

Articles

Often Ignored Facts about the Control of the 2-Oxoglutarate Dehydrogenase Complex

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Information about the control of the activity of the 2-oxoglutarate dehydrogenase complex (OGDHC), a key enzyme in the citric acid cycle, is not well covered in the biochemical education literature, especially as it concerns the allosteric regulation of OGDHC by adenine nucleotide and orthophosphate. From experimental work published during the last 25 years, the following basic view is clear: (a) animal OGDHC is very sensitive to ADP, P_i , and Ca^{2+} ; (b) these positive effectors increase the affinity of OGDHC to 2-oxoglutarate; (c) OGDHC is inhibited by ATP, NADH, and succinyl-CoA; (d) the ATP effect is realized mainly via opposition to ADP activation; (e) NADH, in addition to inhibiting the dihydrolipoamide dehydrogenase component of the enzyme complex (competitively versus NAD^+) decreases the affinity of 2-oxoglutarate dehydrogenase to its substrate; (f) bacterial and plant OGDHC are activated by AMP instead of ADP. These main effects form the basis of short term regulation of OGDHC. It is desirable that such information should reach biochemistry students.

Keywords: 2-Oxoglutarate dehydrogenase, α -ketoglutarate dehydrogenase, allosteric regulation, adenine nucleotides, phosphate, citric acid cycle.

2-Oxoglutarate dehydrogenase complex (OGDHC),¹ along with citrate synthase and NAD^+ -dependent isocitrate dehydrogenase, is considered as a regulatory point in the control of the citric acid cycle [1–10]. The last two enzymes are allosterically regulated by ADP and ATP. The literature has especially emphasized the activation of NAD^+ -isocitrate dehydrogenase by ADP, which increases the affinity of the enzyme for its substrate [1–10]. However, the regulatory role of OGDHC has not been appreciated because biochemical textbooks usually show that the multi-enzyme complex is inhibited only by its end products, succinyl-CoA and NADH. Rarely is it added that ATP inhibits OGDHC [2, 3], whereas Ca^{2+} activates the complex [1, 4, 8].

It should be noted that OGDHC plays a very important role in limiting the flux of the citric acid cycle [11]. A disturbance in its function in nervous tissue leads, for example, to neurodegenerative disorders [12]. The multi-component enzyme system catalyzes the conversion of 2-oxoglutarate, coenzyme A, and NAD^+ into succinyl-CoA, NADH, and CO_2 [1–10]. The structural core of mammalian OGDHC is composed of 24 identical lipoate-containing subunits of dihydrolipoamide succinyltransferase (E2) arranged with octahedral symmetry [13]. Associated

with E2 are six homodimers of thiamine pyrophosphate-containing 2-oxoglutarate dehydrogenase (E1) and six homodimers of FAD-containing dihydrolipoamide dehydrogenase [12, 13]. Control of OGDHC activity is realized mainly via direct allosteric interactions.

Energy-linked Effectors—About 25 years ago investigations of the kinetic properties of OGDHC from pig heart [14], pigeon breast muscle [15], bovine adrenals [16], and kidney [17, 18] showed that the multi-enzyme complex is very sensitive to adenine nucleotides, especially ADP. ADP significantly decreases the K_m (or $S_{0.5}$) for 2-oxoglutarate without any remarkable change in the maximum rate of the reaction catalyzed by OGDHC (Fig. 1). Other studies of OGDHC isolated from varied animal sources [19] and the human heart [20] confirm the effects of ADP. For example, in the case of OGDHC from rat heart mitochondria ADP caused a 7-fold decrease in the $S_{0.5}$ value for 2-oxoglutarate [21], strongly increasing the affinity of OGDHC for main substrate. According to Rodriguez-Zavala *et al.* [22], Mg^{2+} potentiates the effect of ADP because the Mg :ADP complex may be a true activating molecule. There is evidence that ADP is an allosteric activator; desensitization of bovine adrenal OGDHC to ADP without change in the enzyme activity has been reported [23]. 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 OGDHC reaction rate on ADP concentration when the concentration of 2-oxoglutarate was not saturating showed a sigmoid curve, indicating positive homotropic cooperativity of the effector binding

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¹ The abbreviations used are: OGDHC, 2-oxoglutarate dehydrogenase complex; E1, 2-oxoglutarate dehydrogenase; E2, dihydrolipoamide succinyltransferase.

