FEBS Letters 345 (1994) 151–153

FEBS 14039

Activity oscillations predicted for pyruvate dehydrogenase complexes

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Received 15 March 1994; revised version received 8 April 1994

Abstract

A kinetic model for the pyruvate dehydrogenase complex is analyzed. The model takes into account intermediate channeling through the lipoyl network attached to the complex core, as well as inter-related regulatory effects of protein X acetylation and enzyme phosphorylation. The model predicts undamped oscillations of enzyme activity.

Key words: Multi-enzyme complex; Lipoyl network; Oscillation; Kinetic model

1. Introduction

The pyruvate dehydrogenase complex (PDC) is a multi-enzyme complex having a central role in intermediary metabolism [1]. This complex has been intensively studied in many laboratories [2–4], however, its unusual kinetic behavior still needs to be understood. We have recently described a simple kinetic model [5] which explains the unusual types of kinetic 'cooperativity' observed in steady-state kinetics for the PDC [6]. We have taken into account the flexible lipoyl moieties, covalently attached to the complex core, and organized into the network to promote the intermediate transfer between multiple active sites in this complex during the catalysis [7].

In this paper we analyse a slightly modified model, which predicts, in addition to the cooperativity, the unusual time-dependent activity changes. This model for the PDC describes undamped activity oscillations observable under certain conditions in vitro. The critical role in this phenomenon of protein X and the protein kinase is demonstrated.

2. Experimental

We took into account the following experimental data from the literature to formulate the kinetic model.

(1) The mammalian PDC, the most complicated member of the family, contains multiple copies of the enzymic components, E1, E2, E3, as well as the E1-specific kinase, K, the E1-specific phosphatase, and protein X [1]. The stoichiometric relationship between the components may be variable [8–10], so we accepted some averaged relative component levels to satisfy the data available: $[E2]_0 = 10$; $[E1]_0 = 3$; $[X]_0 = 1$; $[K]_0 = 1$; where index 0 denotes the total value.

(2) The lipoyl moieties, attached to the E2 and protein X, serve as two similar but kinetically different participants in channeling acetyl groups and reducing equivalents between multiple active sites [11].

(3) All lipoyl moieties, both attached to the E2 and attached to X, may be acetylated by pyruvate with catalytic participation of the E1 [11], and all those may be deacetylated in the E2 active sites to produce Ac-CoA [12].

(4) By contrast, delivery of reducing equivalents to the E3 occurs preferentially via the lipoyl moiety of protein X [11]. It means that protein X transfers the reducing equivalents from E2 to the E3.

(5) The protein kinase, K, inhibits the E1 component by its phosphorylation [13], and this kinase itself may be activated by acetylated lipoyl moieties [13].

3. Results

We have analysed the following kinetic scheme constructed according to the experimental data available. The scheme for the overall reaction is shown in Fig. 1. As compared with our earlier scheme, presented in [5], the scheme in Fig. 1 is more detailed and includes two overall reaction pathways, one via X-lipoyls only, and another involving E2-lipoyls, as well as two reversible regulatory reactions. The regulatory inactivation of the E1 by the K is shown as the reaction step 7, and E′ refers to the inactivated form of the E1.

The well-known reversible activation of the K by acetylated protein X [14] is shown in Fig. 1 as steps 5 and 6. The E1 participates in steps 0 and 3 in its form E1-TPP-EH, delivering hydroxyethylidene, EH, to the lipoyl moieties, thereby acetylating them. The reaction product, Ac-CoA, is obtained in the E2-catalyzed steps 1 and 4. The following system of differential equations and constraint equations corresponds to the reaction scheme presented in Fig. 1.

