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PHOSPHORYLATION COUPLED TO ACYL-COENZYME A DEHYDROGENASE-LINKED OXIDATION OF FATTY ACIDS BY LIVER AND HEART MITOCHONDRIA

JON BREMER AND E. JACK DAVIS*

Institute of Clinical Biochemistry, Rikshospitalet, University of Oslo, Oslo (Norway)

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SUMMARY

Rat liver and heart mitochondria which oxidised pyruvate, palmitylcarnitine or octanoylcarnitine with phosphorylation to oxygen ratios between 2.3 and 3.0 were used to establish the phosphorylation efficiency accompanying the acyl-coenzyme A dehydrogenase steps of β-oxidation. The P/O ratios observed were always between 1.5 and 2.0. It is concluded that the value of this ratio in the absence of energy losses is probably 2.0.

The data indicate further that the rate of utilisation of fatty acids is not limited by the capacity of the enzymes of β-oxidation in either liver or heart.

The energy yield resulting from the β-oxidation of fatty acids is usually assumed to be 5 moles of ATP per mole of oxygen reduced[1,2]. In analogy with the succinate and α-glycerophosphate oxidase systems, and since the first step of oxidation of each two-carbon fragment is mediated by a flavoprotein, this step is considered to yield two high-energy bonds per two e^- traversing the respiratory chain. This assumption is predicated on the assumption that all electron-transferring flavoproteins have similar redox potentials. That this assumption does not necessarily hold in all cases is illustrated by the fact that lipoamide dehydrogenase probably donates electrons to NAD^+. The reduction potential of this enzyme is very close to that of the NADH–NAD^+ system[3]. The present results are, however, interpreted to support the assumption that the P/O ratio for the flavin-linked steps of β-oxidation of fatty acids is two.

Rat liver and heart mitochondria were prepared according to published procedures[4,5]. Respiration was measured at 25 °C using a Clark oxygen electrode. ADP was standardised spectrophotometrically by enzymic assay for its content of ADP and AMP[6]. Control experiments showed that liver, but not heart mitochondria (as prepared), phosphorylated AMP. Thus, the P/O ratios were calculated with respect to the amounts of ADP *plus* 2 AMP (liver), or only ADP (heart). Respiratory control ratios in the uninhibited system were 12–20 for heart mitochondria oxidising

* On leave of absence from Indiana University School of Medicine, Department of Biochemistry, Indianapolis, Ind., U.S.A.

pyruvate *plus* malate, or palmitylcarnitine, and 7–10 for liver mitochondria oxidising succinate in presence of rotenone. Protein was determined by the method of Lowry *et al.*[7]. Reaction conditions are given in legends to tables and figures. Enzymes, coenzymes and chemicals were purchased from Sigma Chemical Company, St. Louis, Mo.

In order to observe the phosphorylation efficiency of the acyl-CoA dehydrogenase steps of β-oxidation, rotenone was included to prevent electron flow from NADH which is produced in the β-hydroxyacyl-CoA dehydrogenase steps. The oxidised species of NAD-dependent redox substrates were added in order to recycle NAD^+. Oxaloacetate was the only hydrogen acceptor which allowed respiration of heart mitochondria to proceed since, in the latter, a system must also be provided for recycling of CoA. In all cases when oxaloacetate was the added hydrogen acceptor it was present at high concentrations owing to its relatively slow entry into the matrix space of mitochondria. Maximum stimulation of respiration was attained at about 25–50 % of the oxaloacetate concentration used.

