Poulson, R., & Avramovic, O., Eds.) Part B, p 325, Wiley-Interscience, New York.
Sukhareva, B. S., & Braunstein, A. E. (1971) *Mol. Biol. (Engl. Transl.)* *5*, 302–317.
Sukhareva, B. S., Dunathan, H. C., & Braunstein, A. E. (1971) *FEBS Lett.* *15*, 241–244.
Tikhonenko, A. S., Sukhareva, B. S., & Braunstein, A. E. (1968) *Biochim. Biophys. Acta* *167*, 476–479.
Yamada, H., & O'Leary, M. H. (1977) *J. Am. Chem. Soc.* *99*, 1660–1661.

Streptomyces L-Methionine Decarboxylase: Purification and Properties of the Enzyme and Stereochemical Course of Substrate Decarboxylation†

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ABSTRACT: L-Methionine decarboxylase from *Streptomyces* species ATCC 21020 has been purified to near homogeneity, characterized, and compared to the enzyme from the fern *Dryopteris filix-mas* [Stevenson, D. E., Akhtar, M., & Gani, D. (1990) *Biochemistry* (first paper of three in this issue)]. The enzyme catalyzes the decarboxylation of a range of alkylamino acid substrates, but the substrate specificity is different from that for the fern enzyme. In accord with the properties of the fern enzyme, the *Streptomyces* enzyme is also a homodimer of M_r 100 000 ± 5000 and requires PLP for activity. At low pH, where the value of V_{max} for both enzymes is maximal and essentially pH independent, k_{cat} for the *Streptomyces* enzyme with (2*S*)-methionine as substrate is slightly higher (60 s^{-1}) than the value for the eukaryotic protein (50 s^{-1}). The pH optimum for V/K is much higher than that for the fern enzyme although many features of the pH dependence are similar, including the shape of the curve for the pH dependence of K_m. When the decarboxylations of (2*S*)-methionine, (2*S*)-norleucine, and (2*R*)-*S*-ethyl-L-cysteine were conducted on a preparative scale in protium and deuterium oxide, unlabeled and deuteriated amines were formed. ^{1}H NMR spectroscopic analysis of the stereochemistry at C-1 of the camphanamide derivatives of the products [Stevenson, D. E., Akhtar, M., & Gani, D. (1990) *Biochemistry* (first paper of three in this issue)] indicated that each conversion was stereospecific and occurred with retention of configuration at C-2 of the substrates. The *Streptomyces* enzyme does not catalyze abortive transamination reactions and is unable to catalyze the incorporation of tritium from the solvent into the product amine or into (2*R*)-methionine. A monoprotic acid appears to serve as the proton donor in the conversion of the quinoid intermediate to the external product aldimine during decarboxylation. The differences and similarities of the two enzymes are discussed in mechanistic terms.

Dryopteris filix-mas L-methionine decarboxylase catalyzes the decarboxylation of L-methionine and a range of alternative alkylamino acids to give amines and carbon dioxide. The enzyme also catalyzes an occassional abortive transamination event in which the coenzyme is converted to PMP[1] and the amine, prior to release as product, is converted to an aldehyde (Stevenson et al., 1990). Analysis of the mechanism and the stereochemical courses of reactions catalyzed by the decarboxylase (Stevenson et al., 1990) indicated that the enzyme shared many common features with transaminase enzymes. For example, for fern methionine decarboxylase, it was apparent that the coenzyme was protonated at C-4′ on the 4′-*si* face quinoid intermediate derived from the physiological substrate during the abortive transamination reaction and that a polyprotic base, probably the ϵ-ammonium group of the active-site aldimine-forming lysine residue, served as the proton donor. Furthermore, from the retentive mode of decarboxylation and the high chiral integrity of the C-2 deuteriated products derived from the decarboxylation of a range of substrates, it appeared that decarboxylation (carbanion/quinoid intermediate generation) occurred on the same (4′-*si*) face of the coenzyme. This chemistry exactly parallels the well-established mode of catalysis by transaminases (Kirsch et al., 1984).

In order to extend our understanding of the PLP-dependent enzymes as a group and, in particular, to identify common mechanistic and structural features, we wished to compare the chemistry of the fern enzyme to a similar broad substrate specificity enzyme from an evolutionarily distant species. Hagino and Nakayama (1968) described an L-methionine decarboxylase activity in *Streptomyces* strains grown in the presence of L-methionine. Several years later the enzyme was purified 582-fold, in 5% yield, and characterized (Misono et al., 1980). The reported properties of the enzyme indicated that the system was similar to the fern enzyme in many re-

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[1] Abbreviations: PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; NMR, nuclear magnetic resonance; DTT, DL-dithiothreitol; EDTA, ethylenediaminetetraacetic acid; DEAE, (diethylamino)ethyl; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BSA, bovine serum albumin; FPLC, fast protein liquid chromatography; AAT, aspartate aminotransferase; pL, -log [L^+] (Schowen, 1978).

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spects and would be suitable for comparison. More interestingly, two features of the *Streptomyces* enzyme markedly contrasted with the properties of the fern enzyme. First, the reported optimum pH was much higher than that for the fern enzyme, and second, the authors had not noted the occurrence of abortive transamination events. Such events lead to a gradual decrease in the activity of the enzyme that can be detected during assays if exogenous coenzyme is not included in the assay incubation medium.

Here we report on the efficient purification and further characterization of the *Streptomyces* enzyme and on the mechanism, stereochemical course, and pH dependence of decarboxylation. The catalytic and structural features of the fern and *Streptomyces* enzymes are compared with each other and with other PLP-dependent decarboxylases.

