REGULATORY EFFECTS OF MITOCHONDRIAL LIPIDS ON GLUTAMATE DEHYDROGENASE (NAD(P))

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1. Introduction

As previously reported [1] GDH* has been covalently bound to thin films of collagen to mimic the possible influence of mitochondrial membranes since GDH is assumed to be located by the inner mitochondrial membrane in the matrix. Therefore it was important to precise not only the influence of binding itself but also the effects of membrane and mitochondrial lipids on GDH.

This paper reports the activation of GDH by mitochondrial crude lipid fractions and its inhibition by pure phospholipids**.

2. Materials and methods

Bovine liver GDH in 50% glycerol, NAD, ADP, were purchased from Boehringer Mannheim; L-glutamic acid, A grade, from Calbiochem, USA; Bovine serum albumin (BSA) from Koch-Light Laboratories, Ltd; DL-α-lecithin (phosphatidylcholine) and DL-α-cephalin (phosphatidylethanolamine) were purchased from Fluka AG, and phosphatidyl serine from Sigma Chemical Co. Cardiolipin was kindly supplied by Dr. M. Faure (Institut Pasteur, Paris), to whom thanks are addressed.

Pig heart mitochondria were prepared according to Crane et al. [2] and washed, tested for respiratory control ratios, as previously [3]. Crude mitochondrial lipid fractions were obrained according to Folch [4].

Lipids and phospholipids were dissolved in ethanol to test their effects. Ethanol alone had no effects in the same conditions. Initial rates of glutamate oxidation by GDH were determined at 340 nm by spectrophotometric measurements of reduced coenzyme after addition of glutamate in the presence of NAD at saturating level (about 10 K_m).

3. Results

Fig. 1A shows that crude mitochondrial lipids always activated GDH in the range 0–50 μg/ml; the activation was maximal around 20 μg/ml for 5 μg GDH, and then decreased for higher concentrations of lipids.

Fig. 1B shows that the activation is of the mixed type.

In fig. 2 we see that phosphatidylcholine (PC) and phosphatidylethanolamine (PE) inhibition patterns towards GDH were of the same type. These patterns were used to calculate the inhibitor concentration $i_{[50]}$ for 50% inhibition which were:

$i_{[50]}$ = 60 μM for both inhibitors (PC and PE).

* Abbreviations:
GDH: Glutamate dehydrogenase EC 1.4.1.3.
2 ME: 2-mercaptoethanol.

** This work was the subject of a communication at the 7th FEBS Meeting, Varna, 23 September 1971, and is part of the Thesis of Doctorat de Spécialité maintained by J.H. Julliard, on 13 November 1971 (Université Claude Bernard, Lyon, France).

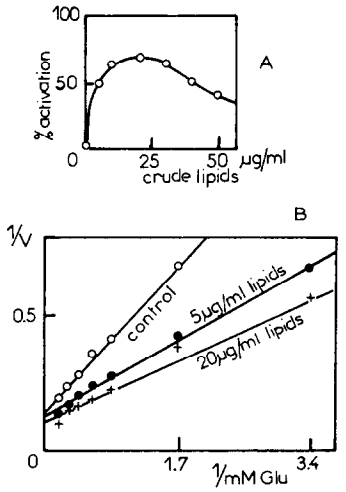

Fig. 1A. Activation of GDH by crude lipids from pig heart mitochondria. Experimental conditions: 20 mM phosphate K buffer, 1 mM 2 ME, 0.5 mM EDTA pH = 8.0, 28°. 0.6 mM NAD, 5 µg/ml GDH, 10 mM glutamate. Fig. 1B. Double reciprocal plot with variable glutamate concentrations. Experimental conditions as in fig. 1A. v is expressed in nmoles of NADH appearing/ml/min.

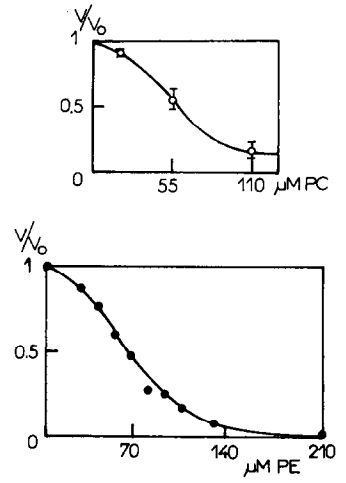

Fig. 2. Inhibition of GDH by phosphatidylcholine(PC) and phosphatidylethanolamine(PE). Experimental conditions: 20 mM phosphate K buffer, 1 mM 2 ME, 0.5 mM EDTA pH = 8.0, 28°. 0.5 mM NAD, 10 mM glutamate, GDH: 1 µg/ml. v is expressed in nmoles NADH appearing/ml/min. v_0 is the control initial velocity.

