THE EFFECT OF ADENINE NUCLEOTIDES UPON THE 2-OXOGLUTARATE DEHYDROGENASE OF BLOWFLY FLIGHT MUSCLE

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1. Introduction

Earlier studies [1] showed that blowfly flight muscle mitochondria only oxidise 2-oxoglutarate at a maximal rate if the majority of the intramitochondrial adenine nucleotide pool consists of ADP. The present work indicates that ATP inhibits a partially purified 2-oxoglutarate dehydrogenase (EC 1.2.4.2) from this tissue, and that ADP and AMP reverse this inhibition. This is believed to be the first report of an effect of adenine nucleotides upon a 2-oxoglutarate dehydrogenase of animal origin.

2. Materials and methods

Blowflies (*Calliphora erythrocephala*) were frozen at the temperature of solid CO_2/acetone and fractionated into heads, abdomens and thoraces. Approx. 25 g of thoraces were used for each preparation of the enzyme, using the method of Sanadi [2]. A slight modification was the use of 10 mM potassium phosphate pH 7.2 for suspension of the mitochondrial membranes, prior to freeze-thawing. The usual yield at this stage (i.e. in the "amber extract") was 15–20 international units. The extract was further purified either by ammonium acetate fractionation [2], which resulted in a heavy contamination by pyruvate dehydrogenase, or by ammonium sulphate precipitation between 29 and 41% saturation, followed by ultracentrifugation of the dissolved precipitate at 100,000 *g* for 2 hr, and extraction of the subsequent pellet in 30 mM potassium phosphate pH 7.2. The results presented were similar whichever method of purification was used. In each case the final yield was rather poor. Glutamate dehydrogenase and succinic thiokinase were undetectable providing that the initial "low speed spin" of the Sanadi method [2] of 2,000 *g* for 30 min was used, and the pellet discarded. This probably serves to remove any intact mitochondria. ATPase (assayed at 2 mM ATP) was present at up to 33% of the oxoglutarate dehydrogenase activity, but at this activity should not have hydrolysed more than 5% (at 0.21 mM) or 0.5% (at 2.1 mM ATP) of the ATP added, during the course of the experiment. All preparations contained some pyruvate dehydrogenase which was found very difficult to remove.

2-Oxoglutarate dehydrogenase activity was determined in a system comprising 0.1 M potassium phosphate, of the pH indicated, 10 mM $MgCl_2$, 5 mM cysteine, 50 μM coenzyme A, 0.2 mM NAD, 0.24 mM thiamine pyrophosphate and the concentrations of 2-oxoglutarate and adenine nucleotides given in the appropriate figure legends. The reduction of NAD was monitored at 340 nm using a Hilger–Gilford spectrophotometer.

In assays of 2-oxoglutarate dehydrogenase (enzyme 1 of the complex) the system comprised 0.1 M potassium phosphate, 0.24 mM thiamine pyrophosphate, 1 mM ferricyanide and varied concentrations of 2-oxoglutarate and adenine nucleotide. Decrease in absorbance at 420 nm was followed.

The temperature was 25° throughout.

3. Results

ATP inhibits 2-oxoglutarate dehydrogenase, by

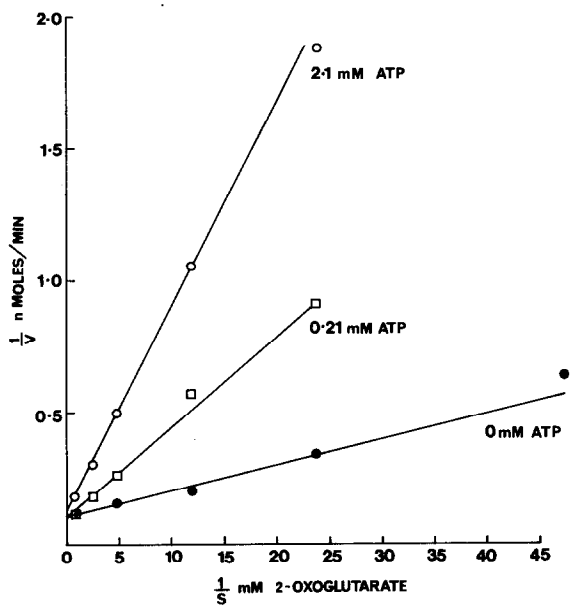

Fig. 1. The competitive nature of the inhibition of the 2-oxoglutarate dehydrogenase complex by ATP. The rate of reduction of NAD was determined, at pH 7.4 and the concentrations of ATP indicated. Extra $MgCl_2$ was added to 2 mM in the incubations involving 2.1 mM ATP.

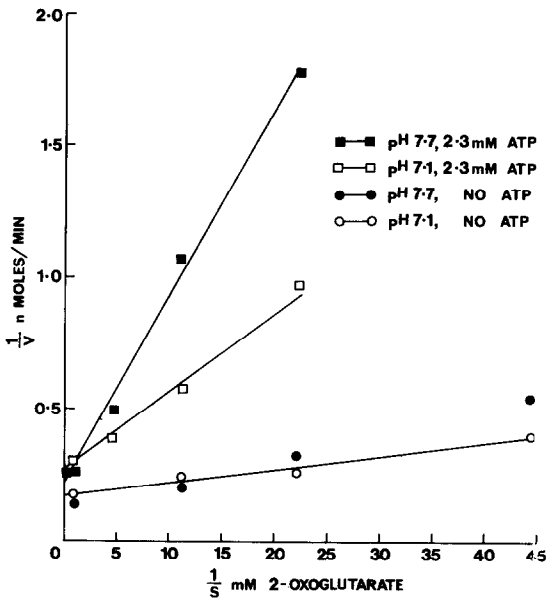

Fig. 2. The effect of pH upon the inhibition by ATP. The pH and the concentrations of ATP were as indicated on the diagram, and the rate is that of NAD reduction.