Materials and Methods

Amino acid substrates, PLP, PMP, buffers, and salts were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.), and [1-^{14}C]-L-amino acid substrates and tritiated water were obtained from Amersham International (Amersham, Bucks, U.K.). Water-miscible scintillant (ES-199) was obtained from Canberra Packard (Pangbourne, Berks, U.K.). Protein standards for electrophoresis and gel exclusion chromatography were obtained from British Drug Houses (Poole, Dorset, U.K.) and Bio-Rad (Watford, Herts, U.K.), respectively. Amberlite IR45(OH) and Dowex 1X8(OH) ion-exchange resins were also obtained from British Drug Houses. (1*S*,4*R*)-(−)-Camphanic acid was obtained from Aldrich (Gillingham, Dorset, U.K.). All other chemicals were of analytical grade or were recrystallized or redistilled before use.

^{1}H NMR spectra were recorded by using a Brucker AM360 instrument, and mass spectra were obtained by using a AEI MS30 spectrometer.

Ammonium sulfate solutions of a given percentage saturation were prepared according to published procedures (Green & Hughes, 1955).

Protein concentrations were determined by the method of Spector (1978) with solutions prepared from crystalline BSA as standards.

Cell Cultures. *Streptomyces* species ATCC 21020 was stored as a spore suspension in 20% glycerol at −30 °C. Seed cultures were grown in four 500-mL shaker flasks each containing 100 mL of medium (115 mM glucose, 1% yeast extract, and 3% tryptic soya broth, w/v). After inoculation with 50 μL of spore suspension, the flasks were incubated with shaking at 30 °C for 20 h. The main fermentation was carried out in a 4-L batch fermenter fitted with a paddle stirrer and an aeration system. After the addition of the seed culture, the medium composition was glucose (300 mM), K_2HPO_4 (3 mM), $MgSO_4 \cdot 7H_2O$ (1 mM), $(NH_4)_2SO_4$ (15 mM), yeast extract (0.3%), and L-methionine (70 mM) at pH 7.0 in a total volume of 4.0 L. The cells were grown with constant aeration and agitation at pH 7.0 and 30 °C for 10 h and were then harvested by centrifugation at 10000*g* to yield 55–65 g (L of fermentation medium)$^{-1}$. The cells were washed with 150 mM NaCl solution and were mixed with an equal volume of extraction buffer (0.5 mM PLP, 2 mM DTT, and 2 mM EDTA in 10 mM potassium phosphate at pH 7.2) prior to storage in 120-g portions at −30 °C.

Enzyme Activity Assays: (*a*) *Electromanometric Assay.* Assay incubations contained substrate (70 mM), PLP (1 mM), and enzyme in a total volume of 2 mL in 0.2 M potassium succinate buffer, pH 5.5, at 37 °C in a Warburg flask. Reactions were initiated by tipping the enzyme (contained in the Warburg side arm) into the substrate solution, after the apparatus had reached thermal equilibrium. The incubations were stirred under a nitrogen atmosphere. The rates of CO_2 evolution were monitored with a resetting diaphragm-cell electromanometer (Mercury M12) connected to a Kipp and Zonen chart recorder. Rates were calibrated against L-methionine (100%) and standardized by comparison with the extent of $^{14}CO_2$ release from (2*S*)-[1-^{14}C]methionine in a similar experiment. Note that at the low pH of the assay (necessary to ensure CO_2 evolution) low initial rates were recorded, ~30% of the values measured radiochemically at pH 6.75.

(*b*) *Radiochemical Assay.* Assay incubations contained L-methionine (20 mM), PLP (1 mM), and enzyme in a total of 300 μL of 0.2 M potassium succinate buffer, pH 6.75. This solution together with (2*S*)-[1-^{14}C]methionine (20000 dpm) was incubated in a scintillation vial (6.0-mL size) at 37 °C. Several identical reactions were initiated by the addition of enzyme and were terminated at zero time and at intervals thereafter by the addition of 8 M sulfuric acid (200 μL). After the reactions stood for 30 min to ensure the complete evolution of CO_2, scintillant (3.0 mL) was added and the residual radioactivity was determined by using a Packard Tri-Carb 300 C scintillation counter. The rate of reaction was calculated by plotting the decrease in radioactivity against time for the seven incubations. Except where indicated, the radiochemical assay was used for the routine determination of activity. One unit of activity converts 1 μmol of L-methionine to products per minute under these assay conditions and is equivalent to 1.06 units as defined by Misono et al. (1980), using a manometric assay at a higher substrate concentration.

Enzyme Purification: (*1*) *Preparation of Crude Extract.* Frozen cells in extraction buffer (240 g) were thawed and sonicated at 5 °C with a Heat Systems-Ultrasonic W220F cell disruptor for 10 min with vigorous stirring. The cell debris was removed by centrifugation (25000*g*, 15 min) to give 196 mL of crude extract.

(*2*) *Protamine Sulfate Treatment.* The crude extract was stirred at 4 °C and a solution of protamine sulfate (300 mg) in extraction buffer (20 mL) was added dropwise over 10 min. After a further 15 min, the suspension was centrifuged (25000*g*, 15 min) and the pellet was discarded.

(*3*) *Ammonium Sulfate Fractionation/Heat Treatment.* The supernatant solution (212 mL) was stirred at 4 °C and solid ammonium sulfate was slowly added to 30% saturation. After 30 min, the precipitated protein was removed by centrifugation (25000*g*, 15 min) and the supernatant solution was treated with additional ammonium sulfate to bring the solution to 50% saturation and was again centrifuged. The pellet was dissolved in buffer A (0.1 mM PLP, 0.5 mM DTT, 0.25 mM EDTA, and 100 mM potassium succinate, pH 5.0; 50 mL) and the solution was heated to 40 °C for 10 min. The precipitated protein was removed by centrifugation (40000*g*, 15 min) and the supernatant solution was desalted on a column of Sephadex G-50 (2.5 × 30 cm) equilibrated with buffer B (0.1 mM PLP, 1 mM DTT, 0.25 mM EDTA, and 50 mM potassium phosphate, pH 6.8). The column was eluted with buffer B, and all protein-containing fractions were pooled. The final volume was adjusted to 80 mL with buffer B.

