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MINI-REVIEW

Short-Term Regulation of the α-Ketoglutarate Dehydrogenase Complex by Energy-Linked and Some Other Effectors

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Abstract—The question of regulation of α-ketoglutarate dehydrogenase complex (KGDHC) has been considered in the biochemical literature very rarely. Moreover, such information is not usually accurate, especially in biochemical textbooks. From the mini-review of research works published during the last 25 years, the following basic view is clear: a) animal KGDHC is very sensitive to ADP, P_i, and Ca^{2+}; b) these positive effectors increase manifold the affinity of KGDHC to α-ketoglutarate; c) KGDHC is inhibited by ATP, NADH, and succinyl-CoA; d) the ATP effect is realized in several ways, probably mainly via opposition versus ADP activation; e) NADH, besides inhibiting dihydrolipoamide dehydrogenase component competitively versus NAD^+, decreases the affinity of α-ketoglutarate dehydrogenase to substrate and inactivates it; f) thioredoxin protects KGDHC from self-inactivation during catalysis; g) bacterial and plant KGDHC is activated by AMP instead of ADP. These main effects form the basis of short-term regulation of KGDHC.

Key words: α-ketoglutarate dehydrogenase, 2-oxoglutarate dehydrogenase, complex, regulation

α-Ketoglutarate dehydrogenase complex (KGDHC) occupies an important position in mitochondrial metabolism as a key enzyme of the tricarboxylic acid cycle [1]. Three enzymes are considered as regulatory points of the cycle: citrate synthase, NAD^+-dependent isocitrate dehydrogenase, and KGDHC [2-10]. The first two are interpreted as allosteric enzymes sensitive to the adenine nucleotide, ADP and ATP. The literature has especially emphasized the activation of NAD^+-isocitrate dehydrogenase by ADP via increasing of the enzyme affinity to substrate [2-9]. However, as to KGDHC, the educational literature usually shows that the multienzyme complex is inhibited by its end products, succinyl-CoA and NADH. Rarely is it added that ATP inhibits KGDHC [2, 3], and Ca^{2+} activates the complex [4, 8]. Unfortunately, such information seems to be very incomplete.

It should be noted that in general the regulation of KGDHC receives less attention than the control of the activity of pyruvate dehydrogenase complex (PDHC), which has a similar structure and catalytic mechanism [11, 12]. Probably the exceptional metabolic position of PDHC and the availability of the attractive mechanism of PDHC regulation via phosphorylation–dephosphorylation have significance. However, KGDHC also plays a very important role as an enzyme limiting the flux of the tricarboxylic acid cycle [13]. Its substrate, α-ketoglutarate, is at the branch point connecting the Krebs cycle with amino acid metabolism. The products of the KGDHC catalyzed reaction, NADH and succinyl-CoA, are important bioenergetic molecules. A disturbance in function of the multienzyme complex in nerve tissue leads, for example, to neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [14]. It is logical that KGDHC has numerous regulatory possibilities.

KGDHC is a multicomponent enzyme system that catalyzes the conversion of α-ketoglutarate, coenzyme A (CoA), and NAD^+ into succinyl-CoA, NADH, and CO_2 [1-10]. The structural core of mammalian KGDHC is composed of 24 identical lipoate-containing subunits of dihydrolipoamide succinyl transferase (E2) arranged with octahedral symmetry [15]. Associated with E2 are six homodimers of thiamine pyrophosphate-containing α-ketoglutarate dehydrogenase (E1) and six homodimers of FAD-containing dihydrolipoamide dehydrogenase (E3) [14, 15].

