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RAT LIVER CYSTEINE SULFINATE DECARBOXYLASE: SOME OBSERVATIONS ABOUT SUBSTRATE SPECIFICITY

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SUMMARY

The procedures in use for the determination of the activity of cysteine sulfinate decarboxylase (L-cysteine sulfinate carboxy-lyase, EC 4.1.1.29) in rat liver homogenate were evaluated. In addition, since we observed that decarboxylation of cysteine sulfinate as well as of cysteate occurred throughout the different purification steps of the enzyme, our results support the hypothesis that a single enzyme is responsible for both decarboxylations. The molecular weight of the enzyme was estimated at 65 000–70 000, using a column of Sephadex G-100 and at 78 000–85 000 using a Biogel P-150 column. A reason for this discrepancy is suggested.

INTRODUCTION

A recent paper[1] dealing with the purification and the properties of rat liver cysteine sulfinate decarboxylase (L-cysteinesulfinate carboxy-lyase EC 4.1.1.29) prompted us to report some results obtained in our laboratory.

We have also worked out a purification procedure for this enzyme from rat liver, which will be described in detail elsewhere. Adult male rats (Wistar) were used since it has been observed that decarboxylation of cysteine sulfinate[2,3] as well as of cysteate[4] by liver extracts was higher in the male than in the female. The first steps of the purification which have already been reported[5,6] consisted of a controlled thermal denaturation of the cytosol carried out in presence of pyridoxal phosphate (pyridoxal-*P*) and a precipation by 30–50% $(NH_4)_2SO_4$ followed by desalting by dialysis or by a run on a column of Sephadex G-25. Further steps included chromatography on a column of Amberlite at pH 5.6, a precipitation by $(NH_4)_2SO_4$ (35–45% saturated), chromatography on DEAE-cellulose at pH 7.4 and on CM-cellulose at pH 5.6.

The main objects of this report are first to evaluate the procedures set up for the determination of enzymic activity by Lin *et al.*[1] and in our laboratory[7], second to underline the similarities as well as the differences when the results of Lin *et al.* are compared to our results regards substrate specificity and molecular weight of the enzyme.

* L-Cysteine sulfinate was purchased from Cyclo Chemical (Los Angeles, U.S.A.), L-cysteate was a product of Calbiochem (Los Angeles, U.S.A.).

EXPERIMENTAL

The determination of enzymic activity was performed as previously reported[7] with slight modifications[5,6] in Warburg flasks equipped with one side arm (or two side arms when acidification at the end of the incubation period was needed).

In a final volume of 3.2 ml (completed as necessary with water) the mixture contained $6 \cdot 10^{-2}$ M phosphate buffer, pH 6.8, 10^{-4} M pyridoxal-*P*, 10^{-3} M dithiothreitol and the enzymic preparation (0.1 to 1.5 ml) in the main compartment; the substrate (10^{-2} M) was added from the side arm to the main compartment after temperature equilibration (37 °C) under a nitrogen atmosphere. All the concentrations given are initial concentrations.

Two differences are to be clearly noted between the two procedures. On the one hand, Lin *et al.*[1] carried out the incubations in air as the gas phase whereas we always used a purified nitrogen atmosphere; on the other hand, Lin *et al.*[1] acidified the mixture at the end of the incubation period whereas we usually did not. However, the second modification may be helpful since it increases the accuracy of the measurement of CO_2 production (without acidification at pH 6.8 in phosphate buffer, approx. 57% of the CO_2 formed was released[7]). The first modification, according to our observations, was detrimental at least when crude homogenates of rat liver were used: Fig. 1 shows the results obtained when decarboxylation of cysteine sulfinate and of cysteate were performed in air and in nitrogen, respectively.