(*4*) *DEAE-cellulose Chromatography.* The desalted protein solution was applied to a column of DEAE-Sephacel (2.5 × 20 cm) equilibrated with buffer B. The column was washed with one column volume of buffer B and was eluted with a gradient of 0–600 mM KCl in buffer B (500 mL at 0.5 mL min^{-1}) (see supplementary material Figure 1). The active fractions were pooled (48 mL), and the protein was precipi-

tated by the addition of ammonium sulfate to 60% saturation. The protein was collected by centrifugation (25000*g*, 15 min).

(*5*) *Ion-Exchange FPLC.* The precipitated protein was dissolved in the minimum quantity of buffer C (0.1 mM PLP, 0.5 mM DTT, and 50 mM potassium succinate, pH 5.6; 10 mL); the solution was centrifuged (45000*g*, 15 min) to remove undissolved protein and was then desalted on a column of Sephadex G-25 (1.5 × 25 cm) equilibrated with buffer C and with buffer C as the eluent. All of the protein-containing fractions were pooled (30 mL). Half of the protein solution was applied to a column of TSK DEAE-5PW (Pharmacia-LKB, 2.15 × 15 cm) equilibrated with buffer C and eluted with a gradient of 50–250 mM KCl in buffer C (80 mL at 2 mL min^{-1}) (see supplementary material Figure 2). The active fractions were pooled (14 mL). The above procedure was repeated for the other half of the protein solution, and then the combined fractions (28 mL) were desalted on the same column of Sephadex G-25 (1.5 × 25 cm) with buffer C. All the protein-containing fractions were pooled and applied to the column of TSK DEAE-5PW equilibrated with buffer C and eluted with a gradient of 100–250 mM KCl in buffer C (80 mL at 2 mL min^{-1}). The active fractions were pooled (10 mL).

(*6*) *Size-Exclusion FPLC.* The pooled active fractions from the above procedure were desalted on Sephadex G-50 (1.5 × 30 cm) equilibrated with 5 mM potassium phosphate buffer, pH 6.5, precipitated by the addition of ammonium sulfate to 60% saturation, and then centrifuged. (The pellet could be stored at −30 °C in this form for several months without loss of activity.) The protein was redissolved in buffer D (0.1 mM PLP, 100 mM KCl, 0.5 mM DTT, and 100 mM potassium phosphate, pH 6.5; 500 μL) and was subjected to FPLC size-exclusion chromatography on Pharmacia-LKB TSK G3000 SWG (2.15 × 30 cm), equilibrated with buffer D, at a flow rate of 0.15 mL min^{-1} (see supplementary material Figure 3). The active fractions were pooled to give 5 mL of essentially homogeneous enzyme (specific activity was routinely 25–30 units mg^{-1}).

Electrophoresis. Electrophoretic analysis of the enzyme was performed on both SDS-containing and nondenaturing polyacrylamide gels as described previously for the fern enzyme (Stevenson et al., 1990) using the methods of Laemmli (1970). The R_f of the active protein on 10% nondenaturing gels, relative to bromophenol blue, was determined by the coincidence of a staining band and activity in gel slices assayed with (2*S*)-[1-^{14}C]methionine as substrate.

Abortive Decarboxylation–Transamination. Several identical incubations contained enzyme, freed of unbound coenzyme, and (2*S*)-[1-^{14}C]methionine in buffer at the appropriate pH at 37 °C in a total volume of 300 μL. Incubations were sequentially terminated over a period of 2–3 h by the addition of sulfuric acid as described previously for the fern enzyme (Stevenson et al., 1990). Control incubations contained added coenzyme.

Synthesis of Chirally Deuteriated Standards. These were prepared as described earlier for the analysis of the stereochemical course of the fern enzyme (Stevenson et al., 1990).

(2*S*)-[2-^{2}H]Methionine was prepared by exchanging deuterium into the azlactone derived from *N*-acetylmethionine followed by hydrolysis and then acylase resolution of the deuteriated antipodes (Stevenson et al., 1990).

Preparation of 3-(Methylthio)-1-aminopropane and Other Product Amines. (2*S*)-Methionine (100 mg, 0.68 mM) was incubated with *Streptomyces* L-methionine decarboxylase (2 units) in 100 mM potassium phosphate buffer at pH 6.75 and 25 °C. After 24 h, the solution was adjusted to pH 11 by the addition of 2 M sodium hydroxide. The aqueous phase was extracted with dichloromethane (3 × 25 mL), and the organic phase was extracted with 500 mM hydrochloric acid (2 × 15 mL). The acid solution was reduced in volume in vacuo to give 3-(methylthio)-1-aminopropane hydrochloride. The crude product was purified on Dowex 1X8(OH) and was then converted to the hydrochloride by the addition of 6 M HCl, in 70% overall yield. The compound showed the expected spectral and analytical properties and was identical with an authentic sample (Stevenson et al., 1990). The amine products of two alternative substrates, norleucine and *S*-ethylcysteine, were isolated in a similar manner in lower yield, 15–50%.

Preparation of Deuteriated Decarboxylation Products. (2*S*)-[2-^{2}H]Methionine, prepared as described previously (Stevenson et al., 1990), was decarboxylated and purified exactly as outlined above to give the deuteriated amine in 70% yield. The deuteriated amine products of methionine and the alternative substrates were prepared as described above for the unlabeled materials with deuterium oxide as the solvent. The isolated yields were similar.