Regulation of KGDHC is generally realized via allosteric interactions. About 25 years ago, kinetic investigations of KGDHC from pig heart [16], pigeon breast muscle [17], and bovine adrenals [18] and kidney [19] showed that the multienzyme complex is very sensitive to

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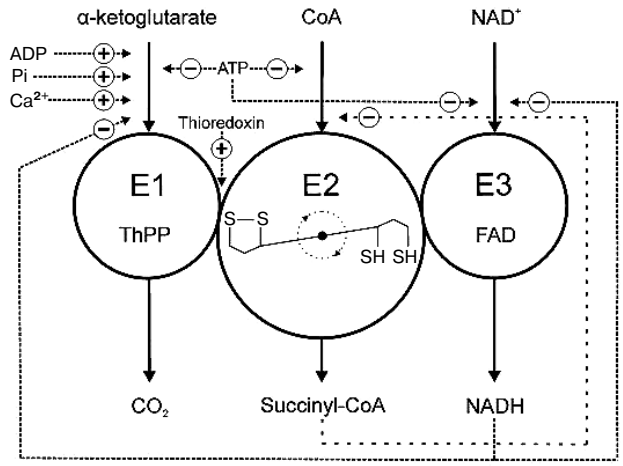
adenine nucleotides, especially ADP. According to the literature, ADP significantly decreases K_m (or $S_{0.5}$) values for α-ketoglutarate without any remarkable change in the maximum rate of the reaction catalyzed by KGDHC. Later studies of KGDHC isolated from varied animal sources [20] and the human heart [21] confirm the effects of ADP. For example, in the case of KGDHC from rat heart mitochondrial extracts ADP caused a 7-fold decrease in $S_{0.5}$ value for α-ketoglutarate [22]. So, this positive effector strongly increases the affinity of KGDHC to its main substrate. Indubitably, it is an allosteric mechanism because ADP and α-ketoglutarate have very different chemical structures. According to Rodriguez-Zavala et al., Mg^{2+} potentiates the effect of ADP because the Mg-ADP complex may be the true activating molecule [23]. There is also additional evidence that ADP action has an allosteric nature: desensitization of α-ketoglutarate dehydrogenase to ADP without change in the enzyme activity has been obtained [24, 25]. For the desensitization, treatment of the enzyme with 2,3-butanedione (a known modifier of arginine) was used. The study of the dependence of the initial KGDHC reaction rate on ADP concentration when the concentration of α-ketoglutarate was nonsaturating showed a sigmoid curve as a kinetic attribute of positive homotropic cooperativity of the effector binding sites [26]. An experiment in which radioactively labeled ADP was incorporated into KGDHC confirmed the phenomenon [27]. The positive cooperativity of ADP binding probably has a regulatory significance because it increases the sensitivity of KGDHC to changes in ADP concentration in the range 0.01-0.20 mM, which is usual in animal tissues [3].

Like ADP, inorganic orthophosphate (P_i) decreases the K_m value for α-ketoglutarate but has no effect on V_{max} [28]. For example, in the presence of P_i the K_m value of KGDHC from bison heart for α-ketoglutarate was about 12 times lower than that in the absence of orthophosphate [29]. P_i can also increase the V_{max} of the reaction catalyzed by pig heart KGDHC [23] but the effect of P_i on K_m for α-ketoglutarate is much larger. It is known that P_i together with adenine nucleotides contributes to phosphorylation potential [ATP]/[ADP]·[P_i], which reflects the energetic state of cells and cell organelles [3]. High concentrations of ADP and P_i together with low ATP level indicate a low phosphorylation potential and energetic state; on the other hand, high ATP concentrations together with low ADP and P_i levels indicate the inverse. Naturally, ATP differs from ADP and P_i with respect to its effect on KGDHC. It has been shown that ATP inhibits KGDHC from pig heart [16] and bovine kidney [19] at suboptimal α-ketoglutarate concentration. In the case of KGDHC from pigeon breast muscle [17] and bovine adrenals [18], the effect of ATP was rather weak at saturating concentrations of all needed cofactors. However, when the dependence of the initial reaction rate on NAD^+ and CoA concentrations was investigated, ATP showed a mixed type of KGDHC inhibition with respect to the substrates-coenzymes [18], probably because all three substances contain the similar adenylate fragment. At nonsaturating concentrations of α-ketoglutarate, ATP also markedly inhibited the KGDHC due to chelation of divalent ions required for activity of the multienzyme complex. Moreover, ATP opposed competitively the activating action of the positive effector, ADP [30]. It should be noted that AMP, which is a strong positive effector for bacterial [31] and plant [32] α-ketoglutarate dehydrogenase, has no influence on animal KGDHC affinity to its substrate. In the latter case components reflecting the phosphorylation potential (P_i, ADP, ATP) rather than the energy charge (according to Atkinson) taking into account AMP [3] are significant. In comparison with adenine nucleotides, guanine nucleotides are remarkably less effective [16].

