Change in α-ketoglutarate dehydrogenase cooperative properties due to dihydrolipoate and NADH

V.I. Bunik, O.A. Buneeva and V.S. Gomazkova

Department of Biochemistry, Moscow State University, Moscow 119899, USSR

Received 20 June 1990

Reducing 2 SH-groups of KGD by dihydrolipoate leads to cooperativity in substrate binding. Cooperative properties of KGD in the KGD complex are modulated by NADH. Physiological significance of these observations is discussed.

α-Ketoglutarate dehydrogenase; Cooperativity; Dihydrolipoate; NADH

1. INTRODUCTION

KGD, a component of the multienzyme complex, catalyzes the first step of KG oxidative decarboxylation. The enzyme is a dimer consisting of identical subunits [1,2]. Recently we have shown two forms of KGD from pigeon breast muscle to exist [2-4]. One of them has the subunits cooperative in catalysis. This has been evidenced by their non-equivalency in substrate binding shown in the chemical modification studies [3] and higher specific activity of the monomer compared to the dimer [5]. This form is characterized by complicated initial velocity plots versus KG [6] and TPP [7] concentrations. The other form of KGD has the subunits non-interacting during the formation of the enzyme-substrate complex [2]. It also has hyperbolic dependencies of the activity versus KG and TPP concentrations [4]. It has been shown that the functional peculiarities described are correlated to the redox state of 2 SH-groups per KGD subunit [4]. Those thiols oxidized, KGD does not reveal the cooperative properties. Reducing the enzyme with DTT has led to the subunit interactions [4,8].

The present work is of interest with respect to the possibility of converting the KGD forms by cellular thiols under conditions approximated to physiological. It has been shown that the most effective agent reducing KGD thiols essential for the cooperativity is dihydrolipoate, one of the intermediates of the overall KGD reaction. The incubation of the KGD complex with NADH has been established to induce the conversion from the non-cooperative form to the cooperative one obviously through reduction of lipoic acid bound by the complex in the reaction catalyzed by the third component of the complex, lipoyl dehydrogenase. The results obtained suggest the reversible oxidation-reduction of the KGD SH-groups essential for the cooperativity being of physiological significance.

Correspondence address: V.I. Bunik, Department of Biochemistry, Moscow State University, Moscow 119899, USSR

Abbreviations: KGD, α-ketoglutarate dehydrogenase; KG, α-ketoglutarate; DTNB, 5,5'-dithio-bis-(2-nitrobenzoic) acid; DTT, dithiothreitol; DEP, diethyl pyrocarbonate; TPP, thiamine pyrophosphate; CoA, coenzyme A

2. MATERIALS AND METHODS

The chemicals used were obtained from the following sources: KG, DTT, DTNB from Serva, potassium hexacyanoferrate from Merck, NADH, NAD^+ from Boehringer, DEP, lipoic acid, sodium borohydride and CoA from Sigma, cysteine and glutathion from Reanal. Dihydrolipoate was obtained by the reduction of lipoic acid with borohydride according to [9]. The compounds used were of the finest grade available.

KGD was isolated from pigeon breast muscle as in [8,10]. KGD activity was determined with the artificial electron acceptor ferricyanide [3]. The KGD complex was obtained simultaneously with the isolation of the individual KGD. After the elution of non-ligated KGD from the column with calcium phosphate on cellulose [10] the non-dissociated complex was isolated according to [11] with the addition of chromatography on Sepharose 6B as the last stage. The activity of the KGD complex was assayed by monitoring the NAD^+ reduction [6]. SH-groups of native KGD and KGD denatured by 8 M urea were titrated with DTNB as in [8]. The reduction of KGD (8-12 mkM) was performed by its incubation with the thiol containing compounds in 0.1 M potassium phosphate buffer pH 7.0. The thiol excess was removed by gel-centrifugation chromatography as in [8]. The enzyme inactivation with DEP was performed according to [3].

3. RESULTS

Fig. 1 shows the results of the incubation of non-cooperative KGD with different thiols. As can be seen, under the conditions used the increase in KGD SH-groups occurs only in the presence of dihydrolipoate. DTT having similar redox properties does not reduce KGD at neutral pH even at 10 mM concentration,

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50 © 1990 Federation of European Biochemical Societies

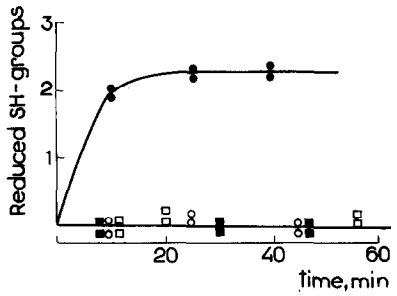

Fig. 1. The number of SH-groups reduced in KGD by dihydrolipoate (•), glutathione (○), cysteine (■) and CoA (□). The concentration of SH-groups of thiols used is 0.2 mM in all the cases.

