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Unusual kinetic behavior predicted for α-keto acid dehydrogenase complexes

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A novel regulation type, which may be observed as an unusual kinetic 'cooperativity', is predicted for the α-keto acid dehydrogenase complexes. The inter-relationship of this regulation with the well-known regulatory effect of enzyme phosphorylation is discussed.

Multienzyme complex; Lipoyl network; Bistability; Phosphorylation

1. INTRODUCTION

The α-keto acid dehydrogenase complexes are usually considered to be classic examples of multienzyme complexes, with the result that their structure–function organization has been intensively studied [1,2]. Nevertheless, their kinetic behaviour and regulation still needs to be understood. An unusual kinetic 'cooperativity' was observed for some of these complexes under certain conditions [3–6]. This cooperativity is difficult to explain on the basis of any known model of allosteric enzymes.

In this paper we have analyzed a simple kinetic model by taking into account the existence of covalently bound flexible lipoyl moieties organized into the network to promote intermediate transfer in these complexes [7]. This model predicts the unusual type of activity regulation.

We have concentrated upon the mammalian pyruvate dehydrogenase complex (PDC), the most complicated member of this family. The obtained results seem to offer a key to understanding the regulation of similar complexes.

2. EXPERIMENTAL

2.1. *The model for pyruvate dehydrogenase complexes*

PDC contains multiple copies of the catalytic components, E1, E2, E3 (the E1-specific kinase, the E1-specific phosphatase, and protein X, respectively [7]). We have taken into account the following experimental data from the literature to formulate the kinetic model.

(1) The lipoyl moieties attached to the E2, as well as the lipoyl moiety attached to protein X, serve as two similar but different substrates for the catalytic components [7–9].

(2) Another substrate is an acetyl group obtained in the first step catalyzed by the E1.

(3) The lipoyl substrates may be acetylated by pyruvate, with catalytic participation of the E1, and deacetylated in the E2 active sites to produce Ac-CoA [7].

(4) The preferential delivery of reducing equivalents from the lipoyl moieties of the E2 to the E3 occurs via the lipoyl moiety of protein X [8,9].

The scheme of this reaction is shown in Fig. 1, and includes two overall reaction pathways. One of them includes X-lipoyls only, however, protein X also participates in the E2 lipoyl pathway (step k_3) and, being acetylated, may limit the reaction rate.

3. RESULTS

The following system of differential equations corresponds to the reaction scheme:

$$u'_0 = k_{+0}[pyr] - k_{-0}u_0 - k_1u_0u_1 - k_1u_0u_4$$

$$u'_1 = k_3u_3u_4 - k_1u_0u_1$$

$$u'_2 = k_1u_0u_1 - k_2u_2$$

$$u'_4 = k_2u_5 - k_1u_0u_4$$

$$u_1 + u_2 + u_3 = 10$$

$$u_4 + u_5 = 1$$

Two constraint equations refer to the stoichiometrically different [7] total E2 and X lipoyl levels conserved.

This system was analyzed and solved to obtain the overall reaction rate dependent on the input (pyruvate)

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Abbreviations: E1, E2, E3, catalytic components of α-keto acid dehydrogenase complexes; PDC, pyruvate dehydrogenase complex; pyr, pyruvate; Ac, acetyl groups.

