Pyridoxal Phosphate as the Coenzyme of the Mammalian Decarboxylase for L-Cysteine Sulphinic and L-Cysteic Acids

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Some time ago an enzyme was found in dog liver which decarboxylates L-cysteic acid (Blaschko, 1942). The product of the enzymic reaction is taurine (Blaschko, Datta & Harris, 1953). The enzyme has since been found in the rat and a number of other mammalian species.

It has always seemed likely that the enzyme was concerned with the *in vivo* formation of taurine, but this has never been fully established. It is known that in the pyridoxine-deficient rat the enzyme is lost (Blaschko, Carter, O'Brien & Sloane Stanley, 1948); at the same time taurine disappears from the rat's urine in which it is normally present (Blaschko *et al.* 1953). Recent work has made it likely that 2-aminoethane sulphinic acid (also called hypotaurine) is an intermediate in taurine synthesis (Bergeret & Chatagner, 1952; Bergeret, Chatagner & Fromageot, 1952; Awapara, 1953; Awapara & Wingo, 1953).

It has recently been shown by Chatagner, Tabechian & Bergeret (1954) that rat liver contains a decarboxylase for L-cysteine sulphinic acid and that the decarboxylation product is hypotaurine; this enzyme has also been shown to disappear in pyridoxine deficiency.

The experiments described in this paper make it likely that one and the same enzyme is responsible for the decarboxylation of both cysteic and cysteine sulphinic acids, a possibility already discussed by Awapara & Wingo (1953). In addition, evidence will be presented that the decarboxylase is a pyridoxal phosphate protein.

METHODS

L-Cysteine sulphinic acid was prepared from L-cystine by the method of Toennies & Lavine (1936). Calcium pyridoxal 5-phosphate was kindly given by Dr K. Folkers, Merck and Co., Rahway, New Jersey, U.S.A.

The rats used in the experiments on pyridoxine deficiency were of the hooded Lister strain obtained freshly weaned from the Laboratory Animals Bureau. The pyridoxine-deficient diet has already been described by Blaschko *et al.* (1953).

In some of the experiments rats were used which had been deficient in pyridoxine for only a few days. In these experiments, all the animals were put on the pyridoxine-supple-

mented diet for 12 days. In this period the animals doubled their body weight; the consequent increase in the size of the liver made it possible to use the material from one animal for the preparation of a sufficient amount of liver extract. Moreover, preliminary experiments had suggested that the enzymic activity in the livers of small rats weighing about 30 g. was low and somewhat variable. The animals and their diet were under the care of Miss M. A. Lewin, to whom I am grateful.

The liver extracts were prepared by freezing the weighed tissue in a cooled mortar; the tissue was thoroughly ground with a little sand and 0.067M sodium phosphate buffer, pH 6.64, was added; the volume of buffer added varied with the amount and activity of the material available. The extracts were centrifuged for 30 min. at 2150 g at 0°.

It is to be noted that the pH of the phosphate buffer differed from that used in previous work from this laboratory. This change from pH 7.4 to 6.64 was adopted in order to reduce the CO₂ retention. Preliminary experiments, carried out using L-cysteine sulphinic acid, showed that the enzymic activity was nearly independent of pH.

The plan of the manometric experiments was similar to that used by Sloane Stanley (1949). The total reaction volume was 2.7 ml.; the initial substrate concentration was 1.48×10^{-3} M. Where stated, 10 µg. of pyridoxal phosphate were added to each flask. The gas phase was N₂ and the temperature was 37.5°. The enzymic activity was expressed as q_{CO_2} , i.e. as µl. CO₂ formed/mg. fresh wt. of tissue/hr., and was calculated from the initial rate of the decarboxylation.

RESULTS

Distribution of activity in normal liver extracts. It was found that liver extract from several animal species could decarboxylate both L-cysteic and L-cysteine sulphinic acids. The results of these experiments are summarized in Table 1. In all these experiments L-cysteine sulphinic acid was decarboxylated at a much faster rate than L-cysteic acid.

A typical experiment with dog liver is shown in Fig. 1. In this experiment the CO₂ formed from L-cysteine sulphinic acid was less than the theoretical amount. This was frequently observed. The cause of the deficit was not investigated, but it must be borne in mind that mammalian liver contains another enzyme, desulphinase, which acts upon L-cysteine sulphinic acid, giving alanine and sulphur dioxide (Fromageot, 1951).

Table 1. *Decarboxylation of L-cysteic and L-cysteine sulphinic acids by extracts of livers of different species*

Initial substrate concn., $1.48 \times 10^{-3} \text{ M}$. Gas phase, N_2 ; temp., 37.5° . The q_{CO_2} is defined as $\mu\text{l. CO}_2$ formed/mg. fresh wt. of tissue/hr. Sex indicated by M. or F. in brackets.

