AN ATP-DEPENDENT SUCCINIC THIOKINASE IN BIRDS AND ITS RELATION TO KETONE-BODY UTILIZATION

Mari L. HAMILTON and J. H. OTTAWAY
Department of Biochemistry, Hugh Robson Building, George Square, Edinburgh EH8 9XD, Scotland

Received 27 November 1980

1. Introduction

Computer simulation of the tricarboxylic acid cycle in mammalian heart mitochondria leads to a prediction that an important control point for the utilization of acetoacetate lies at succinic thiokinase (STK) [1,2], because STK and 3-oxoacid transferase compete for succinyl-CoA. STK has a much higher affinity for this substrate than does the activating enzyme, so if acetoacetate is sole substrate, not enough is activated to keep the cycle running, unless STK is inhibited. It is suggested that maintaining a high mitochondrial GTP/GDP ratio would be an effective way of exercising control [3].

In [4] the nucleotide specificity of STK from a wide range of animal species was examined to assess the relationship between ketone body utilization and GTP control. They found that extracts of pigeon heart appeared to use both ATP and GTP for the conversion of succinate to succinyl-CoA. After a more detailed examination, both of pigeon and chicken tissues, we now report that not only is there a close relationship between the GTP specificity of STK and acetoacetate utilization, but also that the STK of pigeon breast muscle uses only ATP and not GTP. This is the first report of an ATP-dependent succinic thiokinase in vertebrate tissues (cf. [5,6]).

2. Materials and methods

Adult pigeons were obtained commercially, and 6-week-old chickens from the Poultry Research Centre, Roslin, Midlothian. High-speed supernatants of tissue homogenates were prepared as in [4]. Mitochondria were usually prepared in mannitol (0.22 M)/sucrose (0.07 M)/MOPS (0.002 M) medium, but muscle mitochondria were somtimes prepared in the KCl medium in [7]. Proteinase was not used. Mitochondria were aged in the presence of EDTA (0.3 or 3.0 mM) as in [8]. Substrates and inhibitors were purchased from Boehringer Mannheim.

Succinic thiokinase activity was measured at 235 nm according to [5] and nucleoside diphosphate kinase (NDK) was measured using dCTP as phosphoryl donor and ADP as acceptor [8]. Both enzymes were released from mitochondria by sonication, which was found to be more effective than lubrol treatment, especially for STK. Protein was measured by biuret.

3. Results

In 140 000 × *g* supernatants of sonicated homogenates from both pigeon and chicken tissues, the existence of both GTP-dependent and ATP-dependent STK activity was detected (table 1). The ratio of activity with GTP or ATP as substrate varied from tissue to tissue, except that in extracts of pigeon breast muscle, no GTP-dependent esterification of succinate could be detected. No comparable tissue was found in chickens. Because of the possibility that ATP-dependent activity might be an artifact caused by NDK in the extracts, the assays were repeated on fresh mitochondria (twice-washed) and on sonicates of mitochondria aged for 6 h in the presence of EDTA to remove NDK [8]. In fact, while substantial NDK activity was found in crude homogenates and 15 000 × *g* supernatants, no activity could be detected in washed mitochondria, whether fresh or aged, of heart, liver or pigeon breast muscle. Thus in mitochondrial

 Elsevier/North-Holland Biomedical Press

Table 1
STK activity in tissue homogenates

Tissue	GTP	ATP	GTP/ATP ratio
Liver			
Pigeon	4.0 ± 3.4	+[a]	–
Chicken	7.8; 2.3	0[a]	–
Kidney			
Pigeon	19.1 ± 8.9	4.0 ± 0.6	4.8
Chicken	16.0	2.2	7.3
Brain			
Pigeon	5.5; 0.9	6.6; 7.5	0.45
Chicken	0.6; 0.2	3.8; 2.0	0.14
Heart			
Pigeon	3.2 ± 1.8	15.2 ± 4.0	0.21
Chicken	8.2	12.6	0.65
Breast muscle			
Pigeon	0.0 ± 0	24.1 ± 6.3	0
Gizzard muscle			
Chicken	0.3	2.3	0.13

[a] Results unreliable because of a high blank due to an ATP-specific synthesis of coenzyme A thio-ester, presumably from endogenous fatty acids

Values in nmol CoA ester formed . min^{-1} . mg $protein^{-1}$. SD is quoted for the mean of 3 obs.