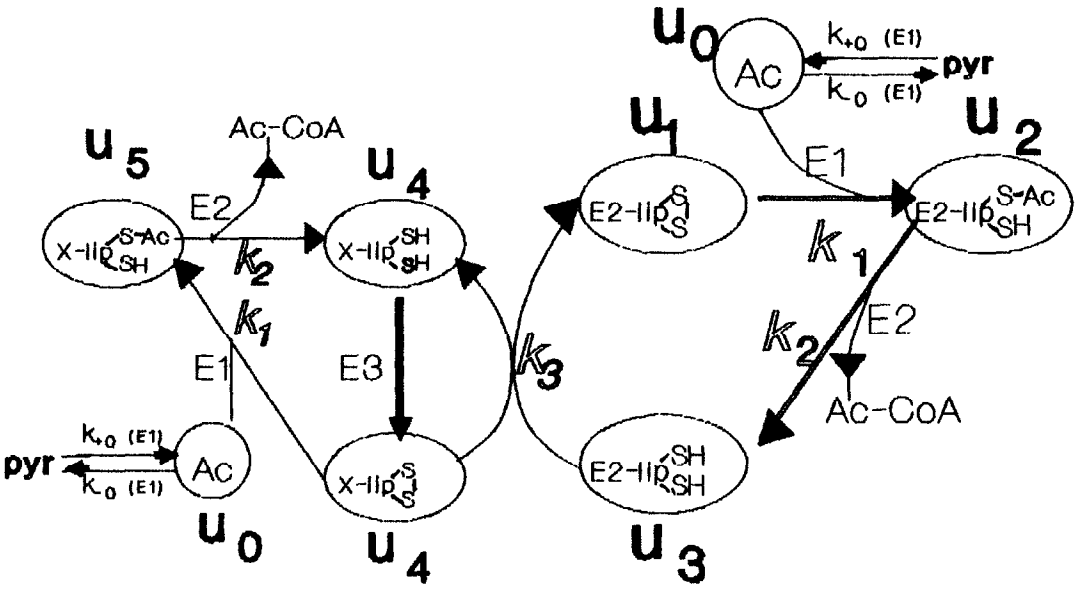

Fig. 1. The scheme for the PDC-catalyzed reaction. Oxidized, reduced, and acetylated lipoyl moieties covalently attached to the E2 and X components, as well as acetyl groups, are considered as the variables, u_0–u_5. Other participants are taken into account in the parameters, k_0–k_3. The thick line corresponds to the rapid E3-catalyzed step. The X-lipoyls, i.e. those participating in the rapid E3-catalyzed step, may be considered as a single variable, u_4, instead of two shown in the scheme. The E1 component catalyzes two first steps (k_0 and k_1) in each of two reaction pathways. Deacetylation (step k_2) proceeds in E2 active sites. Step k_3 shows the reducing equivalent transfer.