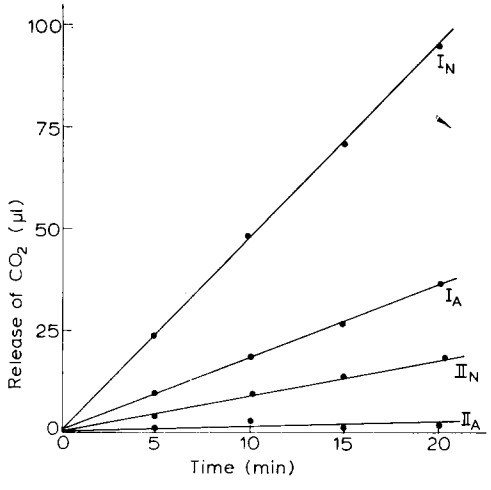

Fig. 1. Decarboxylation of cysteine sulfinate and cysteate by a homogenate of rat liver. Each flask contains 0.5 ml of a homogenate prepared in 8.5% sucrose (1 g of liver for a final volume of 3 ml). The composition of the incubated mixture is described in the text. I_N, decarboxylation of cysteine sulfinate in nitrogen; I_A, decarboxylation of cysteine sulfinate in air; II_N, decarboxylation of cysteate in nitrogen; II_A, decarboxylation of cysteate in air.

Clearly, the evolution of CO_2, cysteine sulfinate being the substrate, was decreased by approx. 60% when air was used instead of nitrogen. When cysteate was the subtrate, no significant release of CO_2 was measured in air unless the mixture was acidified. In addition we observed that, under these conditions, the incu-

TABLE I

RATIO OF CYSTEINE SULFINATE DECARBOXYLATION (A) TO CYSTEATE DECARBOXYLATION (B) THROUGHOUT THE STEPS OF PURIFICATION OF THE ENZYME

The conditions are described in the text.

Step	*A/B*
Whole homogenate	5.6
Supernatant fraction from acid-precipitated homogenate	6.2
$(NH_4)_2SO_4$ fraction (35–45%)	5.9
Sephadex G-100 (pooled)	5.4

bated mixture, devoid of substrate, consumed a volume of air highly sufficient to mask the production of CO_2 from cysteate.

According to previous reports[2,7–11] it is generally agreed that, in rat liver, decarboxylation of cysteine sulfinate and of cysteate is catalyzed by the same enzyme, although the possibility that two distinct enzymes are involved has been suggested[12]. It is relevant to recall, however, that it has been observed that cysteine sulfinate unlike cysteate can reactivate, at least in part, an inactive preparation of the enzyme presumably by reducing disulfide linkages[13].

We observed that the ratio of the decarboxylation of cysteine sulfinate to that of cysteate was constant throughout all the steps of our purification procedure. This ratio, which more or less varies depending upon the experiments, is approx. 6, *i.e.* higher than the ratio (2.4) reported by Lin *et al.*[1] for a homogenate of rat liver.

A purification of the enzyme was carried out following the first steps of the

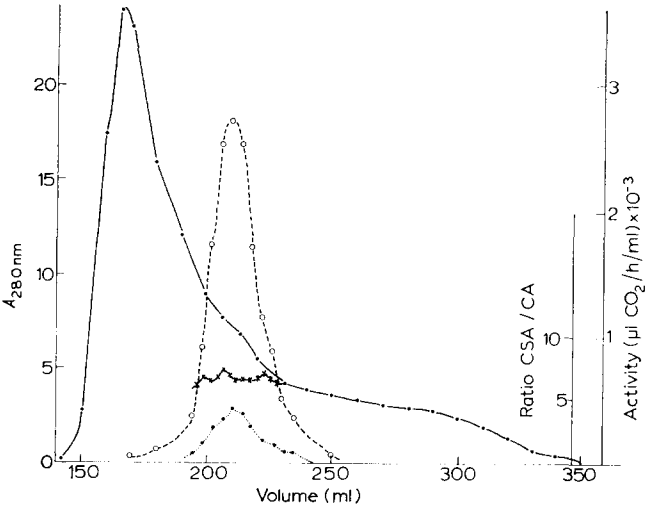

Fig. 2. Decarboxylation of cystein sulfinate and cysteate by the protein fractions eluted from the column of Sephadex G-100. ———, proteins (absorbance at 280 nm); – – –, decarboxylation of cysteine sulfinate; ·····, decarboxylation of cysteate; —×—×—, ratio cysteine sulfinate decarboxylation/cysteate decarboxylation. The conditions are described in the text. CSA, cysteine sulfinate; CA, cysteate.

procedure of Lin *et al.*[1]. The results, which are reported in Table I, indicate that the two activities were never dissociated, even on the column of Sephadex G 100.

