Regulation of pantothenate kinase from various tissues of the rat

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The relative tissue activities of pantothenate kinase range from 1 to 5 nmol·min^{-1}·g^{-1} wet wt for heart, brain, kidney, and liver. The enzyme partially purified from each tissue is inhibited by CoA, but there is a 10-fold greater potency of inhibition of the heart enzyme compared to those from the other tissues. With the heart and liver enzyme, this difference in potency of CoA inhibition may be a reflection of the differing cytosolic CoA concentrations in these tissues. L-Carnitine specifically reversed the inhibition of each enzyme by CoA. It is concluded that L-carnitine may be a regulator of CoA synthesis in each tissue.

Pantothenic acid *Coenzyme synthesis* *Carnitine kinase* *Pantothenate kinase* *Carnitine synthesis* *CoA synthesis*

1. INTRODUCTION

Pantothenate kinase (ATP:D-pantothenate 4'-phosphotransferase, EC 2.7.1.33) was initially found in bacteria [1], rat liver and kidney [2] and identified as the first enzyme in the pathway of CoA synthesis [3]. Recently, the enzyme was shown to be the rate-limiting step for CoA synthesis in perfused hearts [4]. There are no reports of the relative activities of pantothenate kinase in different tissues, but some properties have been described for the isolated enzyme. CoA is an inhibitor of pantothenate kinase from liver and kidney [5–7] and may therefore be a feedback inhibitor of its own synthesis. In studies of the enzyme from heart, we have also found CoA to be an inhibitor (unpublished), but with a much greater potency of inhibition than is reported for the liver and kidney enzyme. In addition, we found that L-carnitine could specifically reverse the inhibition of the heart enzyme by CoA. These properties of the heart enzyme could suggest tissue-specific variation in the properties of pantothenate kinase. Thus the aims of our experiments were to measure the relative activities of pantothenate kinase in different tissues of the rat, determine the potencies of inhibition by CoA, and examine the effects of L-carnitine on this inhibition.

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2. MATERIALS AND METHODS

2.1. *Materials*

Substrates, creatine kinase, DTT, CoA and Dowex 1X8-400 (chloride form) were from Sigma. L- and D-carnitine (inner salts) were from Sigma Tau, Italy. PD 10 desalting columns were from Pharmacia. D-[^{3}H]Pantothenate (1.1 Ci/mmol) and ACS 11 scintillation fluid were from Amersham. All other chemicals were reagent grade or better.

2.2. *Tissue extraction*

1–4 g of heart, brain, liver or kidney from male Sprague-Dawley rats (200–300 g) were extracted in 4 vols cold buffer using a Polytron (Brinkman Instruments) at 40% maximum speed. The buffer

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consisted of 20 mM Mops, 0.5 mM EDTA, 2 mM DTT and 0.25 M sucrose, pH 7. Homogenates were centrifuged at 30000 × *g* for 40 min, and the supernatants collected. In a separate experiment, the individual supernatants were fractionated between 35 and 50% saturation with solid $(NH_4)_2SO_4$, the precipitate redissolved and desalted by gel filtration on PD 10 columns.

2.3. *Partial purification of pantothenate kinase*

Tissues from 8 rats were pooled, and extracted and centrifuged as described above. The supernatants were treated with 0.035 vol. of 2% protamine sulfate solution [5] and the precipitates removed by centrifugation. The protamine-treated supernatants were then fractionated between 35 and 50% saturation with solid $(NH_4)_2SO_4$, and the fractions redissolved and precipitated to 50% saturation. The final precipitates were desalted after dissolution in extraction buffer, and stored in batches at −20°C. The apparent purification and recoveries were: heart, 6-fold and 82%; brain, 11-fold and 50%; kidney, 2.5-fold and 36%; and liver, 2-fold and 36%.

