Biochimica et Biophysica Acta, 590 (1980) 285—289

BBA 47829

RELATION BETWEEN EXTRA- AND INTRAMITOCHONDRIAL ATP/ADP RATIOS IN RAT LIVER MITOCHONDRIA

FRITZ BRAWAND, GEORGES FOLLY and PAUL WALTER

Department of Biochemistry, University of Basel, Vesalianum, Vesalgasse 1, CH-4051 Basel (Switzerland)

(Received August 28th, 1979)

Key words: Pyruvate carboxylase: Atractylate; ATP/ADP ratio; Adenine nucleotide translocase; Respiratory state; (Mitochondria)

Summary

Changes of the extra- and intramitochondrial ATP/ADP ratios as a function of the respiratory state were measured in incubations with rat liver mitochondria. ATPase or creatine/creatine kinase was used to change the extramitochondrial ATP/ADP ratio; the separation of the mitochondrial pellet was performed by a Millipore filtration technique. Under all conditions tested, the intramitochondrial ratio changed in the same direction as the extramitochondrial one, except in the presence of atractylate where this correlation was not observed. Furthermore, it could be shown that the oxygen uptake and pyruvate carboxylase activity correlated with the intramitochondrial ATP/ADP ratio and not with the extramitochondrial one. These results do not support the proposal that the adenine nucleotide translocase is rate limiting for respiration.

Introduction

In our earlier studies with liver mitochondria on the regulation of pyruvate carboxylase, we had observed a close correlation between changes of the extra- and intramitochondrial ATP/ADP ratios [1—3]. The creatine/creatine kinase system had been used for the variation of the mitochondrial ATP/ADP ratio in these experiments. Klingenberg [4] had also shown a positive correlation between changes in the ratios of extra- and intramitochondrial ATP/ADP ratios using the hexokinase/glucose system for the variation of the respiratory state. Yet in both the hexokinase/glucose and in the creatine kinase/creatine system, the concentration of phosphate is not constant and may become rate limiting for phosphorylation during the incubation. The group of Davis [5,6] solved

this problem by the use of purified ATPase. They observed a positive correlation between the amount of ATPase added, oxygen uptake and the extramitochondrial ATP/ADP ratios, however, no changes in the intramitochondrial ratios were found. Together with other observations, these results were interpreted to indicate that the mitochondrial adenine nucleotide translocase is the rate-determining step for respiration [7,8]. These findings would also seem to exclude a regulation of matrix enzymes by matrix ATP/ADP changes as a function of extramitochondrial adenine nucleotide variations.

The results in this paper confirm that the mitochondrial ATP/ADP ratios change as a function of the extramitochondrial ratios under various experimental conditions and also under those used by the group of Davis [6,7]. Possible reasons for the observed discrepancies between the two laboratories will be discussed.

Materials and Methods

Rat liver mitochondria were isolated according to Johnson and Lardy [9] in 0.25 M mannitol/0.07 M sucrose [10] and suspended in the same medium at a concentration of 1 g liver per ml. Male albino Wistar rats from the Swiss Vitamin Institute were used. Purified mitochondrial ATPase from bovine heart mitochondria was isolated by a modification (Davis, E.J., personal communication) of the method described by Penefsky [11]. Part of the ATPase was a gift of E.J. Davis. Separation of mitochondria from the incubation medium was attained by filtration through Millipore filters according to a procedure described earlier in detail [12]. The enzymatic measurements of citrate, malate, ATP and ADP were performed as described earlier [2]. Inorganic phosphate was determined colorimetrically according to a modification of Lowry and Lopez [13]. If phosphate was measured, mannitol was omitted [14].

Results and Discussion

In the experiments of Table I, oxygen uptake and adenine nucleotides were measured under state 3 and 4 conditions as defined by Chance and Williams [15]. The results show that a statistically significant change of 0.8 in the ATP_i/ADP_i ratio could be observed between the two states.

TABLE I

ATP/ADP RATIOS IN STATE 3 AND STATE 4

Mitochondria (2.5 mg protein/ml) were incubated in 3 ml of isotonic medium containing 10 mM potassium glutamate, 2 mM potassium malate, 12 mM potassium phosphate, pH 7.4, 2 mM ATP, 33 mM Tris-HCl, pH 7.4, 2.5 mM $MgCl_2$ at 30°C. State 3 was initiated by the addition of 800 μM ADP. After the ADP had been utilized, the state 4 measurements were performed. Oxygen uptake was recorded with the Clark electrode. A and B, mean values of seven experiments ± S.E. *P* (A and B) <0.001 (unpaired Student's *t*-test). o, extramitochondrial; i, intramitochondrial.

