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Purification and Characterization of the Crown Gall Specific Enzyme Nopaline Synthase†

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ABSTRACT: Nopaline synthase of sunflower (*Helianthus annuus* L.) crown gall tissue induced by *Agrobacterium tumefaciens* strain C58 or T37 (nopaline utilizers) was purified to homogeneity as judged by analytical disc gel electrophoresis. The native enzyme elutes from a column of Ultrogel AcA 34 as a single peak with an estimated molecular weight of 158 000. The dissociated enzyme migrates on $NaDodSO_4$–polyacrylamide gels as a single band with a molecular weight of 40 000. Thus, the native enzyme appears to be composed of four equal-weight subunits. Nopaline synthesizing activity is found exclusively in crown gall tissues induced by strains of *A. tumefaciens* that utilize nopaline (e.g., C58 and T37). We found the same tissue specificity for the purified protein that we believe represents nopaline synthase. The results of kinetic studies of the purified enzyme are consistent with a ter-bi rapid-equilibrium random-order mechanism. Nopaline synthase is probably responsible for the in vivo synthesis of both N^2-(1,3-dicarboxypropyl)arginine (nopaline) and N^2-(1,3-dicarboxypropyl)ornithine (ornaline) in crown gall tissues since substrate specificities and K_m values do not change during purification.

Cells of a variety of dicotyledonous plants are transformed to crown gall tumor cells by transfer of DNA from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* (E. F. Sm. and Town.) Conn. to the host (Chilton et al., 1977). Although Ti plasmids are quite large [(112–156) × 10^6 daltons] (Zaenen et al., 1974), only a relatively small portion of their DNA [(3–5) × 10^6 daltons] is stably associated with tumor cells (Chilton et al., 1977). The transferred DNA (T-DNA) appears to be functional, since several investigators (Drummond et al., 1977; Gurley et al., 1979; Ledeboer, 1978) have demonstrated that tumor tissue synthesizes RNA that hybridizes to T-DNA. However, the question of whether the RNA transcribed from T-DNA actually codes for structural gene products is still unresolved.

Tumors incited by most strains of *A. tumefaciens* can be assigned to two groups (octopine type and nopaline type) based on the crown gall specific amino acid derivatives that they synthesize. Octopine-type tumors synthesize N^2-(1-carboxyethyl) amino acid derivatives of arginine (octopine) (Mênagê & Morel, 1964), of lysine (lysopine) (Biemann et al., 1960), of ornithine (octopinic acid) (Goldmann-Mênagê, 1970), and of histidine (histopine) (Kemp, 1977). The nopaline-type tumors, in contrast, synthesize N^2-(1,3-dicarboxypropyl)arginine (nopaline) (Goldmann-Mênagê, 1970) and N^2-(1,3-dicarboxypropyl)ornithine (ornaline) (Kemp, 1978). It is the Ti plasmid, not the plant species, that determines whether N^2-(1-carboxyethyl) amino acids or N^2-(1,3-dicarboxypropyl) amino acids are synthesized by the plant tumor (Petit et al., 1970; Bomhoff et al., 1976; Montoya et al., 1977).

The obvious candidates for structural gene products of T-DNA are the enzymes that synthesize the unusual amino

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acid derivatives. One enzyme, octopine synthase (Hack & Kemp, 1977; Otten et al., 1977), catalyzes the synthesis of the four known members of the octopine family from pyruvate, NADPH, and either arginine, ornithine, lysine, or histidine. This enzyme is found exclusively in octopine-type crown gall tumors, such as those incited by *A. tumefaciens* strains B6 and 15955. Octopine synthase has been purified to homogeneity (Kemp et al., 1979).

A second enzyme that is a candidate for the expression of a T-DNA gene is nopaline synthase (Sutton et al., 1978; Goldmann, 1977; Scott & Firmin, 1978). This enzyme catalyzes the synthesis of nopaline and ornaline from α-ketoglutarate, NADPH, and either arginine or ornithine. Nopaline synthase activity is found exclusively in nopaline-type crown gall tumors, such as those incited by *A. tumefaciens* strains T37 and C58.

This report describes procedures for purifying nopaline synthase to homogeneity and contains a partial physical and kinetic characterization of the enzyme.

