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QUARTERLY

Effect of Ca^{2+} on the activity of mitochondrial NADP-specific isocitrate dehydrogenase from rabbit adrenals

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Mitochondrial NADP-specific isocitrate dehydrogenase (ICDH) partially purified from rabbit adrenals is sensitive to low concentrations of Ca^{2+}. In the absence of Ca^{2+} (addition of 1 mM EGTA) the K_m of ICDH for DL-isocitrate was 143 μM, and in the presence of about 10 μM Ca^{2+} ($EGTA-Ca^{2+}$ buffer) it was decreased to 55 μM. The K_m value of ICDH for NADP was similar in the absence (4.4 μM) and in the presence (5.0 μM) of Ca^{2+}. The K_m of mitochondrial NADP-ICDH for DL-isocitrate was higher than that of cytoplasmic NADP-ICDH from the same source.

NADP-specific D-isocitrate dehydrogenase (EC 1.1.1.42) is present in both the cytoplasm and mitochondria of mammalian cells [1, 2]. The extramitochondrial and intramitochondrial NADP-ICDH isoenzymes are encoded by different genes [2, 3].

NADP-ICDH is an important supplier of reducing equivalents for steroidogenesis in adrenal glands [4]. However, so far only cytoplasmic NADP-ICDH has been isolated from rabbit [5] and bovine [6] adrenals and investigated in detail. The object of the present study is partially purified NADP-ICDH from rabbit adrenal glands. Information about the basic kinetic properties of mitochondrial NADP-ICDH will make possible their comparison with the properties of the cytoplasmic izoenzyme in the aspect of activity regulation.

MATERIALS AND METHODS

Adrenal glands of 20 rabbits were used to obtain the NADP-ICDH preparation. The tissue was homogenized for 2 min in a glass homogenizer with a teflon pestle. The isolation medium was 0.25 M sucrose containing 0.1 mM EDTA. The homogenate was centrifuged at 800 × *g* for 10 min to remove nuclei and tissue debris. Mitochondria from the supernatant were pelleted by centrifugation at 9 500 × *g* for 10 min. The pellet was washed and centrifuged 3 times. The mitochondrial paste thus obtained was resuspended in 50 mM Mops buffer, pH 7.0, containing 0.1 mM EDTA, 0.5 mM dithiothreitol and 2% Triton X-100. After 1 h incubation the preparation was centrifuged at 20 000 × *g* for 20 min and a clear colored mitochondrial extract was obtained.

NADP-ICDH was purified from the mitochondrial extract by the method used for purification of the cytoplasmic enzyme from rabbit adrenals [5] except that the last purification step, ion-exchange chromatography on DEAE Sephadex A-50, was replaced by gel filtration on CL-Sepharose 6B. The partially purified NADP-ICDH preparation had specific activity of 4.1 U/mg protein. One unit of the enzyme

Abbreviation used: NADP-ICDH, NADP-specific isocitrate dehydrogenase.

activity is defined as 1 μmole of NADPH formed per minute and was based on the initial rate.

The initial rate of the ICDH-catalyzed reaction was recorded by measuring NADPH formation at 340 nm with spectrophotometer model DU-640 (Beckman) at 30°C. The basic assay medium contained 50 mM Mops buffer, pH 7.6, 2 mM $MgCl_2$, 1 mM EGTA or 1 mM EGTA plus 1 mM $CaCl_2$ and various DL-isocitrate and NADP concentrations; 3.0 mM DL-isocitrate and 0.5 mM NADP were used as saturating concentrations. The reaction was started by addition of 1.2 μg of the ICDH preparation. The assays were carried out in triplicate and analysed by the Beckman Enzyme Mechanism Program.

DL-Isocitrate (Na_3-salt) was obtained from Serva (Germany), NADP from Boehringer (Germany), Mops and EGTA from Sigma (U.S.A.).

RESULTS AND DISCUSSION

The kinetic study over a broad range of DL-isocitrate concentrations showed that the dependence of the initial rate of the mitochondrial NADP-ICDH-catalysed reaction on isocitrate concentration followed Michaelis-Menten kinetics as indicated by a straight line in the Lineweaver-Burk plot (Fig. 1). The K_m value, as calculated from this and other plots, is about 55 μM. Interestingly, the $S_{0.5}$ constant of cytoplasmic NADP-ICDH from rabbit adrenal [5] for DL-isocitrate is considerably lower (about 7.6 μM) than the K_m of the mitochondrial NADP-ICDH isoenzyme under the same conditions. Moreover, at law substrate concentrations, cytoplasmic isocitrate dehydrogenase showed a positive cooperativity [5] whereas mitochondrial ICDH did not.

Addition of 1 mM EGTA to the assay mixtures containing 2 mM Mg^{2+} and lacking added Ca^{2+} increased the K_m value of mitochondrial NADP-ICDH for DL-isocitrate about threefold, i.e. up to K_m = 143 μM (Fig. 2). Addition of 1 mM EGTA in the absence of added Ca^{2+} decreased free Ca^{2+} concentration below 1 nM, and such a mixture will be referred to as nominally Ca^{2+}-free medium [7, 8]. Therefore, for the next assays in the presence of about 10 μM Ca^{2+}, the Ca^{2+}-EGTA buffer was used [8, 9]. Under these conditions the K_m value decreased to 55 μM (Fig. 2). This indicates that at this low Ca^{2+} concentration, characteristic for mitochondria under some conditions [8], the apparent affinity of NADP-ICDH for the substrate was considerably increased.