2.4. *Pantothenate kinase assay*

The reaction mixture (0.1 ml) consisted of 50 mM triethanolamine, 10 mM DTT, 4 mM ATP, 6 mM $MgCl_2$, 10 mM creatine phosphate, 15 U/ml creatine kinase, and 55 μM D-pantothenate containing 5 μCi D-[^{3}H]pantothenate/ml, at pH 7. Other additions are as shown in the tables and figure. Solutions of CoA were made in the presence of 10 mM DTT to prevent oxidation. The reaction at 37°C was for 20–40 min and was stopped by the addition of 0.2 ml cold methanol. A portion (0.2 ml) of the supernatant after centrifugation was transferred to a small anion-exchange resin column and washed with 1 ml deionised water. The column (5 × 50 mM, Kontes) contained Dowex 1X8 to a height of 15 mm. Labeled pantothenate was eluted with 4 ml of 0.1 M NaCl and then labeled 4′-phosphopantothenate was collected directly into a scintillation vial by elution with 2 ml of 0.25 M HCl. Radioactivity in vials was measured after the addition of 15 ml ACS 11. Progress curves were linear for the time of reaction and activity was calculated from the fractional conversion of substrate to product.

3. RESULTS AND DISCUSSION

Pantothenate kinase is located in the cytosol [7,8] and activities measured in this fraction range from 1 to 5 $nmol \cdot min^{-1} \cdot g^{-1}$ wet wt for heart, brain, kidney and liver (table 1). The activity of the kinase in crude supernatant extracts from heart is suppressed since ammonium sulfate fractionation of the supernatant reveals a markedly higher activity (table 1, columns 1,3). The addition of L-carnitine to assays of the crude supernatant extracts of heart also leads to a greater expression of kinase activity (table 1, column 2) and it will be shown that this apparent activation is likely to be due to a reversal of inhibition. The activity of the kinase expressed in crude supernatant extracts from brain, kidney and liver is not markedly affected by the presence of L-carnitine in the assays (table 1, columns 1,2) and for kidney and liver, activities revealed after ammonium sulfate fractionation are similar to those of the crude supernatants (table 1, column 3). These tissue activities of the kinase are more than sufficient to account for the rates of CoA synthesis measured in vivo. Based on the rate of incorporation of labeled pantothenate after injection into rats, the rates of CoA synthesis

Table 1

The relative activities of pantothenate kinase in various tissues of the rat

Tissue	Enzyme activity ($nmol \cdot min^{-1} \cdot g^{-1}$ wet wt)		
	Crude supernatant ± 10 mM L-carnitine		Ammonium sulfate fraction
	−	+	
Heart	0.44 ± 0.06	1.33 ± 0.15	2.02 ± 0.24
Brain	1.38 ± 0.05	1.56 ± 0.08	ND
Kidney	2.09 ± 0.18	2.66 ± 0.19	2.67 ± 0.15
Liver	3.71 ± 0.22	5.29 ± 0.25	4.08 ± 0.12

Kinase activity was assayed in crude supernatant extracts of tissue with and without addition of L-carnitine to the assays. Kinase activity was also assayed in extracts after ammonium sulfate fractionation to remove soluble metabolites. Activities are given as mean ± SE and n = 5 for crude supernatants, n = 4 for ammonium sulfate fractions. ND, not determined

range from 0.03 to 0.23 nmol·min^{-1}·g^{-1} for heart, kidney and liver [9,10].

Previously, it was reported that pantothenate kinase partially purified from liver and kidney is inhibited by CoA [5–7]. The kinase partially purified from heart and brain is also inhibited by CoA, but there is a more than 10-fold greater potency of inhibition of the heart enzyme compared to that from the other tissues (fig.1). Under the conditions of assay, concentrations of CoA giving 50% inhibition (I_{50}) are 0.08 μM for the heart enzyme compared to 1.7, 2.2 and 3.1 μM, respectively, for the enzyme from kidney, liver and brain. It is likely that pantothenate kinase is strongly inhibited in vivo by the CoA that is present in the cytosol. For instance, the total CoA concentration of the cytosol of heart is 14 μM [11] and that of the liver 186 μM [12]. These cytosolic CoA concentrations are 100-fold higher than the I_{50} for the inhibition of the respective enzymes. A proportion of this cytosolic CoA will be present as acyl esters, but these are also inhibitors of the kinase from each tissue ([6,7] and unpublished). The similarity in the ratio of cytosolic CoA concentration to I_{50} for heart and liver but the markedly differing I_{50} probably indicates tissue-specific variation in this particular property of pantothenate kinase.

