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Short Communications

Partial Purification of Cysteine Synthase (*O*-Acetylserine Sulfhydrylase) from Onion (*Allium cepa*)

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In a previous investigation on the sulfur metabolism of *Allium* plant tissues it was found that cysteine derivatives can be formed enzymatically by condensation of various thiols with serine.[1] It was proposed that this reaction is mediated by the second member in a two-enzyme system which forms cysteine from serine. Most workers in this field now agree that cysteine is formed by the following reactions:
Acetyl-CoA + serine ⟶ *O*-acetylserine + CoA
O-Acetylserine + sulfide ⟶ L-cysteine + acetate.

The first step is catalyzed by serine acetyl transferase (EN 1972 2.3.1.30). The enzyme of the second step is known in the literature as *O*-acetylserine sulfhydrylase. However, according to the 1972 Enzyme Nomenclature it should be named cysteine synthase (EN 1972 4.2.99.8), not to be confused with cysteine synthase (EN 1964 4.2.1.22). In this paper the 1972 Nomenclature will be followed. The enzyme reacts not only with sulfide but also with methyl mercaptan and ethyl mercaptan, forming *S*-methylcysteine and *S*-ethylcysteine, respectively (for literature, see Ref. 1). In order to study further the mechanism of the biosynthesis of cysteine derivatives in onion, an attempt has now been made to purify the cysteine synthase of onion. Becker and coworkers have purified the cysteine synthase of *Salmonella typhimurium*,[2] and their procedure has been applied to the onion enzyme. However, the much lower enzyme content in onion and the presence of large amounts of polysaccharides necessitated the design of other initial purification steps. Also, it was found beneficial to raise the pH throughout the purification procedure.

Experimental. Materials. The onions used were different batches of freshly harvested or stored bulbs obtained from the local market. *O*-Acetyl-L-serine was synthesized by acetylation of L-serine in perchloric acid/acetic anhydride/acetic acid solution.[3] Other reagents were of commercial origin.

Enzyme assay. Cysteine formation was measured by the assay of Becker *et al.*[2]

Purification of cysteine synthase. The initial purification steps were performed at temperatures between 0 and +4 °C. The peeled and sliced onion (3 kg) was homogenized for 30 s at medium speed in a 5 l Waring Blendor with 1500 ml extraction buffer (0.1 M Tris-HCl, pH 8.2, containing 0.01 M 2-mercaptoethanol). A certain amount of solid Tris (about 14.5 g) was added immediately before the homogenization to establish a pH of 8.2 when the homogenization process was finished. The required amount of Tris varied from batch to batch of onion and was estimated from a small scale experiment. The slurry was strained through cheesecloth and centrifuged in a refrigerated centrifuge for 20 min at 10 400 *g*. One gram activated charcoal (BDH, for decolorizing purposes) was added per 100 ml supernatant, and the mixture was stirred for 5 min in an ice-bath and immediately centrifuged as above. Two grams moist Ca-phosphate gel was added per 100 ml supernatant, and the mixture was stirred for 10 min and centrifuged as above.[4] After this step all operations were carried out at room temperature. The pH of the supernatant was re-adjusted to 8.2 with 1 M ammonia, and solid ammonium sulfate was added to 40 % saturation. After stirring for 45 min and centrifugation, the precipitate was discarded and the supernatant was made 60 % saturated with ammonium sulfate. The solution was stirred for one hour and allowed to stand for another hour and centrifuged. The precipitate was dissolved in water and dialyzed overnight against 0.1 M Tris-HCl containing 0.01 M 2-mercaptoethanol.

First Sephadex filtration. The dialyzed enzyme was applied to a Sephadex G-100 column (52 cm × 2.7 cm ⌀), equilibrated with 0.1 M Tris-HCl – 0.01 M 2-mercaptoethanol, and eluted with the same buffer. Fractions of 5 ml were collected and the active fractions were pooled.

DEAE-Cellulose chromatography. The pooled enzyme was applied to a DEAE-cellulose column (14 × 1 cm ⌀), equilibrated with 0.1 M Tris-HCl – 0.01 M 2-mercaptoethanol, and was eluted with a 1 liter linear gradient of 0 to 0.35 M NaCl in the same buffer. Fractions of 5 ml were collected, and the fractions with the highest activities were pooled.

