THE EFFECTS OF Ca^{2+} AND ADP ON THE ACTIVITY OF NAD-LINKED ISOCITRATE DEHYDROGENASE OF MUSCLE

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1. Introduction

The NAD-linked isocitrate dehydrogenase (ICDH) (E.C. 1.1.1.41) is considered to be a regulatory enzyme for the tricarboxylic acid cycle in muscle [1]. According to the general systematic approach to metabolic control [2], the properties of this enzyme should provide a basis for a theory of control of its activity. In previous work on ICDH from bovine heart, rat heart and locust flight muscle, ADP has been shown to be a specific activator and to decrease the apparent K_m for isocitrate [3,1]. Thus the increase in ADP concentration caused by a stimulation of mechanical activity would be expected to enhance the rate of the cycle.

It is well established that Ca^{2+} is important in controlling the mechanical activity of muscle. The concentration of Ca^{2+} in the sarcoplasm is normally maintained at a very low level by an active uptake of Ca^{2+} into the sarcoplasmic reticulum. Nervous stimulation of the muscle causes a release of Ca^{2+} from the reticulum and hence an increase in the sarcoplasmic Ca^{2+} concentration from 10^{-8} to 10^{-6} M approximately. This change is sufficient to activate the myofibrillar ATPase and hence to initiate contraction. A similar change in Ca^{2+} concentration activates mitochondrial glycerol-1-phosphate dehydrogenase from insect flight muscle [4,5]. As this enzyme regulates the activity of the glycerol-1-phosphate cycle in insect flight muscles, this suggested that other important mitochondrial enzymes might be influenced by Ca^{2+}. Therefore the effects of this ion on the activity of NAD-linked ICDH were studied; it has been found to modify the effects of ADP on this enzyme.

2. Methods

Crude extracts of muscle were prepared by homogenization in a Silverson homogenizer (vertebrate muscle) or a ground-glass hand homogenizer (insect muscle) with a medium containing: 50 mM PIPES, 10 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM ADP, 10 mM mercaptoethanol, 0.5 mM EDTA and 0.5 mM EGTA at pH 7.9.

Mitochondria were prepared by careful homogenization of insect flight muscle in a medium containing: 0.25 M sucrose, 20 mM Tris, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM ADP, 1 mM EDTA, 1 mM EGTA and 2 mg/ml Nagarse (a proteolytic enzyme) at pH 7.9. After homogenization the solution was centrifuged at 10,000 *g* for 10 min; the mitochondrial pellet was resuspended in the sucrose medium (without the Nagarse).

All preparations were sonicated prior to assay. The NAD- (or NADP-) linked ICDH activity was measured by following the reduction of NAD (or NADP) at 340 nm in a Gilford recording spectrophotometer. The reaction medium contained: 20 mM PIPES, 2 mM NAD (or NADP), 5 mM $MnCl_2$, 2.5 μg/ml, antimycin A, at pH 7.1. Immediately after addition of the muscle extract the reaction was initiated by addition of a citrate/isocitrate (concentration ratio 15/1) mixture.

3. Results

Previous studies had shown that NAD-linked ICDH was specifically activated by ADP, and this has been

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Table 1
The effects of Ca^{2+} and ADP on the activity of ICDH from muscles from different animals.

Animal	Muscle	Concn. of DL-isocitrate (mM)	Concn. of Ca^{2+} (M)	ICDH activity (μmoles/min/g fresh muscle)						
				Concentration ADP added to cuvette (mM)						
				0	0.02	0.1	0.2	0.5	1.0	2.0
Locust	Flight	3.0	10^{-9}	10.0	10.6	15.4	16.7	16.7	14.8	15.4
(*Locusta*		3.0	10^{-5}	0.3	2.0	10.2	12.8	16.7	14.4	15.4
migratoria)		3.0	10^{-3}	1.0	2.9	13.1	17.9	16.0	16.0	16.6
Blowfly	Flight	0.33	10^{-9}	28.9	22.8	27.0	25.7	28.9	29.5	24.4
(*Sarcophaga*		0.33	10^{-5}	< 0.1	2.6	37.2	38.5	38.5	36.6	33.4
barbata)		0.33	10^{-3}	< 0.1	3.2	37.4	40.5	45.0	45.0	–
Water bug	Flight	1.0	10^{-9}	11.9	12.2	12.6	12.6	14.9	15.0	12.9
(*Lethocerus*		1.0	10^{-5}	3.6	4.2	10.1	12.8	17.4	18.4	18.4
cordofanus)		1.0	10^{-3}	2.0	6.1	10.4	13.0	16.2	19.5	–
Rat	Heart	0.6	10^{-9}	1.0	–	1.3	1.3	1.3	1.3	1.3
		0.6	10^{-5}	0.1	–	0.4	0.4	0.8	1.3	1.3