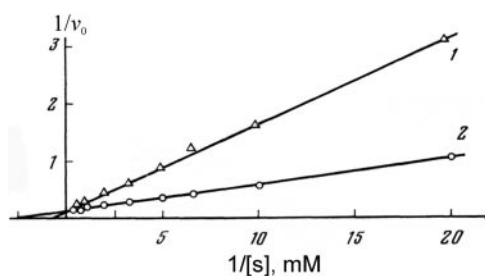


FIG. 1. Lineweaver-Burk plot showing the effect of ADP on bovine adrenal OGDHC activity with varying 2-oxoglutarate concentrations (from Ref. 27). 1, in the absence of ADP, the K_m for 2-oxoglutarate = 1.7 mM. 2, in the presence of 1.0 mM ADP; the K_m for 2-oxoglutarate = 0.4 mM.

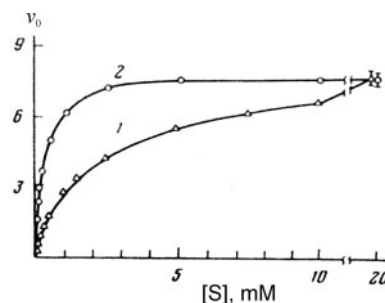


FIG. 2. The initial rate of bovine adrenal OGDHC reaction as a function of 2-oxoglutarate concentration (from Ref. 27). 1, in the absence of orthophosphate (P_i), the K_m for 2-oxoglutarate = 2.0 mM. 2, in the presence of 5.0 mM orthophosphate, the K_m for 2-oxoglutarate = 0.3 mM.

sites [24]. An experiment in which radioactively labeled ADP was incorporated into OGDHC confirmed this effect [25]. The positive cooperativity of ADP binding probably has a regulatory significance because it increases the sensitivity of OGDHC to changes of ADP concentration in the range 0.01–0.20 mM, changes that usually take place in animal tissues [3, 26].

Like ADP, inorganic orthophosphate (P_i) decreases the K_m value for 2-oxoglutarate but has no effect on V_{max} [17, 27] (Fig. 2). For example, in the presence of P_i the K_m value of OGDHC from bison heart for 2-oxoglutarate was about 12 times lower than that in the absence of orthophosphate [28]. P_i also increases the V_{max} of the reaction catalyzed by pig heart OGDHC [22], but the effect of P_i on K_m for 2-oxoglutarate is much larger. It is known that P_i , together with adenine nucleotides, contributes to the phosphorylation potential, $[ATP]/[ADP]$ [P_i], which reflects the energetic state of cells and cell organelles [3, 29]. High concentrations of ADP and P_i , together with low ATP, 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 [29]. Naturally, ATP differs from ADP and P_i with respect to its effect on OGDHC.

It has been shown that ATP inhibits OGDHC from pig heart [14] and bovine kidney [17] at a suboptimal 2-oxoglutarate concentration. In the case of OGDHC from pigeon breast muscle [15] and bovine adrenals [16] 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 inhibition of OGDHC with respect to the substrates-coenzymes [16]. This was probably because all three substances contain the similar adenylate fragment. At unsaturating concentrations of 2-oxoglutarate, ATP also markedly inhibits OGDHC because of chelation of divalent ions required for activity of the multienzyme complex [22, 30]. Moreover, ATP competitively opposes the activating action of the positive effector, ADP [30]. It should be noted that AMP, which is a strong positive effector of OGDHC from bacteria [31] and plants [32] has no influence on the affinity of animal OGDHC for its substrate. In the last case, components reflecting the phosphorylation potential, $[ATP]/[ADP]$ [P_i], rather than the energy charge, $([ATP] + 0.5 [ADP]) / ([ATP] + [ADP] + [AMP])$, taking into account AMP instead P_i [3, 33], are essential. For example, when muscles are

intensively working, they use ATP at a great rate, which leads to the production of large amounts of ADP and P_i . In this case, the ATP/ADP ratio and “phosphorylation potential” are low. Because OGDHC is very sensitive to ADP and P_i , it is really activated, showing high affinity to 2-oxoglutarate. When cells are resting and contain high concentrations of ATP, but low ADP and P_i , OGDHC is inhibited, indicating a decrease in its affinity for its substrate. This control of OGDHC activity is effective because the concentration of 2-oxoglutarate in animal tissues [26] is significantly lower than required to saturate OGDHC. It should be noted that physiological concentrations of P_i (about 2–5 mM), which are found in cells [26, 34], exert a significant activation of OGDHC [22].

