On the Involvement of Ca^{2+} and Mn^{2+} in the Regulation of Mitochondrial Glutamic Dehydrogenase from Blastocladiella.

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The intact mitochondrion is a biochemical machine which catalyzes energy transductions in various ways. One such transduction involves the translocation of ions across the mitochondrial membranes (see reviews by Lehninger, Carafoli, and Rossi, 1967; Pullman and Schatz, 1967). The mechanism of this mitochondrial ion translocation and its relationship to oxidative phosphorylation is, at present, based on two major hypothesis: (1) the chemical coupling theory and (2) the H^+ pump or "chemiosmotic" concept. Current lines of experimentation to test these theories have so far failed to favour one or the other hypothesis. Recent reports by Slater (1967), Rossi, et al., (1967) have revealed several weak points in the chemiosmotic theory of Mitchell (see 1967 ref. for a revised concept).

One important aspect of ion translocation in the mitochondria that has not received much attention is the probable involvement of ions on regulatory enzymes within and associated with the mitochondrial membrane. Most of the work in this area is focused on the membrane-bound ATPase.

This communication is intended to show that the NAD-specific glutamic dehydrogenase from the 'unicellular' aquatic mold, *Blastocladiella emersonii*, may be regulated by cations (notably, Ca^{2+} and Mn^{2+}) and by the adenylates, ATP, ADP, and AMP. This enzyme is localized on the surface of the mitochondria (Jackson, Thesis, University of Manitoba, 1968). A complete report on the allosteric control exerted by adenylates on this glutamic dehydrogenase has appeared (LéJohn and Jackson, 1968).

EXPERIMENTAL

The method used in isolating *Blastocladiella emersonii* glutamic dehydrogenase is in the report of LéJohn and Jackson, 1968. A highly purified preparation with a specific activity 330 times greater than that of the crude extract has been used with identical results. Reaction rates of enzyme activity were determined by means of a Gilford model 2000 recording spectrophotometer. One unit of enzyme activity is equivalent to 0.001 O.D. change at 340 mμ min^{-1}.

RESULTS

Cationic Effects. From Table I, it is evident that only Ca^{2+} and Mn^{2+} were capable of activating the reductive amination reaction catalyzed by *B. emersonii* glutamic dehydrogenase. Other cations, particularly, Zn^{2+} and Cu^{2+} were inhibitory. It is pertinent to mention that Cu^{2+} was not inhibitory in the oxidative deamination reaction (LéJohn, J. Biol. Chem. In Press).

TABLE I

The Effect of Cations on the Reductive Amination of α-Ketoglutarate by Blastocladiella Glutamic Dehydrogenase.

The reaction system contained 0.166 mM NADH; 1.66 mM α-Ketoglutarate; 500 mM NH_4^+; 0.2 M Tris-chloride buffer, pH 7; 5 μg of enzyme protein. All solutions were made up to pH 7 just before use.

Addition	Concn. (mM)	$A_{340\ m\mu}\ min^{-1}$	Inhibition (%)	Activation (%)
None	-	0.040	-	-
AMP	1	0.088	-	120
Ca^{++}	3.33	0.075	-	85
Mn^{++}	3.33	0.065	-	65
Mg^{++}	3.33	0.044	-	10
Zn^{++}	3.33	0.008	80	-
Cu^{++}	3.33	0.000	100	-
Co^{++}	3.33	0.034	12	-

Kinetic Evaluation. To determine in what way Ca^{2+} activated the enzyme, substrate levels were varied in the presence of fixed amounts of Ca^{2+}. From Fig. 1a and b, it is clear that Ca^{2+} activated the reductive amination of α-ketoglutarate markedly at low levels of NADH. Because of the sharp threshold level of the effective substrate

concentrations (evident in the substrate inhibition profiles), these reciprocal (reaction rate-substrate concentration) plots cannot be properly analyzed. Nevertheless, one may surmise that Ca^{2+} was affecting either the free enzyme or the enzyme-NADH complex. The plots of activation by AMP (Fig. 1a and b) and ATP inhibition (Fig. 1a) have been included to emphasize the effectiveness of Ca^{2+}. Activation by ADP parallel the response shown by AMP.