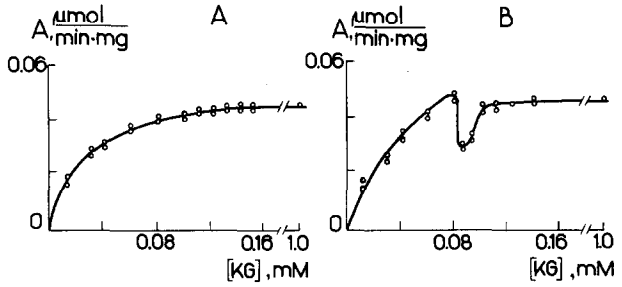

Fig. 3. The plots of the KGD activity versus KG concentration before (A) and after (B) the reduction by dihydrolipoate.

which argues for the specificity of the dihydrolipoate effect.

The chemical modification of KGD by DEP allow to see the subunit interactions when KG binding [3] indicates the cooperativity of the active sites of KGD reduced by dihydrolipoate. Fig. 2A shows, that before the reduction all the KGD subunits are equivalent both in the absence of KG (line 1) and after the enzyme-substrate complex formation (line 2). The treatment with dihydrolipoate brings about the biphasic inactivation by DEP in the presence of KG (Fig. 2B, curve 2). It indicates that only one half of the subunits of the reduced KGD is able to form the enzyme-substrate complex rapidly. Decreased rate of their inactivation corresponds to the slow phase of the process. The other half of the subunits is inactivated with such a rate only after 5 min preincubation with KG (Fig. 2B, line 3). This is evident about their interaction with the substrate being slow enough. The inactivation of reduced KGD deleted from the KG excess by gel-filtration is biphasic, which indicates the tight binding of KG in one half of the active sites of the reduced enzyme. Thus, reducing KGD by dihydrolipoate leads to the cooperativity of the KGD active sites in the substrate binding, which is displayed in the different rates of formation and different stabilities of enzyme-substrate complexes in two subunits of the KGD dimer.

Reducing KGD by dihydrolipoate changes also the plots of initial velocity vs KG concentration: hyperbola is transformed into a curve possessing the maximum and minimum (Fig. 3). Such a complex curve has been shown to be inherent in cooperative KGD [6,8].

The titration of the KGD SH-groups revealed that the change in the cooperative properties due to dihydrolipoate, as in the experiments with DTT [8], were correlated with the reduction of 2 thiols: the most reactive to DTNB in native KGD one and a buried one.

The specificity of dihydrolipoate in the reduction of KGD thiols essential for the cooperativity suggested to us that conversion of the KGD forms might be possible in the KGD complex, if lipoic acid of the complex is reduced. To test this assumption we studied the change in the properties of integration into the complex KGD due to NADH, that reduces lipoic acid bound by the complex through the reaction catalyzed by the third component of the KGD complex, lipoyl dehydrogenase. The multienzyme complex was incubated with 10 mkM NADH for 30 min at room temperature. Decreasing KGD activitity of the complex by DEP is shown in Fig.

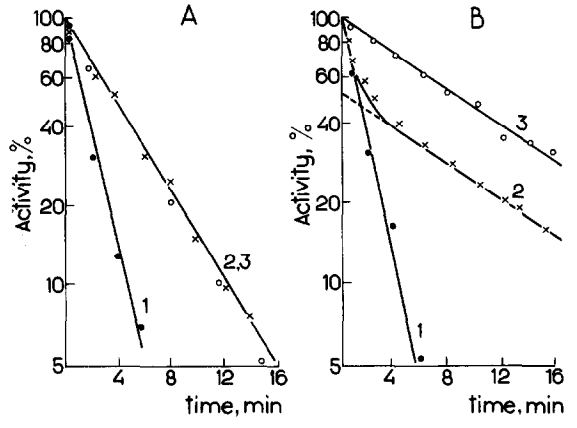

Fig. 2. The KGD inactivation by DEP (0.4 mM) before (A) and after (B) the reduction by dihydrolipoate. 1, without KG, 2, with 1 mM KG, 3, 1 mM KG was preincubated with KGD for 5 min.

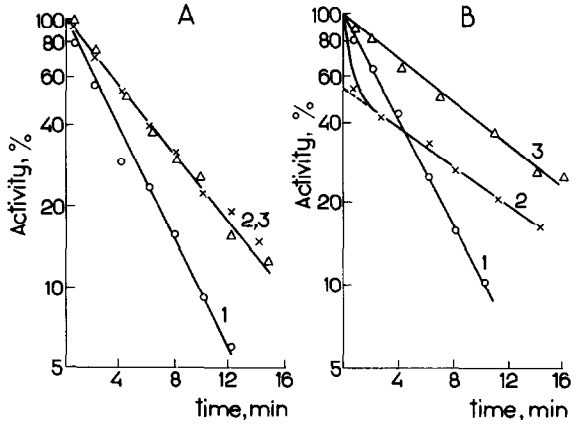

Fig. 4. The inactivation of KGD in the complex by DEP (0.4 mM) before (A) and after (B) the NADH treatment. 1, without KG, 2, with 1 mM KG, 3, 1 mM KG was preincubated with the KGD complex for 5 min.

