BBA 46127

REGULATION MECHANISM FOR FATTY ACID AND α-KETOGLUTARATE OXIDATIONS*

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(Received December 29th, 1970)

SUMMARY

In uncoupled mitochondria the activation of fatty acid within the inner membrane matrix space by the ATP-dependent acyl-CoA synthetase produces a considerable accumulation of AMP which remains sequestered within the same space, inhibiting the acyl-CoA synthetase. Addition of α-ketoglutarate induces an immediate transformation of endogenous AMP into ATP and a simultaneous fatty acid oxidation.

As AMP is reconverted into ATP, through the substrate-level phosphorylation promoted by α-ketoglutarate oxidation, the speed of α-ketoglutarate oxidation is slowed down. Addition of oleate stimulates α-ketoglutarate oxidation promoting, through the formation of AMP, a continuous generation of GDP which is necessary for α-ketoglutarate oxidation.

A regulatory mechanism by which α-ketoglutarate and fatty acid oxidation are mutually controlled through changes in the intramitochondrial adenine nucleotide pool is proposed.

INTRODUCTION

Fatty acid activation in mitochondria occurs in two different sites, one in the outer membrane, the other in the inner membrane matrix space[1-6].

In order to reach the oxidation site which is located within the inner membrane, fatty acids activated by the ATP-dependent acyl-CoA synthetase, located in the external site, require carnitine as a carrier: carnitine-dependent oxidation[1-6].

Responsible for the internal activation are two acyl-CoA synthetases: (Acid: CoA ligase (AMP), EC 6.2.1.3) one ATP-dependent[1-6], the other GTP-dependent[2,7-9]; the former is inhibited by AMP[6,10], the latter by inorganic phosphate[6,9].

In the present paper a regulatory mechanism of the internal ATP-dependent acyl-CoA synthetase is described. It has been found that such a regulation is mediated by the composition of the internal pool of adenine nucleotides and precisely by the ATP/AMP ratio. Furthermore, it was possible to demonstrate a close interrelationship between fatty acid and α-ketoglutarate oxidation, the latter also being regulated by the endogenous ATP/AMP ratio.

* Part of these results has been presented at the "Colloquium on Bioenergetics", Pugnochiuso, Italy, September 1970.

When the ATP/AMP ratio is high, fatty acid oxidation is facilitated and α-ketoglutarate oxidation inhibited; when the ratio is low, fatty acid oxidation is depressed and α-ketoglutarate oxidation stimulated.

EXPERIMENTAL PROCEDURE

Liver mitochondria were prepared from Wistar strain albino rats essentially by the procedure of SCHNEIDER[11]. Oxygen uptake was measured with a Clark oxygen electrode as described by KIELLEY AND BRONK[12]. The concentrations of ATP, ADP and AMP in mitochondria were measured enzymically as previously described[13]. Protein concentration was determined by the biuret reaction[14]. The enzymes used for analysis were purchased from Boehringer. [$^{14}C_{18}$]Oleate and α-[5-^{14}C]ketoglutarate were obtained from Radiochemical Centre, Amersham.

RESULTS

As shown in Fig. 1, in the presence of DNP* oligomycin and malonate, oleate oxidation in liver mitochondria declined very rapidly, and was restored by the addition of a catalytic amount of α-ketoglutarate.

An inspection of the adenine nucleotide pattern before and after α-ketoglutarate addition revealed that the stimulation of fatty acid oxidation by α-ketoglutarate was concomitant with a sharp change in the endogenous adenine nucleotide pool (Fig. 1).

