ON THE PURIFICATION OF CYSTEINESULFINIC ACID DECARBOXYLASE AND ITS SUBSTRATE SPECIFICITY

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The occurrence of an animal enzyme that decarboxylates cysteinesulfinic acid was first reported by CHATAGNER AND BERGERET[1]. This enzyme, which is found in the liver of some mammalian species, is dependent on pyridoxal phosphate[2,3] and sulfhydryl groups[4] for activity. It has been suggested[3–5] that cysteic acid is also decarboxylated by the same enzyme. The present communication describes a purification procedure for this highly active animal decarboxylase and a study of its activity with regard to cysteic acid.

METHODS

The decarboxylase activity was assayed in Warburg manometers at 35°C in a nitrogen atmosphere. The main compartment of the flasks contained the enzyme, 0.4 ml 0.5 *M* phosphate buffer of pH 6.8, 0.30 ml 0.02 *M* glutathione, 0.10 ml 0.1% pyridoxal phosphate, 0.20 ml 0.10 *M* versene of pH 6.8 and water to 3.0 ml. The reaction was started by adding 0.20 ml 0.16 *M* sodium cysteinesulfinate from a side arm, and readings were taken every 5 minutes during a 30 minute period. The reaction was of zero order under these conditions. The necessity of including pyridoxal phosphate and a thiol compound in the assay system has previously[4] been demonstrated in this laboratory. Versene was included in the present work as it was found that crude preparations displayed a 20–30% higher activity in its presence. No activating effect of versene was, however, observed with the most purified preparations. The amount of CO_2 evolved was obtained from the manometer readings by the use of a flask constant, corrected for the retention of CO_2 according to BURRIS[6]. Protein was determined by a modification[7] of the turbidimetric technique of BÜCHER[8]. Specific activities of the different preparations are given in the conventional measure Q_{CO_2}, meaning μl CO_2 evolved per mg protein per hour.

PURIFICATION OF THE ENZYME

In preliminary experiments livers from different animals were assayed for activity. In confirmation of previous reports it was found that rat and dog liver were very active. Beef liver was much less active and horse liver completely inactive. HOPE[3] has previously reported that guinea pig liver has a moderate activity, rabbit liver a very low activity and cat liver no activity. HOPE also reported that male rat liver was more active than female rate liver, and the same sex difference was now observed in dog liver. Consequently we decided to use male dog liver as the starting material.

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The livers could be stored for a few days at —20°C without significant loss of activity. If acetone powder was prepared from the livers, most of the activity was destroyed. All operations during the purification procedure were carried out at +4°C, as significant inactivation of the enzyme occurred at room temperature.

Extraction

The best procedure was to homogenize the liver with 2.5 volumes/weight of distilled water in a Turmix blender and acidify the homogenate to pH 5.6. Some protein impurities were denatured at this pH and after centrifugation a clear extract was obtained.

Ammonium sulfate fractionation

The extract was then fractionated with ammonium sulfate (added as the solid salt). Optimum purification and yield were obtained at pH 5.6 and at this pH the enzyme was precipitated between 1.4 to 1.8 *M* salt concentration. The precipitate was taken up in distilled water($^1/_5$ of the extract volume) and the enzyme again fractionated with ammonium sulfate. Precipitation was carried out stepwise and the enzyme obtained from 1.0–1.3 *M* salt concentration.

Dialysis

Before the following adsorption steps the salt content of the preparation had to be reduced by dialysis. This was carried out at +4°C during one night against a large volume of pre-chilled distilled water. Purification was achieved by this step, as impurities were denatured and could be removed by centrifugation. A certain inactivation, however, always occurred in this step and could not be prevented by carrying out the dialysis against dilute NaCl or Na_2HPO_4.

Calcium phosphate gel adsorption and elution

Preliminary experiments were carried out on columns, prepared according to TISELIUS *et al.*[9]. Elution was carried out stepwise, as recommended by these authors, with phosphate buffer of pH 6.8. The enzyme was strongly adsorbed on the columns from 0.001 *M* phosphate buffer, but some impurities were eluted at this ionic strength. The enzyme was eluted by 0.05 *M* buffer and only one protein component was observed in the elution curve. A significant amount of impurities was retained by the column at this buffer concentration. These results indicated that "batch-wise" treatment of the enzyme with calcium phosphate gel should give practically the same purification, and this was found to be the case. This treatment was carried out as follows: The dialysed enzyme solution was made 0.001 *M* with respect to phosphate buffer of pH 6.8 and to each 5 ml of enzyme 2 ml calcium phosphate gel (26 mg dry weight/ml)[10] were added. The adsorbed enzyme was centrifuged down and eluted twice with 0.2 volume (referring to that of the dialysed enzyme) of 0.05 *M* phosphate buffer pH 6.8. After this step the enzyme was found to be purified about 300-fold compared with the starting material. The results from a typical purification experiment are shown in Table I. The preparation obtained in the last steps was found to be very unstable. It could be partially stabilized by the presence of 0.01% pyridoxal phosphate, but even in this case about 20% inactivation was observed after 24 hours storage at +4°C. No stabilizing effect was observed with versene or glutathione.