Materials and Methods

Tissue Cultures. The establishment of primary sunflower crown gall tissue cultures (PSCG)[1] from plants inoculated with various strains of *A. tumefaciens* and of habituated sunflower stem section tissue cultures (HSSS) was described previously (Kemp, 1978). Tissue cultures were maintained at 27 °C in the dark on Linsmaier & Skoog's medium (1965) lacking phytohormones. Following a short lag after transfer, tissues grew exponentially for about 4 weeks. They were harvested for experimentation after 3 weeks.

Enzyme Assay. The reaction mixture (0.6 mL) contained 0.1 M potassium phosphate (pH 6.5), 14 mM 2-mercaptoethanol, 16 mM sodium α-ketoglutarate, 0.17 mM NADPH, either 6.7 mM L-arginine hydrochloride or 33 mM L-ornithine hydrochloride, and 20–50 μL of enzyme preparation. The progress of the reaction was followed at 25 °C by the decrease in A_{340}. The activity is expressed in nanokatals (nkat). One katal is defined as the amount of enzyme which catalyzes the oxidation of 1 mol of NADPH per s under these assay conditions.

Protein Determinations. Protein concentrations were determined by a modified Lowry procedure (Rutter, 1967) using bovine serum albumin as a standard.

Subunit Molecular Weight Determinations. Sodium dodecyl sulfate gel electrophoresis was performed according to the method of Weber & Osborn (1969) with the modification that 7.5% acrylamide gels were used. The gels were stained with Coomassie Brilliant Blue R-250 as described by Jendrisak & Burgess (1975). The subunit molecular weight of nopaline synthase was estimated from the linear calibration curve (Weber & Osborn, 1969) obtained from bovine serum albumin (66 000), ovalbumin (45 000), pepsin (35 000), bovine trypsinogen (24 000), β-lactoglobulin (18 400), and lysozyme (14 000).

Gel Filtration. The molecular weight of native nopaline synthase was estimated by gel filtration on a column (1.5 × 100 cm) of Ultrogel[2] AcA 34 (LKB) equilibrated with 0.1 M Tris-HCl (pH 7.5) and 14 mM 2-mercaptoethanol. The column was calibrated with aldolase (160 000) and alcohol dehydrogenase (150 000), and the molecular weight of native nopaline synthase was estimated (Andrews, 1964).

Table I: Purification of Nopaline Synthase from PSCG-T37 Tissue

fraction	total enzyme[a] (nkat)	total protein[a] (mg)	sp act. (nkat/mg)	yield (%)
gel-filtered extract	112	79	1.4	100
$(NH_4)_2SO_4$ precipitation	88	12.5	7	79
hydroxylapatite	67	1.85	36	60
affinity chromatography	38	0.086	442	34
Sephacryl S-200	32.5	0.074	439	29

[a] A total of 20 g fresh weight of PSCG-T37 tissue was used.

Procedures for Purification of Nopaline Synthase. All operations were carried out at 4 °C. PSCG-T37 or PSCG-C58 tissues (20 g fresh weight) were homogenized with a Duall homogenizer in 1.5 volumes (w/v) of extraction buffer [0.1 M Tris-HCl (pH 8.0), 0.5 M sucrose, 1 mM EDTA, and 14 mM 2-mercaptoethanol] (Sutton et al., 1978). After centrifugation for 10 min at 18000*g*, the clear supernatant (40 mL) was passed through a column (5 × 28 cm) of Sephadex G-25 equilibrated with 10 mM Tris-HCl (pH 7.5) containing 14 mM 2-mercaptoethanol. The straw-colored fractions that eluted at the void volume were combined (gel-filtered extract).

The gel-filtered extract was brought to 40% $(NH_4)_2SO_4$ saturation (0.226 g mL^{-1} of extract). After removal of precipitated proteins by centrifugation, the supernatant was adjusted to 55% saturation (an addition of 89 mg of $(NH_4)_2SO_4$ mL^{-1}). The precipitated material was recovered by centrifugation and dissolved in 4 mL of 10 mM Tris-HCl (pH 7.5) containing 14 mM 2-mercaptoethanol. This solution was passed through a column (1 × 26 cm) of Sephadex G-25 as described above (ammonium sulfate fraction).