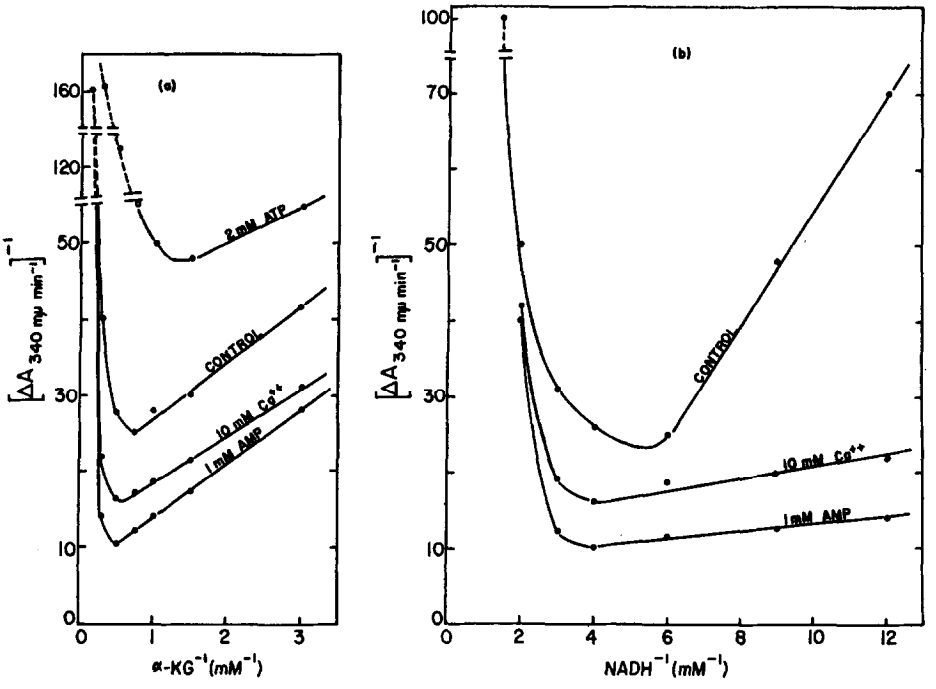

Fig. 1. *Lineweaver-Burk representation of the effect of Ca^{++}, AMP, ADP, and ATP on the reductive amination of α-ketoglutarate by Blastocladiella glutamic dehydrogenase.* In (a), α-ketoglutarate was the varied substrate at a fixed concentration of NADH (0.166 mM); NH_4^+ (500 mM); 5 μg enzyme protein; 0.15 M Tris-chloride buffer, pH 7; additions as shown on the plots. In (b), conditions as for (a) except that NADH was the varied substrate and α-ketoglutarate was held fixed at 1.66 mM concentration. Additions as shown.

On the contrary, Ca^{2+} participated as an inhibitor of the oxidative deamination of glutamate (Fig. 2). At 10 mM Ca^{2+} concentration, approximately 50% inhibition is observed. High concentrations of NAD^+ appeared to be incapable of reducing the inhibitory action of Ca^{2+}. The response, therefore, may be

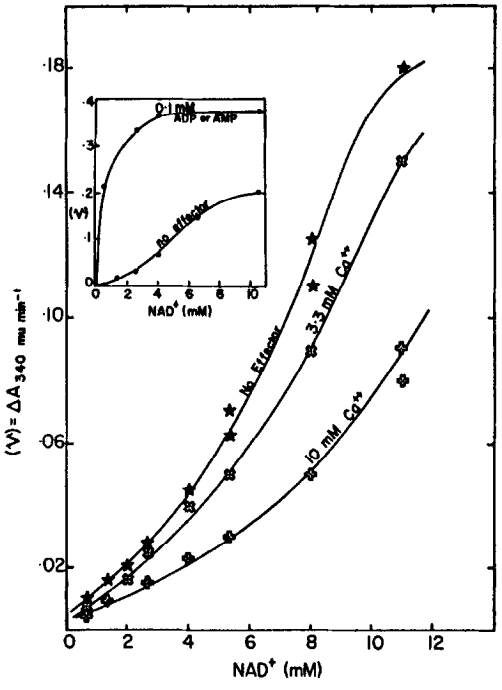

Fig. 2. *Rate-concentration representation of the effect of Ca^{++} on the oxidative deamination of glutamate by Blastocladiella glutamic dehydrogenase.* The reaction system contained 16.66 mM glutamate; 4 mM NAD^+; 18 μg enzyme protein; 0.15 M Tris-chloride buffer, pH 9, and Ca^{++} as indicated. Inset. Rate-concentration profile of activation by AMP or ADP (100 μM level) in the system described above.

noncooperative. This will have to await further evaluation. The inset of Fig. 2 emphasizes the intense activation caused by AMP and ADP in this reaction.