elevating the K_m for 2-oxoglutarate (fig. 1). Thus, the K_m in the absence of nucleotide was 0.09 mM whereas in the presence of 0.21 mM ATP it was 0.32 mM and in the presence of 2.1 mM ATP, 0.46 mM. The former 2 figures indicate a K_i for ATP of 91 μM. The effect is more pronounced at alkaline than at neutral pH, as shown in fig. 2. This does not simply reflect a lower concentration of ATP^{4-} at pH 7.0, as raising the ATP concentration to 4.2 mM gave no greater inhibition. The difference in K_m for 2-oxoglutarate in the absence of ATP seen in comparing figs. 1 and 2 reflects storage and freeze-thawing of the enzyme used. The inhibitory effect of ATP was considerably reduced when the ferricyanide assay was used, under otherwise identical conditions.

The inhibition by 2 mM ATP is reversed by ADP or AMP (fig. 3). Neither of these nucleotides activates the enzyme, at non-saturating levels of 2-oxoglutarate, in the absence of ATP. *In vivo* the total concentration of adenine nucleotide within the mitochondrion remains constant but the phosphorylation state varies, conditions reproduced in the experiment of fig. 4. It is seen that maximal activity is dependent on from 25–100% ADP, depending on the pH.

GTP was found to be much less inhibitory than ATP and GDP totally ineffective in reversing the inhibition due to ATP.

4. Discussion

The fact that 2-oxoglutarate dehydrogenase, an enzyme catalysing a reaction with a large negative standard free energy change, is sensitive to the phosphorylation state of the adenine nucleotide pool may be of significance in connection with the control of this segment of the tricarboxylic acid cycle. Atkinson [3] has produced a quantitative measure of phosphorylation state in the parameter "energy charge", and investigated the behaviour of several enzymes with respect to this parameter. This approach was simplified here (fig. 4) by the omission of AMP, for the reason that AMP is not translocated across the mitochondrial membrane [4], and there is no obvious

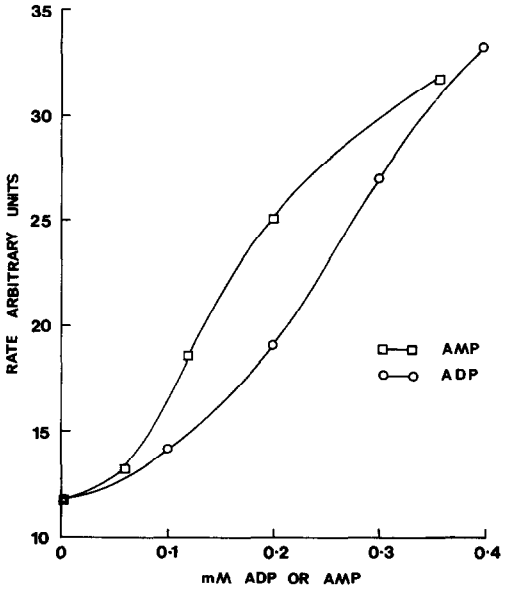

Fig. 3. The reversal of ATP inhibition by either ADP or AMP. The rate of NAD reduction was determined at pH 7.4, in the presence of 80 μM 2-oxoglutarate and 4 mM ATP. The corresponding rate in the absence of any nucleotide was 39 arbitrary units.

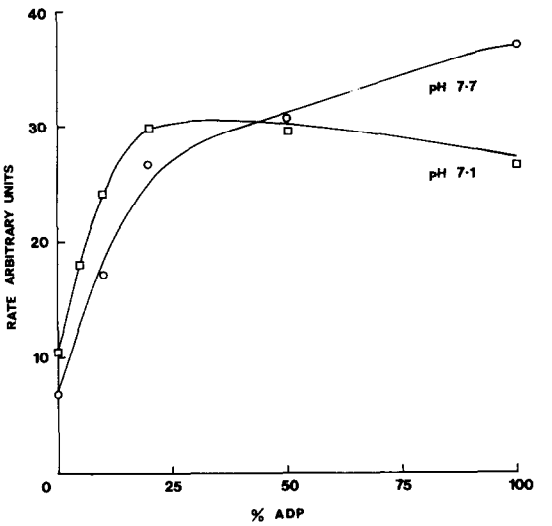

Fig. 4. The dependence of the activity of the 2-oxoglutarate dehydrogenase complex upon the composition of a mixture of ADP and ATP, total concentration 4.5 mM. The concentration of 2-oxoglutarate was 80 μM, and the pH as indicated.

source of AMP within the fly flight muscle mitochondrion.

The inhibition of 2-oxoglutarate dehydrogenase does not seem to reflect a phosphorylation of the enzyme, as is found for pyruvate dehydrogenases from animal sources [5, 6] in that there is no time-dependent variation in activity when the enzyme, at any stage of purification after freeze-thawing, is incubated under the conditions of Wieland and Siess [6].

The locus of action of ATP within the complex has not been identified, although the competition with respect to 2-oxoglutarate might suggest the oxoglutarate dehydrogenase, enzyme 1. In this case, one would expect a similar effect in the ferricyanide-linked assay, as has indeed been found for the inhibition of bacterial pyruvate dehydrogenase by GTP [7]. In fact, ATP inhibition is greatly reduced under these conditions.

References

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