Species	No. of observations	L-Cysteic acid (q_{CO_2})	L-Cysteine sulphinic acid (q_{CO_2})	q_{CO_2} L-Cysteic acid	
				q_{CO_2} L-Cysteine sulphinic acid	
Dog	1	0.18	1.24	0.15	
Rat (M.)	5	0.12	1.01	0.12	
Rat (F.)	6	0.10	0.76	0.13	
Mouse (M.)	1	—	0.40	—	
Guinea pig (F.)	1	0.07	0.52	0.14	
Rabbit (M.)	1	0.02	0.04	0.50	
Cat	4	0	0	—

Fig. 1. Decarboxylation of L-cysteic and L-cysteine sulphinic acids by an extract of dog liver. Each manometer flask contained extract derived from 500 mg. of fresh tissue. Curve 1, $1.48 \times 10^{-3} \text{ M}$ L-cysteic acid; curve 2, $1.48 \times 10^{-3} \text{ M}$ L-cysteine sulphinic acid. Gas phase: N_2 . Temp., 37.5° .

Table 1 also shows that dog liver, which is known to have the highest activity with L-cysteic acid as substrate, was also highly active with L-cysteine sulphinic acid. In the cat, where no L-cysteic acid decarboxylase could be demonstrated, no CO_2 was formed from L-cysteine sulphinic acid. The results with rat liver confirm Sloane Stanley's (1949) observation that the mean L-cysteic acid decarboxylase activity is higher in males than in females. The results in Table 2 show that the same is true for L-cysteine sulphinic acid.

Competition experiments. The great difference in the rate of decarboxylation of the two amino acids made the study of substrate competition difficult, but an additive formation of CO_2 never occurred when the two acids were added together. In an experiment with a rat-liver extract, in which $10 \mu\text{g.}$ of pyridoxal phosphate were added to each manometer flask, the amounts of CO_2 formed in the first 6 min. were: with $1.85 \times 10^{-3} \text{ M}$ L-cysteic acid, $4 \mu\text{l.}$; with $1.85 \times 10^{-3} \text{ M}$ L-cysteine sulphinic acid, $58 \mu\text{l.}$; with both (each $1.85 \times 10^{-3} \text{ M}$), $44 \mu\text{l.}$

Observations on pyridoxine-deficient rats. The earlier observations that decarboxylase activity for both L-cysteic and L-cysteine sulphinic acids disappear from the liver of rats fed on a pyridoxine-deficient diet were confirmed. The results of these experiments are summarized in Table 3.

It can be seen that the ability to decarboxylate the two amino acids was lost very rapidly; at the end of a fortnight no activity remained. Most of the activity towards both substrates was lost in a few days.

The action of synthetic co-decarboxylase (pyridoxal 5-phosphate) on liver extracts. It was noted that the addition of synthetic pyridoxal phosphate to liver extracts from normal rats sometimes increased the decarboxylase activity. This is shown in Table 3; the increases in activity for the group of seven normal rats were 0, 3, 25, 17, 20, 39 and 0%, respectively, with L-cysteine sulphinic acid as substrate.

This observation, together with the loss of enzymic activity in pyridoxine deficiency, sug-

Table 2. *Rate of decarboxylation of L-cysteine sulphinic acid in male and female rat-liver extracts*

Experimental details as in Table 1.

	q_{CO_2}	
	Males	Females
	1.08	0.72
	1.02	0.74
	1.08	0.75
	0.93	0.88
	0.94	0.81
	—	0.66
Mean	1.01	0.76
S.E.M. ($\pm$)	0.033	0.031

Table 3. *Decarboxylase activity in normal and vitamin B₆-deficient rat-liver extracts*

Where pyridoxal phosphate was added, 10 μ g. were used in a total volume of 2.7 ml. Other experimental details as in Table 1.

Sex	Days on vitamin B ₆ -deficient diet	Without added pyridoxal phosphate		With pyridoxal phosphate added	
		L-Cysteic acid (gco ₂)	L-Cysteine sulphinic acid (gco ₂)	L-Cysteic acid (gco ₂)	L-Cysteine sulphinic acid (gco ₂)
F.	0	0.11	0.75	0.11	0.75
M.	0	0.12	1.02	0.12	1.05
F.	0	0.14	0.72	0.14	0.90
M.	0	0.14	1.08	0.16	1.26
F.	0	0.06	0.74	0.08	0.89
F.	0	0.12	0.88	0.11	1.22
M.	0	0.13	0.94	0.12	0.94
F.	2	0.05	0.28	0.06	0.53
M.	3	0.08	0.60	0.12	1.05
F.	3	0.02	0.16	0.05	0.31
F.	3	0.02	0.24	0.09	0.72
F.	4	0	0.07	0.05	0.23
F.	4	0	0.06	0.03	0.18
F.	4	0	0.04	0.03	0.095
M.	11	0	0.01	0	0.08
M.	14	0	0	0	0