The desalted ammonium sulfate fraction (8 mL) was applied to a column (1.5 × 5 cm) of hydroxylapatite previously equilibrated with 0.05 M potassium phosphate (pH 6.8) containing 14 mM 2-mercaptoethanol. The column was washed with 20 mL of equilibration buffer, and then the enzyme activity was eluted with 0.1 M potassium phosphate (pH 6.8) containing 14 mM 2-mercaptoethanol. The fractions containing enzyme activity were desalted on a Sephadex G-25 column as described above (hydroxylapatite fraction).

The hydroxylapatite fraction (8 mL) was further purified by adsorbing the enzyme activity to a column (1.5 × 7 cm) of Cibacron Blue F3GA coupled to cross-linked agarose (Bio-Rad Laboratories) equilibrated with 10 mM Tris-HCl (pH 7.5) containing 14 mM 2-mercaptoethanol. The enzyme activity was eluted from the gel with 0.1 mM NADPH. The fractions containing activity were combined (6 mL), and the preparation was stored at 4 °C (affinity chromatography fraction).

Removal of the last traces of contaminating protein was accomplished by gel filtration of the preparation on a column (1.5 × 100 cm) of Sephacryl S-200 equilibrated with 10 mM Tris-HCl and 14 mM 2-mercaptoethanol. Fractions containing enzyme activity were pooled and stored at 4 °C (purified enzyme).

Results

Purification of Nopaline Synthase. Nopaline synthase was purified to homogeneity in five steps (Table I). Analytical disc gel electrophoresis (Laemmli, 1970) was used to evaluate the degree of homogeneity at each purification step (Figure 1). Purification through affinity chromatography resulted

[1] Abbreviations: PSCG-T37, primary sunflower crown gall tissue culture incited by *Agrobacterium tumefaciens* strain T37; PSCG-C58, incited by strain C58; HSSS, habituated sunflower stem section tissue culture isolated as growing tissue from normal sunflower callus culture after planting on phytohormone-free medium; α-kg, α-ketoglutarate.

[2] Mention of companies or commercial products does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

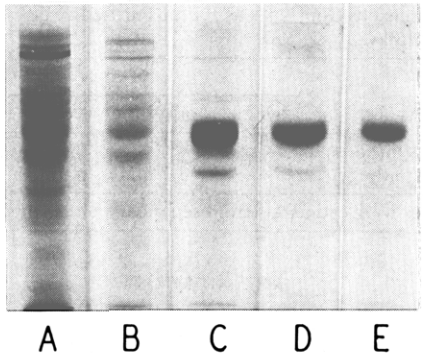

FIGURE 1: $NaDodSO_4$–polyacrylamide disc gels of nopaline synthase at each stage of purification. (A) Protein (30 μg) from the ammonium sulfate step; (B) protein (10 μg) from the hydroxylapatite step; (C) protein (20 μg) from the affinity chromatography step; (D) protein (10 μg) from the Sephacryl step (broad cut); (E) protein (7 μg) from the Sephacryl step (back 75% of the elution curve). After electrophoresis the gels were stained with Coomassie Blue and photographed. For gels A–C and E, the top of the figure is the top of the running gel and the bottom of the figure is the dye band. Gel D is from a separate preparation, run for a shorter length of time.

in an enzyme preparation that was about 92% homogeneous as judged by gel scans. In the final step (Sephacryl), the elution curve of nopaline synthase partly overlaps that of the contaminating proteins. However, the purity of nopaline synthase is routinely greater than 99% if only the fractions from the back 75% of the activity curve are combined (Figure 1) after Sephacryl chromatography. The purified enzyme activity can be preserved for several weeks by storage at 4 °C in 0.01 M Tris (pH 7.5), 14 mM 2-mercaptoethanol, and 0.1 mM NADPH.

Deviations from these procedures can cause a rapid loss of activity. Examples include leaving out 2-mercaptoethanol, freezing the preparation, and subjecting the preparation to mechanical stress or aeration.

Molecular Weight Determination and Subunit Composition. The catalytic activity of purified nopaline synthase eluted from a column of Ultrogel AcA 34 as a single peak just after aldolase but well in advance of alcohol dehydrogenase. From the elution volumes of the protein standards, we estimated the molecular weight of native nopaline synthase to be 158 000. By comparison of the electrophoretic mobility of nopaline synthase to that of protein standards during $NaDodSO_4$–polyacrylamide gel electrophoresis, the dissociated enzyme migrates as a single band corresponding to a molecular weight of 40 000.