Other Effectors—NADH can inhibit both dihydrolipoamide dehydrogenase and E1 components of OGDHC [15, 18, 24]. The first is based on competition of NADH versus NAD^+ , and the second mechanism is allosteric. NADH increases the K_m for 2-oxoglutarate and slightly decreases the V_{max} of OGDHC catalyzed reactions [15, 24]. In addition, the presence of NADH leads to an extension of the 2-oxoglutarate concentration range, causing positive cooperativity of the substrate binding sites in the absence of P_i and ADP [24]. So, in the control of OGDHC by its end product, NADH, negative feedback is 2-fold. The other end product of 2-oxoglutarate dehydrogenase reaction, succinyl-CoA, also has an inhibitory effect on OGDHC [1–4]. This inhibition is competitive versus coenzyme A and is realized mainly in active centers of dihydrolipoamide succinyltransferase [35].

Besides ADP and P_i , calcium ions (Ca^{2+}) are activators for OGDHC [14, 17, 19, 21, 22, 36]. Ca^{2+} was shown to activate E1 of OGDHC by markedly decreasing the K_m of the enzyme for its substrate. It should be noted that low (micromolar) Ca^{2+} concentrations are effective. Ca^{2+} binds to pig OGDHC with a stoichiometry of 3–4 mol of Ca^{2+} /1 mol of the complex [21]. According to McCormack and Denton [19], Ca^{2+} is an important regulator of intramitochondrial oxidative metabolism in vertebrate tissues because it activates both OGDHC and NAD^+ -isocitrate dehydrogenase.

Manganese ions appreciably decrease the $S_{0.5}$ of bison heart OGDHC for 2-oxoglutarate without any notable changes in the maximum reaction rate [37]. OGDHC was sensitive to Mn^{2+} in a wide range of concentrations be-

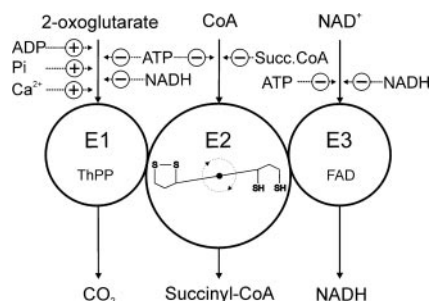


FIG. 3. Short term regulation of animal 2-oxoglutarate dehydrogenase complex.

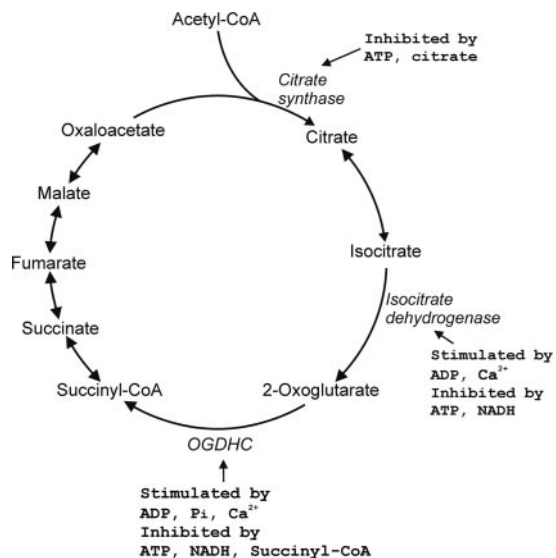


FIG. 4. The main control points of the citric acid cycle.

ginning with $1-2 \times 10^{-6}$ M. However, calcium ions seem to be more physiological regulators of OGDHC than Mn^{2+} and other divalent cations because Ca^{2+} has appropriate systems for its transfer into and out of mitochondria [1–4, 21]. It should be added that H^+ ions also favor the higher affinity of OGDHC for 2-oxoglutarate [18, 19, 38].

Recently, the influence of thiols on OGDHC has received wide attention [39] because a redox state of lipoate groups in E2 is very important for OGDHC activity.

CONCLUSION

This short communication is not a complete and detailed presentation of the theme. It is intended to suggest that OGDHC has a number of important regulatory mechanisms. Participation of the only main effectors in the short term regulation of OGDHC is summarized in Fig. 3. The necessary minimum knowledge of the control OGDHC activity for students of biochemistry should contain the following information: ADP, P_i , and Ca^{2+} are strong positive effectors increasing the affinity of the E1 for its substrate, whereas ATP, NADH, and succinyl-CoA are inhibitors of OGDHC. So, OGDHC is an important regulatory step of the citric acid cycle and is equal to citrate synthase and isocitrate dehydrogenase (Fig. 4). It should be noted that in general nature of the control of all three enzymes by energy-linked cell compounds is similar, but OGDHC is especially sensitive to the state of adenine nucleotide phosphorylation.

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