Effect of Protons. One other interesting aspect of this unique behaviour of activation by cations in one direction of the reaction and inhibition in the other, was revealed during a study of the influence of pH on the enzymic activity. What appears to be similar to the Bohr proton effect observed for haemoglobin (Wyman, 1948, 1963) has been elucidated in our studies on the molecular nature of this enzyme (LéJohn and Jackson, 1968). The results of Table II demonstrate that high pH levels favour Ca^{2+} activation of the reductive amination of α-ketoglutarate and, at the same time, emphasize Ca^{2+} inhibition

of the oxidative deamination of glutamate. The effects shown by Mn^{2+} were similar, though slightly less effective.

TABLE II

The Effect of Ca^{++} and Mn^{++} on (a) the Reductive Amination of α-Ketoglutarate: (b) the Oxidative Deamination of Glutamate by Blastocladiella Glutamic Dehydrogenase.

The reaction system for (a) is as outlined in Table I. 15 µg enzyme protein was used and buffers set at the specified pH.

Addition	Reaction Rate* (pH) (6)	(7)	(8)	(9)
None	0.006	0.125	0.054	0.015
10 mM Ca^{++}	0.010	0.322	0.190	0.045
10 mM Mn^{++}	0.005	0.264	0.188	-
% Activation by Ca^{++}	0	255	350	300
% Activation by Mn^{++}	0	200	350	-

The reaction system for (b) consisted of 4 mM NAD^{+}; 16.66 mM Glutamate; 40 µg enzyme protein and buffers at the specified pH.

Addition	Reaction Rate* (pH) (7)	(8)	(8.6)	(9.5)
None	0.038	0.140	0.084	0.068
10 mM Ca^{++}	0.040	0.142	0.060	0.028
% Inhibition by Ca^{++}	0	0	30	60

*Reaction Rate = $\Delta A_{340\ m\mu}\ min^{-1}$

DISCUSSION AND MODEL

It has been reported (LéJohn, J. Biol. Chem. In Press) that this glutamic dehydrogenase from *B. emersonii* display a '*polarized*' inhibition of the oxidative deamination of glutamate by citrate and some members of the citric acid cycle without affecting the reductive amination of α-ketoglutarate. This inhibition could be relieved by a variety of divalent cations (Ca^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, included). This communication has shown that in the absence of the inhibitory metabolites (citrate etc.) higher concentrations of some cations can simultaneously activate and inhibit the enzyme.

It is well-known that H^+ are extruded during cation uptake in the mitochondria, particularly, Ca^{2+}, Mn^{2+}, and Sr^{2+}. This is related to the hydrolysis of ATP by ATPase (see review by Lehninger, et al., 1967). The findings reported here and elsewhere appear to support a mechanism whereby this glutamic dehydrogenase is directly regulated by ion translocations through the mitochondrial membrane. Under conditions of active citric acid cycle oxidations, the mitochondria may be regarded as highly "energized". This would stimulate entry of cations with the concomitant hydrolysis of ATP. Valuable citric acid cycle intermediates may then be funnelled into a glutamate 'sink' since the reductive amination of α-ketoglutarate would be maximally activated. During ATP synthesis, there may be an extrusion of cations in exchange for H^+. The oxidative deamination of glutamate could then be released from metabolite inhibition by a change in the adenylate concentrations and pH. These studies have been extended to another mitochondrial enzyme, an NAD-specific isocitrate dehydrogenase found in this mold. The findings, which show similar regulation by protons is to be published.

ACKNOWLEDGMENT

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REFERENCES

1. Lehninger, A.L., Carafoli, E., and Rossi, C.S., Advanc. Enzymol., 29, 259, (1967).
2. LeJohn, H.B., and Jackson, S., J. Biol. Chem., 243, 3447 (1968).
3. Mitchell, P., Advance. Enzymol., 29, 33 (1967).
4. Pullman, M.E., and Schatz, G., Annu. Rev. Biochem. 36, 539 (1967).
5. Rossi, C.S., Siliprandi, N., Carafoli, E., Bielawski, J., and Lehninger, A.L., Europ. J. Biochem., 2, 332 (1967).
6. Slater, E.C., Europ. J. Biochem., 1, 317 (1967).
7. Wyman, J., Advanc. Protein Chem., 4, 407, (1948).
8. Wyman, J., Cold Spring Harbor Symp. Quant. Biol., 28, 483 (1963).