$$v_1 = k_i u_5;\ v_0 = k_0 u_0 u_1;\ v_1 = k_1([E_2]_0 - u_1 - u_2);$$
$$v_2 = k_2 u_2 u_3;\ v_3 = k_3 u_0 u_3;\ v_4 = k_4([X]_0 - u_3);$$
$$v_5 = k_5([K]_0 - v_4)\,([X]_0 - u_3);\ v_6 = k_6 u_4;$$
$$v_7 = k_7 u_0 u_4;\ v_8 = k_8([E_1]_0 - u_0 - u_5);$$
$$du_0/dt = v_i - v_0 - v_3 - v_7 + v_8;\ du_1/dt = v_2 - v_0;$$
$$du_2/dt = v_1 - v_2;\ du_3/dt = v_4 - v_3;$$
$$du_4/dt = v_5 - v_6;\ du_5/dt = v_0 - v_i; \quad (1)$$

This system has been analysed and solved to obtain the overall reaction rate dependent on time and parame-

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Abbreviations: E1, pyruvate dehydrogenase (EC 1.2.4.1), E2, dihydrolipoamide acetyltransferase (EC 2.3.1.12), E3, dihydrolipoamide dehydrogenase (EC 1.8.1.4), catalytic components of pyruvate dehydrogenase complexes; PDC, pyruvate dehydrogenase complex; pyr, pyruvate; Ac, acetyl groups.

SSDI 0014-5793(94)00425-U

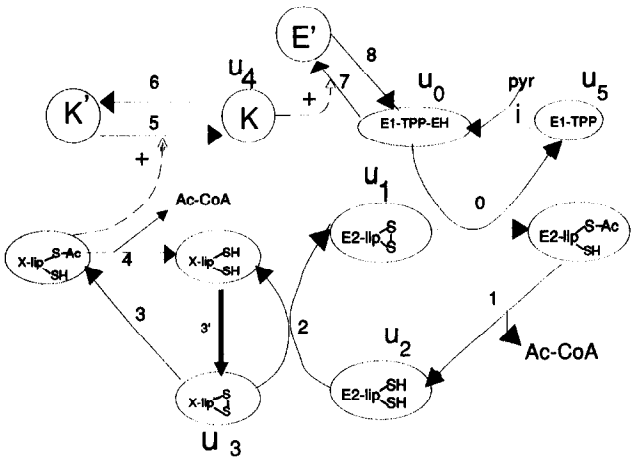

Fig. 1. The scheme of the PDC-catalyzed reaction. Oxidized, reduced, and acetylated lipoyl moieties, covalently attached to the E2 and X components, as well as activated and inactivated forms of the E1 and the kinase, K, are considered the variables, u_0–u_5. Other participants are taken into account in rate constants for steps, i, 0–8. The thick line corresponds to the rapid E3-catalyzed step. Two X-lipoyls participating in this step are considered as a single variable, u_3, because the quantity of reduced X-lipoyl is negligibly small. The E1 catalyzes two first steps in each of two reaction pathways. Step 2 shows the reducing equivalent transfer between lipoyls.

ters. The results are shown with the curves in Figs. 2 and 3. It can be seen from the curves that the oscillatory kinetic behavior may arise for certain parameter values. These parameter values, corresponding to undamped oscillations, have been found by means of the characteristic polynomial analysis performed for the linearised system [1] similar to the method of our earlier works [5,15]. The analysis shows that the parameter domain, corresponding to undamped oscillations, is rather narrow in this case. However, it may be experimentally reached simply by suitably decreasing the substrate level, usually used in great excess. In accordance with data available [9,12] and after some transformations to be applied to the steps in Fig. 1, the following parameter values have been used in our calculations:

$$k_0 = k_3 = 40\ M^{-1}\cdot s^{-1},\ k_2 = 30\ M^{-1}\cdot s^{-1},\ k_7 = 0.26\ M^{-1}\cdot s^{-1} \quad (2)$$

The rate constants for the deacetylation steps, 1 and 4, have been chosen rather small, as may be the case at unsaturating CoA levels:

$$k_1 = 7\ s^{-1};\ k_4 = 2\ s^{-1} \quad (3)$$

We chose the parameters k_1 and k_4 to be rather small and different in their values with the purpose of obtaining oscillations, shown in Figs. 2 and 3. The difference in these values is explainable, because the deacetylation of the E2 and protein X may be reasonably supposed to be different in their rates. Other parameter values used in the calculations are shown in the figure legends.