Interesting results have been obtained in studies of the regulatory possibility of NADH. It was stated that NADH can inhibit KGDHC on both dihydrolipoamide dehydrogenase (E3) and α-ketoglutarate dehydrogenase (E1) component levels [17, 19, 26]. The first way is based rather on competition of NADH versus NAD^+, but the second mechanism certainly has an allosteric nature. Namely, NADH increases K_m value for α-ketoglutarate and slightly decreases V_{max} of the KGDHC catalyzed reaction [17, 26]. In addition, the presence of NADH in a reaction medium leads to an extension of the α-ketoglutarate concentration range, into which the positive cooperativity of the substrate binding sites takes place in the absence of P_i and ADP [26]. Strong inhibitory effect of NADH on the activity of the α-ketoglutarate dehydrogenase component (E1) resolved from bovine kidney KGDHC was shown by Lawlis and Roche [19]. On the other hand, NAD^+ prevented NADH inhibition of the component E1. The results constitute evidence that besides ADP and ATP, NADH and NAD^+ bind to the α-ketoglutarate dehydrogenase component too [19].

Very interesting results were obtained by Bunik et al. in studies of the possible mechanism of NADH influence on the function of KGDHC from pigeon breast muscle [33]. The authors established that NADH even inactivates E1 component of KGDHC during catalysis in the presence of α-ketoglutarate and CoA. The effect of NADH is realized via reduction of lipoate of the E2 component in the backward reactions, and this induces time-dependent inactivation of E1 and cooperativity among the active centers of the first component of KGDHC. This leads to hysteretic behavior of KGDHC, and a complication of its kinetics. Dihydrolipoate-mediated inactivation of E1 is observed at low NAD^+ concentration, and it is irreversible [33]. Recent studies have shown that generation of intrinsic radical species is accompanied by inactivation of the enzyme [34]. The irreversible character of the NADH inhibition is not very "elastic" as a regulatory mechanism, yet it seems to be real. In any case, NADH strongly influences the activity of KGDHC. Thus, in reg-



Short-term regulation of animal α-ketoglutarate dehydrogenase complex by its main effectors

ulation of KGDHC by its end product, NADH, the principle of negative feedback is realized twofold: short feedback exists on the dihydrolipoamide dehydrogenase level, and a longer one is on the α-ketoglutarate dehydrogenase level. The other end product of the α-ketoglutarate dehydrogenase reaction, succinyl-CoA, has an inhibitory effect on KGDHC too [1-4]. The inhibition is competitive versus CoA and realized mainly in active centers of dihydrolipoamide succinyl transferase [35].

Besides ADP and P_i, calcium ions are strong activators for KGDHC from varied vertebrate tissues [16, 19, 20, 22, 28, 36], but not for the multienzyme complex from insect flight muscle, or potato, or *E. coli* [20]. Ca^{2+} was shown to activate the α-ketoglutarate dehydrogenase component of the complex by markedly decreasing the K_m value of the enzyme for its substrate. It should be noted that low micromolar Ca^{2+} concentrations are effective. Ca^{2+} binds to pig KGDHC with stoichiometry of 3-4 mol of Ca^{2+} per 1 mol of the complex [22]. According to McCormack and Denton, these observations indicate that Ca^{2+} is an important regulator of intramitochondrial oxidative metabolism, at least in vertebrate tissues, because it activates both KGDHC and NAD^+-isocitrate dehydrogenase [20].

