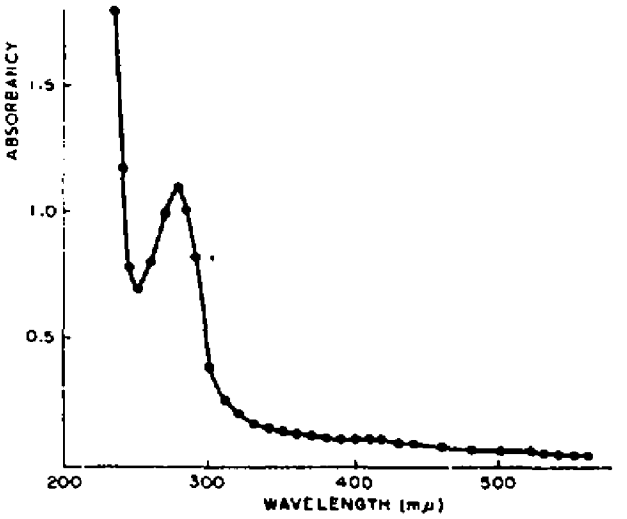

Fig. 1. Absorption spectrum of purified nitrate reductase from *A. fischeri*.

Ultracentrifugal studies indicated the presence of at least two components, a slow moving fraction with an $s_{20,w}$ value of about 1.5 and a faster-moving component with an $s_{20,w}$ value of about 4.5. Though it was not determined which of the two components represents the enzyme, these results indicate that nitrate reductase from *A. fischeri* is a much smaller molecule than the enzyme from *Escherichia coli*[3] which is reported to have a $s_{20,w}$ value of 25 corresponding to a molecular weight of about 1 000 000.

National Chemical Laboratory,
Poona (India)

J. C. SADANA*
R. RAMA RAO
M. D. JOSHI

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* On leave 1961–62. Present address: College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York 32, N.Y. (U.S.A.).

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γ, δ-Dioxovalerate aminotransferase activity in *Rhodopseudomonas spheroides*

It was previously reported[1] that L-α-alanine-γ,δ-dioxovalerate aminotransferase (transaminase) activity is present in *Rhodopseudomonas spheroides* extracts, and that in contrast to an enzyme detected in mammalian tissues[2–4], L-α-alanine and β-alanine are from 5–7 times more effective than L-glutamic acid. Transamination

between δ-aminolaevulic acid and pyruvate has been observed in cell-free extracts of *Corynebacterium diphtheriae*[5]. Details for the assay of enzyme activity in *Rps. spheroides* extracts by measuring δ-aminolaevulic acid formation, and the effect of growth conditions on activity, have been reported[6].

It is the purpose of this communication to report the partial purification of the transaminase, and present information on substrate specificity and some other properties of the enzyme.

Cell-free extracts of *Rps. spheroides* were prepared as previously described[6]. These extracts were treated by heating at 50° for 10 min, the addition of protamine sulphate to a concentration of 0.33% at 4°, and fractionation with $(NH_4)_2SO_4$ to 30% saturation at 25°. Material precipitated at each step was discarded. The precipitate obtained by 40% saturation of the supernatant with $(NH_4)_2SO_4$ was retained. Approx. 40% of the activity present in the original extracts was obtained in the 30–40% saturated fraction, which had a specific activity 10 times that of the cell-free extracts. All the above steps were carried out in 0.1 M KH_2PO_4-K_2HPO_4 buffer (pH 7). A further 2.5-fold purification was achieved by column chromatography on DEAE-cellulose. During concentration-gradient elution with 0.05–0.3 M phosphate buffer (pH 7) peak enzyme activity was recorded in fractions eluting from the column at a buffer concentration of approx. 0.17 M. Of the enzyme applied to columns, about 50% is recovered in the peak fractions. In a number of purifications, it has been found necessary to dialyse enzyme preparations against 1 mM β-mercaptoethanol, and this is now done routinely before activity assays are carried out. L-α-Alanine is used as the amino-group donor in such assays, and all measurements are made at the pH optimum, *i.e.* pH 7. The enzyme is stable for several months when stored at −15°.

Enzyme activity measured at a protein concentration of less than 0.5 mg/ml using crude cell-free extracts is not inhibited by bovine plasma albumin at a concentration of 7.5 mg/ml during pre-incubation with dioxovalerate for 22 min at 37°. At twice this albumin concentration inhibition was 13%. Using protein from enzymically inactive fractions obtained during column chromatography of enzyme preparations, no inhibition was caused by preincubation of dioxovalerate with 5 mg protein/ml.

