nitrogen group, but not those of sulfhydryl group, is to run the reaction in strong acid and thus protonate the nitrogen atom.

The oxidation of dopa can be performed by several common oxidizing reagents, but the relatively poor stability of the product complicates matters. The electrochemical oxidation of catecholic compounds has been studied from a mechanistic point of view[8], but its preparative use has not been explored. We studied the cyclic voltamogram of dopa and 5-S-cysteinyldopa at pH 1, and found that both compounds have peaks with E_{pa}-values close to +0.7 V vs saturated calomel electrode on the first anodic sweep. Some excess of cysteine is advantageous for good yields of monocysteinyldopas which indicates a simultaneous oxidation of cysteine although the E_{pa}-value of cysteine is reported to be higher than +0.9 V in 1 M H_2SO_4[9]. Various types of anode electrode material were tested since the oxidation products of dopa are to some degree polymerized on the electrode surface even in strong acid, and we preferred the platinum electrode.

From our enzymatic studies on the oxidation of dopa in the presence of cysteine, we knew that in addition to 5-S-cysteinyldopa, 2-S-cysteinyldopa and 2,5-S,S-dicysteinyldopa are also formed in substantial amounts. As 2,5-S,S-dicysteinyldopa is formed by an oxidation of the monocysteinyldopas followed by the addition of cysteine, an increased amount of 2,5-S,S-dicysteinyldopa is to be expected when an excess amount of charge, Q, is passed through the cell. The amounts of dopa, 5-S-cysteinyldopa, 2-S-cysteinyldopa and 2,5-S,S-dicysteinyldopa were determined by HPLC[10] at different stages of the reaction. After the theoretical amount of charge (2 F per mole dopa) had been passed through the cell, a large amount of dopa (about 30%) was left. 1 F/mole of dopa more increased the yield of 5-S-cysteinyldopa by 10%. Further oxidation lowers the yields of monocysteinyldopas, but is advantageous if 2,5-S,S-dicysteinyldopa is desired.

The preparative electrochemical synthesis of cysteinyldopas is a fast and inexpensive method. It is simple to reproduce and large amounts of either mono- or dicysteinyldopa can easily be prepared.

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Aminotransferase study of *Neurospora crassa ser-3* versus wild type ST4A

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Summary. The *Neurospora crassa* serine auxotroph *ser-3* and the wild type ST4A were compared with respect to their phosphoserine and serine aminotransferase activities. The results obtained indicate no deficiency of either of these enzymes in the mutant strain.

Three pathways for the biosynthesis of serine are known in microorganisms. The 1st pathway involves the glycolytic intermediate 3-phosphoglycerate which is oxidized to 3-phosphohydroxypyruvate followed by transamination to L-P-serine. In the last step, L-P-serine is hydrolyzed to L-serine[3]. The 2nd pathway is nonphosphorylated. It begins with glycerate formed by the dephosphorylation of 3-phosphoglycerate. The glycerate is then oxidized to hydroxypyruvate and transaminated to serine[4]. The 3rd pathway involves C_1 fragments, glycine and the enzyme transhydroxymethyltransferase (EC 2.1.2.1)[5].

In *Neurospora crassa* both phosphorylated and nonphosphorylated pathways for L-serine synthesis are functioning to some extent in the wild type strain Li-la[6]. Preliminary results obtained by Sojka using the same procedures described herein[7] suggested that the serine auxotroph of *N. crassa ser-3* (47903), was blocked in the phosphorylated pathway at the transamination step. The purpose of this study was to determine whether the parallel reaction in the nonphosphorylated pathway, transamination of hydroxypyruvate, was also affected, indicating that a common transaminase is used in the 2 pathways, or whether the defect affected a specific phosphoserine transaminase.

Materials and methods. Stock cultures of *N. crassa* wild type ST4A were maintained on Vogel's minimal medium N slopes[8] containing 2% (w/v) sucrose and 2% (w/v) agar; *ser-3* cultures were supplemented with 10 mM L-serine. To obtain a dense conidial suspension, 50 ml of sterile Westergaard and Mitchell medium[9] was added to 7-day-old cultures grown on 20 ml of agarized medium in 125-ml Erlenmeyer flasks. The conidial suspension was used to inoculate 700 ml of the same liquid medium contained in a 2-l Fernbach flask and the resultant culture was incubated with shaking for 48 h in a 32 °C waterbath. All remaining procedures were carried out in a 4 °C cold room. The mycelial pad was harvested, rinsed, weighed, and ground in

Assay conditions

	A	B	C
2-keto [5-^{14}C] Glutarate	0.046 µmole/ml	1.0	1.0
KH_2PO_4/KOH pH 8.0	1.0 M	0.5	0.5
Pyridoxal phosphate	10 µg/ml	0.2	0.2
L-Serine	1 mg/ml	0.0	2.0
O-L-Phosphoserine	20 µmoles/ml	2.0	0.0
Protein extract	0.4 mg/ml	1.0	1.0
Water	-	0.3	0.3

A, Concentration of solution used; B, volume used for L-phosphoserine aminotransferase assay; C, volume used for L-serine aminotransferase assay; total volume, 5.0 ml. Temperature of incubation of the reaction mixture, 32 °C; specific activity of 2-keto[5-^{14}C] glutarate, 10.3 Ci/mole.