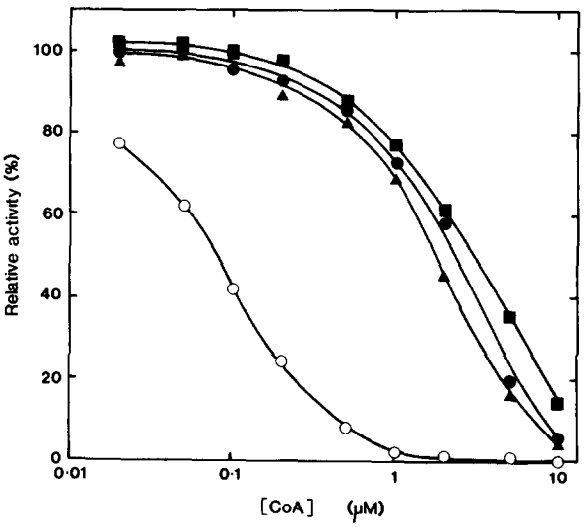

Fig.1. Inhibition of pantothenate kinase by CoA. Pantothenate kinase isolated from (○) heart, (▲) kidney, (●) liver and (■) brain was assayed in the presence of increasing concentrations of CoA. Activities relative to the control activity are plotted vs a log scale of CoA concentration. The control specific activities were heart, 0.35; brain, 0.62; kidney, 0.16; and liver, 0.11 nmol·min^{-1}·mg^{-1} protein.

L-Carnitine, the naturally occurring isomer, is found in varying quantities in every tissue examined [13]. By itself, L-carnitine has no effect on the activity of pantothenate kinase partially purified from heart, brain, kidney and liver (table 2). However, it appears to reverse specifically the inhibition by CoA of the enzyme from each source (table 2). The specificity of this property is shown by the lack of any effect of D-carnitine on the enzyme whether it is inhibited by CoA or not (table 2).

The physiological significance of this effect of L-carnitine will require a more detailed study of this property of pantothenate kinase than is provided here. However, the cellular function of L-

Table 2

Effect of L-carnitine on the inhibition of pantothenate kinase from various tissues by CoA

Tissue	CoA (μM)	Relative activity (%)			
		Added carnitine (mM)			
		L-Carnitine			D-Carnitine
		0	1	10	10
Heart	0	100	104	103	97
	0.1	44	56	64	43
	0.25	23	34	43	19
Brain	0	100	101	100	101
	2.5	61	64	70	60
	5.0	36	43	48	37
Kidney	0	100	101	101	105
	2.5	33	62	72	35
Liver	0	100	106	105	105
	2.5	48	61	71	47

The kinase partially purified from each tissue was assayed in the absence and presence of CoA, and with the concentrations of carnitine indicated. Activities are given relative to that obtained in the absence of additions, and are the mean of 2 experiments. The control specific activities were: heart, 0.35; brain, 0.64; kidney, 0.15; and liver, 0.11 nmol·min^{-1}·mg^{-1} protein

carnitine and CoA are linked through the exchange of acyl units between the 2 cofactors so that carnitine can facilitate the translocation of the acyl unit across the inner mitochondrial membrane [14]. The apparent universality of the effect of L-carnitine on CoA inhibition of pantothenate kinase suggests that there is a second link between carnitine and CoA in that carnitine may act to regulate the synthesis of CoA.

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