Tissue Specificity of the Nopaline Synthase Protein. We previously demonstrated that nopaline synthesizing activity is found exclusively in nopaline-type crown gall tissues; no activity was detected in habituated tissue (HSSS) or in octopine-type crown gall tissues (Sutton et al., 1978). The following experiment was designed to determine if the same specificity holds for the purified protein we believe to be nopaline synthase (Figure 1, gel E). Starting with HSSS tissue instead of crown gall tissue, we purified the proteins as outlined above. The early steps of the purification produced analytical disc gel staining patterns very similar to those for PSCG-T37 tissue (Figure 1). However, after the affinity chromatography step very little total protein remained and no band was visible on gels where nopaline synthase was expected to migrate [Figure 2 (---)]. In order to ensure that nopaline synthase could have been isolated from HSSS tissue if it had been present, we again purified proteins from HSSS, this time mixed with a small amount of PSCG-T37 tissue (20 parts by weight HSSS to 1 part by weight PSCG-T37). The progress of the purification was followed at each step by analytical disc gels and enzyme activity. As expected, approximately 30% of the activity remained after the affinity chromatography step (Table I), and a faint protein band in the position of nopaline synthase was visible in the gel [Figure 2 (···)]. The amount of nopaline synthase in the mixture of HSSS and PSCG-T37 was estimated by scanning gels of purified enzyme preparations. The relative area of the presumed synthase peak in the mixed preparation [9; Figure 2 (···)] was compared with the area of the corresponding peak in gels containing nopaline synthase from an equal amount of PSCG-T37 (11; data not shown) or from 20 times the amount of PSCG-T37 [230; Figure 2 (—)]. From these results we conclude that the amount of synthase protein measured in the mixed tissue preparation was nearly equal to the amount of nopaline synthase expected from the quantity of PSCG-T37 tissue in the mixture.

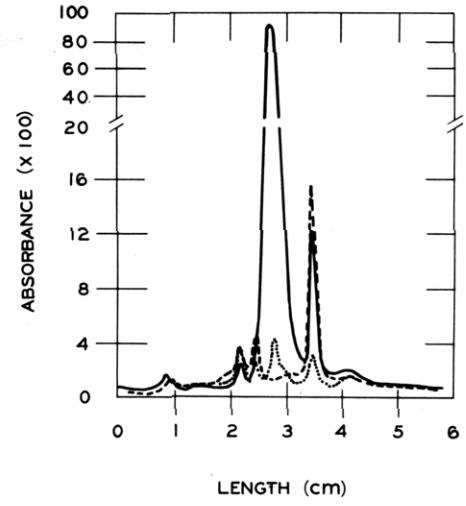

FIGURE 2: Scans of stained $NaDodSO_4$–polyacrylamide disc gels after electrophoresis of affinity chromatography purified protein preparations. The gels were scanned at 650 nm in a Gilford Model 240 spectrophotometer. Each gel received the total amount of protein purified from 5 g fresh weight of tissue. The approximate amounts of total protein were 20 μg for PSCG-T37 (—) (see gel C in Figure 1), 4 μg for HSSS (---), and 3 μg for a mixture of 5 g of HSSS and 0.25 g of PSCG-T37 (···). Nopaline synthase runs at 2.7 cm.

pH Optimum for Nopaline Synthase. The pH optima for nopaline and ornaline synthesis catalyzed by purified nopaline synthase are 6.0 and 6.5, respectively. The pH curves for both reactions are superimposable from pH 8.2 to 6.5. Then the curve for ornaline synthesis decreases rapidly to zero by pH 5 whereas the curve for nopaline synthesis continues to rise slightly to its maximum at pH 6 before starting its decrease. Further kinetic analyses were performed at pH 6.5 where the catalysis of both reactions was near maximal.