In the same way as Ca^{2+}, manganese ions appreciably decreased the $S_{0.5}$ value of bison heart KGDHC for α-ketoglutarate without any notable changes in the maximum reaction rate [37]. The KGDHC was sensitive to Mn^{2+} in a wide range of its concentrations beginning with 1-2 μM. However, calcium ions seem to be more obvious regulators of KGDHC than Mn^{2+} and other cations (Sr^{2+}, Zn^{2+}, Cu^+, K^+) because it is Ca^{2+}, which as a second messenger, is transferred into and out of mitochondria under hormonal control [1-4, 22]. It should be added that H^+ ions also favor the higher affinity of KGDHC to α-ketoglutarate [19, 20, 38].

Recently, control of KGDHC function by thiols has received wide attention [39, 40] because the redox state of the complex-bound lipoate seems to be very important. Namely, mitochondrial thioredoxin has been shown to protect KGDHC from self-inactivation during catalysis at low NAD^+ concentrations [40]. The protective function of thioredoxin may be essential in aspect of short-term control of KGDHC activity. On this theme, a splendid review was published by Bunik in 2003 [41].

There are also publications about the role of thiamine pyrophosphate (ThPP) in the regulation of KGDHC from European bison heart [42]. In this case, KGDHC was saturated with endogenous tightly bound ThPP as coenzyme, but the addition of exogenous ThPP to the reaction medium led to a marked increase in KGDHC affinity to α-ketoglutarate. Moreover, exogenous ThPP changed the circular dichroism spectrum and lowered the fluorescence emission [43], perhaps indicating its allosteric action. Further studies are required to determine whether the action occurs through binding of ThPP and ADP at partially shared sites. The ThPP effect calls for investigation of KGDHC from other sources in this aspect.

This mini-review does not aspire to be a very detailed presentation of the theme, but it should sufficiently convince that KGDHC has rich regulatory mechanisms. Participation of the main effectors in regulation of KGDHC is summarized in the figure. The necessary minimum of knowledge about control of KGDHC activity for persons studying biochemistry must contain the following information: ADP, P_i, and Ca^{2+} are positive effectors increasing the affinity of the α-ketoglutarate dehydrogenase component to its substrate, while ATP, NADH, and succinyl-CoA are inhibitors of KGDHC. Moreover, mitochondrial thioredoxin efficiently protects KGDHC from its para-catalytic inactivation. It would be good if the authors of biochemical textbooks and academic lecturers remembered these facts.

REFERENCES

1. Khailova, L. S., and Gomazkova, V. S. (1986) *Biokhimiya*, **51**, 2054-2074.
2. Campbell, M. K., and Farrell, S. O. (2003) *Biochemistry*, 4th Edn., Thomson Learning, USA.
3. Stryer, L. (2002) *Biochemistry*, 5th Edn., W. H. Freeman, New York.
4. Nelson, D. L., and Cox, M. M. (2000) *Lehninger Principles of Biochemistry*, 3rd Edn., Worth Publishers, USA.
5. Zubay, G. L. (1998) *Biochemistry*, 4th Edn., Mc Graw-Hill, Boston.
6. Grisham, C. M., and Garrett, R. H. (1998) *Biochemistry*, 2nd Edn., Brooks Cole, USA.
7. Matthews, H. R., Freedland, R. A., and Miesfeld, R. L. (1997) *Biochemistry: a Short Course*, John Wiley & Sons, USA.

