Purification and Properties of NADP-dependent Glutamate Dehydrogenase from *Streptomyces fradiae*

By IVANA VANČUROVÁ,\textsuperscript{1,*†} ALEŠ VANČURA,\textsuperscript{2} JINDŘICH VOLC,\textsuperscript{1} JAN KOPECKÝ,\textsuperscript{2} JIRÍ NEUŽIL,\textsuperscript{1} GABRIELA BASAROVÁ\textsuperscript{2} and VLADISLAV BĚHAL\textsuperscript{1}

\textsuperscript{1}Institute of Microbiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia
\textsuperscript{2}Prague Institute of Chemical Technology, Prague, Czechoslovakia

(Received 26 June 1989; revised 21 August 1989; accepted 4 September 1989)

*Streptomyces fradiae* has two chromatographically distinct forms of glutamate dehydrogenase (GDH): one GDH utilizes NAD as coenzyme, the other uses NADP. The intracellular level of both GDHs is strongly regulated by the nitrogen source in the growth medium. NADP-dependent GDH was purified to homogeneity from crude extracts of *S. fradiae*. The *M*, of the native enzyme was determined to be 200,000 by size-exclusion high-performance liquid chromatography whereas after sodium dodecyl sulphate-polyacrylamide gel electrophoresis one major band of *M*, 49,000 was found, suggesting that the enzyme is a tetramer. The enzyme was highly specific for the substrates 2-oxoglutarate and L-glutamate, and required NADP, which could not be replaced by NAD, as a cofactor. The pH optimum was 9.2 for oxidative deamination of glutamate and 8.4 for reductive amination of 2-oxoglutarate. The Michaelis constants (*K*ₘ) were 28.6 mM for L-glutamate and 0.12 mM for NADP. *K*ₘ values for reductive amination were 1.54 mM for 2-oxoglutarate, 0.07 mM for NADPH and 30.8 mM for NH₃. The enzyme activity was significantly reduced by adenine nucleotides, particularly ATP.

**INTRODUCTION**

Amino acid dehydrogenases have been isolated from a wide variety of microbial sources. In streptomycetes, these enzymes have been studied with regard to their significance in synthesis of some antibiotics (Ômura et al., 1983), and in connection with branched-chain fatty acid and amino acid synthesis (Vančura et al., 1987, 1989a) and ammonium ion utilization (Aharonowitz & Friedrich, 1980; Braña et al., 1986). Valine dehydrogenase (VDH; EC 1.4.1.8) from *Streptomyces aureofaciens* (Vančurová et al., 1988a), *S. fradiae* (Vančura et al., 1988a), *S. coelicolor* (Navarrete et al., 1989) and *S. cinnamonensis* (Priestly & Robinson, 1989) and alanine dehydrogenase (ADH; EC 1.4.1.1) from *S. phaeochromogenes* (Itoh & Morikawa, 1983), *S. aureofaciens* (Vančurová et al., 1988b) and *S. fradiae* (Vančura et al., 1989b) have been isolated and characterized.

Glutamate dehydrogenases [GDH; L-glutamate : NAD(P) oxidoreductase; EC 1.4.1.2–4] catalyse the reversible reductive amination of 2-oxoglutarate to glutamate and thus play an important role in the assimilation of ammonia and catabolism of glutamate. There are at least three types of glutamate dehydrogenases, which differ in their coenzyme specificity: those specific for either NAD or NADP and those that can use both coenzymes. A catabolic role has

\* Present address: Institute of Microbiology, Czechoslovak Academy of Sciences, Department of Biogenesis of Natural Substances, Videfská 1083, 142 20 Prague 4, Czechoslovakia.

**Abbreviations**: ADH, alanine dehydrogenase; BSA, bovine serum albumin; FPLC, fast protein liquid chromatography; GDH, glutamate dehydrogenase; VDH, valine dehydrogenase.
been assigned to the NAD-dependent GDH (EC 1.4.1.2) while the NADP-specific enzyme (EC 1.4.1.4) is thought to participate in glutamate biosynthesis (Ferguson & Sims, 1971). Most bacteria, however, are thought to contain only one species of GDH. Moreover, some members of the genera *Bacillus* and *Streptomyces* have been reported to lack GDH activity (Phibbs & Bernlohr, 1971; Aharonowitz & Friedrich, 1980; Vančurová et al., 1988c).