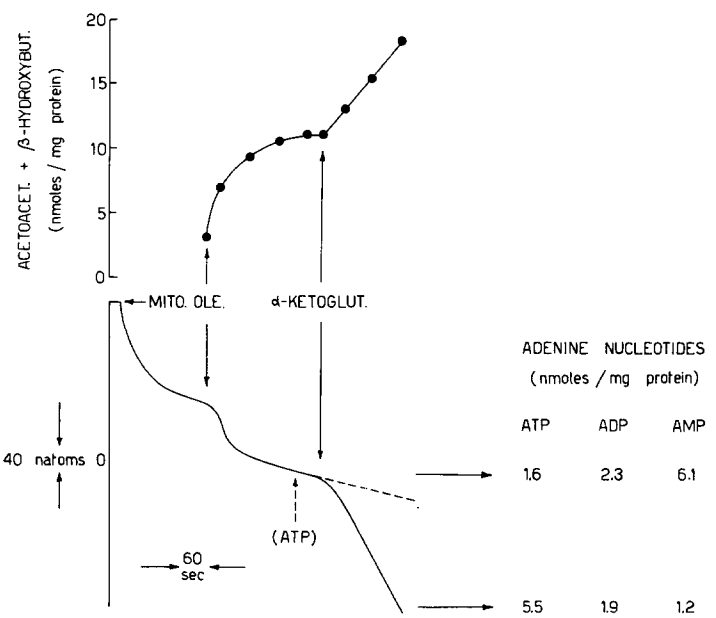

Fig. 1. Oleate oxidation and changes in adenine nucleotides in rat liver mitochondria. The incubation system contained 16 mM phosphate buffer (pH 7.4), 26 mM NaCl, 58 mM KCl, 6 mM $MgCl_2$, 1.25 mM malonate, 0.05 mM dinitrophenol (DNP) and 10 μg of oligomycin. Total vol. 2.0 ml. At the points indicated by arrows 10 mg of mitochondrial proteins (MITO), 0.2 mM oleate (OLE) and 4 mM α-ketoglutarate (α-KETOGLUT) were added.

* DNP = dinitrophenol.

In particular it can be observed that addition of α-ketoglutarate induced an immediate increase of the ATP/AMP ratio.

Evidence that α-ketoglutarate was activating oleate oxidation and not just sering as an oxidizable substrate was provided by the formation of ketone bodies which paralleled the oxygen uptake evoked by addition of α-ketoglutarate (see the upper curve of Fig. 1). Furthermore using [$^{14}C_{18}$]oleate as oxidizable substrate, the amount of the [^{14}C]-labelled acid-soluble metabolites plus evolved $^{14}CO_2$ was increased more than 2-fold upon addition of α-ketoglutarate.

TABLE I

ENDOGENOUS ADENINE NUCLEOTIDES IN RAT LIVER MITOCHONDRIA DURING OXIDATIVE PHOSPHORYLATION IN THE ABSENCE AND IN THE PRESENCE OF ADDED ADP

Incubation system as in Fig. 2. Where ADP was added the mitochondria were diluted after incubation with 15-fold excess ice-cold 0.25 M sucrose. The endogenous adenine nucleotides were determined in the mitochondrial pellets recovered after centrifugation for 5 min at 20000 × *g*.

Additions	*Incubation time (min)*	*Adenine nucleotides (nmoles/mg protein)*		
		ATP	*ADP*	*AMP*
None	0	0.9	4.3	3.4
Succinate	2	5.6	2.5	1.8
α-Ketoglutarate	2	6.0	2.7	1.3
Succinate + ADP + atractyloside	2	6.0	2.4	1.5
α-Ketoglutarate + ADP + atractyloside	2	5.9	2.7	1.4
Succinate + ADP	2	1.7	4.4	3.6
α-Ketoglutarate + ADP	2	2.6	5.4	1.6

The fundamental role of α-ketoglutarate oxidation for the phosphorylation of intramitochondrial AMP (ref. 15) appears also from the results reported in Table I. These results show that in coupled mitochondria incubated in the absence of added ADP (State 4) or in the presence of added ADP *plus* atractyloside both succinate (and any other oxidizable substrate, not shown in Table I) and α-ketoglutarate promoted a remarkable removal of endogenous AMP. However, in the presence of added ADP (State 3) only α-ketoglutarate oxidation was efficient in phosphorylating intramitochondrial AMP. Hence, it could be argued that, since State 3 presumably is the condition *in vitro* which most approaches the physiological situation of mitochondria within the cell, α-ketoglutarate oxidation is critical for the phosphorylation of intramitochondrial AMP and consequently for allowing favourable conditions for fatty acid activation.

The experiment shown in Fig. 2 shows that in DNP- and oligomycin-treated liver mitochondria α-ketoglutarate oxidation rapidly slowed down and could be restored by addition of oleate, while palmitylcarnitine had no effect.

Data relative to mitochondrial adenine nucleotides show that during α-ketoglutarate oxidation AMP was quantitatively transformed into ATP (Fig. 2). From the same figure it can also be observed that the AMP decrease was concomitant with the decline of α-ketoglutarate oxidation.

