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

supplied giving an unknown actual concentration in the aqueous phase because of the wide variation in water solubility and volatility of the thiols used. After 30 min acetic acid was added to stop the reaction, and the mixture was dried in a vacuum desiccator. The reaction products were dissolved in water and subjected to thin layer chromatography with methyl ethyl ketone/pyridine/water/acetic acid (70 + 15 + 15 + 2 v/v) followed by autoradiography.[1,5]

Results. When the enzymes from onion and *E. coli* were incubated with labeled *O*-acetylserine and one of the thiols methyl, ethyl, propyl, allyl, or benzyl mercaptan, distinct spots of *S*-methylcysteine, *S*-ethylcysteine, *S*-propylcysteine, *S*-allylcysteine, and *S*-benzylcysteine, respectively, appeared on the autoradiograms. When butyl mercaptan was tested the reaction was only weak, which may reflect the low water solubility of this compound.

Discussion. Although the biosynthesis of *trans*-(+)-*S*-(propen-1-yl)cysteine sulfoxide from *S*-(2-carboxypropyl)cysteine in onion has been elucidated the origin of the side chain in other *S*-substituted cysteine derivatives has remained unknown.[1] At present, there is no evidence about the origin of the allyl side chain in *S*-allylcysteine sulfoxide in garlic. The side chain of *S*-propylcysteine sulfoxide, a minor component in onion, may be formed by hydrogenation of propenylcysteine sulfoxide, although this has not yet been experimentally proved. The metabolism of *S*-methylcysteine and its sulfoxide has been studied in several higher plants (for literature, see the reviews of Thompson [6] and Granroth [1] and some more recent publications [7–11]). This compound can be formed either by methylation of cysteine or by thiomethylation of serine, but the former reaction seems to be the normal one.

The demonstration in this investigation of the non-specific action of cysteine synthase from two different sources suggests that the ability to form cysteine derivatives from thiols and serine is wide-spread and may be an important detoxification mechanism. In onion sulfur metabolism it explains the side-chain recycling mechanism previously observed.[1] This is a reaction in which the thioalkyl moiety of an *S*-substituted cysteine derivative is combined with serine to form a new molecule with the same structure as the original. This reaction is sometimes confusing and makes the investigation of onion sulfur metabolism more intricate.

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The N-Terminal Amino Acid Sequence of *Bacillus subtilis* α-Amylase

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While it has been reported that native α-amylase isolated from *Bacillus subtilis* has a weight of 48 000 daltons, this weight is reduced to approximately 24 000 in the presence of 6 M guanidine.HCl, *i.e.* two subunits of equal weight are obtained.[1] Unequivocal proof that the primary structure of these two subunits is identical will have to come from sequence studies. Preliminary results, which are reported here, suggest that the enzyme molecule contains only one kind of polypeptide chain.

Two commercial preparations of the α-amylase, one manufactured by Novo Industries,** the other procured from Sigma Chemical Company and labeled Type II-A were examined. Both products exhibited identical elution volumes upon chromatography on Sephadex G-100. (Employing 0.025 M sodium acetate buffer, pH 6.0, as elution medium, a major fraction and a minor-slower migrating one were obtained. Utilizing 0.025 M ethylenediaminetetraacetate buf-

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