In the genus *Streptomyces*, NAD-dependent GDH from *S. noursei* (Gráf et al., 1977) and NAD-dependent GDH from *S. venezuelae* (Shapiro & Vining, 1983) have been described. However, none of these enzymes has been purified so far, and their molecular structure and properties are not well established. Here we report the presence of two GDH activities in *S. fradiae*, a bacterium that produces the oligoketide antibiotic tylosin, and describe the purification procedure and some properties of the NADP-specific enzyme.

### METHODS

**Materials.** NAD, NADP and NADPH were obtained from Reanal, Budapest, Hungary. Tris, Bistris, amino acids, 2-oxo acids and NADPH were purchased from Sigma. DEAE 52-Cellulose was from Serva and the Mono Q HR 5/5 prepacked fast protein liquid chromatography (FPLC) column from Pharmacia. All other chemicals were of the highest purity available.

**Micro-organism and growth conditions.** *Streptomyces fradiae* 30/3 was from the collection of the Research Institute of Biofactors and Veterinary Drugs in Kouim near Prague (Vančurová et al., 1988a).

The composition of the sucrose/mineral salts medium used for the study of GDH regulation, and other cultivation conditions, were as described previously (Vančurová et al., 1988b). Nitrogen sources were sterilized separately and used at the concentrations indicated in the text. The complex medium described by Vančurová et al. (1988a) was used for the isolation of GDH.

**Enzyme and protein assay.** The ammonium-assimilating activity of GDH was measured as the decrease of NAD(P)H absorbance at 340 nm. The reaction mixture (1 ml) contained 0.1 M-Tris/HCl buffer, 10 mM-sodium 2-oxoglutarate, 0.2 mM-NAD(P)H and 0.1 mM-NH$_4$Cl. The assay was done at pH 8.4 and 30 °C.

The oxidative deamination activity of GDH was measured as the increase of NAD(P)H absorbance at 340 nm. The reaction mixture (1 ml) contained 0.1 M-glycine/KCl/KOH buffer, 50 mM-sodium L-glutamate and 0.5 mM-NAD(P); the final pH was 9.2, temperature 30°C. One enzyme activity unit (U) was defined as the amount required to convert 1 nmol substrate min$^{-1}$. Unless otherwise stated, the enzyme activity was measured in the oxidative deaminating system with 50 mM-L-glutamate.

The $K_m$ values of the purified NADP-GDH in the reductive amination system were obtained under standard assay conditions by means of double-reciprocal plots of reaction velocity versus substrate concentration. In the oxidative deamination system the $K_m$ values for L-glutamate and NADP were obtained from secondary plots of intercepts versus reciprocal concentrations of the substrate. NADP was used as a variable substrate in the presence of several fixed concentrations of L-glutamate. The lines were calculated on the basis of eight points by the method of least squares. For $K_m$ determination, concentrations of individual substrates were changed over these ranges: L-glutamate, 0.20 to 85 mM; NADP, 0.02 to 0.5 mM; 2-oxoglutarate, 0.2 to 10 mM; NADPH, 0.01 to 0.2 mM; NH$_4$Cl, 5 to 100 mM. While changing concentration of the substrate in question, the concentrations of the other substrates in the reductive amination system were maintained at saturation level (maximal value of the tested concentration range).

Protein was determined by the absorbance method of Whitaker & Granum (1980) and by the Lowry method with bovine serum albumin (BSA) as standard. All spectrophotometric measurements were made with a Cary 118 C (Varian) spectrophotometer.

**Enzyme purification.** Step 1. Preparation of crude extract. A 72 h mycelium grown in complex medium was separated from the fermentation broth by centrifugation (4000 g, 4 °C, 15 min), washed with ice-cold distilled water and centrifuged again (20000 g, 4 °C, 30 min). The mycelium was disintegrated in a Biox X-Press at −25 °C and a pressure of 300 MPa. Broken mycelium (wet weight, 30 g) was suspended in 40 ml 0.01 M-Tris/HCl buffer, pH 7.4 (buffer A), and after 40 min the homogenate was centrifuged (22000 g, 4 °C, 40 min).