Furthermore, we observed (Fig. 2) that the ratio of the two decarboxylations did not change throughout the peak of active protein eluted from the Sephadex G-100 column.

It has not been possible to compare the specific activities determined respectively by Lin *et al.*[1] and by ourselves, owing to the tremendous difference in protein concentration in the liver homogenates: Lin *et al.* reported that they obtained 24.6 g of protein in a homogenate prepared from the livers of three rats, whereas we observed that from ten rats (131 g of liver) we obtained 26.5 g of protein in the homogenate; the determination of protein being performed with Folin–Ciocalteu reagent and using bovine serum albumin as standard protein. Our figure is in keeping with the generally agreed percentage of protein in mammalian liver (20% of the wet weight).

It is of interest to recall that it has been reported[8,11,14] that when the two substrates cysteine sulfinate and cysteate were simultaneously added, at saturating concentrations, to an extract of rat liver[8] or to the soluble liver fraction[14] or to a purified preparation of the enzyme[11], an inhibition of the release of CO_2 was observed instead of an expected addition of the two productions of CO_2 if two specific enzymes were involved. For instance, Fig. 3 (taken from ref. 14) shows the results obtained with the cytosol.

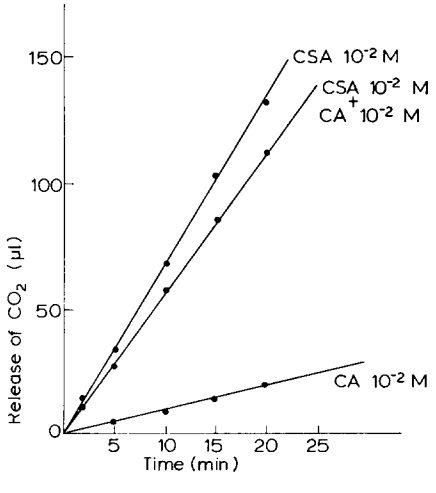

Fig. 3. Decarboxylation of cysteine sulfinate, of cysteate and of a mixture of the two substrates by the cytosol of rat liver. Each flask contains 0.5 ml of a supernatant obtained by ultracentrifugation (105 000 × *g*, 45 min) of a homogenate of rat liver in sucrose (8.5%). The composition of the incubation mixture is described in the text. (Taken from ref. 14.) CSA, cysteine sulfinate; CA, cysteate.

In conclusion, until a clear-cut separation of both decarboxylase activities is achieved, there is no evidence that in rat liver the two decarboxylations are not catalyzed by a single enzyme.

We estimated the molecular weight of the purified enzyme, according to the method of Andrews[15], using a column of Biogel P-150 (polyacrylamide) and a column

of Sephadex G-100 (polydextran). In both cases the columns were calibrated using proteins of known molecular weight (bovine serum albumin, monomer and dimer, and ovalbumin, monomer and dimer). Furthermore, the enzymic activity towards cysteine sulfinate from the eluted fractions of our preparation was checked.

The results obtained with the column of Sephadex G-100 were in agreement with those reported by Lin *et al.*[1]. Indeed, an estimated molecular weight of 65 000–70 000 was determined. However, we observed that on the column of Biogel P 150, the protein associated with the enzymic activity was eluted earlier, and by this technique a molecular weight of 78 000–83 000 was estimated.

Since it is known[16] that the elution of "aromatic" proteins is delayed on Sephadex G-100 and since such a feature may be suggested for cysteine sulfinate decarboxylase as a result of its susceptibility to chymotrypsin[6], it seems highly necessary to determine the molecular weight of cysteine sulfinate decarboxylase by other methods before a conclusion can be reached.

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