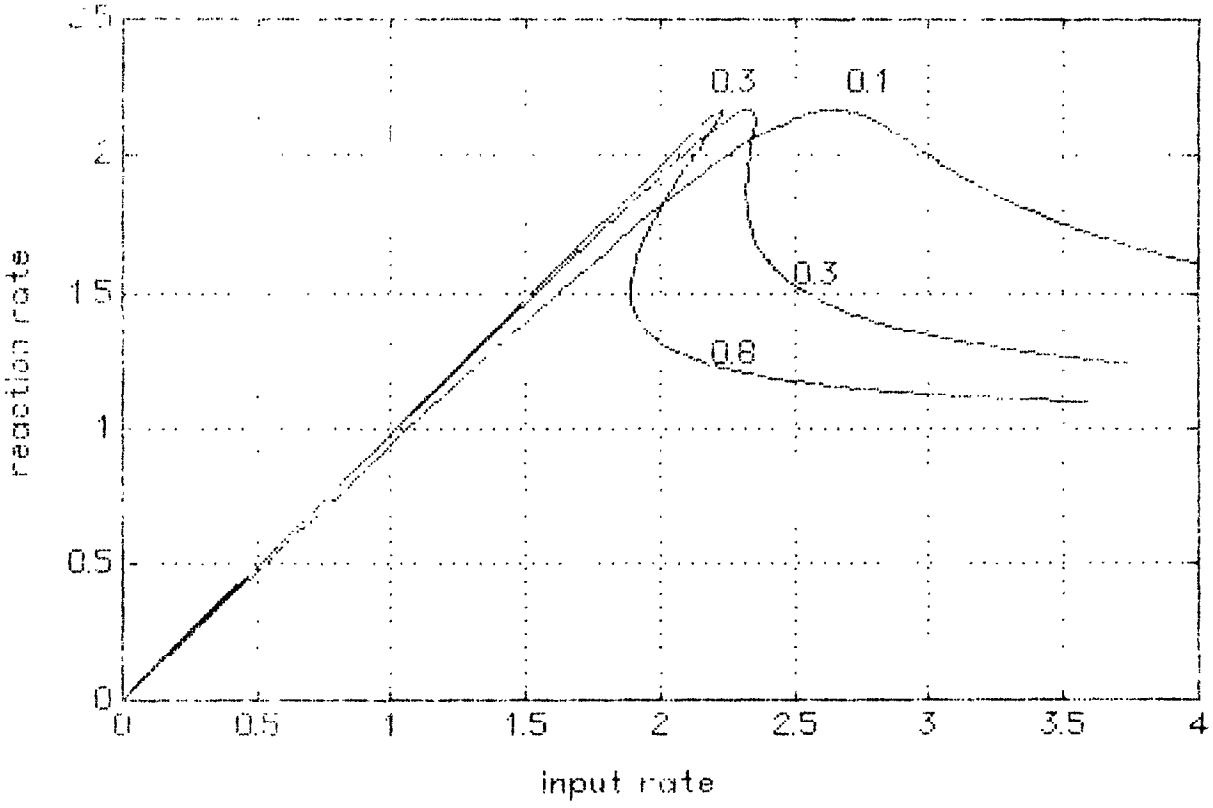

Fig. 2. Computed PDC activity as a function of relative input (pyruvate) levels. The numbers, shown on curves, representing the changeable k_1 parameter, simulate the protein kinase effect, opposite at the low and high pyruvate levels. Other parameters are the following: $k_{-0} = 0.05$, $k_3 = 0.5$, $k_2 = 1.0$.