Step 2. Ion-exchange chromatography on DEAE-cellulose. The cell extract (55 ml) was applied to a DEAE-cellulose bed (2.6 by 34 cm) pre-equilibrated with buffer A. After the column had been washed with the same buffer (300 ml), the adsorbed material was eluted with a linear gradient (330 ml) of 0 to 1.5 M-KCl in buffer A at a flow rate of 22 ml h$^{-1}$, and 5 ml fractions were collected. The operations were performed at 4 °C.

Step 3. First ion-exchange chromatography on Mono Q HR 5/5 FPLC column. Fractions containing NADP-GDH activity were pooled (30 ml) and transferred into buffer B (0.02 M-Bistris/HCl buffer, pH 6.3) in an Amicon model 52 UF cell, membrane PM-10. Four 10 ml samples (about 10 mg protein each) were each applied via a 10 ml Superloop to a Mono Q HR 5/5 column equilibrated with buffer B. After the column had been washed with the
same buffer (10 ml), the elution was continued with a linear gradient (22 ml) of 0 to 40% buffer C (1 M-NaCl in buffer B). Fractions of 0.8 ml were collected at a flow rate of 1 ml min⁻¹; temperature was 20 °C.

Step 4. Second ion-exchange chromatography on Mono Q HR 5/5 column. The active fractions from step 3 were combined (2.4 ml), desalted on a Pharmacia PD-10 column and subjected to repeated chromatography on a Mono Q column as for step 3 except that a linear gradient (12 ml) of 5 to 30% buffer C was used. The adsorbed material was eluted with a flow rate of 1 ml min⁻¹ and 0.5 ml fractions were collected.

Analytical methods. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was done as described previously (Vančurová et al., 1988a). The M₉ of the enzyme subunits was estimated with BSA (M₉ 67000), egg albumin (45000), chymotrypsinogen A (25000) and lysozyme from chicken egg white (144000) as standard proteins (Serva).

Size-exclusion high-performance liquid chromatography (HPLC) of the purified NADP-GDH was performed with 0.1 M-sodium phosphate buffer, pH 7.0, on a TSK G 3000 SW column (7.5 by 300 mm) at a flow rate of 0.5 ml min⁻¹. M₉ standards (Kit MS II, Serva) were used to calibrate the column.

Growth was determined gravimetrically.

RESULTS

Occurrence of GDH activities in S. fradiae under different cultivation conditions

Two GDH activities, NAD-specific and NADP-specific, were detected in cell-free extracts of 72 h cultures of S. fradiae 30/3 grown in minimal synthetic medium with various nitrogen sources (Table 1). Maximal specific activity of both GDHs was found in medium with 25 mM-NH₄⁺ as the nitrogen source. Increasing the concentration of NH₄⁺ or the use of amino acids (glutamate, glutamine, alanine or aspartate) as the nitrogen source led to a decrease in the specific activity of both NAD- and NADP-dependent GDH.

The GDH activities specific for NAD and NADP detected in the cell-free extracts of S. fradiae differed markedly in their Kₘ values for L-glutamate and NH₄⁺. The Kₘ values of NAD-GDH, determined in the crude extract, for L-glutamate and NH₄⁺ were 1.5 ± 0.1 mM and 90 ± 3 mM, respectively, while in the case of NADP-GDH they were 29 ± 1 mM and 31 ± 1 mM, respectively (means ± se; n = 4).

Purification of NADP-GDH

NADP-specific GDH was isolated from a 72 h culture of S. fradiae 30/3 cultivated in complex medium. Previous work has shown that GDH of S. fradiae cannot be efficiently purified by means of hydrophobic interaction chromatography, which was used for the isolation of VDH and ADH (Vančura et al., 1988a, 1989b). Therefore, ion-exchange chromatography on a DEAE-cellulose column was employed as a first purification step. During chromatography on DEAE-cellulose VDH, ADH, NAD-GDH and NADP-GDH were eluted together by using 0.8 M-KCl. The dehydrogenases were separated later during FPLC on a Mono Q column. The purified NADP-GDH fraction did not contain either NAD-associated GDH activity or VDH and ADH activities. The NADP-GDH was purified 560-fold with a 32% recovery (Table 2); the purification was repeated three times.