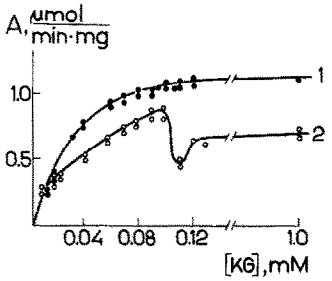

Fig. 5. The plots of the activity of KGD in the complex versus KG concentration before (1) and after (2) the NADH treatment of the complex.

4. It is obvious, that before NADH treatment all the active sites are equivalent both in the free state and in the presence of KG (Fig. 4A). The incubation of the complex with NADH results in the cooperativity of the KGD subunits in the substrate binding (Fig. 4B). Fig. 5 shows that the incubation with NADH leads also to the complicated plots of the KGD activity vs KG concentration, indicative of the cooperative form of the enzyme.

Comparing the data shown in Figs. 2 and 4 and Figs. 3 and 5 we may conclude that the reduction of individual KGD by dihydrolipoate induces the same changes in its cooperative properties as the incubation of KGD in the complex with NADH. The control experiments have shown that the same treatment of individual KGD with NADH has no influence on the activity and interaction of the enzyme active sites. Thus, it may be concluded that the effect of NADH upon KGD in the complex involves the reducing action of dihydrolipoate, arising from NADH oxidation by lipoyl dehydrogenase.

4. DISCUSSION

The in vivo modification of cysteine residues of proteins through the reaction of thiol-disulfide exchange with cellular thiols and disulfides draws the attention of researchers as a possible way of regulating enzymes in response to altering the energy status of a cell [12,13]. The KGD complex catalyzing the formation of macroergic compound - succinyl CoA - and NADH could be regulated in such a way. The results described here show that the cooperative properties of KGD catalyzing the first and rate-limiting stage of KG oxidative decarboxylation are controlled by NADH. Thus, the functional peculiarities of KGD may depend on the redox state of the cell.

Figs 2 and 4 show, that as a result of the increase in reductive equivalents in the medium one of the dimer subunits binds the substrate slowly enough compared to the rate of KGD reaction. Therefore at the particular moment of time only one of the dimer subunits is catalytically competent. It must lead to the decrease in the specific activity of KGD. In fact, the maximal velocity of cooperative KGD after the incubation of the KGD complex with NADH is about two-fold less, than that of the initial, non-cooperative, enzyme (Fig. 5). This is consistent with our findings on immobilized cooperative KGD: the dimer has been shown to be less active than the independently functioning monomer [5]. The absence of the change in the maximal velocity after reducing individual KGD (Fig. 3) may be explained by the concomitant reduction of non-specifically oxidized thiols proceeding to some extent in this case and increasing the KGD activity. The decrease in KGD activity due to NADH - the overall reaction product - is justified from the physiological point of view and reflects feedback regulation. But it is not the single consequence of converting the non-cooperative form into the cooperative one. As described here and in previous publications [3,7,8], the enzyme with interacting sites is able to bind the substrate and the cofactor more tightly, which may be important for KGD functioning in the cell. Finally, complicated kinetic behavior (Figs 3,5), inherent also in KGD integrated into the complex, may be the evidence of additional possibilities of KGD regulation by changing the substrate concentration.

Thus, reversible oxidation-reduction of the KGD thiols is the regulatory mechanism of switching the cooperativity of KGD active sites essential for a number of KGD properties. The results obtained argue for the concentration of NADH to be a factor determining the realization of such a mechanism in the cell.

REFERENCES

[1] Severin, S.E., Gomazkova, V.S. and Krasovskaja, O.E. (1974), Dokl. Acad. Nauk SSSR 218, 719-721.
[2] Gomazkova, V.S., Bunik, V.I. and Buneeva, O.A. (1987), Biochimija 52, 1144-1149.
[3] Bunik, V.I. and Gomazkova, V.S. (1985), Biochimija 50, 1668-1675.
[4] Bunik, V.I., Buneeva, O.A., Lvova, N.B. and Gomazkova, V.S. (1989), Biochem. Int. 18, 561-571.
[5] Gomazkova, V.S., Kologrivova, O.A. and Severin, S.E. (1985), Biochem. Int. 10, 917-925.
[6] Severin, S.E., Gomazkova, V.S., Krasovskaja, O.E. and Stafeeva, O.A. (1978), Biochimija 43, 2241-2248.
[7] Gomazkova, V.S., Stafeeva, O.A., Laufer, A.I. and Severin, S.E. (1981), Dokl. Acad. Nauk SSSR 259, 477-480.
[8] Bunik, V.I., Buneeva, O.A. and Gomazkova, V.S. (1990), Biochem. Int. (in press).
[9] Suzuki, K. and Reed, L.J. (1963) J. Biol. Chem. 238, 4021-4025.
[10] Severin, S.E. and Gomazkova, V.S. (1971), Biochimija 36, 1099-1106.
[11] Massey, V. (1960), Biochim. Biophys. Acta 38, 447-460.
[12] Gilbert, H.F. (1984), Meth. Enz. 107, 330-351.
[13] Ziegler, D.M. (1985), Annu. Rev. Biochem. 54, 305-329.