Initial Rate Studies and Kinetic Constants for Nopaline Synthase. The initial reaction rate was determined at varying concentrations of one substrate, with fixed concentrations of the other two. The resulting primary plots of the reciprocal initial rate against the reciprocal of the concentration of each substrate are linear over a wide range of concentrations (Figure 3). Therefore, the initial rate behavior of nopaline synthase can be described by the general empirical equation presented by Dalziel (1969) for enzyme-catalyzed reactions involving three substrates. The equation is

$$\frac{e}{V_0} = \phi_0 + \frac{\phi_A}{[A]} + \frac{\phi_B}{[B]} + \frac{\phi_C}{[C]} + \frac{\phi_{AB}}{[A][B]} + \frac{\phi_{AC}}{[A][C]} + \frac{\phi_{BC}}{[B][C]} + \frac{\phi_{ABC}}{[A][B][C]}$$

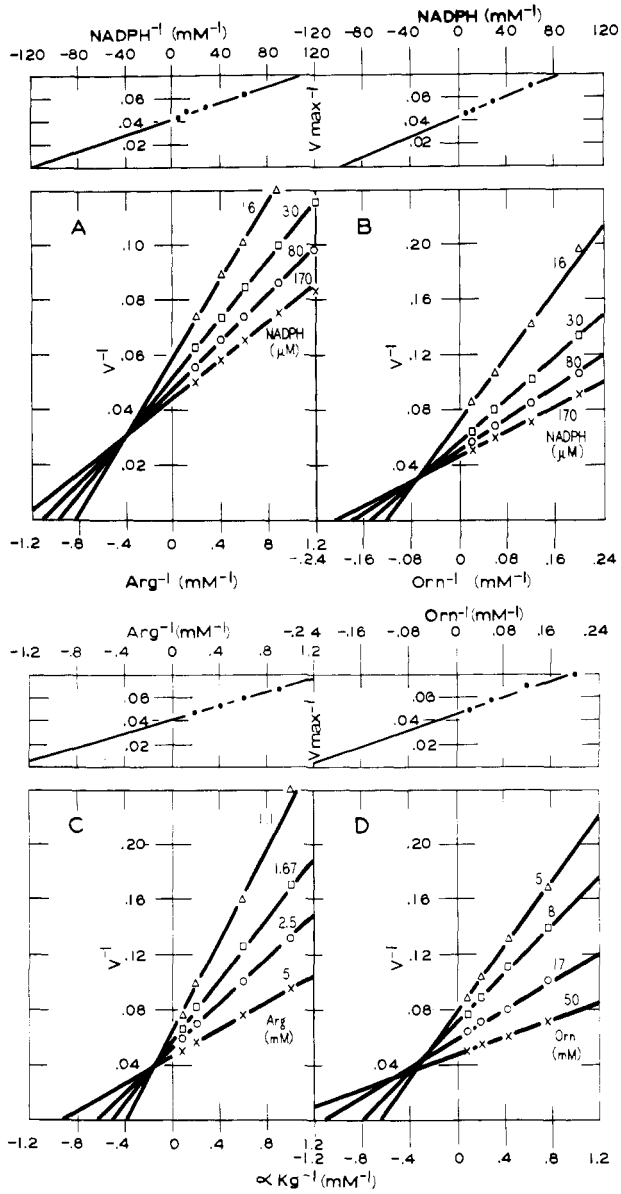

FIGURE 3: Double-reciprocal plots of initial rates at varying concentrations of one substrate, fixed concentrations of a second substrate, and a constant saturating concentration of the third substrate for the reactions catalyzed by purified nopaline synthase. The top half of each plot represents a replot of the ordinate intercept (V_{max}^{-1}) against the reciprocal of the fixed substrate concentration. (A) Arginine varied, NADPH fixed, and α-kg at a saturating concentration of 13 mM; (B) ornithine varied, NADPH fixed, and α-kg at a saturating concentration of 13 mM; (C) α-kg varied, arginine fixed, and NADPH at a saturating concentration of 0.17 mM; (D) α-kg varied, ornithine fixed, and NADPH at a saturating concentration of 0.17 mM.

where e is enzyme concentration, V_0 is initial rate; ϕ_0, ϕ_A, etc. are kinetic coefficients, and [A], [B], and [C] are substrate concentrations.

The concentration of a second substrate was also varied to generate families of primary double-reciprocal plots (Figure 3). The intercepts and slopes of the members of these families were replotted against the reciprocal of the concentration of the second substrate, thus generating secondary plots (top part of Figure 3). All replots were again linear for all combinations of substrates as predicted by the equation.