Derivatization of the Decarboxylation Products. The amines derived from methionine were each converted to their (−)-camphanamide derivatives by the water-soluble carbodiimide method described previously (Stevenson et al., 1990). The labeled and unlabeled amines derived from norleucine and *S*-ethylcysteine were converted to their camphanamide derivatives by the acid chloride method. The enzymically formed amine derivatives were each examined by ^{1}H NMR spectroscopy, and the spectra were compared with spectra obtained from synthetic samples and those obtained from the fern enzyme decarboxylation products.

Decarboxylation in 50% Deuterium Oxide. (2*S*)-Methionine (50 mg, 0.34 mmol) was incubated with enzyme (2 units) in 50 mM potassium succinate buffer containing exactly 50 molar % deuterium oxide (allowing for exchangeable hydrogens) at pH 6.0 and 7.0 at 37 °C in a total volume of 10 mL. The partially deuteriated amine was isolated as the hydrochloride and purified as described above. The deuterium content was assessed by integration of the 360-MHz ^{1}H NMR spectrum, with the remaining signals serving as an internal reference.

Results

L-Methionine decarboxylase from *Streptomyces* species ATCC 21020 was purified 636-fold in six steps in 20% yield, as outlined in Table I. This is a significant improvement on the methods of Misono et al. (1980), who obtained a preparation of comparable activity after a 582-fold purification in nine steps in 5.6% yield from *Streptomyces* species 590, although it is not clear that the proteins are identical. The R_f of the active protein was ~0.5 on 10% nondenaturing gels at pH 8.3, relative to bromophenol blue, as determined by the coincidence of a staining band and activity in gel slices assayed with (2*S*)-[1-^{14}C]methionine. SDS–PAGE analysis of the purified enzyme showed a single band, which when rerun against protein standards gave an M_r value of 59 000 ± 3000. The protein was ~90% homogeneous as judged by SDS–PAGE, since at high loadings several other faint bands representing up to 10% of the total protein were present.

The enzyme eluted as a single band when subjected to FPLC on a precalibrated TSK G3000 SWG gel-exclusion chromatography column with a retention volume corresponding to M_r 100 000 ± 5000, as shown in Figure 1. This value is slightly lower than that reported for the 590 strain, M_r 130 000, as determined by size-exclusion chromatography on Sephadex

Table I: Summary of Purification of L-Methionine Decarboxylase from *Streptomyces* Sp ATCC 21020

stage	protein (mg mL^{-1})	tot. protein (mg)	sp act. (units mL^{-1})	tot. act. (units)	purification factor	yield (%)
crude extract	14.3	2803	0.044	124.7	1	100
protamine sulfate treatment	12	2544	0.053	134.8	1.2	108
30–50% ammonium sulfate + pH 5.0 treatment	6.4	512	0.227	116	5.2	93
DEAE-cellulose (0–0.6 M KCl)	2.05	98.4	0.874	86	19.8	69
TSK DEAE 5PW I (0.05–0.25 M KCl)	0.165	4.62	12.5	57.7	284	46
TSK DEAE 5PW II (0.1–0.25 M KCl)	0.22	2.2	21	46	477	37
TSK G3000 SWG gel permeation	0.18	0.9	28	25	636	20

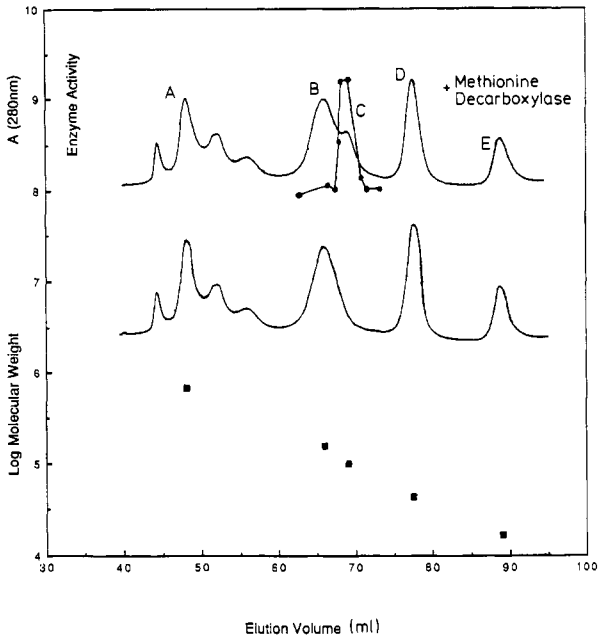

FIGURE 1: Determination of protein M_r. A sample of purified enzyme was mixed with standard proteins (shown in the lower trace) and was chromatographed on TSK G3000 SWG (2.15 × 30 cm), equilibrated with 0.1 M potassium phosphate, pH 6.5, at a flow rate of 0.1 mL min^{-1}. Upper trace: A, bovine thyroglobulin (M_r 670 000); B, bovine δ-globulin (M_r 158 000); C, L-methionine decarboxylase; D, chicken ovalbumin (M_r 44 000); E, horse myoglobin (M_r 17 000).

G-150 (Misono et al., 1980) but is very similar to the value reported for the fern enzyme, M_r 100 000 ± 5000. Thus, the protein has a homodimeric structure and in this respect also is similar to the fern enzyme (Stevenson et al., 1990).

The relative rates of reaction for a range of substrates are given in Table II. The kinetic parameters K_m and V_{max} were determined for (2*S*)-methionine over the pH range 5.0–9.25 by using the radiochemical assay; see Figure 2. Initial rate data was analyzed by the method of Eisenthal and Cornish-Bowden (1974). The maximum turnover value for the enzyme is 120 mol s^{-1} (mol of enzyme)$^{-1}$ (k_{cat} = 60 s^{-1}), about 15% higher than the value for the fern enzyme. We were unable to demonstrate the occurrence of an abortive decarboxylation–transamination reaction for the *Streptomyces* enzyme, and the activity of the decarboxylase remained constant during sustained substrate turnover in the absence of excess exogenous coenzyme. This result completely contrasts that for the fern enzyme (Stevenson et al., 1990).