Fig. 2 shows the time-dependent activity of the PDC as calculated for different fixed parameter k_i values. Parameter k_i depends here on the pyruvate level, which is accepted to be a time-independent parameter. It is seen from Fig. 2 that both increased and decreased pyruvate levels (k_i = 10 and k_i = 20) induce similar time-dependent kinetic curves with a similar drop to the steady-state activity level. However, the intermediary pyruvate level (k_i = 14) induces drastically different kinetic behavior with undamped activity oscillations around a higher activity level. This unusual phenomenon is in good agreement with highly dispersed steady-state activity values observed for the PDC at the definite pyruvate level only [6].

Fig. 3 demonstrates another unusual phenomenon

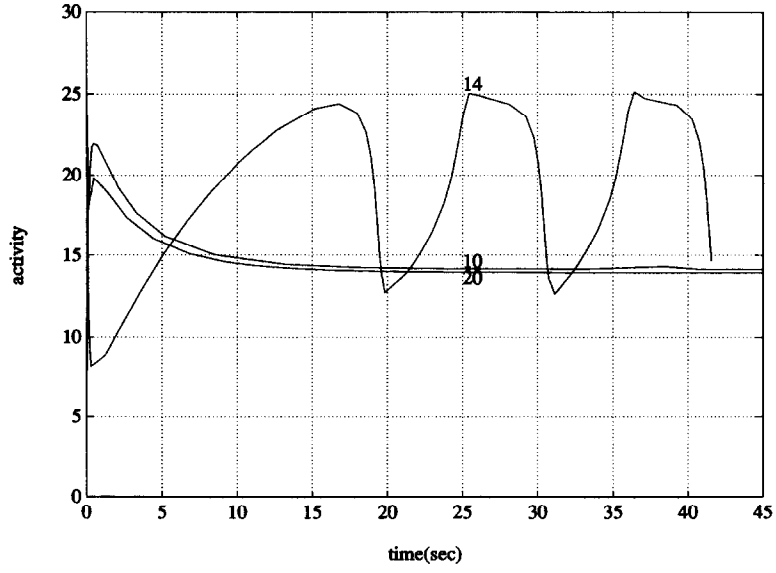

Fig. 2. Computed PDC activity (v_1 + v_4) as a function of time for different fixed k_i values, shown on the curves. Other parameters are the following: $k_0 = k_3 = 40$, $k_1 = 7$, $k_4 = 2$, $k_5 = 0.2$, $k_6 = 0.2$, $k_7 = 0.26$, $k_8 = 0.06$. The dimension scale for the first order and second order rate constants is s^{-1} and $M^{-1}\cdot s^{-1}$, respectively.

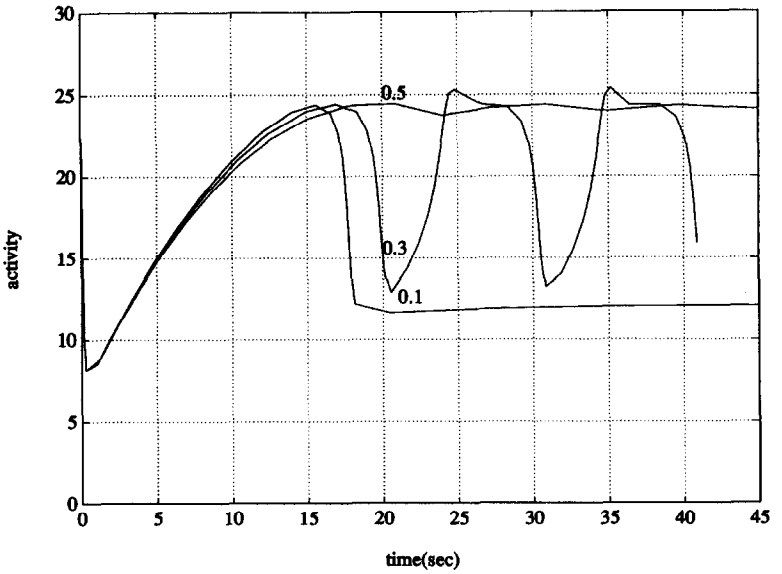

Fig. 3. Computed PDC activity as a function of time for different fixed k_7 values, shown on the curves. Other parameters are the following: $k_i = 14$; $k_0 = k_3 = 40$, $k_1 = 7$, $k_4 = 2$, $k_5 = 0.2$, $k_6 = 0.2$, $k_8 = 0.06$. The dimension scale for the first order and second order rate constants is s^{-1} and $M^{-1} \cdot s^{-1}$, respectively.