	ATP_o/ADP_o	ATP_i/ADP_i
A. State 3	18.4 ± 2.2	1.9 ± 0.06
B. State 4	245 ± 13.5	2.7 ± 0.14

In Tables II and III, the incubation medium of Davis and Lumeng [7] was used. Increasing amounts of ATPase were added in order to adjust defined steady states between state 3 and state 4. In Table II, the extramitochondrial ratio was progressively diminished from 193 in state 4 to about 30 and respiration approached about 90% of state 3. Even though ATPase addition was always less than that required for state 3 respiration, a gradual decrease of the mitochondrial ATP/ADP ratio was observed with increasing ATPase addition. It is interesting to note that mitochondrial ratios are sensitive to alterations even at very high extramitochondrial ratios. This finding is not consistent with the proposal that the translocation of ADP through the inner membrane is rate limiting. It could be argued that this decrease might be due to a concentration-dependent adsorption of ADP to the mitochondria or to the filter during the separation procedure. However, in the presence of atractylate at high external ADP concentrations (870 nmol/3 ml), the mitochondrial ADP content reached the low value of state 4 conditions (74 nmol/pellet). The results in Table III furthermore show that the oxygen uptake in the presence of ATPase and atractylate corresponded to the intramitochondrial ATP/ADP ratio or to the phosphorylation state. Also when pyruvate carboxylation was measured (Table IV), addition of atractylate clearly showed a positive correlation of enzyme activity with the matrix nucleotide ratio rather than with the extramitochondrial one.

In the experiments of the group of Davis, intramitochondrial ATP/ADP ratios remained insensitive to state 4—3 transitions [16] in heart mitochondria or to additions of ATPase in liver mitochondria [5—7]. Only when a 9-fold excess of ATPase over that required for state 3 respiration was added, was the matrix ATP/ADP ratio appreciably diminished [7]. The reason for this discrepancy between their results and ours is very likely due to the different methods employed for the separation and measurement of the intramitochondrial adenine nucleotides. In the presence of high extramitochondrial concentrations, the centrifugation method used by the group of Davis has the dis-

TABLE II

ATP/ADP RATIOS AS A FUNCTION OF RESPIRATORY STATES ADJUSTED BY ATPase

Mitochondria (4.4 mg protein/ml) were incubated at 30°C for 5 min in 3 ml of isotonic medium containing 66 mM KCl, 33 mM Tris-HCl, pH 7.4, 2 mM potassium phosphate, pH 7.4, 2 mM ATP, 2 mM $MgCl_2$, 10 mM potassium glutamate and 2 mM potassium malate in the presence of different amounts of ATPase. Nucleotides were measured after Millipore filtration and are given as nmol per filtrate or pellet, respectively. The ATP_i:ADP_i values represent geometric means ± S.D. of four incubations with the same mitochondrial preparation. *P* values (unpaired Student's *t*-test) are smaller than 0.001 for the following pairs: b vs. a, d vs. a, e vs. a, f vs. a, d vs. b, e vs. c; for f vs. e $P < 0.005$; for c vs. a $P < 0.01$. Extramitochondrial values are averages of two incubations. For the subscripts o and i see Table I.

% of state 3 respiration	ATP_o/ADP_o	ATP_i/ADP_i
—	5400/28 = 193	96/75 = 1.28 ± 0.02 (a)
25	5120/44 = 119	96/81 = 1.19 ± 0.02 (b)
34	5030/50 = 102	95/84 = 1.13 ± 0.08 (c)
75	5300/72 = 73	95/88 = 1.08 ± 0.03 (d)
90	4870/146 = 33	94/113 = 0.83 ± 0.11 (e)
90 + 15 μM atractyloside	3970/871 = 4.7	86/74 = 1.16 ± 0.07 (f)

TABLE III

OXYGEN UPTAKE AS A FUNCTION OF THE PHOSPHORYLATION STATE

Incubation conditions were similar to those in Table II. 5 mg/ml mitochondrial protein was incubated. The amount of ATPase was the same in the presence and absence of atractyloside. Matrix water was taken as 1 μl/mg protein. ATP_i:ADP_i values represent geometric means ± S.D. of five incubations with the same mitochondrial preparation. *P* values are for b vs. a and c vs. a less than 0.001, for c vs. b less than 0.005 (unpaired Student's *t*-test). All other values are averages of two incubations. For the subscripts o and i see Table I.