The stereochemical course for the decarboxylation of (2*S*)-methionine and a range of alternative hydrophobic amino acid substrates were determined by conducting incubations in protium oxide and deuterium oxide, respectively. The amine products were each isolated and converted to their corresponding *N*-camphanamide derivatives for analysis by ^{1}H NMR spectroscopy. Comparison of the ^{1}H NMR spectra of the derivatives with compounds synthesized by alternative procedures (Stevenson et al., 1990) revealed that the decarboxylation of each substrate proceeded with retention of configuration at C-2 of the substrate; see Figure 3.

Table II: Comparison of Substrate Specificity of Methionine Decarboxylase Enzymes

substrate	relative rate: *Streptomyces*[a] (70 mM)	Misono et al., 1980[b] (22 mM)	fern[c] (70 mM)
L-methionine	100.0	100.0	100.0
L-ethionine	7.8	19.0	0
S-ethyl-L-cysteine	29.3	61.0	12.0
L-norvaline	22.0	34.0	55.0
L-leucine	5.3	15.0	40.0
L-isoleucine	24.2	29.0	35.0
L-norleucine	45.3	63.0	40.0
L-valine	<5		16.0
L-alanine	<5		0
L-*allo*-isoleucine	<5		0
O-ethyl-L-serine	<5		<5

[a] pH 6.75. [b] pH 6.8. [c] pH 4.8 (Stevenson et al., 1990).

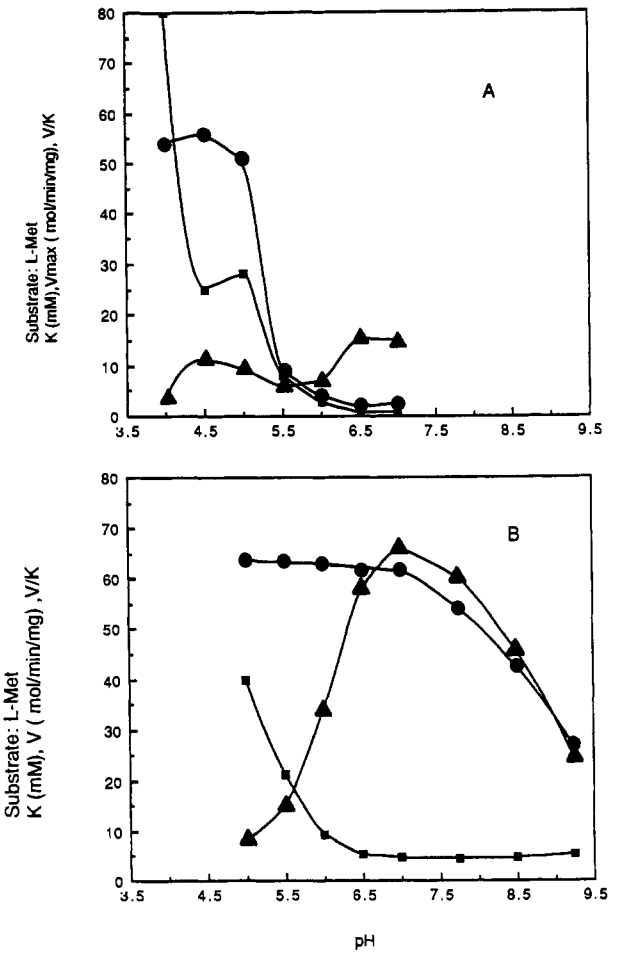

FIGURE 2: Comparison of the variation of the kinetic parameters V (●), V/K ×5 (▲), and K (■) for (A) *D. filix-mas* (Stevenson et al., 1990) and (B) *Streptomyces* L-methionine decarboxylases.

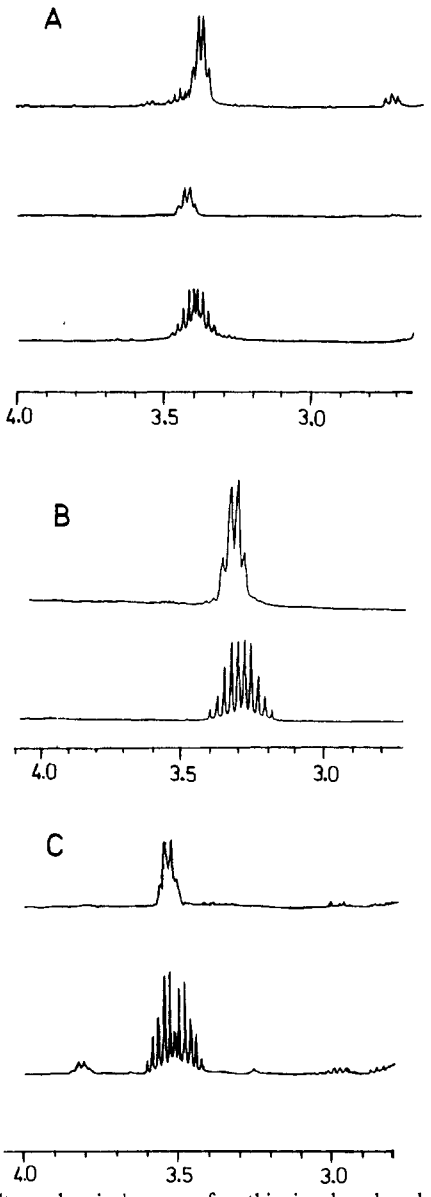

FIGURE 3: Stereochemical course of methionine decarboxylase reaction with (A) methionine, (B) norleucine, and (C) *S*-ethylcysteine as substrate. The lower trace of each set of ^{1}H NMR spectra corresponds to the (−)-camphanoyl derivative of the amine produced from the incubation of the amino acid with the enzyme in protium oxide. The middle trace of (A) and the upper trace of each pair from (B) and (C) is for the derivative of the amine produced upon the incubation of the amino acid with the enzyme in deuterium oxide. The upper trace of set A is for the derivative of the amine produced upon the incubation of (2*S*)-[2-^{2}H]methionine with the enzyme in protium oxide. (See Materials and Methods for details.)