Purified GDH was homogeneous according to SDS-PAGE and size-exclusion HPLC on a TSK G 3000 SW column. The fraction corresponding to the only peak after HPLC exhibited NADP-associated GDH activity.

The purified GDH did not lose activity when stored at 4 °C in 0.2 M-Tris/HCl buffer, pH 7.4, for 24 h or at −25 °C in the same buffer for two months.

M₉ and subunit structure

The M₉ of native NADP-GDH was determined to be 200 000 ± 5000 by size-exclusion HPLC. The M₉ of the denatured GDH as determined by SDS-PAGE was approximately 49 000 (Fig. 1). Thus, NADP-GDH of S. fradiae appears to be a tetrameric enzyme.

pH optima and temperature characteristics

NADP-GDH of S. fradiae exhibited maximum activity at pH 8.4 for the reductive amination of 2-oxoglutarate in the presence of 0.2 M-Tris/HCl buffer. The pH optimum for the oxidative deamination of L-glutamate in 0.2 M glycine/KCl/KOH buffer was 9.2.
Table 1. Specific activities of GDHs from S. fradiae cultivated with different sources of nitrogen

<table>
<thead>
<tr>
<th>Nitrogen source</th>
<th>Concn (mM)</th>
<th>NAD-</th>
<th>NADP-</th>
<th>GDH activity* [U (mg protein)^-1]</th>
<th>Ratio NADP-GDH/ NAD-GDH</th>
<th>Doubling time (h)</th>
<th>Maximum growth (mg ml^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH4+</td>
<td>25</td>
<td>6.98</td>
<td>8.57</td>
<td>1.2</td>
<td>26.4</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>NH4+</td>
<td>100</td>
<td>1.75</td>
<td>5.87</td>
<td>3.4</td>
<td>13.4</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>25</td>
<td>1.43</td>
<td>7.30</td>
<td>3.1</td>
<td>15.1</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>25</td>
<td>2.38</td>
<td>6.92</td>
<td>3.1</td>
<td>18.3</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>25</td>
<td>3.55</td>
<td>6.75</td>
<td>3.1</td>
<td>18.7</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>25</td>
<td>1.87</td>
<td>6.35</td>
<td>3.1</td>
<td>19.1</td>
<td>6.1</td>
<td></td>
</tr>
</tbody>
</table>

* A 72 h culture. The data represents the means of three experiments. Standard deviations were all within 10% of the mean values.

Table 2. Purification of NADP-GDH

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein (mg)</th>
<th>Total activity (U)</th>
<th>Specific activity (U mg^-1)</th>
<th>Purification (-fold)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>350.0</td>
<td>1020</td>
<td>3</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>43.4</td>
<td>912</td>
<td>21</td>
<td>7</td>
<td>87</td>
</tr>
<tr>
<td>1. Mono Q</td>
<td>3.8</td>
<td>768</td>
<td>204</td>
<td>68</td>
<td>73</td>
</tr>
<tr>
<td>2. Mono Q</td>
<td>0.2</td>
<td>336</td>
<td>1680*</td>
<td>560</td>
<td>32</td>
</tr>
</tbody>
</table>

* The specific activity of NADP-GDH of 1680 U (mg protein)^-1, obtained in the oxidative deamination system, corresponds to 10920 U (mg protein)^-1 in the reductive amination system.

At the optimum pH of individual reactions and saturating concentrations of all substrates (maximal concentrations of tested concentration ranges as given in Methods), the amination rate was 6.5 times higher than the deamination rate.

The optimum temperature of NADP-GDH activity under standard conditions was 60°C, for both reductive amination and oxidative deamination.

Substrate and coenzyme specificity

Among the amino acids tested, only L-glutamate was a substrate for the oxidative deamination reaction of NADP-GDH, none of the following amino acids giving a detectable reaction: L-alanine, L-valine, L-isoleucine, L-leucine, D-glutamate, glycine, L-threonine, L-serine, L-cysteine, L-methionine, L-aspartate, L-glutamine and L-asparagine.