All members of each family of primary plots for nopaline synthase catalyzed reactions intersect at a point above the abscissa and to the left of the ordinate (Figure 3). Primary plots with this characteristic produce secondary plots (replots of either intercepts or slopes) whose ordinate intercept and slope are greater than zero (top parts of Figure 3). For the experimental results presented in Figure 3, one substrate was always kept at saturating levels. Therefore, an estimate of the substrate K_m values was made by extrapolating the lines of the secondary intercept plots and calculating K_m as -1/abscissa intercept (Table II).

Table II: Michaelis Constants (K_m) for Purified Nopaline Synthase

	K_m[a]			
catalytic act.	arginine	ornithine	α-kg	NADPH
nopaline synthesis	0.74 mM		0.9 mM	8 μM
ornaline synthesis		3.7 mM	0.9 mM	9 μM

[a] K_m values were estimated from secondary plots (Figure 3).

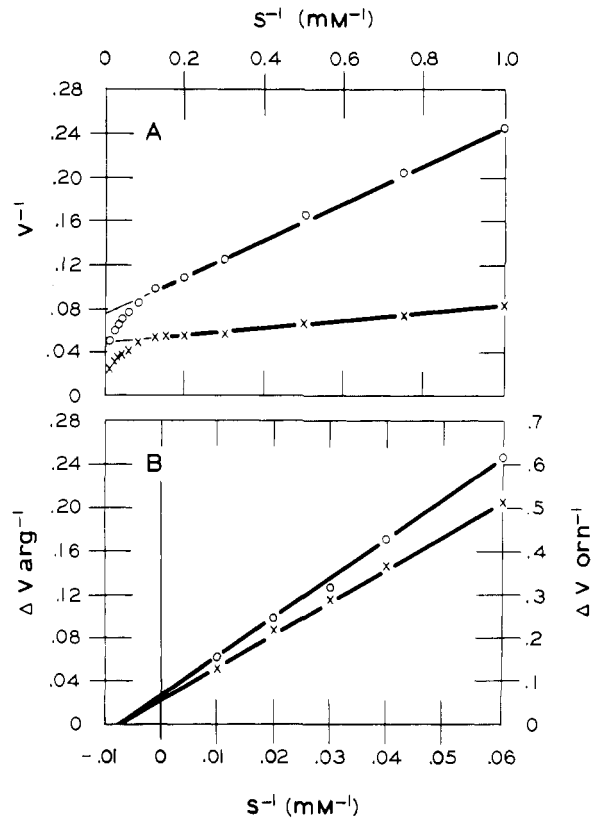

FIGURE 4: Double-reciprocal plots of initial rates at varying concentrations of arginine (×) or ornithine (○) and saturating concentrations of α-kg (13 mM) and NADPH (0.17 mM). (A) Reciprocal of the observed initial rates (V^{-1}); (B) reciprocal of the observed initial rates minus the extrapolated rate (ΔV^{-1}). Extrapolated rates were estimated from the position of the line drawn through the low substrate concentration points in (A).

We attempted to generate families of secondary plots by repeating the experiment presented in Figure 3 at various nonsaturating levels of the third substrate. The results were not conclusive but suggested that families of secondary plots also intersect in the upper left quandrant. The significance of such a result as it relates to Dalziel's equation will be considered under Discussion.

Kinetic Data at High Concentrations of Substrates. In the previous section we stated that double-reciprocal plots of initial rates against substrate concentration are linear over a wide range of concentrations. The range included concentrations as low as $K_m/2$ and as high as $10K_m$. At yet higher concentrations of α-kg or NADPH substrate, inhibition appeared. This is not uncommon for pyridine nucleotide requiring enzymes. For some enzyme preparations a rather unexpected

result occurred at high concentrations of arginine or ornithine (Figure 4A). Initial velocity experiments yielded rates at high concentrations that were greater than would be expected by extrapolation of rates at low amino acid concentrations. Such results, when observed, have been interpreted as substrate activation or negative cooperativity. However, our data do not fit the equations for either substrate activation as presented by Frieden (1959) or negative cooperativity as discussed by Levitzki & Koshland (1969). The data do fit the model of two independent reactions superimposed on one another, each obeying Michaelis–Menten kinetics. This is illustrated in Figure 4B where the reciprocals of the differences between observed and extrapolated rates are plotted against the reciprocals of substrate concentrations. It is interesting to note that the K_m values of arginine and ornithine for the "low-affinity" reaction are the same, 0.2 M.