When (2*S*)-methionine was incubated with the enzyme in 50 molar % deuterium oxide in water at pL 6.0 and 7.0, the isolated amine products each contained 48% ± 5% deuterium in the *pro*-1*R* position, indicating that a monoprotic acid acts as a proton donor during the decarboxylation process.

Discussion

L-Methionine decarboxylase from *Streptomyces* species ATCC 21020 is a PLP-dependent homodimeric protein and in this respect is similar to the eukaryotic enzyme from *D. filix-mas*. The molecular weights of the proteins are very similar, and both enzymes catalyze the decarboxylation of a range of hydrophobic amino acids in addition to the physiological substrate, (2*S*)-methionine. The substrate specificities for the two enzymes are different, and the fern enzyme is capable of decarboxylating substrates possessing larger substituents at C-3. For example, (2*S*)-valine is a good substrate for the fern enzyme but barely reacts with the *Streptomyces* enzyme, as shown in Table II. Both enzymes catalyze decarboxylation with retention of configuration at C-2 of the substrate, in accord with the reported stereochemical courses for all other PLP-dependent L-α-amino acid decarboxylases that have been examined (Floss & Vederas, 1982; Gani, 1985; Stevenson et al., 1986). Furthermore, a monoprotic conjugate acid appears to be responsible for the protonation of the quinoid intermediate at C$^\alpha$, also in accord with the results of other studies (Yamada & O'Leary, 1977). The imidazolium side chain of an active-site histidine residue might fulfill this role [see Akhtar et al. (1990)].

Table III: Comparison of Properties of Methionine Decarboxylase Enzymes

	fern, *Dryopteris filix-mas*	*Streptomyces* species
protein M_r	100 000	100 000
subunit M_r	57 000	59 000
	homodimeric	homodimeric
coenzyme	PLP	PLP
pH optimum with 20 mM L-methionine as substrate	5.0	6.9
k_{cat} (s^{-1}) at optimum pH for V_{max} (L-methionine as substrate)	100	120
substrates[a]	methionine	methionine
	S-ethylcysteine	*S*-ethylcysteine
	norvaline	norvaline
	leucine	leucine
	isoleucine	isoleucine
	norleucine	norleucine
	valine	ethionine
stereochemical course of decarboxylation	retention	retention
conjugate acid type	monoprotic	monoprotic
abortive reaction	yes	no

[a] See Table II.

The pH dependence of the kinetic parameters for methionine is different for the two enzymes. However, these differences are slight and may be completely accounted for by a single change in the pK_a value of one active-site functionality, vide infra. This change in ionization is reflected upon V_{max} and titrates at approximately pH 8.5 for the prokaryotic enzyme instead of pH 5.3 (Stevenson et al., 1990). The pH dependencies of the K_m values for methionine are essentially identical for the two enzymes, and therefore, the pH dependence of V/K is quite different. Below pH 5.0, where the V_{max} for both enzymes is maximal and essentially pH independent, the value of k_{cat} for the *Streptomyces* enzyme is 60 s^{-1}, whereas the value for the fern enzyme is 50 s^{-1} (Stevenson et al., 1990). Table III summarizes the properties of the two enzymes.

Major differences between the two enzymes also exist. While the fern enzyme catalyzes transamination reactions with a range of substrates in acidic or neutral solution, the *Streptomyces* enzyme does not. Like the fern enzyme, the *Streptomyces* enzyme is unable to catalyze the exchange of hydrogen from the C-1 position of the product amine, 3-(methylthio)-1-aminopropane, with solvent hydrogen. Note that it is not possible to exclude the occurrence of *actual* enzyme-catalyzed proton removal from the product aldimine when the conjugate acid of the active-site base is monoprotic and inaccessible to solvent, since these events are invisible (Akhtar et al., 1990).

The difference in the ability of the enzymes to partake in transamination reactions is of particular interest for several reasons. One could question whether the *Streptomyces* enzyme's inability to effect transamination of the coenzyme

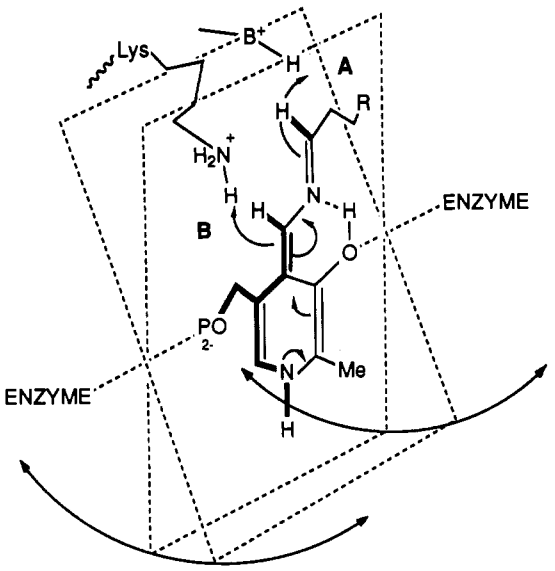

FIGURE 4: Possible pivoting about the 3′-*O*-phenolic and 5′-hydroxymethyl positions of the quinoid intermediate due to weak (or nonexistent) interaction between the pyridine heteroatom and a protein anchor. Such motion may provide a mechanism for abortive transamination in some decarboxylases.

reflects the operation of a more perfect catalyst, i.e., one that does not become involved in side reactions, or whether the abortive transamination reaction observed for the fern enzyme reflects a physiological requirement to be able to turn off production of the amine when high concentrations accumulate. The latter scenario now seems unlikely to be correct, given that extremely high concentrations of the product amine are required to facilitate decarboxylase-mediated coenzyme transamination (Akhtar et al., 1990). Nevertheless, whatever the reasons, the question of fundamental importance is what is the difference in the active-site structures of the two enzymes?