That the oxidation of α-ketoglutarate was, at least in part, responsible for the

increased oxygen uptake consecutive to the addition of oleate was also clearly shown by using as oxidizable substrate α-[5-^{14}C]ketoglutarate.

With this labelled substrate it was observed that upon addition of oleate the evolution of $^{14}CO_2$ was significantly increased. Indirect evidence was provided by the increase of ADP (see Fig. 2). The mitochondria being incubated in the presence of DNP and oligomycin the rise of endogenous ADP can only be explained with the reaction catalyzed by the GTP–AMP phosphotransferase.

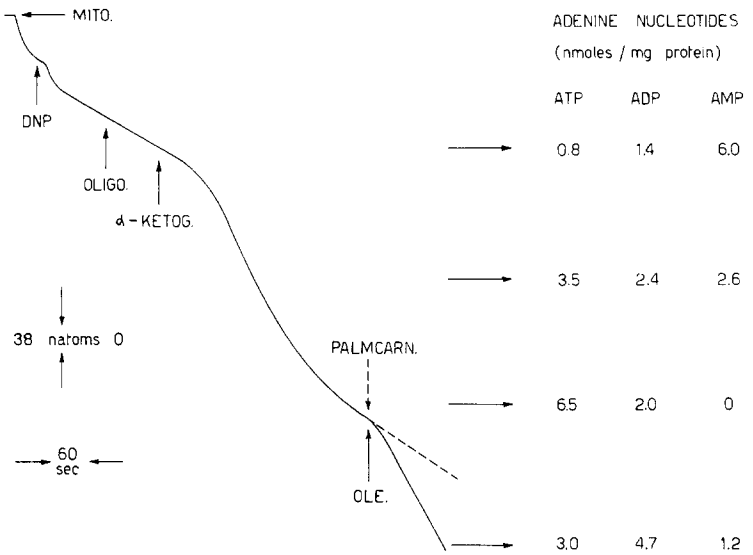

Fig. 2. Oxidation of α-ketoglutarate and of oleate in rat liver mitochondria. The incubation system contained 16 mM phosphate buffer (pH 7.4), 26 mM NaCl, 58 mM KCl and 6 mM $MgCl_2$. At the points indicated by arrows 10 mg of mitochondrial proteins (MITO), 0.05 mM dinitrophenol (DNP), 10 μg of oligomycin (OLIGO), 4 mM α-ketoglutarate (α-KETOG), 0.5 mM palmitylcarnitine (PALMCARN.) and 0.2 mM oleate (OLE) were added.

DISCUSSION

In the presence of DNP and oligomycin and in the absence of added adenine nucleotides the only source of energy for the activation of fatty acid was the substrate-level phosphorylation supported by α-ketoglutarate oxidation. Furthermore, the presence in all the experiments of 16 mM phosphate rules out the involvement of the GTP-dependent acyl-CoA synthetase which is inhibited by P_i[6,9].

From the results reported in Fig. 1 it is evident that the stimulation of oleate oxidation by α-ketoglutarate is concomitant with an increase of the ATP/AMP ratio. It must be underlined here that the only possibility for AMP phosphorylation within the inner membrane matrix space is represented by the reaction catalyzed by GTP–AMP transphosphorylase[15–17]; in fact, adenylate kinase is located in the outer mitochondrial space[17]. Furthermore, internal AMP cannot exchange with external adenine nucleotides as demonstrated by PFAFF AND KLINGENBERG[18]; this circumstance explains why in our experiments added ATP was unable to modify the pattern of mitochondrial adenine nucleotides and to stimulate the oxidation of oleate.

Also in coupled mitochondria α-ketoglutarate oxidation appears to be important for the promotion of fatty acid activation by internal ATP-dependent acyl-CoA synthetase. In State 3, only α-ketoglutarate, but not succinate or other substrates, was efficient in decreasing the intramitochondrial level of AMP.

This result clearly indicates that the rate of exchange between internal ATP produced in the phosphorylation linked to the respiratory chain and external ADP is so high to prevent the reaction:

$$ATP + GDP \leftrightharpoons ADP + GTP$$

In other words, it seems that in State 3 GTP is substantially produced only *via* the substrate-level phosphorylation. In contrast, in State 4 GTP is produced also from ATP through the above transphosphorylation reaction.