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TABLE I

PURIFICATION OF CYSTEINESULFINIC ACID DECARBOXYLASE

Stage	*Total volume* ml	*Total activity* $\mu l\, CO_2 \times 10^3/h$	*Recovery* %	*Specific activity* Q_{CO_2}
Liver	—	—	—	4.4
Extract	262	158	100	18.8
Ammonium sulfate fractionation (1.4–1.8 *M*)	63	97.2	62	112
Ammonium sulfate fractionation (1.0–1.3 *M*)	14	34.9	22	297
Calcium phosphate gel eluate	10	15.3	9.7	1650

Cysteic acid as substrate

The activity of the preparations with respect to cysteinesulfinic acid and cysteic acid was compared during the different stages of purification. The results are given in Table II. It is evident that the activity as regards on cysteic acid is significantly reduced with respect to that shown with the sulfinic acid after the last purification step. As the purified enzyme was rather unstable, the possibility was considered that it

TABLE II

DECARBOXYLASE ACTIVITY DURING PURIFICATION

*Stage**	*Activity*		
	Cysteinesulfinic acid	*Cysteic acid*	
	Q_{CO_2}	Q_{CO_2}	*Relative to sulphinic acid* %
Extract	14	2.2	16
Ammonium sulfate fractionation, dialysed	432	55	12.7
Calcium phosphate gel eluate	1320	41	3.1

* These results were obtained with a different preparation than that presented in Table I.

was protected by cysteinesulfinic acid, but not by cysteic acid in the assay system. Such a phenomenon would of course simulate a relative decrease in cysteic acid decarboxylase. However, the purified enzyme also gave a zero order reaction with cysteic acid, which demonstrates that no inactivation occurred in the assay system.

DISCUSSION

The purified cysteinesulfinic acid decarboxylase is apparently the most active animal decarboxylase yet obtained. Its specific activity is, however, only about 7% of that of the most active bacterial decarboxylases[11]. The lability of the purified enzyme prevented further purification experiments, and progress in this direction probably must await a method for stabilizing the enzyme. It has been suggested[12] that decarboxylases are not only dependent on pyridoxal phosphate, but also on a metal ion for their activity. As the inactivation of the cysteinesulfinic acid decarboxylase frequently encountered in the present work, could be explained by a removal of a bound metal from the enzyme during the purification, attempts were made to demonstrate the presence of a metal in the enzyme. No inhibition was, however, observed with chelating compounds, such as *o*-phenanthroline or hydroxyquinoline (the experimental procedure was similar to that used by VALLEE AND HOCH[13] for alcohol dehydrogenase). The slight

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activating effect of versene on crude preparations has already been mentioned. Inactivated preparations were not reactivated by addition of Zn^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+} or Al^{3+}. No positive evidence for the presence of a metal in the enzyme was thus obtained.

The 5-fold decrease in activity with cysteic acid, relative to that with cysteinesulfinic acid, obtained after purification, indicates that the two compounds are decarboxylated by different enzymes. This is in disagreement with the conclusions arrived at by HOPE[3] and DAVISON[4] from other experiments. It is of some interest that these workers did not obtain a summation of activity when the two substrates were added together to their enzyme preparations. This was taken as evidence for the presence of one single enzyme acting on both substrates. However, another explanation is also possible. Two separate decarboxylases may be involved, but one enzyme (or both) is inhibited by the substrate of the other enzyme. In such a case, no summation of activities can of course be expected. In fact, the data presented by HOPE and DAVISON support this interpretation, as the decarboxylation obtained with a mixture of cysteinesulfinic acid and cysteic acid was even less than that obtained with the sulfinic acid alone. The sole conclusion that can be drawn from this experiment, is that cysteic acid inhibits the decarboxylation of cysteinesulfinic acid. Whether one or two enzymes are involved cannot be decided. Another possibility, which has to be considered, is that cysteic acid is enzymically reduced to cysteinesulfinic acid before decarboxylation. As cysteinesulfinic acid is enzymically decarboxylated to hypotaurine and cysteic acid to taurine[14] this explanation appears very unlikely.

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SUMMARY

Cysteinesulfinic acid decarboxylase from dog liver has been purified about 300-fold. Its decarboxylating action on cysteic acid decreases during purification.

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