The most obvious differences between the two enzymes are the values of the titrations for V_{max} and the inability of the *Streptomyces* enzyme to catalyze abortive transamination. Since these two properties might be related, it is useful to consider why V_{max} titrates at different pH values for the two enzymes.

Given that transaldimination and C–C bond cleavage are both partially rate limiting for both enzymes [Akhtar et al., 1990; also see Abell and O'Leary (1988a,b)] and that similar functional groups are available at the active site of each enzyme, the decrease in V_{max} at high pH for both enzymes may result from the deprotonation of the pyridinium N-atom of the coenzyme. Deprotonation will reduce the ability of the coenzyme to act as an electron sink, thus retarding C–C bond cleavage, and will also reduce the electrophilicity at C-4′, thereby slowing both transaldimination processes. Indeed, there is good evidence to suggest that the pyridinium N-atom titrates at ~pH 5.3 for the fern enzyme (Akhtar et al., 1990) and slightly above 5.7 for *E. coli* glutamate decarboxylase (Fonda, 1972; O'Leary et al., 1981). Tobias et al. have recently shown that, in free solution at 25 °C and at an ionic strength (μ) of 1.0, the pK_a values for the deprotonation of the pyridinium N-atom of a range of PLP-α-amino acid Schiff bases are ~6.0 (Tobias et al., 1984). Thus, the values of the titrations derived from kinetic data are close to those expected for the methionine and glutamic acid PLP-aldimines.

While it is accepted that molecular acid dissoication constants can be perturbed from their free solution values by the local environment of the active site, it is difficult to imagine why the coenzyme in the *Streptomyces* enzyme should titrate at ~3 pK_a units higher than in the fern enzyme if the active-site structures are indeed similar. Nevertheless, other examples of PLP-dependent enzymes that possess coenzyme aldimines for which N-1 deprotonation occurs at high pH are known. For instance, at pH 8.3 it is thought that the internal aldimines of both cytosolic and mitochondrial AAT exist in dipolar ionic forms in which the pyridine ring is protonated (Kallen et al., 1985). These species absorb at 363 and 354 nm, respectively, close to the expected absorption maximum value of 357 nm derived from studies of *N*-methyl-PLP (Chen, 1981). The pH profiles for these transaminase enzymes indicate that V_{max} is constant in the region 5.6–9.8 (Velick & Vavra, 1962; Kiick & Cook, 1983; Martinez-Carrion et al., 1975), and since the coenzyme must be protonated in the active forms, the *external aldimines* must possess pK_a values greater than 9.8. For both cytosolic and mitochondrial AAT, the protonated pyridinium heteroatom forms a hydrogen bond with the β-carboxylate group of aspartate residue 222 (Kirsch et al., 1984), and presumably this interaction accounts for the high pK_a value of N-1. A similar hydrogen-bonding interaction may account for the high pK_a value derived from the titration of V_{max} for the *Streptomyces* enzyme, since such an interaction would decrease the acidity at N-1 of the coenzyme.

The above analysis suggests that the fern enzyme does not contain a negatively charged functional group, such as the ω-carboxylate group of an aspartate or glutamate residue, that can form hydrogen bonds to the pyridinium heteroatom of the coenzyme but that the *Streptomyces* enzyme does. Whether the lack of a strong interaction between the fern protein and the lower part of the coenzyme leads to pivoting about the 3′-*O*-phenolic and 5′-hydroxymethyl positions of the quinoid intermediate and, consequently, increased motion in the region of the proton donor(s) remains to be determined. Clearly, any such differential flexibility in this region of the active site could account for the differences in the ability of the enzymes to effect abortive transamination of the coenzyme, as illustrated in Figure 4.

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SUPPLEMENTARY MATERIAL AVAILABLE

Three figures showing the elution profile of L-methionine decarboxylase from (1) DEAE-Sephacel, (2) TSK DEAE-5PW, and (3) TSK G3000 SWG (3 pages). Ordering information is given on any current masthead page.

Registry No. L-Methionine decarboxylase, 37290-50-9; (2*S*)-methionine, 63-68-3.

REFERENCES

Abell, L. M., & O'Leary, M. H. (1988a) *Biochemistry 27*, 3325–3330.

Abell, L. M., & O'Leary, M. H. (1988b) *Biochemistry 27*, 5927–5933.

Akhtar, M., Stevenson, D. E., & Gani, D. (1990) *Biochemistry* (second paper of three in this issue).

Chen, V. J. (1981) Ph.D. Dissertation, Iowa State University, Ames, IA.

Eisenthal, R., & Cornish-Bowden, A. (1974) *Biochem. J. 139*, 715–720.

Floss, H. G., & Vederas, J. C. (1982) in *Stereochemistry* (Tamm, C., Ed.) p 161 Elsevier, Amsterdam, The Netherlands.