Table 2
Nucleotide specificity of STK in mitochondrial extracts

	GTP	ATP	ITP	GTP/ATP
Breast muscle	0.0	69.3	0.0	0
	0.0	22.5	0.0	0
	0.0	22.7	0.0	0
	0.0	19.8[a]	–	0
Heart muscle	4.3[b]	12.0	–	0.36
	12.0[b]	16.9[b]	ND	0.71
	2.8[b]	12.0[a]	–	0.23
	6.5	62.9	7.7	0.10
	5.0	36.7[a]	3.8	0.14
Liver	30.1	13.9	–	2.2
	2.8	0	–	∞
	0.8	0[a]	–	∞
Kidney	17.9	0.7	10.9	25.6

[a] Aged mitochondria; [b] Chicken

Values are in nmol/CoA esterified . min^{-1} . mg $protein^{-1}$

sonicates, utilization of ATP through NDK can be ruled out, but the pattern of nucleotide specificity, and the GTP/ATP activity ratio in mitochondria from various tissues, remains (see table 2) very much as it is in total tissue homogenates.

Succinic thiokinase from mammalian sources uses ITP as well as GTP [5]. It was found that extracts from those tissues which could use GTP could also use ITP, but extracts of pigeon breast mitochondria could not. This provides additional evidence that the breast muscle enzyme differs from that in other avian tissues. An O_2 electrode was used to measure oxidation of acetoacetate and other substrates in coupled mitochondria of pigeon breast muscle, heart, kidney and liver. Liver mitochondria did not oxidize acetoacetate at all. Heart muscle mitochondria were able to oxidize acetoacetate rapidly in the absence of other substrates in the medium. There was no detectable oxidation of endogenous substrate either in the presence or absence of added ADP in these mitochondria. Oxygen consumption in the presence of acetoacetate was ~50% of the rate in the presence of succinate, oxoglutarate or glutamate (fig.1b). Kidney mitochondria behaved similarly, although they oxidized acetoacetate more slowly.

Breast muscle mitochondria were completely unable to utilize acetoacetate (fig.1a), unless a low concentration of oxoglutarate was also added. Oxoglutarate itself was oxidized very readily and its concentration had to be reduced to ~0.1 mM before acetoacetate oxidation could be detected. The significance of these findings is discussed below.

4. Discussion

The results in table 1 and 2 confirm the findings in [4], and show that in several avian tissues the STK reaction can be supported by GTP, ITP and ATP. In the breast muscle of the pigeon, however, only ATP can be utilized, and this is the first observation of an ATP-dependent succinic thiokinase in vertebrate tissues. Hansford [6] purified an ATP-dependent STK from blowfly flight muscle.