8. Devlin, T. M. (1997) *Textbook of Biochemistry*, 4th Edn., Wiley-Liss, USA.
9. Hames, B. D., Hooper, N. M., and Houghton, J. D. (1997) *Instant Notes in Biochemistry*, Bios Scientific Publishers, Oxford, England.
10. Price, N. C., and Stevens, L. (2001) *Fundamentals of Enzymology*, 3rd Edn., University Press, Oxford.
11. Patel, M. S., and Korotchkina, L. G. (2003) *BAMBED*, **31**, 5-15.
12. Harris, R. A., Bowker-Kinley, M. M., Huang, B., and Wu, P. (2002) *Advan. Enzyme Regul.*, **42**, 249-259.
13. Cooney, G. J., Taegtmeyer, H., and Newsholme, E. A. (1981) *Biochem. J.*, **200**, 701-703.
14. Gibson, G. E., Park, L. C. H., Sheu, K.-F. R., Blass, J. P., and Calingasan, N. Y. (2000) *Neurochem. Int.*, **36**, 97-112.
15. Yeaman, S. J. (1989) *Biochem. J.*, **257**, 625-632.
16. McCormack, J. G., and Denton R. M. (1979) *Biochem. J.*, **180**, 533-544.
17. Gomazkova, V. S., and Krasovskaya, O. E. (1979) *Biokhimiya*, **44**, 1126-1136.
18. Strumilo, S. A., Vinogradov, V. V., and Senkevich, S. B. (1980) *Ukr. Biochem. J.*, **52**, 321-324.
19. Lawlis, B. V., and Roche, T. E. (1981) *Biochemistry*, **20**, 2512-2524.
20. McCormack, J. G., and Denton, R. M. (1981) *Biochem. J.*, **196**, 619-624.
21. Ostrovtsova, S. A., and Strumilo, S. A. (1990*) Biomed. Biochim. Acta*, **49**, 515-517.
22. Nichols, B. J., Rigoulet, M., and Denton, R. M. (1994) *Biochem. J.*, **303**, 461-465.
23. Rodriguez-Zavala, J. S., Pardo, J. P., and Moreno-Sanchez, R. (2000) *Arch. Biochem. Biophys.*, **379**, 78-84.
24. Stafeeva, O. A., Gomazkova, V. S., and Severin, S. E. (1980) *Doklady AN USSR*, **125**, 497-499.
25. Strumilo, S. A., Taranda, N. I., and Vinogradov, V. V. (1984) *Biomed. Biochim. Acta*, **43**, 237-240.
26. Strumilo, S. A., Taranda, N. I., and Vinogradov, V. V. (1982) *Biokhimiya*, **47**, 724-732.
27. Strumilo, S. A., Taranda, N. I., and Vinogradov, V. V. (1983) *Doklady AN BSSR*, **27**, 269-271.
28. Strumilo, S. A., Taranda, N. I., and Vinogradov, V. V. (1981) *Biokhimiya*, **46**, 156-161.
29. Markiewicz, J., and Strumilo, S. (1995) *Acta Biochim. Polon.*, **42**, 339-346.
30. Strumilo, S. A. (1988) *Voprosy Med. Khim.*, **34**, 2-7.
31. Weitzman, P. D. J. (1972) *FEBS Lett.*, **22**, 323-326.
32. Wedding, R. T., and Black, M. K. (1971) *J. Biol. Chem.*, **246**, 1638-1643.
33. Bunik, V. I., Buneeva, O. A., and Gomazkova, V. S. (1990) *FEBS Lett.*, **269**, 252-254.
34. Bunik, V., and Sievers, C. (2002) *Eur. J. Biochem.*, **269**, 5004-5015.
35. Smith, C. M., Bryla, J., and Williamson, J. R. (1974) *J. Biol. Chem.*, **249**, 1497-1505.
36. Strumilo, S. A. (1983) *Ukr. Biochem. J.*, **55**, 415-419.
37. Markiewicz, J., and Strumilo, S. (1997) *Biochem. Arch.*, **13**, 127-129.
38. Pawelczyk, T., and Angielski, S. (1984) *Acta Biochim. Polon.*, **31**, 289-305.
39. Bunik, V. I., Buneeva, O. A., and Gomazkova, V. S. (1990) *Biochem. Int.*, **21**, 873-881.
40. Bunik, V., Follmann, H., and Bisswanger, H. (1997) *Biol. Chem.*, **378**, 1125-1130.
41. Bunik, V. I. (2003) *Eur. J. Biochem.*, **270**, 1036-1042.
42. Strumilo, S., and Markiewicz, J. (1995) *Biochem. Mol. Biol. Int.*, **37**, 101-106.
43. Strumilo, S., Czygier, M., Kondracikowska, J., Dobrzyn, P., and Czerniecki, J. (2002) *J. Mol. Struct.*, **614**, 221-226.