Fonda, M. L. (1972) *Biochemistry 11*, 1304-1309.
Gani, D. (1985) *Annu. Rep. Prog. Chem., Sect. B 82*, 287-310.
Green, A. A., & Hughes, H. L. (1955) *Methods Enzymol. 1*, 67-70.
Hagino, H., & Nakayoma, K. (1968) *Agric. Biol. Chem. 32*, 727-733.
Kallen, R. G., Korpela, T., Martell, A. E., Matsushima, Y., Metzler, C. M., Metzler, D. E., Morozov, Y. V., Ralston, I. M., Savin, F. A., Torchinsky, Y. M., & Ueno, H. (1985) in *Transaminases* (Christen, P., & Metzler, D. E., Eds.) p 37, Wiley-Interscience, New York.
Kiick, D. M., & Cook, P. F. (1983) *Biochemistry 22*, 375-382.
Kirsch, J. F., Eichele, G., Ford, G. C., Vincent, M. G., Jansonius, J. N., Gehring, H., & Christen, P. (1984) *J. Mol. Biol. 174*, 497-525.
Laemmli, U. K. (1970) *Nature (London) 227*, 680-685.
Martinez-Carrion, M., Cheng, S., Stankewicz, M. J., & Relimpio, A. (1975) in *Isoenzymes* (Markers, C. L., Ed.) Vol. II, p 567, Academic Press, New York.
Misono, H., Kawabata, Y., Toyosato, M., Yamamoto, T., & Soda, K. (1980) *Bull. Inst. Chem. Res. Kyoto Univ. 58*, 323-333.
O'Leary, M. H., Yamada, H., & Yapp, C. J. (1981) *Biochemistry 20*, 1476-1481.
Schowen, K. B. J. (1978) in *Transition States in Biochemical Processes* (Gandour, R. D., & Schowen, R. L., Eds.) p 225, Plenum Press, New York.
Spector, T. (1978) *Anal. Biochem. 86*, 142-146.
Stevenson, D. E., Akhtar, M., & Gani, D. (1986) *Tetrahedron Lett. 27*, 5661-5664.
Stevenson, D. E., Akhtar, M., & Gani, D. (1990) *Biochemistry* (first paper of three in this issue).
Tobias, P. S., Cermosky, D., & Kallen, R. G. (1984) as cited in Kallen et al. (1985).
Velick, S. F., & Vavra, J. (1962) *J. Biol. Chem. 237*, 2109-2122.
Yamada, H., & O'Leary, M. H. (1977) *J. Am. Chem. Soc. 99*, 1660-1661.

Inhibition of RecA Protein Promoted ATP Hydrolysis. 1. ATPγS and ADP Are Antagonistic Inhibitors†

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ABSTRACT: ADP and adenosine 5′-*O*-(3-thiotriphosphate) (ATPγS) inhibit recA protein promoted ATP hydrolysis by fundamentally different mechanisms. In both cases, at least two modes of inhibition are observed. For ADP, the first mode is competitive inhibition. The second mode is manifested by dissociation of recA protein from DNA. These are readily distinguished in a comparison of ATP hydrolyses that are activated by (a) DNA and (b) high (≈2 M) salt concentrations. Competitive inhibition with a significant degree of cooperativity is observed under both sets of conditions, although the DNA-dependent activity is more sensitive to ADP than the high-salt reaction. The reaction in the presence of poly(deoxythymidylic acid) or duplex DNA ceases when about 60% of the available ATP is hydrolyzed, reflecting an ADP-mediated dissociation of recA protein from the DNA that is governed by the ADP/ATP ratio. In contrast, ATP hydrolysis proceeds nearly to completion at high salt concentrations. At high concentrations of ATP and ATPγS, ATPγS also acts as a competitive inhibitor. At low concentrations of ATPγS and ATP, however, ATPγS activates ATP hydrolysis. These patterns are observed for recA-mediated ATP hydrolysis with either high salt concentrations or a poly(deoxythymidylic acid) [poly(dT)] cofactor, although the activation is observed at much lower ATP and ATPγS concentrations when poly(dT) is used. ATPγS can also relieve the inhibitory effect of ADP under some conditions. ATPγS and ADP are antagonistic inhibitors, reinforcing the idea that they stabilize different conformations of the protein and suggesting that these conformations are mutually exclusive. The ATPγS (ATP) conformation is active in ATP hydrolysis. The ADP conformation is inactive.

The recA protein of *Escherichia coli* promotes DNA strand exchange reactions in vitro that mimic key steps in homologous genetic recombination in vivo [for reviews, see Cox and Lehman (1987) and Radding (1988)]. The active species in this reaction is a nucleoprotein filament of recA protein containing one recA monomer for every three nucleotides or base pairs of DNA. The DNA in this filament is held in an extended (5.1 Å per base pair) and underwound (18 bp/turn) conformation in the presence of ATP or adenosine 5′-*O*-(3-thiotriphosphate) (ATPγS).[1] RecA protein has a DNA-dependent ATPase activity producing ADP and P_i (Roberts et al., 1978; Weinstock et al., 1981a). ATP hydrolysis is required in this system for DNA strand exchange (Cox & Lehman, 1981). The precise molecular role of ATP hydrolysis is unclear. RecA

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[1] Abbreviations: ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB, single-stranded binding protein; EDTA, ethylenediaminetetraacetate; DTT, 1,4-dithiothreitol; PEP, phosphoenolpyruvate; etheno-ssDNA, modified ssDNA containing 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine; poly(dT), poly(deoxythymidylic acid); FI, supercoiled closed circular form of a DNA molecule as isolated from *E. coli* cells; FII, nicked circular form of the same DNA molecule; FIII, linear form of the same DNA molecule; FX, closed circular form of the same DNA molecule but underwound by approximately 40%; PEI, poly(ethylenimine); AMP-PNP, adenylyl β,γ-imidotriphosphate.

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