Additions	ATP_o/ADP_o	$[P]_o$ (mM)	$ATP_o/ADP_o \cdot P_o$ (M^{-1})	ATP_i/ADP_i	$[P]_i$ (mM)	$ATP_i/ADP_i \cdot P_i$ (M^{-1})	O_2 uptake (natoms O/mg per min)
Control	103	2.1	49 050	1.40 ± 0.05 (a)	16	87.5	17
+ ATPase (77% state 3)	44	2.3	19 130	1.16 ± 0.04 (b)	16	72.5	100
+ ATPase + atractylosyde (15 μM)	2.8	2.9	966	1.26 ± 0.03 (c)	16	78.8	28

advantage that large correction factors have to be employed in order to correct for ATP and ADP adhering to the pellet [7]. This large factor may at least in part be responsible for the method being not sensitive enough to pick up the small difference occurring at the different metabolic conditions. This lack of sensitivity may also explain why Davis and Lumeng [6] were unable to observe a meaningful difference in the intramitochondrial ATP/ADP ratios after addition of atractyloside or palmityl-CoA.

Our results are in agreement with those of Klingenberg [17] who showed that the mitochondrial nucleotides are on the main path of oxydative phosphorylation and that intra- and extramitochondrial adenine nucleotide pools are coupled through the translocase. Our findings do not support the concept of the adenine nucleotide translocase being a limiting step for respiration in liver mitochondria.

TABLE IV

PYRUVATE CARBOXYLATION AS A FUNCTION OF ATP/ADP RATIOS

Mitochondria (4.4 mg protein/ml) were incubated at 37°C for 10 min in 3 ml of isotonic medium containing 4 mM ATP, 10 mM $MgSO_4$, 6.6 mM potassium phosphate, pH 7.4, 10 mM $KHCO_3$, 6.6 mM triethanolamine buffer, pH 7.4. 0.6 μmol of pyruvate was added initially and 0.5 μmol/min by infusion during the incubation. Creatine kinase was added in all incubations. The sum of citrate + malate formed was taken as a measure for pyruvate carboxylation [1]. All values are averages of two incubations. For subscripts o and i see Table I.

Additions	ATP_o/ADP_o	ATP_i/ADP_i	Pyruvate carboxylation (nmol/min per mg)
1.6 mM creatine	87	0.51	23.8
40 mM creatine	8	0.17	6.1
13 mM creatine + 100 μM atractyloside	2.7	0.79	41.8

Acknowledgements

This work was supported by grants of the Swiss National Science Foundation. The excellent technical assistance of Miss L. Lehmann, Miss S. Studer and Miss S. Simmen is gratefully acknowledged. We also thank Dr. G. Schöttli for the preparation of the ATPase.

References

1 Walter, P. and Stucki, J.W. (1970) Eur. J. Biochem. 12, 508—519
2 Stucki, J.W., Brawand, F. and Walter, P. (1972) Eur. J. Biochem. 27, 181—191
3 Walter, P., Mörikofer-Zwez, S. and Brawand, F. (1974) in Regulation of Hepatic Metabolism (Lundquist, F. and Tygstrup, N., eds.), Vol. 6, pp. 79—91, Munksgaard, Copenhagen
4 Klingenberg, M. (1972) in Mitochondria: Biogenesis and Bioenergetics. Biomembranes: Molecular Arrangements and Transport Mechanisms, Proceedings of the 8th FEBS Meeting, Vol. 28, pp. 147—162, Amsterdam, North-Holland
5 Davis, E.J., Lumeng, L. and Bottoms, D. (1974) FEBS Lett. 39, 9—12
6 Davis, E.J. and Lumeng, L. (1974) FEBS Lett. 48, 250—252
7 Davis, E.J. and Lumeng, L. (1975) J. Biol. Chem. 250, 2275—2282
8 Davis, E.J. and Davis-van Thienen, W.I.A. (1978) Biochem. Biophys. Res. Commun. 83, 1260—1266
9 Johnson, D. and Lardy, H.A. (1967) Methods Enzymol. 10, 94—96
10 Graven, S.N., Lardy, H.A. and Rutter, A. (1966) Biochemistry 5, 1735—1742
11 Penefsky, H.S. (1967) Methods Enzymol. 10, 522—526
12 Brawand, F. and Walter, P. (1974) Anal. Biochem. 62, 485—498
13 Lowry, O.H. and Lopez, J.A. (1946) J. Biol. Chem. 162, 421—428
14 Ho, C.H. and Pande, S.V. (1974) Anal. Biochem. 60, 413—416
15 Chance, B. and Williams, G.N. (1955) J. Biol. Chem. 217, 409—427
16 Chao, D.L. and Davis, E.J. (1972) Biochemistry 11, 1943—1952
17 Klingenberg, M. (1977) in Structure and Function of Energy-Transducing Membranes (van Dam, K. and van Gelder, B.F., eds.), Vol. 14, pp. 275—282, Elsevier, Amsterdam