ROLE OF CITRATE CARRIER IN CITRATE MEDIATED SUCCINATE (+ ROTENONE) OXIDATION

J. POPINIGIS, M. WOŹNIAK, C. NOWICKA and P. GMAJ
Department of Biochemistry and Department of Clinical Biochemistry, Medical School, Gdańsk, Poland

Received 19 March 1973

1. Introduction

In 1970 Popinigis et al. [1] described conditions in which mitochondrial succinate (+ rotenone) oxidation become dependent on the extra added citrate. It has been observed, that when succinate (+ rotenone) oxidation was inhibited by the addition of protamine, introduction of citrate into the incubation medium restored mitochondrial respiration. The explanation offered was that in these conditions citrate acts as a proton conductor across the mitochondrial membrane.

On the other hand, findings that the citrate was noncompetitive with respect to succinate in these conditions [2], were in discordance with the well established competitive manner of carrier dependent entry of anionic substrates into the mitochondria [3, 4], and put in doubt the proposed citrate movement.

Therefore, the possible role of citrate in restoration of protamine inhibition on succinate (+ rotenone) oxidation was re-examined using inhibitors of citrate entry into the mitochondria: benzene 1,2,3 tricarboxylic acid and high concentrations of sucrose. Results obtained indicate that the ability of citrate to reverse protamine inhibition increased when the citrate entry into the mitochondria via citrate translocator was inhibited.

2. Materials and methods

Rat liver mitochondria were prepared and suspended in 0.25 M sucrose + 3 mM Tris chloride, according to Loewenstein et al. [5], but with omission of the last (digitonin) step. Oxygen consumption was measured with the Clark oxygen electrode E 5037 Radiometer and PHM 27 pH meter with gas monitor Radiometer. pH was measured in the same vessel with the combined glass calomel electrode GR 232 Radiometer and N 512 pH meter Elpo.

Both oxygen and pH were registered with two synchronized recorders type I 37/N from Zip.

Protamine sulfate ex herring (Koch-Light) was used as a pH 7.2 Tris solution. Solutions of sucrose were prepared from Analar (Koch-Light) sucrose and were passed through the Amberlite IRC-50 before use.

Benzene 1,2,3 tricarboxylaic acid (Aldrich), was a generous gift of Dr. J.M. Smoly.

Detailed experimental conditions are described in the legend to fig. 1.

3. Results

The ability of citrate to restore protamine inhibited succinate (+ rotenone) oxidation was found to be dependent on the composition of the medium:

Exp. A was performed in the medium containing 0.25 M sucrose. It can be seen that the addition of protamine inhibited both oxygen uptake and oxidative phosphorylation measured by the proton uptake. Addition of citrate to the incubation medium induced a fast phase of proton appearance in the medium (not shown) followed by a steady state of proton concentration, with the concomitant restoration of mitochondrial respiration.

Exp. B was carried out in the medium in which the sucrose concentration was increased to 0.4 M. It can be seen that this high concentration of sucrose inhibited