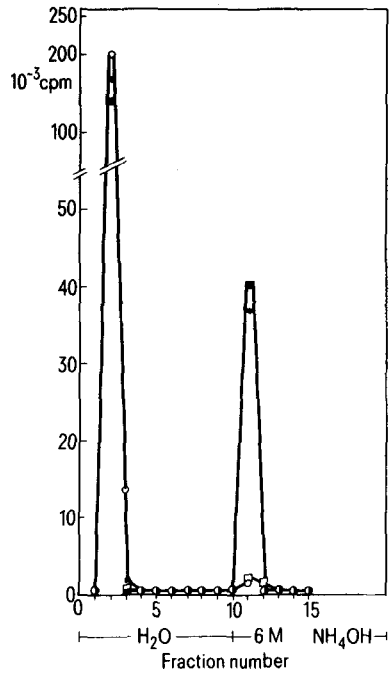

Figure 1. Dowex 50 (H^+) chromatography of the products formed in the L-phosphoserine aminotransferase assay. Results are reported as radioactivity in 1.0 ml of the 5.0 ml fraction. Symbols: ○, wild type ST4A incubated for 0 min; ●, wild type ST4A incubated for 30 min; □, *ser-3* incubated for 0 min; ■, *ser-3* incubated for 30 min; ◑, all symbols superimposed; I, 2-keto[5-^{14}C]glutarate; II, [5-^{14}C]glutamate. Specific assay conditions are found in the table. Temperature of assay was 32 °C.

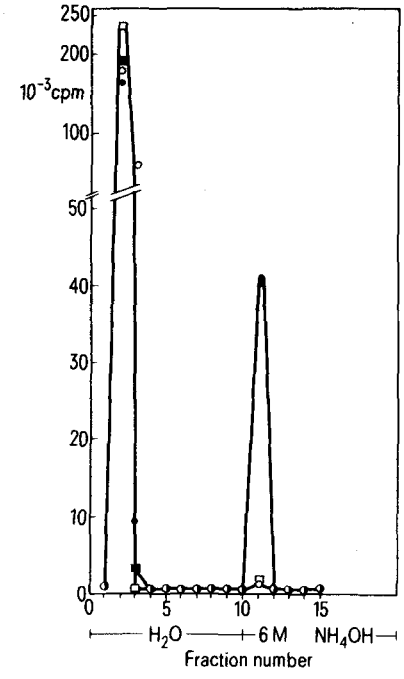

Figure 2. Dowex 50 (H^+) chromatography of the products formed in the L-serine aminotransferase assay. Symbols are the same as for figure 1.

a cold mortar and pestle with sea sand, then extracted with 5 volumes (ml/g wet weight) 0.01 M KH_2PO_4/KOH buffer, pH 8.0. The extracts were centrifuged at 12,350 ×g for 15 min. The supernatant was passed through a 1.5×30 cm Bio-Gel P-10 column and fractions were assayed for protein content by the biuret method[10]. The fractions with the highest readings were combined for the assay. The aminotransferase assay was a slight modification of Umbarger's method[11]. The reaction was carried out in reverse of the direction leading to serine in the proposed pathways. Either L-serine or L-P-serine acted as the amino donor. The 2-keto [^{14}C] glutarate [10.3 Ci/mole] acted as the amino acceptor. The amount of labelled glutamate formed measured the extent of interconversion between labelled keto and amino acid and hence the extent of enzyme activity. As shown in the table, the enzyme reaction mixture contained either L-phosphoserine or L-serine, 2-keto [5-^{14}C] glutarate, and pyridoxal-5'-phosphate in KH_2PO_4/KOH pH 8 buffer. The reaction was stopped by adding 0.1 ml of 50% (w/v) trichloroacetic acid to 1.0 ml samples of the reaction mixture. The supernatant from a low speed centrifugation was decanted and saved. Ion exchange chromatography using Dowex 50W H^+ form contained in a column made from a Pasteur pipette plugged with a miniscule amount of glass wool was used to separate the reaction products. After application of the 0.6-ml sample to the resin, it was washed with 10 ml of distilled water. The amino acids were eluted by the addition of 10 ml of 6 M NH_4OH. A 1-ml sample of each 5.0-ml fraction collected was placed in a plastic vial with 15 ml of scintillation fluid. Samples were dark adapted for several hours to minimize extraneous fluorescence, and then were analyzed with a Beckman 3000 Liquid Scintillation Counter.

Results and discussion. Qualitatively similar results were obtained with 3 separate extracts of wild type ST4A; in 2 cases, extracts of *ser-3* were run in parallel with the wild type. The elution profiles given in figures 1 and 2 are from one of the latter experiments. Figure 1 shows the results obtained with L-phosphoserine as a substrate, while figure 2 shows the results obtained with L-serine as a substrate. The profiles are strikingly similar for the 2 substrates and for the wild type and mutant extracts. Assays using denatured protein from the wild type and mutant showed no labelled glutamate formed after 30 min in either case. We conclude that the mutant *ser-3* has wild type levels of both phosphoserine transaminase and serine transaminase, and that its serine requirement is not the result of a deficiency of phosphoserine transaminase.

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