Similarly, in the reductive amination system absolute substrate specificity for 2-oxoglutarate was found: NADP was not produced with pyruvate, oxaloacetate, 2-oxobutyrate, 2-oxoisovalerate, 2-oxoisocaproate or 2-oxo-3-methyl-n-valerate.

No NADPH oxidation was observed when ammonia was replaced by glutamine or NH2OH even if a high concentration of the purified enzyme was used in the reaction mixture. This proves that the purified NADP-GDH was not contaminated by glutamate synthase. NADP-GDH exhibited no activity when NADPH and NADP were substituted for NADH and NAD, respectively.

The Km values of purified NADP-GDH of S. fradiae were obtained as described in Methods. Typical Michaelis–Menten kinetics was found for all substrates. The Km value for L-glutamate was 28.6 ± 0.8 mM and for NADP 0.12 ± 0.005 mM. For reductive amination the Km value for 2-oxoglutarate was 1.54 ± 0.05 mM, for NADPH 0.07 ± 0.002 mM and for NH2 30.8 mM ± 0.9 mM (means ± SE, n = 4).
Glutamate dehydrogenase of S. fradiae

Fig. 1. Densitometric evaluation of SDS-PAGE of NADP-dependent GDH. Trace 1 shows colour densities of protein bands (stained with Coomassie Blue R-250) from crude cell-free extract sample. Trace 2, purified enzyme. Trace 3, marker proteins (left to right): BSA (M, 67000), egg albumin (45000), chymotrypsinogen A (25000) and lysozyme from chicken egg white (14400).

Table 3. Effect of nucleotides on NADP-GDH activity

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Conc (mM)</th>
<th>Oxidative deamination</th>
<th>Reductive amination</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>AMP</td>
<td>1·0</td>
<td>100</td>
<td>96·6</td>
</tr>
<tr>
<td></td>
<td>2·0</td>
<td>95·4</td>
<td>89·3</td>
</tr>
<tr>
<td>ADP</td>
<td>0·5</td>
<td>78·4</td>
<td>87·5</td>
</tr>
<tr>
<td></td>
<td>1·0</td>
<td>43·2</td>
<td>77·2</td>
</tr>
<tr>
<td></td>
<td>2·0</td>
<td>23·0</td>
<td>69·4</td>
</tr>
<tr>
<td>ATP</td>
<td>0·5</td>
<td>52·5</td>
<td>97·8</td>
</tr>
<tr>
<td></td>
<td>1·0</td>
<td>0</td>
<td>90·3</td>
</tr>
<tr>
<td></td>
<td>2·0</td>
<td>0</td>
<td>78·2</td>
</tr>
</tbody>
</table>

* Relative activity of 100% corresponds to a specific activity of 1680 U (mg protein)⁻¹ in the oxidative deamination system and to 10920 U (mg protein)⁻¹ in the reductive amination system. The data represent the means of three experiments. Standard errors were within 5% of the mean values.

Effectors

NADP-dependent GDH was inhibited by AMP, ADP and ATP, in both the reductive amination and the oxidative deamination system (Table 3). The most pronounced effect was shown by ATP for the oxidative deamination reaction: it completely inhibited the enzyme...
activity. With ATP as the inhibitor, in both the oxidative deamination and the reductive amination system, the double-reciprocal plots of reaction velocity against NADP or NADPH concentration at saturating concentrations of other substrates (maximal concentrations of tested concentration ranges as given in Methods) showed competitive inhibition (data not shown).

The enzyme was also inhibited by inhibitors of -SH groups, 4-chloromercuribenzoate (0-01 mM) and HgCl₂ (0-01 mM), to 35% and 27% of the original enzyme activity, respectively. Metal ions influenced NADP-GDH activity only insignificantly. None of the following compounds at 1 mM exhibited a marked effect on NADP-GDH activity: adenine, adenosine, guanosine, cytosine, thymine, FAD, FMN, CoA, acetyl-CoA, thiamin pyrophosphate and EDTA.