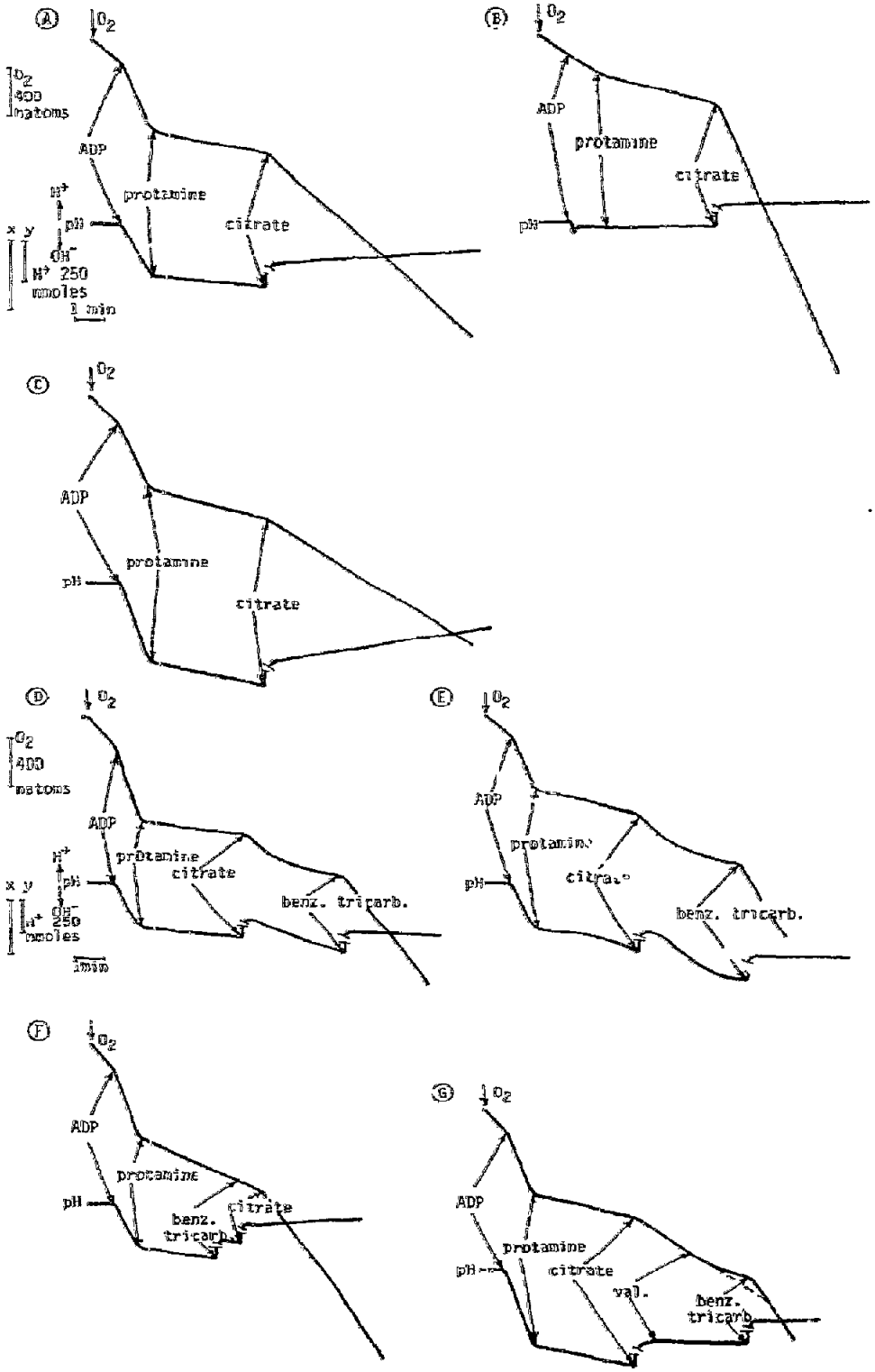

Fig. 1. Citrate mediated succinate (+ rotenone) oxidation. The effect of sucrose and benzene 1,2,3 tricarboxylate. Rat liver mitochondria (4 mg of protein in 0.05 ml of 0.25 M sucrose + 3 mM Tris chloride) were added into 2.5 ml of the pH 7.2 media containing: 10 mM KCl, 3 mM $MgSO_4$, 2.5 mM potassium phosphate, 5 mM Tris succinate, 3 μg of rotenone and in exp: A, 250 mM sucrose; B, 400 mM sucrose; C, 100 mM sucrose; D, 100 mM sucrose + 75 mM KCl; E, 100 mM sucrose + 150 mM KCl; F, 100 mM sucrose + 75 mM KCl: G, 100 mM sucrose. After 1 min of preincubation of mitochondria in the above medium, oxygen uptake and changes in proton concentration in the medium were recorded simultaneously. Additions: 1 mM ADP, 500 μg protamine, 10 mM Tris citrate, 10 mM Tris benzene 1,2,3 tricarboxylate, 1 μg valinomycin. Marks (x) and (y) indicate changes in pH upon addition of 250 nmoles of protons as a HCl solution in the absence (x) or after addition of citrate (y). Fast changes in proton concentration in the medium which occurred upon addition of citrate are omitted.

(in most experiments completely) ADP induced state 4 → state 3 transition in both oxygen consumption and proton uptake. Addition of protamine inhibited mitochondrial respiration and this inhibition could be overcome by the addition of citrate. The rate of this citrate mediated respiration was about 5-fold higher than the rate of respiration in state 4.

On the contrary, when the same experiment was performed in the medium containing 0.1 M sucrose (exp. C), the ability of citrate to reverse protamine inhibition was reduced.

Sucrose could not be replaced by KCl. It may be seen in exp. D and E that in the medium with the same osmolarity as in exp. A or B, but in which sucrose was partially replaced by equivalent concentrations of KCl, the effect of citrate on oxygen consumption was biphasic after an initial substantial increase, the rate of respiration decreased rapidly to the level of protamine inhibition. The addition of benzene 1,2,3 tricarboxylate resulted in further stimulation in the rate of respiration. Introduction of benzene 1,2,3 tricarboxylate into the incubation medium before addition of citrate prevented the decline phase in citrate mediated respiration (exp. F). A biphasic effect of citrate was observed also in low KCl concentration, but in the presence of valinomycin (exp. G). Benzene 1,2,3 tricarboxylate reversed this valinomycin induced inhibition of respiration.

4. Discussion

It is known that the high concentrations of sucrose can suppress citrate, and other Krebs cycle intermediates, oxidation in mitochondria [6], probably through the inhibition of anion entry into the mitochondria [7]. Similarly, benzene 1,2,3 tricarboxylic acid was described as a potent inhibitor of citrate entry into the mitochondria [8, 9].