Substrate Specificity of Nopaline Synthase. We previously demonstrated that crude preparations of PSCG-T37 tissue will synthesize nopaline or ornaline from α-ketoglutarate, NADPH, and arginine or ornithine, respectively (Sutton et al., 1978). No other amino or keto acids tested were active. The substrate specificity and K_m values for nopaline and ornaline synthesis do not change during purification of nopaline synthase. Both the crude enzyme activity and the purified enzyme can use either NADPH or NADH. Further, the ratio of NADPH/NADH activity does not change during purification. Since the K_m for NADH (1 mM) is 2 orders of magnitude larger than the K_m for NADPH, NADH is probably not an important source of electrons in vivo for nopaline or ornaline synthesis.

Discussion

A protein was purified from two nopaline-synthesizing crown gall tissue culture lines, PSCG-T37 and PSCG-C58. We conclude that this protein is nopaline synthase for the following reasons: (1) the specific enzymic activity does not change during further attempts at purification; (2) the protein migrates as a single band on $NaDodSO_4$–polyacrylamide gels; (3) the protein shows the same tissue specificity as nopaline synthesizing activity (Sutton et al., 1978). The nopaline synthase protein is present in both PSCG-T37 and PSCG-C58 tissues at a concentration of 13 μg of protein per g fresh weight. On the other hand, nopaline synthase protein could not be detected in either HSSS tissue (Figure 2) or the octopine-type tissue PSCG-B_6 (data not shown). The same tissue specificity was reported by Sutton et al. (1978) for nopaline synthesizing activity.

Recently we concluded that a single enzyme catalyzed the synthesis of both nopaline and ornaline (Sutton et al., 1978). This conclusion is confirmed by our observation that nopaline and ornaline synthesizing activities copurify with no change in their kinetic parameters.

An initial velocity kinetic analysis of nopaline synthase is consistent with a ter-bi rapid-equilibrium random-order mechanism as discussed by Dalziel (1969). The criterion for this mechanism is that none of the parameters of Dalziel's equation are zero. If any of the parameters are zero, the mechanism will be either ordered quaternary complex or enzyme substitution (ping pong). This will be reflected in the characteristics of the primary and secondary plots. These characteristics include a family of parallel plots or a family of plots that all intersect on the ordinate. A "ping pong" mechanism can be ruled out since all of the primary plots intersect in the upper left quadrant. However, it is much harder to discriminate between the random and the ordered quaternary complex mechanisms.

The former requires that all secondary plots intersect at a point other than the ordinate. As pointed out by Dalziel, it is sometimes difficult to determine whether this is the case. This is expecially so for an enzyme like nopaline synthase that has a low K_m for NADPH (10 μM). One way to determine the mechanism conclusively would be to make the kinetic measurements with a sensitive fluorometric method.

The biphasic nature of the reciprocal plots at high concentrations of either arginine or ornithine suggests that two separate reactions are taking place simultaneously. One is a high-affinity reaction with K_m values for arginine and ornithine of 0.74 and 3.7 mM, respectively; the other is a low-affinity reaction with the same apparent K_m (0.2 M) for both arginine and ornithine. It is possible that nopaline synthase may have two sets of active sites with low and high affinities for the substrates.

Nopaline synthase has a molecular weight of 158 000 and is composed of four equal-weight subunits. If the four subunits are identical, then the size of the structural gene coding for nopaline synthase may be as small as 0.72×10^6 daltons. However, if all of the subunits of the enzyme are different, it may require as much as 2.9×10^6 daltons of DNA to code for this protein. Van Vliet (1979) has located a (2×10^6)-dalton restriction endonuclease-generated fragment of pTi-C58 that is necessary for nopaline production in crown gall tissue. However, it is not known whether this fragment actually codes for the nopaline synthase protein. An answer to this question must wait until active nopaline synthase is synthesized from the pTi-C58 or until antibodies made against nopaline synthase are shown to cross-react with a polypeptide synthesized from pTi-C58.