Fig. 2. Decarboxylation of L-cysteic and L-cysteine sulphinic acids by rat-liver extracts. *A*, Female rat deficient in pyridoxine for 3 days; *B*, female rat litter mate on supplemented diet. Each manometer flask contained extract derived from 250 mg. of fresh tissue. Curve 1, 1.48×10^{-3} M L-cysteic acid; curve 2, 1.48×10^{-3} M L-cysteic acid plus 10 μ g. pyridoxal phosphate; curve 3, 1.48×10^{-3} M L-cysteine sulphinic acid; curve 4, 1.48×10^{-3} M L-cysteine sulphinic acid plus 10 μ g. pyridoxal phosphate. Gas phase: N₂. Temp., 37.5°.

gested that the decarboxylation was catalysed by an enzyme containing pyridoxal phosphate. Experiments were therefore carried out with liver extracts from pyridoxine-deficient rats in which pyridoxal phosphate was added in order to find out whether the enzymic activity could be re-established.

Reactivations with pyridoxal phosphate could not be obtained with liver extracts from animals which had been on the deficient diet for 2 weeks or more, and only very slight effects were seen after the first week. However, an effect of pyridoxal phosphate added *in vitro* was observed in a number of experiments in which the rats were killed during the first week of deficiency, i.e. on the second to the fourth day. At this time the activity towards both amino acids was found to be markedly reduced. Fig. 2 shows one of these experiments in which the rates of decarboxylation of both amino acids were measured with and without added pyridoxal phosphate; the experimental animal, a female, had been on the pyridoxine-deficient diet for 3 days. The rates of decarboxylation of the two acids by the pyridoxine-supplemented control animal, a female litter mate, are also shown.

DISCUSSION

The experiments described give strong support to the suggestion of Awapara & Wingo (1953) that the enzyme which acts on L-cysteic acid is also responsible for the decarboxylation of L-cysteine sulphinic acid. In all species examined the rate of decarboxylation of the sulphinic acid is greater than that of cysteic acid. This makes it likely that L-cysteine sulphinic acid is the chief substrate for the enzyme in the living organism. The observations also explain why L-cysteic acid can act as a precursor of taurine (Virtue & Doster-Virtue, 1939).

One fact concerning taurine synthesis which remains unexplained is the absence of the decarboxylase in the liver of animals like the cat, which

forms taurocholic acid. A number of explanations are possible. Either there exists a different pathway which does not require the presence of L-cysteine sulphinic acid, or the decarboxylase occurs in an organ other than the liver, or these animals depend upon the taurine taken in with the diet. At present there is no means of distinguishing between these possibilities. The problem of taurine formation has become of greater interest since it has now been established that free taurine occurs in the central nervous system (Roberts, Frankel & Harman, 1950).

The activating effect of pyridoxal phosphate on liver extracts from pyridoxine-deficient animals establishes the chemical nature of the coenzyme. Earlier attempts at reactivation had been unsuccessful; this can now be understood as it has been shown that reactivation is possible only during the first few days of deficiency. This result can best be interpreted by assuming that in the pyridoxine-deficient rat the depletion in apoenzyme follows the loss of coenzyme with only a brief time lag. It would seem that reactivation is possible only while some enzymic activity remains; the enzymic activity is rarely more than trebled.

Successful reactivation of the enzyme L-dopa decarboxylase of the pyridoxine-deficient rat liver has previously been reported (Blaschko, 1950); this enzyme disappears at a slower rate than L-cysteic acid decarboxylase and reactivation can be more easily demonstrated. It thus appears that in the deficient animal there exists a time sequence with which the different pyridoxal phosphate enzymes disappear. The work here reported shows that there are three different sequences of this kind. The first is the disappearance of coenzyme bound to apoenzyme; the second, the disappearance of the enzyme protein; the third sequence is the disappearance of co-decarboxylase in the liver. It has already been shown that with both decarboxylases the loss of enzymic activity is either complete or almost complete when large amounts of coenzyme are still present (Blaschko *et al.* 1948).

SUMMARY

1. The decarboxylation of L-cysteic and L-cysteine sulphinic acids by extracts of mammalian liver has been studied; the evidence reported suggests that one enzyme is responsible for the decarboxylation of both these amino acids.

2. Enzymic activity is rapidly lost in pyridoxine-deficient rats; reactivation by the addition of pyridoxal 5-phosphate *in vitro* has been obtained in the early stages of the deficiency; this observation establishes pyridoxal phosphate as the coenzyme of the decarboxylase.

3. The biosynthesis of taurine is discussed in the light of these findings.

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Comirin: Isolation and Properties

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Comirin, a new fungistatic antibiotic, is produced by a bacterium found accidentally on a medium used for the cultivation of a species of *Aspergillus*. The bacterium was identified as a new species, related to *Pseudomonas fluorescens*, and given the name of *P. antimycetica* n.sp. (Thaysen & Thaysen, 1953).

The characteristics of the bacterium and the culture conditions for comirin production will be reported separately (Thaysen & Thaysen, in preparation). Seven days' growth in peptone (0.5%)—sucrose (0.5%) medium was generally used.

In the following pages, the method of assay elaborated for following the efficiency of the ex-