All the results obtained both in uncoupled as well as in coupled mitochondria are consistent with the interpretation that α-ketoglutarate stimulates fatty acid oxidation through an increase of the ATP/AMP ratio.

The results reported in Fig. 2 show that during α-ketoglutarate oxidation AMP is progressively and quantitatively transformed into ATP. It is reasonable to assume that the disappearance of AMP is responsible for the inhibition of α-ketoglutarate oxidation. In fact, as it can be deduced from the following reactions, catalyzed by GTP–AMP and GTP–ADP phosphotransferase, respectively, AMP plays an important role in the regeneration of GDP from GTP.

$$GTP + AMP \leftrightharpoons GDP + ADP \quad (1)$$

$$GTP + ADP \leftrightharpoons GDP + ATP \quad (2)$$

$$2GTP + AMP \leftrightharpoons 2GDP + ATP$$

AMP through Reaction 1 produces GDP and ADP, the latter displaces the equilibrium of Reaction 2 towards the formation of additional GDP.

Consequently, it appears clear that fatty acid activation stimulates α-ketoglutarate oxidation by promoting, through the formation of AMP, a continuous generation of GDP which is necessary for α-ketoglutarate oxidation. The dependence of endogenous AMP phosphorylation on the oxidation of α-ketoglutarate has been previously recognized by HELDT AND SCHWALBACH[15].

This would explain why palmitylcarnitine, unlike oleate, is without effect on α-ketoglutarate oxidation (see Fig. 2). In fact, it is well known that the oxidation of acyl-carnitine, already activated substrate, occurs without formation of AMP. It could be outlined that also palmitylcarnitine is not oxidized.

The observation that under these conditions arsenate restores α-ketoglutarate oxidation (results not shown) indicates that the observed block of α-ketoglutarate oxidation is consequent to succinyl-CoA accumulation. Very probably the accumulation of succinyl-CoA involves, in turn, a shortage of free CoA necessary for palmitylcarnitine oxidation. The situation is different when oleate is the substrate. In this case the preliminary activation step, through the formation of acyl-AMP and its enzymatic hydrolysis[19], makes AMP available for succinyl-CoA utilization. Consequently more CoA becomes available for oleate oxidation.

In the light of the present results, the intramitochondrial ATP/AMP ratio appears to be an important regulatory system both for fatty acid and α-ketoglutarate

oxidation. When the ATP/AMP ratio is high, fatty acid activation and oxidation is stimulated; when the ATP/AMP ratio is low, α-ketoglutarate oxidation and the substrate-level phosphorylation is stimulated (see Fig. 3).

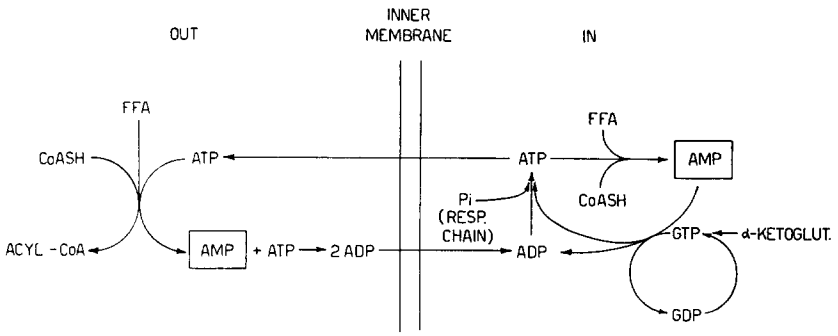

Fig. 3. Scheme for mitochondrial adenine nucleotides changes during fatty acid (FFA) and α-ketoglutaric (α-KETOGLUT) oxidation. FFA, free fatty acids.

AMP produced outside the inner membrane during the external activation of fatty acid does not seem to have any regulatory role, since it is removed, as it is formed, by the very active adenylate kinase, which is absent in the internal space[10]. Such an interpretation is summarized in the scheme of Fig. 3.

These results provide a significant example of a mutual influence between different oxidizable substrates through modifications of the mitochondrial adenine nucleotide pool.

From a general point of view it could be argued that while the oxidation of some substrates modifies the adenine nucleotide pattern, the latter could be discriminatory in promoting the oxidation of some among the available substrates.

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