Fig. 1 (a–c) demonstrates the stimulation of respiration effected by the addition

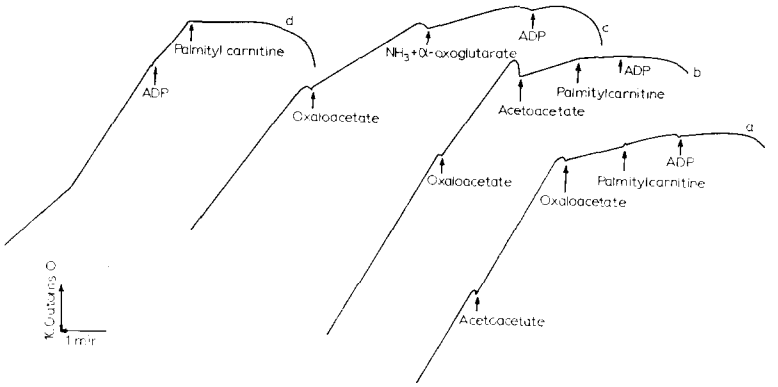

Fig. 1. Stimulation of respiration by oxaloacetate and ADP control of palmitylcarnitine oxidation by rat liver mitochondria in the presence of rotenone. Conditions were as described in Table I, except 3.0 (a–c) or 0.5 (d) μmoles of ADP were added where indicated.

of oxaloacetate, acetoacetate or α-oxoglutarate *plus* ammonia to liver mitochondria incubated in the presence of palmitylcarnitine, ADP and rotenone. Similar results were obtained on addition of oxaloacetate to heart mitochondria under the same conditions. Fig. 1d shows the ADP control of respiration in presence of rotenone and hydrogen acceptor.

Table I summarises the data obtained on respiration rates and phosphorylation efficiencies of rat liver and heart mitochondria incubated with various substrates. As seen from these data, P/O ratios between 2.3 and 2.7 were always observed with substrates which are mainly NAD-linked, whereas the oxidation of succinate, as well as the acyl-coenzyme A dehydrogenase steps of β-oxidation of palmitate or octanoate were accompanied by phosphorylation which gave P/O ratios between 1.5 and 1.8. It is unlikely that succinate formed during incubations with acylcarnitines contributed appreciably to the respiration since in control experiments the oxidation of added succinate was almost completely suppressed by the concentration of oxaloacetate

added in the experiments. The observed P/O with succinate as substrate was usually slightly higher than that obtained with fatty acids in presence of rotenone. Presumably, this is due to the presence of energy 'leaks' which are exaggerated in the latter case as a result of the relatively low rates of respiration; *i.e.* the absolute rate of phosphorylation is much lower than that which obtains with succinate as substrate. In accord with this suggestion, when the rate of oxidation of succinate was limited by malonate, the P/O ratio was slightly lowered (Table I). Also consistent with this argument is the fact that respiration due to both the acyl-CoA dehydrogenase of β-oxidation and succinate dehydrogenase is apparently more loosely coupled to phos-

TABLE I

OXIDATIVE PHOSPHORYLATION AND RESPIRATION OF RAT LIVER AND HEART MITOCHONDRIA INCUBATED WITH VARIOUS SUBSTRATES

Incubations were carried out in a Clark electrode chamber at 25 °C. The reaction mixtures for heart mitochondria contained 250 mM sucrose, 10 mM Tris–HCl, 0.5% bovine serum albumin and 10 mM potassium phosphate. For liver mitochondria, reaction mixtures contained 100 mM KCl, 10 mM potassium phosphate, 0.5% serum albumin and 10 mM *N*-tris(hydroxymethyl)-methyl-2-aminoethane sulphonic acid buffer. Final pH was 7.4 and the volume was 2.5 ml. Substrates and inhibitors, when present, were in the following amounts except where indicated otherwise: palmityl-(−)-carnitine, 200 nmoles; octanoyl-(−)-carnitine, 1.0 μmole; sodium pyruvate, 12.5 μmoles; potassium succinate, 12.5 μmoles; potassium malonate, 10 μmoles; potassium α-oxoglutarate, 10 μmoles; lithium acetoacetate, 25 μmoles; potassium oxaloacetate, 25 μmoles; NH_4Cl, 10 μmoles; potassium malate, 10 μmoles; and rotenone, 10 nmoles. ADP (500 nmoles) was added to initiate rapid respiration, and calculations of P/O ratios were based upon the amount of oxygen consumed during the period of rapid respiration which accompanied the phosphorylation of added ADP. Present in the incubations were 2.5 or 5.0 mg of mitochondrial protein (liver) in the absence and presence of rotenone and hydrogen acceptor (or when succinate *plus* malonate were present); or heart mitochondria, 1.25 and 2.5 mg of protein, respectively. Values are expressed as the mean ± S.E. The number of determinations is given in parentheses.