Second Sephadex filtration. The pooled enzyme from the previous step was dialyzed against polyethylene glycol 6000 (Shell) until the volume was reduced to about 2 ml, and then applied to

a Sephadex G-100 column (55 × 1.7 cm ⌀), equilibrated with 0.1 M Tris-HCl, pH 7.4. The column was eluted with the same buffer. The enzyme peak was pooled and stored at −18 °C.

Results and discussion. The initial adsorption steps clear the enzyme extract from many interfering substances, and gel filtration is a powerful means of further purification. In a Sephadex separation the shape of the UV profile varied with the batch of onion, but the enzyme activity always was eluted between two major UV peaks. In further purification on DEAE-cellulose the enzyme was eluted approximately in the same way as the *Salmonella* enzyme when purified on a DEAE-Sephadex column.[2] Chromatography on Sephadex G-100 also indicates similarity of enzyme from both sources. Like the *Salmonella* enzyme the purified onion enzyme is fairly stable at room temperature.

The enzyme activity varied much between different batches of onion, investigated at different times of the year, the reason for this remaining unknown. In a favourable case *ca.* 900 enzyme units were obtained from 3 kg onion (measured from the pooled enzyme after the first Sephadex fractionation). The result of the purification could not be presented in terms of specific activity since protein determination was unreliable in the crude extract and too low after the chromatographic steps. Protein determination as well as further characterization of the enzyme would have required a considerable scale-up of the whole isolation procedure. Onion is a poor source of the enzyme compared with microorganisms such as *Salmonella* in which the synthesis of this enzyme can be derepressed by growth in a medium containing L-djenkolic acid as the sole sulfur source.[5]

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Synthesis of *S*-Substituted Cysteine Derivatives by the Cysteine Synthase (*O*-Acetylserine Sulfhydrylase) of Onion (*Allium cepa*) and *Escherichia coli*

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Previous investigations showed that *Allium* plants can form a wide variety of cysteine derivatives from externally supplied thiols and serine.[1] It was proposed that this reaction is mediated by the non-specific action of cysteine synthase (EN 1972 4.2.99.8). This enzyme was previously known as *O*-acetylserine sulfhydrylase. It is the second member of the two-enzyme system which normally forms cysteine from serine. The cysteine synthase of onion has now been partially purified.[2] In this investigation we have found that cysteine synthase is non-specific with regard to a wide range of thiols, and there is no difference in this respect between enzyme of plant and bacterial origin.

Materials and methods. Substrate. O-Acetyl-L-serine was synthesized by acetylation of L-serine in perchloric acid/acetic anhydride/acetic acid solution.[3] Labeled substrate was prepared on a micro scale from L-serine-C14(U) by the same method. The labeled substrate (specific activity 10 μCi/μmol) was divided in aliquots, vacuum dried and stored dry at −18 °C until use.

Enzyme. The partial purification of onion cysteine synthase is described elsewhere.[2] The enzyme was partially purified from *E. coli* using essentially a small-scale modification of the procedure of Becker *et al.*[4] The starting material was 1 g frozen cells of *E. coli* ATCC 4157, grown in a glucose/inorganic salt medium, and kindly provided by Mr. Seppo Vilkki, Phil. Lic. The cells were mixed with 9 ml buffer (0.05 M Tris-HCl pH 7.8 and 0.01 M 2-mercaptoethanol) and 13 ml ballotini glass beads and disintegrated by continuous circulation for 2 min at 0 °C in a silicone tube agitated by a fast peristaltic pump. The enzyme was purified by streptomycin precipitation and ammonium sulfate fractionation as described by Becker *et al.*[4] After this step the enzyme was dialyzed against 0.05 M Tris-HCl buffer pH 7.6. The specific activity was 1.46 units per mg protein.

Reaction. O-Acetyl-L-serine labeled with L-serine-C14(U) was incubated with the enzyme for 30 min in small, conical, stoppered test tubes under conditions similar to those described in the assay for cysteine synthase activity,[4] with the sulfide ion replaced by one of the following thiols: methyl, ethyl, propyl, allyl, butyl, or benzyl mercaptan. One μl of each thiol was