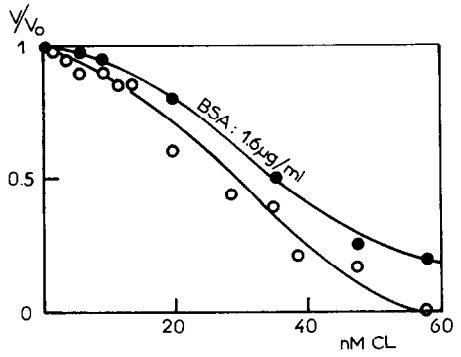

Fig. 3. Inhibition of GDH by cardiolipin and effect of BSA. Experimental conditions: 20 mM phosphate K buffer, 1 mM 2 ME, 0.5 mM EDTA pH = 8.0, 28°. 0.4 mM NAD, 10 mM glutamate, GDH 0.3 µg/ml. v and v_0 are expressed as in fig. 2.

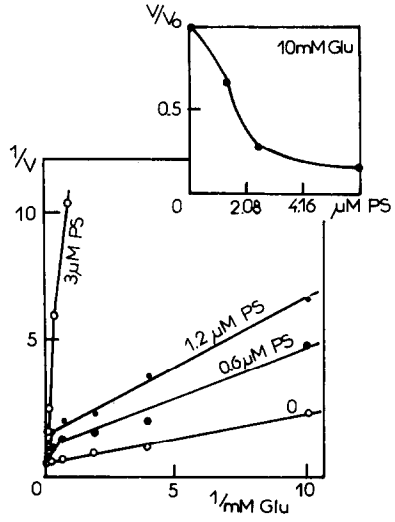

Fig. 4. Inhibition of GDH by phosphatidylserine(PS). Experimental conditions: 20 mM phosphate K buffer, 1 mM 2 ME, 0.5 mM EDTA pH = 8.0, 28°. Insert: 10 mM glutamate, 0.5 mM NAD, v and v_0 expressed as in fig. 2. Double reciprocal plot with variable glutamate concentrations: 0.5 mM NAD, GDH: 1 µg/ml. v is expressed as in fig. 2.

Both these phospholipids were abundant in pig heart mitochondria [5].

Fig. 3 shows that cardiolipin (CL), a very specific constituent of inner mitochondrial membranes, especially abundant in pig heart mitochondria (18% of total mitochondrial phospholipids [5]), strongly inhibited GDH at very low concentration since its $i_{[50]}$ = 24 nM. BSA (1.6 μg/0.3 μg GDH/ml) partially released the inhibition without modification of the inhibition pattern. On the contrary, 0.5 mM ADP, which is a positive effector of GDH did not affect CL inhibition.

Fig. 4 shows that phosphatidylserine(PS) which could not be detected in pig heart mitochondria [5] or in rat liver mitochondria [6] but seemed to be present as traces in beef liver inner mitochondrial membranes [7], could also inhibit GDH with a similar pattern and a $i_{[50]}$ = 2 μM. Double reciprocal plot as a function of glutamate concentration shows that fairly high concentrations of glutamate could release PS inhibition. As stated above, this release was not observed with the other phospholipids tested.

4. Conclusions

All phospholipids tested, constituents or not of mitochondrial membranes, strongly inhibited GDH. The possible regulatory role of cardiolipin towards GDH in situ i.e. in the mitochondrial matrix, should be suggested. Since this phospholipid is one of the most important constituents of inner mitochondrial membrane, it can interfere with GDH on the matrix side; its very low $i_{[50]}$ = 24 nM renders possible such an inhibitory interaction in situ and could be invoked to explain the so-called "latency" of GDH in situ to which some authors refer.

The fact that the crude mitochondrial lipid fraction, which contained all the inhibitory phospholipids, could activate GDH cannot be explained yet. A very sophisticated analysis of this crude fraction will be necessary to understand the regulatory system.

Addendum: A few days ago, we were informed that after our communication at the 7th FEBS Meeting at Varna (September, 1971) and after the maintaining of Dr. Julliard's thesis (13 November, 1971), Dr. G.H. Dodd presented an oral communication describing results partially similar to some of ours [8].

Acknowledgements

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References

[1] J.H. Julliard, C. Godinot and D.C. Gautheron, FEBS Letters 14 (1971) 185.
[2] F.L. Crane, J.F. Glenn and D.E. Green, Biochim. Biophys. Acta 22 (1956) 476.
[3] C. Godinot, C. Vial, B. Font and D. Gautheron, European J. Biochem. 8 (1969) 385.
[4] J. Folch, M. Lees and G.H. Sloane-Stanley, J. Biol. Chem. 226 (1957) 497.
[5] J. Comte, D.C. Gautheron, F. Peypoux and G. Michel, Lipids 6 (1971) 882.
[6] A. Colbeau, J. Nachbaur and P.M. Vignais, Biochim. Biophys. Acta 249 (1971) 462.
[7] M. Levy and M.T. Sauner, Chem. Phys. Lipids 2 (1968) 291.
[8] G.H. Dodd, Oral communication to the 521st Meeting of the Biochemical Society, Biochem. J. 127 (1972) 38 Proceedings.