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Purification and Properties of Strictosidine Synthetase (an Enzyme Condensing Tryptamine and Secologanin) from *Catharanthus roseus* Cultured Cells†

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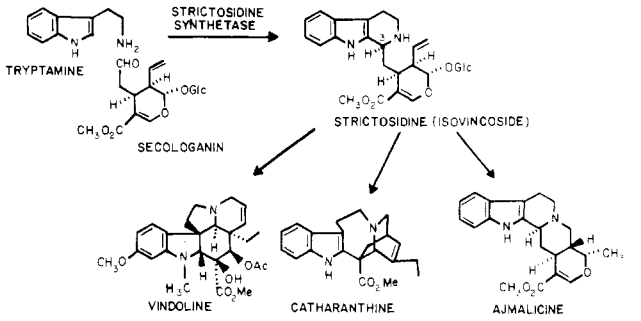
ABSTRACT: Strictosidine synthetase, which catalyzes the condensation of tryptamine with secologanin to form strictosidine (isovincoside), was purified 740-fold to homogeneity from cultured cells of *Catharanthus roseus* in 10% yield. The specific activity is 5.85 nkat/mg. The molecular weight as estimated by gel filtration is 38 000. The isoelectric point is 4.6. Apparent K_m values for tryptamine and secologanin are 0.83 and 0.46 mM, respectively. The enzyme shows a broad pH optimum between 5.0 and 7.5. The product of the enzymic reaction is exclusively strictosidine, while no trace of its epimer vincoside can be detected. Sulfhydryl inhibitors have no effect on the enzyme. End products in the biosynthetic pathway of indole alkaloids such as ajmalicine, vindoline, and catharanthine do not inhibit the activity of strictosidine synthetase.

Catharanthus roseus (periwinkle) produces more than 100 indole alkaloids (Scott, 1970), many of which are pharmacologically active, the most notable of these being vinblastine and vincristine, the antitumor alkaloids. As the contents of these alkaloids in plant cells are extremely low, various, but so far unsuccessful, attempts have been made to produce these compounds from plant tissue culture. It has therefore become important to understand the regulatory mechanism of the biosynthesis of indole alkaloids in higher plants. There were few enzymological studies of the biosynthesis of the indole alkaloids until the discovery of a cell-free system from *C. roseus*, which yielded some important information concerning the formation of indole alkaloids, at both early (Scott & Lee, 1975; Scott et al., 1977a; Stöckigt et al., 1976; Stöckigt & Zenk, 1977a,b) and final stages (Baxter et al., 1979; Stuart et al., 1978). The first step in the biosynthesis of indole alkaloids in *C. roseus* is a stereospecific enzymic condensation of tryptamine and secologanin to form strictosidine with H-3α(*S*) configuration (Scheme I) (Blackstock et al., 1971; DeSilva et al., 1971). Earlier assignments (Battersby et al., 1968, 1969) of vincoside, the H-3β(*R*) isomer, as the precursor for indole alkaloids have been proven (Scott et al., 1977a; Stöckigt & Zenk, 1977a,b; Battersby et al., 1978) to be incorrect. Later, it was found that this compound is a common precursor for monoterpenoid indole alkaloids with both H-3α(*S*) and H-3β(*R*) configuration (Rueffer et al., 1978). The first enzyme in the biosynthetic pathway normally serves as a site of control (Umbarger, 1956; Yates & Pardee, 1956) including secondary metabolic pathways such as the biosynthesis of ergot alkaloids (Heinstein et al., 1971; Lee et al., 1976). In this paper, we describe the purification of strictosidine synthetase from cultured cells of *C. roseus* and some properties of this enzyme, which, as the first committed synthetase enzyme on the pathway, forges the link between the amino acid and mevalonoid derived segments of the alkaloids of *C. roseus*.

Scheme I



Experimental Procedures

Cell Culture. Cultured cells induced from seedlings of *Catharanthus roseus* G. Don (Apocynaceae) were maintained in SH liquid medium (Schenk & Hildebrandt, 1972) at about 28 °C under dim light with transfer intervals of 2 weeks.

Assay of Strictosidine Synthetase. The activity of strictosidine synthetase was determined by the formation of

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