleftharpoons}} (F_1S_1).$$

The critical fragment, shown by thick lines, now becomes of lower order, therefore it may produce self-oscillations.

An additional reaction sustains critical phenomena, because it is reversible, and adds a new cycle rather than irreversible efflux. A resultant fragment of highest (fourth) order may induce multiple steady states. The reversible efflux, through F_1, inhibited by S_1, must be the slowest in the graph to produce self-oscillations. Moreover, if we take into account all equalities of steady-state influxes and effluxes, the following oscillatory condition is obtained:

$$\left(\frac{k_{+6}}{k_{-6}} - \frac{k_{+5}}{k_{-5}}\right)\frac{k_3}{k_1} + \frac{k_{+5}}{k_{-5}} > 0 \qquad (2)$$

The opposite inequality assures hysteresis. The

following concentrational condition must also be fulfilled [6] in the single unstable steady state (to produce self-oscillations):

$$\frac{[S_1]}{[S_2]} > 1 + \frac{[F_1]}{[S_2]} + \frac{[F_1]}{[F_1S_2]} \quad (3)$$

In conclusion, critical phenomena in biochemical systems may be studied in terms of actual kinetic interactions without the need to adopt empirical equations. The procedure of searching for critical fragments which takes account of the relations existing between steady-state fluxes in complex systems may be algorithmically programmed for computers [9].

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