DISCUSSION

Although secondary metabolism has been extensively studied in *Streptomyces*, very little is known about the enzymology of nitrogen metabolism in these bacteria. GDH activity has been detected in cell-free extracts of *S. noursei* (Gräfe et al., 1977), *S. venezuelae* (Shapiro & Vining, 1983) and more recently also in *S. coelicolor* (Fisher, 1988). The enzyme was not demonstrated in *S. clavuligerus* (Braña et al., 1986) or *S. aureofaciens* (Vančurová et al., 1988c).

In the present study, two GDH activities were detected in *S. fradiae* cell-free extracts: NAD-specific and NADP-specific. The two GDH activities were associated with two distinct protein fractions which differed in their chromatographic behaviour and also in their *Kₚ* values for NH₄⁺ and glutamate. The existence of two separate proteins with NAD- and NADP-linked GDH activities is also suggested by the fact that the purified NADP-GDH of *S. fradiae* did not exhibit any NAD-linked activity. These data are similar to those reported for *Thiobacillus novellus* (Le' John et al., 1968), *Hydrogenomonas* H16 (Kramer, 1970), *Micrococcus aerogenes* (Kew & Woolfolk, 1970), *Nitrobacter agilis* (Kumar & Nicholas, 1984) and *Halobacterium halobium* (Bonete et al., 1987), where NAD- and NADP-specific activities of GDH were also associated with two distinct proteins.

On the basis of the *Kₚ* values of the two GDHs of *S. fradiae*, determined in crude enzyme extract, one may presume a catabolic role of NAD-GDH and a biosynthetic function of NADP-GDH. However, this does not correlate with the ratio of NADP- and NAD-dependent activities of GDH present in mycelium grown in medium with glutamate as the nitrogen source. Whereas the NADP-GDH/NAD-GDH ratio was balanced in mycelium grown in medium with NH₄⁺ (25 or 100 mM) and or with 25 mM-aspartate, the NADP-dependent GDH activity was three times higher than the NAD-linked activity when the micro-organism was grown in medium with 25 mM-glutamate or glutamine. These results point to a biosynthetic function of NADP-GDH of *S. fradiae*, while the NAD-specific GDH appears to be capable of functioning in either direction, i.e. amination of 2-oxoglutarate to glutamate and deamination of glutamate to 2-oxoglutarate.

L-Amino acid dehydrogenases in streptomycetes described so far, and also GDHs from different microbial sources, vary greatly in their molecular structure and catalytic properties. Most of the GDHs isolated from micro-organisms are hexamers with a subunit *M*, of about 50 000. However, the NADP-dependent GDH of *S. fradiae* was found to be a tetramer with a subunit *M*, of 49 000. In this respect the enzyme resembles ADH of the same organism, which is composed of four subunits of *M*, 51 000 (Vančura et al., 1989b), and also NADP-dependent GDH of *Halobacterium* from the Dead Sea, which consists of four subunits of *M*, 53 500 (Leicht et al., 1978).

In comparison with the NADP-specific GDHs of *Bacillus licheniformis* (Phibbs & Bernlohr, 1971), *Salmonella typhimurium* (Coulton & Kapoor, 1972), *Halobacterium* from the Dead Sea (Leicht et al., 1978) and *Nitrobacter agilis* (Kumar & Nicholas, 1984), the NADP-dependent GDH from *S. fradiae* had relatively low affinity for ammonia. Variation in the specific activities of pure enzymes from different bacteria is also striking; from this point of view NADP-GDH of *S. fradiae* can be compared with the enzymes of *N. agilis* (Kumar & Nicholas, 1984) and *B. licheniformis* (Phibbs & Bernlohr, 1971). These findings may support the view that NADP-GDH does not serve as the main enzyme of ammonia assimilation in *S. fradiae*. A possible
physiological function of L-amino acid dehydrogenases in streptomyces might be to participate in the maintenance of the intracellular balance between amino and corresponding oxo acids, which is particularly important for the synthesis of oligoketide and β-lactam antibiotics.

We thank Professor C. R. Hutchinson and Professor J. A. Robinson for providing, prior to publication, the manuscripts of their papers describing valine dehydrogenases from S. coelicolor and S. cinnamonensis.

REFERENCES