confirmed in the present work. However, the results in table 1 (using crude extracts of muscle) show that the effect of ADP was dependent upon the concentration of Ca^{2+}, which was controlled by the use of Ca^{2+}-EGTA buffers [6]. At a minimal Ca^{2+} concentration (approximately 10^{-9} M) the enzyme was maximally active (at the given substrate concentration) in the absence of any added ADP, so that addition of ADP up to a final concentration of 2 mM had no further significant effect on the enzyme activity. However, at 10^{-5} M Ca^{2+} and in the absence of added ADP the activity was extremely low, but was increased by the addition of ADP (table 1). It is interesting that raising the Ca^{2+} concentration from 10^{-5} to 10^{-3} M had no further effect on the activity in the absence of added ADP, and did not change the response of the enzyme to increasing ADP concentrations (table 1). This would suggest that the Ca^{2+} effect is maximal at 10^{-5} M, and that the effects of Ca^{2+} and ADP are independent. These effects of Ca^{2+} were observed with NAD-linked ICDH in crude extracts of flight muscles from locust, water bug and blowfly and in crude extracts of rat heart muscle. Similar effects of Ca^{2+} and ADP were also observed when the enzyme was assayed with mitochondria prepared from insect flight muscles as described in Methods.

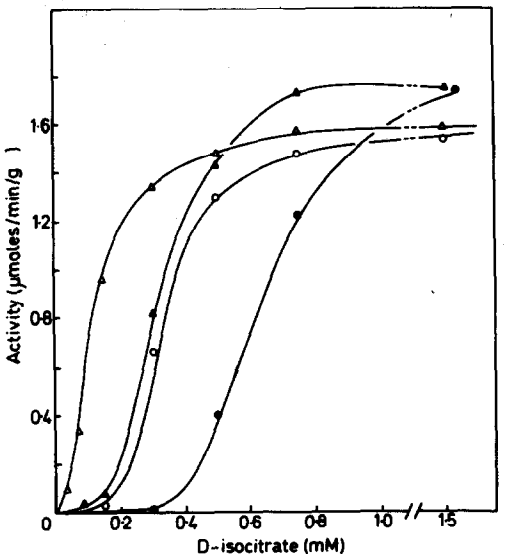

Fig. 1. The effect of the concentration of isocitrate on the activity of NAD-linked ICDH activity from rat heart in the presence of various concentrations of ADP. Enzyme assays were performed on crude extracts of heart muscle. △–△ 10^{-9} M Ca^{2+} and 2 mM ADP; ▲–▲ 10^{-5} M Ca^{2+} and 2 mM ADP; ○–○ 10^{-9} Ca^{2+} in the absence of added ADP; ●–● 10^{-5} M Ca^{2+} in the absence of added ADP.

It had been shown previously that ADP modifies the response of ICDH to isocitrate [3,1]. In the absence of ADP the plot of activity against isocitrate concentration is sigmoid; addition of ADP results in a curve which approaches the hyperbolic, but there is no marked effect on the maximal activity of the enzyme. Fig. 1 shows the effects of 10^{-9} and 10^{-5} M Ca^{2+} and ADP, on the response of ICDH from rat heart muscle to isocitrate. In the presence of 2 mM ADP and a minimal Ca^{2+} concentration (10^{-9} M) the curve is almost hyperbolic; lowering the ADP concentration, or raising the Ca^{2+} concentration to 10^{-5} M, increased the sigmoid nature of the curves. Thus ADP and Ca^{2+} appear to affect the K_m of ICDH for isocitrate: raising the ADP concentration decreased the K_m whereas raising the concentration of Ca^{2+} increased it. These curves provide further support for the suggestion that the effects of Ca^{2+} and ADP on the enzyme are independent.

No effects of Ca^{2+} or ADP were observed with the NADP-linked ICDH from pigeon pectoral muscle.

4. Discussion

The fact that NAD-linked ICDH is activated by ADP has provided a basis for a theory of control of the tricarboxylic acid cycle. The present work has extended the knowledge of the properties of ICDH to show that the effect of ADP is dependent upon the Ca^{2+} concentration. Therefore the theory of the control of the cycle should now be modified to include the effect of Ca^{2+}. The NAD-linked ICDH is considered to be located within the matrix of the mitochondria [7]. For Ca^{2+} to play a role in the regulation of this enzyme (and therefore of the cycle) it would be expected that during contraction of the muscle, the mitochondrial Ca^{2+} concentration would be low ($< 10^{-5}$ M), and conversely during relaxation of the muscle it would be high. This implicates mitochon dria in the regulation of the intracellular distribution of Ca^{2+} in muscle, and suggests that they play a complementary role to the sarcoplasmic reticulum. Indeed such a possibility has been suggested by a number of workers [8,9], and it may be particularly important in muscles in which the content of mitochondria is high, but where the reticulum is poorly developed (e.g. heart muscle, asynchronous insect flight muscles) [9,10].

It should be noted that the theory of control of ICDH by Ca^{2+} does not replace the theory of ADP control. The two effects, which are independent at the enzymatic level, probably provide a concerted regulatory mechanism which ensures that the activity of ICDH is extremely low during rest, yet is maximal when the muscle is mechanically active.

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