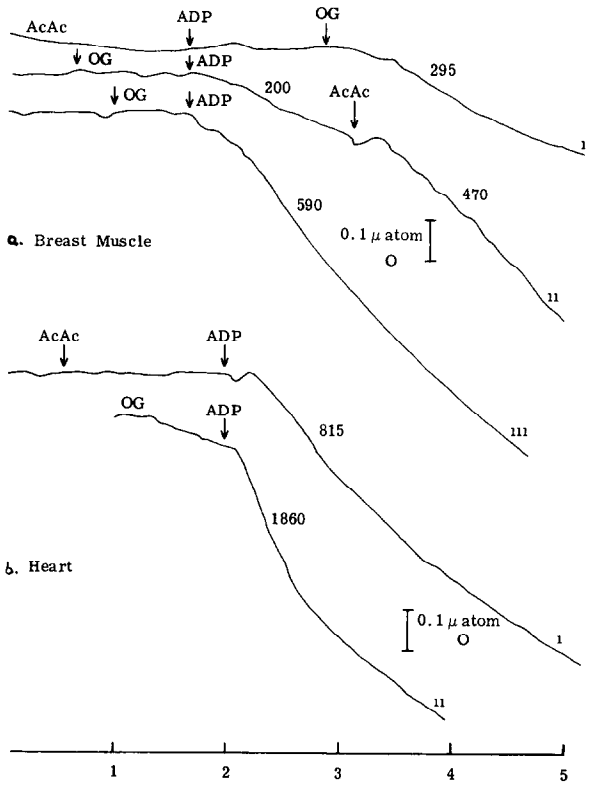

Fig.1. O_2 uptake by pigeon muscle mitochondria. The numbers are μatom O disappearing . min^{-1} . mg $protein^{-1}$ at the points indicated. In the upper curves (i, ii) oxoglutarate was 0.3 mmol/l; in the other experiments in which it was used, oxoglutarate was 30 mmol/l.

In [2] it was predicted that, given the same ratio of K_m-values for STK and 3-oxoacid transferase as holds for the mammalian enzymes, mitochondria containing an ATP-dependent STK would be unable to activate acetoacetate at a significant rate, since the mitochondrial ATP/ADP ratio is generally <1. Because the mitochondrial GTP/GDP ratio is thought to be much greater, for reasons not well understood [3], tissues with a GTP-dependent STK should be able to utilize acetoacetate rapidly, if they possess the CoA transferase.

These predictions are substantially validated by the observations reported here. Homogenates of pigeon breast muscle can utilize acetoacetate [9], but washed mitochondria from this tissue cannot (fig.1a), unless they are also supplied with a continuous precursor of succinyl-CoA (oxoglutarate). Breast muscle STK is entirely ATP-dependent. On the other hand, pigeon heart muscle mitochondria utilize acetoacetate readily (fig.1b) in the absence of any other substrate. As table 2 shows, heart muscle mitochondria have an active GTP-dependent STK. The actual level of acetoacetate utilization in a particular tissue must depend on the concentration of the transferase; although liver and kidney both have an active GTP-dependent STK, liver mitochondria do not oxidize acetoacetate, presumably because they lack the transferase, while kidney mitochondria do so only slowly. In vivo, the evidence suggests that kidney and brain are the main tissues using ketone bodies in birds [10]. Nevertheless product inhibition control by nucleotide triphosphate/diphosphate ratios is now firmly established as a mechanism by which ketone body utilization is regulated.

It is not yet clear how many STK variants there are in avian tissues. The results shown in table 2 could be due to the presence of two enzymes, or of a single enzyme with broad specificity. Attempts to answer this question by using differential enzyme inhibitors will be reported elsewhere; they are at present inconclusive. The strongest evidence for the presence of two enzymes in heart and kidney mitochondria is the consistent 100-fold divergence in the ratio between observed activity with GTP or ATP in the two tissues. We are not yet convinced that the ATP-dependent enzyme in these tissues is identical with the enzyme found in pigeon breast muscle, and presumably also in the pectoral muscle of other flying birds.

References

[1] Ottaway, J. H. and McMinn, C. L. (1979) Biochem. Soc. Trans. 7, 411–412.
[2] Ottaway, J. H. and McMinn, C. L. (1980) FEBS Symp. 60, 69–82.
[3] Ottaway, J. H., Sanderson, C. L. and McClellan, J. A. (1981) Int. J. Biochem. in press.
[4] McClellan, J. A. and Ottaway, J. H. (1980) Comp. Biochem. Physiol. 67B, 679–684.
[5] Cha, S. (1969) Methods Enzymol. 13, 62–69.
[6] Hansford, R. G. (1973) FEBS Lett. 31, 317–320.
[7] Chappell, J. B. and Perry, S. V. (1954) Nature 173, 1094–1095.
[8] Pedersen, P. L. (1973) J. Biol. Chem. 248, 3956–3962.
[9] Krebs, H. A. (1961) Biochem. J. 80, 225–233.
[10] Bailey, E. and Horne, J. A. (1972) Comp. Biochem. Physiol. 42B, 659–669.