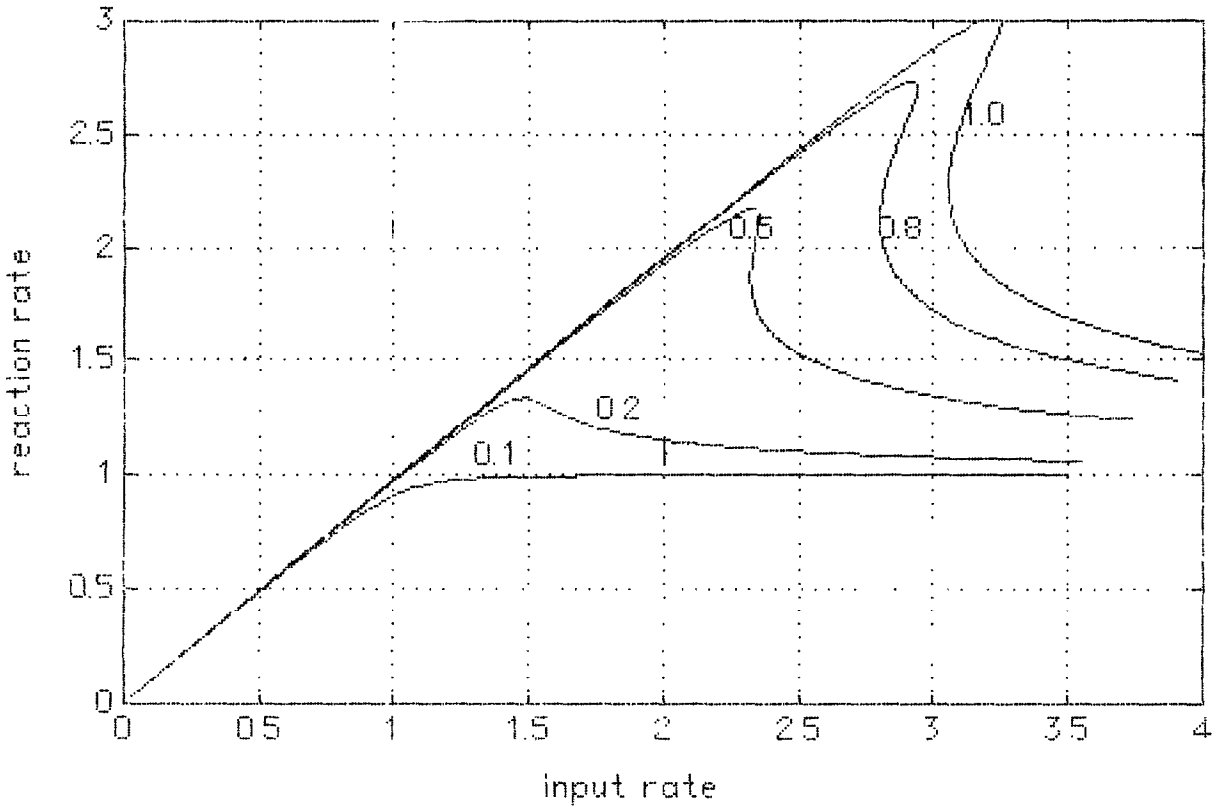

Fig. 3. Computed PDC activity as a function of relative input (pyruvate) levels. The numbers, shown on curves, representing the changeable k_3 parameter, simulate the intensity of lipoyl moiety interactions. Other parameters are the following: $k_{-0} = 0.05$, $k_1 = 0.3$, $k_2 = 1.0$.

levels. The steady-state substrate levels, u_i, were computed by putting all the derivatives in the equations as equal to zero. The result is shown with the curves in Figs. 2 and 3.

The steep activity changes shown on the curves are similar to that observed for some multi-enzyme complexes [3–5] and can be explained here as the bistability transitions. It should be noted, however, that the bistability may simply be an unusually dispersed activity value.

By means of our specific computer program, similar to that used in [10], we obtained the parameter values corresponding to the bistability phenomenon. Surprisingly, the relationship between the parameters obtained was close to the real one, as known from the literature [4,5].

It can be seen that the bistability phenomenon diminishes with decreased k_1 parameters. This effect may simulate the kinase action. The kinase, incorporated into the complex, inhibits the E1-catalyzed acetylation step, k_1 [7]. It was shown [7] that the kinase is activated by small, and inhibited by greater, pyruvate levels. Fig. 2 shows that the protein kinase, by decreasing the k_1 parameter, inhibits the PDC activity at low pyruvate levels but paradoxically activates it at higher pyruvate levels. Thus, PDC regulation via phosphorylation obtains here its new interpretation.

Fig. 3 illustrates the activity dependence on the parameter, k_3, which refers to the intensity of the electron transfer between different lipoyl moieties. In the real systems k_3 seems to be greater than the rate-limiting parameter, k_1. The bistability phenomenon disappears if $k_3 = k_1$.

4. DISCUSSION

The unusual kinetic behaviour of these complexes is explained by dividing the lipoyl moieties into two non-equivalent classes differently located in the complex (electron donators and accepters). Only one of these interacts preferentially with the E3 component to transfer electrons to NAD^+, but both may interact with the catalytic components, E1 and E2. It may be supposed that protein X in the PDC was evolutionarily specialized to bear the lipoyl moieties, interacting with the E3 component. Two kinetically different classes of lipoyl moieties were also observed in the complexes not containing protein X [4,5]. One of them may play the protein X role, thus, these complexes may be regulated in a similar way.

Protein X, being acetylated, limits the flux through the PDC to escape the acetyl-CoA overproduction under highly raised pyruvate levels. This may be the way to stabilize the energy-generating network. The

transition to the stabilized production may occur in a very steep 'cooperative' manner. Moreover, all the regulatory signals may very effectively change the kinetic curves.

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REFERENCES

[1] Perham, R.N. and Packman, L.C. (1989) Ann. NY Acad. Sci. 573, 1–20.
[2] Yeaman, S.J. (1989) Biochem. J. 257, 625–632.
[3] Bunik, V.I., Buneeva, O.A. and Gomazkova, V.S. (1990) FEBS Lett. 269, 252–254.
[4] Waskiewicz, D.E. and Hammes, G.G. (1984) Biochemistry 23, 3136–3143.
[5] Akiyama, S.K. and Hammes, G.G. (1981) Biochemistry 20, 1491–1497.
[6] Bisswanger, H. (1984) J. Biol. Chem. 259, 2457–2465.
[7] Patel, M.S. and Roche, T.E. (1990) FASEB J. 4, 3224–3233.
[8] Gopalakrishnan, S., Rahmatullah, M., Radke, G.A., Power-Greenwood, S. and Roche, T. (1989) Biochem. Biophys. Res. Commun. 160, 715–721.
[9] Lawson, J.E., Xiao-Da Niu and Reed, L.J. (1991) Biochemistry 30, 11249–11254.
[10] Goldstein, B.N. and Ivanova, A.N. (1987) FEBS Lett. 217, 212–215.