predicted by the model calculations performed for the different fixed k_7 values. The changes in the parameter k_7 values simulate the changes in the regulatory activity of the protein kinase, K. It is clearly seen from Fig. 3 that small changes in the kinase activity can induce drastic changes in the kinetic curves. The oscillatory behavior (at $k_7 = 0.3$) is replaced by the activity evolution in time to the higher stable activity level (at $k_7 = 0.5$), or to the lower stable level (at $k_7 = 0.1$). Therefore, two very different stable PDC activity levels, or stable PDC activity oscillations, may be easily obtained, induced by small signals acting through the protein kinase.

It should be noted that the increased kinase activity may induce, in this case, the increased activity of the complex. This paradoxical phenomenon is a result of two interconnected regulatory effects, the protein X acetylation and the kinase-induced phosphorylation.

4. Discussion

The pyruvate dehydrogenase complex (PDC) has recently been shown [6] to reveal unusual types of kinetic behavior, as a result of its substrate channeling through the branched lipoyl network, covalently attached to its subunits. The strongly 'co-operative' transition from one stable kinetic behavior of the PDC to another under the raised pyruvate level has been supposed to stabilize the energy-generating metabolism [5]. This paper, by including into the model the regulatory steps, predicts the unusual time-dependent behavior for the PDC, which is strongly dependent on the external regulatory signals, acting through the protein kinase as well as through the changed substrate levels. The predicted undamped activity oscillations with the period of about 10 s may be observed under suitable conditions in vitro.

The inter-relationship of the two PDC activity regulations, the faster protein X acetylation and the slower enzyme phosphorylation, do induce the undamped oscillations due to the differences in their characteristic times.

References

[1] Patel, M.S. and Roche, T.E. (1990) FASEB J. 4, 3224–3233.
[2] Severin, S.E., Khailova, L.S. and Gomazkova, V.S. (1986) Adv. Enz. Regul. 25, 347–375.
[3] Perham, R.N. and Packman, L.C. (1989) Ann. NY Acad. Sci. 573, 1–20.
[4] Yeaman, S.J. (1989) Biochem. J. 257, 625–632.
[5] Goldstein, B.N. and Selivanov, V.A. (1993) FEBS Lett. 319, 267–270.
[6] Khailova, L.S., Korochkina, L.G. and Severin, S.E. (1989) Ann. NY Acad. Sci. 573, 36–54.
[7] Collins, J.H. and Reed, L.J. (1977) Proc. Natl. Acad. Sci. USA 74, 4223–4227.
[8] Jilka, J.M., Rahmatullah, M., Kazemi, M. and Roche, T.E. (1986) J. Biol. Chem. 261, 1858–1867.
[9] Cate, R.L., Roche, T.E. and Davis, L.C. (1980) J. Biol. Chem. 255, 7556–7562.
[10] Perham, R.N. (1991) Biochemistry 30, 8501–8512.
[11] Rahmatullah, M., Radke, G.A., Andrews, P.C. and Roche, T.E. (1990) J. Biol. Chem. 265, 14512–14517.
[12] Akiyama, S.K. and Hammes, G.G. (1980) Biochemistry 19, 4208–4213.
[13] Roche, T.E. and Cate, R.L. (1976) Biochem. Biophys. Res. Commun. 72, 1375–1383.
[14] Rahmatullah, M. and Roche, T.E. (1987) J. Biol. Chem. 262, 10265–10271.
[15] Selivanov, V.A., Goldstein, B.N. and Khailova, L.S. (1993) Molecularnaya Biologia 27, 1070–1077.