The next step of the experiment was to study the dependence of NADP-ICDH activity on Ca^{2+} concentrations at fixed nonsaturating (50 μM) and almost saturating (500 μM) concentrations of DL-isocitrate. As shown in Fig. 3, Ca^{2+} concentrations above 10 μM increased the enzyme activity only slightly. Thus, it appears that, mitochondrial NADP-ICDH is sensitive to Ca^{2+} in the range from 0 to 10 μM at low level of DL-isocitrate. This effect was similar to the behaviour of three other mitochondrial enzymes: phosphates pyruvate dehydrogenase [10], NAD-specific isocitrate dehydrogenase [7, 11] and 2-oxoglutarate dehydrogenase [8, 11, 12] from various animal sources. However, the ef-

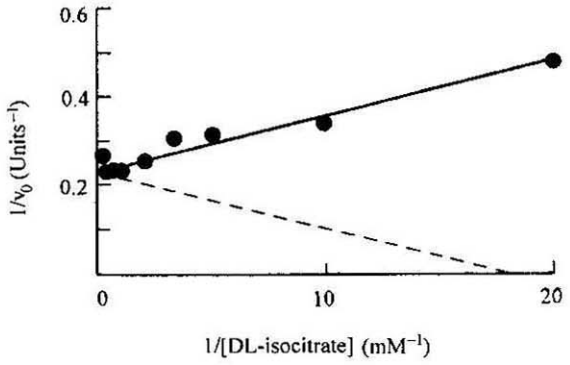

Fig. 1. Lineweaver-Burk plot of the dependence of the initial rate of NADP-ICDH-catalysed reaction on DL-isocitrate concentration without addition of Ca^{2+} or Ca^{2+}-EGTA.

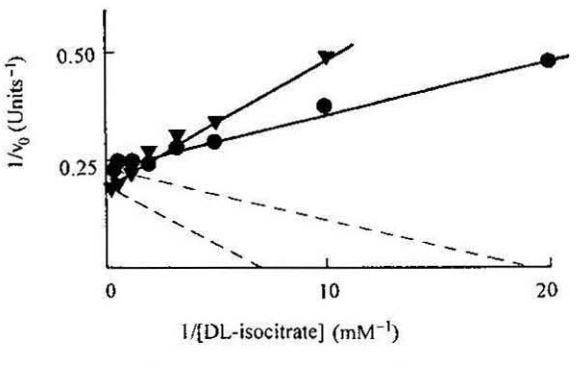

Fig. 2. Double reciprocal plot of the dependence of the initial rate of NADP-ICDH-catalysed reaction on DL-isocitrate concentration in the presence of: 1 mM EGTA (▲) and 1 mM Ca^{2+} + 1 mM EGTA (●).

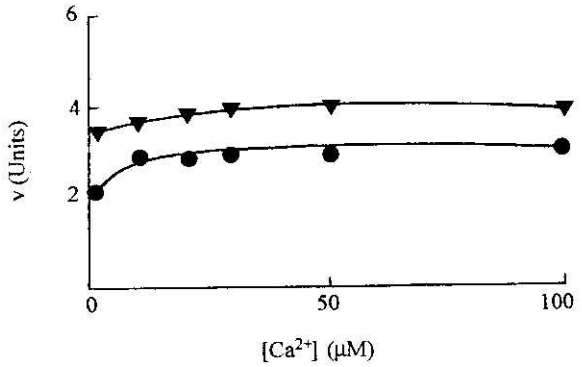

Fig. 3. Dependence of the NADP-ICDH reaction rate on Ca^{2+} concentration at DL*-isocitrate concentrations of: 500 μM (▲) and 50 μM (●).*

fects of micromolar Ca^{2+} concentrations on the two latter enzymes are more pronounced, diminishing the $S_{0.5}$ values for their respective substrates, isocitrate and 2-oxoglutarate [7, 8, 12). It should be added that, NAD-isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase can also be greatly activated byADP [8, 11].

A possible effect of ADP on mitochondrial NADP-ICDH from rabbit adrenals was also examined, and it was found that this enzyme was not sensitive to ADP in the range of 10–500 μM (not shown).

Figure 4 presents Lineweaver-Burk plots of NADP-ICDH for NADP. As can be seen, the K_m is almost the same in the absence (4.4 μM) and in the presence (5.0 μM) of about 10 μM Ca^{2+}. These K_m values are of the same order of magnitude as the $S_{0.5}$ value of cytoplasmic NADP-ICDH for NADP [5].

Analysis of all the data leads to the conclusion that the mitochondrial NADP-isocitrate dehydrogenase from rabbit adrenal is sensitive to

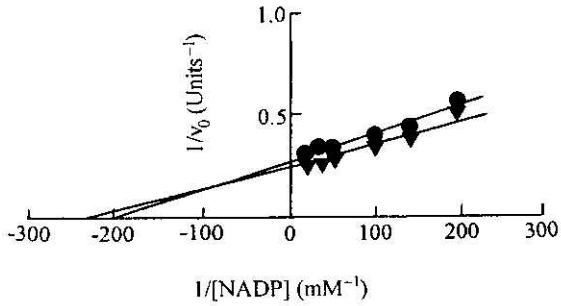

Fig. 4. Double reciprocal plot of the dependence of the initial rate of NADP-ICDH-catalysed reaction on NADP concentration; in the presence of 1 mM EGTA (▲) and 1 mM Ca^{2+} + 1 mM EGTA (●).

Ca^{2+}, which increase the affinity of the enzyme for its substrate but not towards its coenzyme. This seems to be of importance in the regulatory aspect.

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