Presented results indicate that the ability of citrate to reverse the inhibitory effect of protamine on succinate (+ rotenone) oxidation increased with the increase of sucrose concentration in the medium (exp. A–C).

In the medium with low sucrose and high KCl concentrations (exp. D, E and F), the stimulatory effect of citrate on respiration was dependent on the presence of benzene 1,2,3 tricarboxylate. Because the membrane level of action of sucrose [10] and benzene 1,2,3 tricarboxylate [8, 9] seems to be well established, it can be concluded that citrate enhanced mitochondrial respiration when the citrate transport into the mitochondria was inhibited.

On the other hand we can not exclude that the described citrate induced changes in the rate of respiration are organized directly at the level of anion binding (or adsorption [11]) to mitochondrial proteins. This possibility is also suggested by the observations that the high concentrations of sucrose and protamine showed opposite effects on mitochondrial structure and ATPase activity. Sucrose at concentrations higher than 0.25 M inhibited ATPase activity [12] and induced aggregated state of matrix [13]. On the contrary, protamine in concentrations inhibiting succinate (+ rotenone) oxidation induced marked expansion of the matrix space [14, 15] and significantly increased the activity of Mg dependent ATPase [16]. Therefore, it can be considered that the protamine effect is regulated through the changes in the ability of mitochondria to bind anions.

On the other hand it is of interest that the citrate restored succinate (+ rotenone) oxidation inhibited by: progesterone [17, 18], black pigment dispersing hormone [19], polyglutamate [20], maleate [21], or even chlorophyll [22]. This indicates that the phenomena described may be of a wider significance.

References

[1] J. Popinigis, W. Rzeczycki and J. Swieczyński, FEBS Letters 8 (1970) 149–152.
[2] J. Popinigis, J.M. Somly, R.F. Brucker and C.H. Williams, Abstr. Commun. Fed. Eur. Biochem. Soc. (1972) 634.
[3] E. Quagliariello and F. Palmaieri, in: The energy level and metabolic control in mitochondria, eds. S. Papa, J.M. Tager, E. Quagliariello and E.C. Slater (Adriatica Editrice, Bari, 1969) pp. 68–72.
[4] E.J. Harris, in: The energy level and metabolic control in mitochondria, eds. S. Papa, J.M. Tager, E. Quagliariello and C. Slater (Adriatica Editrice, Bari, 1969) pp. 135–140.
[5] J. Loewenstein, H.R. Scholte and E.M. Wit Peeters, Biochim. Biophys. Acta 223 (1970) 432–436.
[6] D. Johnson and H. Lardy, Nature 181 (1958) 701–702.
[7] J.B. Chappell and K.N. Haarhoff, in: Biochemistry of mitochondria, eds. E.C. Slater, Z. Kaniuga and L. Wojtczak (Academic Press and PWN, London, New York, Warszawa, 1967) pp. 75–91.
[8] B.H. Robinson, G.R. Williams, M.L. Halperin and C.C. Leznoff, European J. Biochem. 20 (1971) 65–71.

[9] B.H. Robinson, FEBS Letters 16 (1971) 267–271.
[10] G. Zimmer, A.D. Keith and L. Packer, Arch. Biochem. Biophys. 152 (1972) 105–113.
[11] R.N. Zahlten, A.A. Hochberg, F.W. Stratman and H.A. Lardy, FEBS Letters 21 (1972) 11–13.
[12] R. Cereijo-Santalo, Arch. Biochem. Biophys. 150 (1972) 542–547.
[13] D.E. Green and R.A. Harris, in: The physiology and biochemistry of muscle as a food, eds. J. Briskey, R.G. Cassens and B.B. Marsh (The University of Wisconsin Press, Madison, Milwaukee and London, 1970) pp. 239–271.
[14] J. Popinigis, T. Wrzolkowa and J. Swieczyński, Biochim. Biophys. Acta 245 (1971) 70–79.
[15] J. Popinigis, Y. Takahashi, T. Wakabayashi, R.M. Hull and C.H. Williams, FEBS Letters 19 (1971) 221–224.
[16] J. Popinigis, unpublished observations.
[17] J. Swierczyński and Z. Aleksandrowicz, FEBS Letters 11 (1970) 229–232.
[18] Z. Aleksandrowicz, J. Swierczyński and L. Zelewski, European J. Biochem. 31 (1972) 300–307.
[19] E. Skorkowski, J. Swierczyński and Z. Aleksandrowicz, Comp. Biochem. Physiol. (1973) in press.
[20] J. Popinigis and C. Nowicka, unpublished observations.
[21] C. Nowicka, P. Gmaj and S. Angielski, in preparation.
[22] D. Ciesielski, M. Woźniak, J. Popinigis and M. Zydowo, in preparation.