Substrate	*Other additions*	*P/O*	*Respiration rate (natoms O/mg protein per min)*	
			State 3	*State 4*
Liver				
Palmitylcarnitine	Malate	2.48 ± 0.05 (6)	104 ± 2 *	15 ± 0
Palmitylcarnitine	None	2.32 ± 0.04 (4)	104 ± 3	15 ± 0
Palmitylcarnitine	Malonate	2.27 ± 0.05 (4)	77 ± 2 *	13 ± 1
Octanoylcarnitine	Malonate	2.30 ± 0.06 (4)	79 ± 2	16 ± 2
Succinate	Rotenone	1.76 ± 0.02 (7)	156 ± 4	23 ± 1
Succinate (5 μmoles)	Rotenone, malonate (1.25 μmoles)	1.36 (2)	27	18
Succinate (7.5 μmoles)	Rotenone, malonate (0.5 μmole)	1.55 ± 0.03 (4)	120 ± 4	24 ± 1
Palmitylcarnitine	Rotenone, oxaloacetate	1.61 ± 0.03 (9)	47 ± 1	26 ± 1
Palmitylcarnitine	Rotenone, α-oxoglutarate, NH_3	1.20 (2)	30	15
Palmitylcarnitine	Rotenone, acetoacetate	— (2)	33	27
Heart				
Pyruvate	Malate	2.65 ± 0.04 (5)	254 ± 12	13 ± 1
Palmitylcarnitine	Malate	2.56 ± 0.03 (3)	268 ± 15	19 ± 1
Octanoylcarnitine	Malate	2.31 (2)	260	13
Palmitylcarnitine	Rotenone, oxaloacetate	1.85 ± 0.08 (4)	50 ± 2	24 ± 3
Octanoylcarnitine	Rotenone, oxaloacetate	1.67 ± 0.04 (4)	54 ± 0	30 ± 0

* In some other experiments the effect of malonate on the rate of respiration in presence of palmitylcarnitine was very small. This may be due to biological variability in the capacity of a rate-limiting step in β-oxidation or ketone body production.

phorylation than is that of NAD-linked substrates. In the presence of energy-utilising reactions which are probably going on in all mitochondrial preparations ("ATPases"), State-4 (ref. 8) respiration supported by the oxidation of substrates yielding lower P/O ratios would be stimulated more than that of substrates yielding higher ratios. Indeed, the State-4 respiration supported by succinate or the flavoprotein-linked portion of β-oxidation is about 1.5 times as rapid as that supported by NAD-linked substrates (Table I).

The complete combustion of octanoate proceeds with the reduction of 22 atoms of oxygen per mole of fatty acid, only 6 of which are used in β-oxidation. Since octanoate yields 4 acetyl groups per three cycles of β-oxidation, and there is no possible fate of acetyl-CoA in heart mitochondria other than its entry into the citric acid cycle, it follows that the rate of acetyl group production under State-3 conditions was around 48 nmoles of acetyl/mg of protein per min, whereas in the rotenone-inhibited state in presence of oxaloacetate, the rate of acetyl production can be calculated to be as high as 72. The corresponding values for palmitate oxidation can be calculated in a similar manner to be 40 and 57, respectively. These should be considered minimum values for the capacity of β-oxidation since it is possible that either hydrogen or acetyl acceptor was limiting under the conditions of these experiments. These data do not support the recent suggestion by Pande[9] that the ability of heart mitochondria to oxidise acyl groups is limited by the capacity of the β-oxidation cycle.

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