The ability of β-alanine to replace α-alanine as the amino-group donor is lost during 10-fold purification of the transaminase. α-Amino-*n*-butyric, α-amino-isobutyric, β-amino-*n*-butyric, and β-amino-isobutyric acids are inactive. Serine, taurine, glutamine, asparagine, glutamic and aspartic acids, lysine and cysteine all have negligible activity. Glycine and ornithine have less than 10% of the activity of α-alanine. Cysteine causes 90% inhibition of activity when an equimolar amount is added simultaneously with the alanine substrate. Lysine and ornithine both cause approx. 20% inhibition under similar circumstances. The ω-amino monocarboxylic acids γ-amino-*n*-butyric, δ-amino-*n*-valeric, and ε-amino-*n*-caproic, transaminate at 122, 95, and 103% of the rate of α-alanine using 25-fold purified enzyme. Transaminase activities with alanine and the ω-amino acids referred to above are all inhibited approx. 20% by equimolar concentrations of lysine or ornithine. At a 5-fold diamino acid concentration, lysine inhibits in all cases by 60%, whereas inhibition by ornithine is only slightly increased. Equimolar amounts of cadaverine and β-alanine caused 14 and 8% inhibition, respectively.

Alanine-dioxovalerate transaminase appears to be a sulphydryl protein, and is inhibited by a number of reagents reacting with sulfydryl groups. Thus, iodoacetate and β-iodopropionate reduce activity by 30% when pre-incubated with enzyme at 10^{-3} M, for 30 min at 37°, before the addition of substrates. Under identical conditions, inhibition by iodoacetamide or chloroacetamide is negligible. *p*-Chloromercuribenzoate inhibits by 78% at $1.36 \cdot 10^{-5}$ M. At a concentration of 10^{-3} M, thioglycollate inhibits by 74%, and at 10^{-5} M by 21%. Glutathione or β-mercaptoethanol restore the activity of aged enzyme preparations optimally at 10^{-3} M.

Aminothiol inhibition has been observed with a number of pyridoxal phosphate-dependent enzymes[7-10], and alanine–dioxovalerate transamination is inhibited by cysteamine, penicillamine, homocysteine and cysteine. In the case of cysteamine and cysteine, activity is restored by stoicheiometric amounts of added pyridoxal phosphate. Inhibition by the carbonyl reagent isonicotinic acid hydrazide[11] is progressive with time of preincubation with enzyme, whereas inhibition by amino-oxyacetic acid[12] appears to be complete within 2 min when preincubated at a concentration of 10^{-5} M. The prosthetic group appears to be tightly bound to alanine-dioxovalerate apotransaminase, and added pyridoxal phosphate has no effect on activity.

Preliminary screening of dioxovalerate analogues has shown that inhibition is effected by a wide variety of α-oxocarboxylic acids, oxo-aldehydes, dicarboxylic acids and other dioxo compounds. Measurements were made using alanine at a concentration 30-fold greater than that of the dioxo substrate. The following compounds inhibit, by the percentage of control activity shown, when preincubated with enzyme for 10 min at a concentration equimolar to that of dioxovalerate: Glyoxylic acid, 86%; pyruvic acid, 56%; oxaloacetic acid, 21%; α-oxoglutarate, 5%; glyoxal, 18%; methylglyoxal, 91%; oxalic acid, 5%; succinic acid, 14%; diacetyl, 98%; acetylacetone, 32%; acetonylacetone, 31%; ethyl acetoacetate, 40%; benzoylacetone, 55%; and *o*-phthalaldehyde, 49%. Considerably greater inhibition occurs in all cases at higher relative concentrations of substrate analogue. The amount of monomer present in reagents known to undergo some degree of polymerisation has not been determined.

Substrate inhibition occurs when dioxovalerate concentrations exceed 1.3 mM, *i.e.* one-tenth the alanine concentration used in the standard activity assay procedure[6]. When the alanine concentrations are 0.04 and 0.12 M, the dioxovalerate concentrations at which inhibition becomes apparent are 1.7 and 2.0 mM, respectively. Using a range of substrate concentrations which avoid inhibition by excess, apparent Michaelis constants were obtained by the kinetic procedure of VELICK AND VAVRA[13]. For alanine and dioxovalerate, the K_m values were approx. 8.0 and 0.4 mM, respectively. The measurement of transamination using δ-aminolaevulate and pyruvate as substrates is hindered by the strongly inhibitory effect of pyruvate, but techniques are being developed to overcome this difficulty.

Department of Chemical Pathology,
St. Mary's Hospital Medical School,
London (Great Britain)

A. NEUBERGER
J. M. TURNER

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PN 10028 **Crystalline L-aspartate 4-carboxy-lyase**

L-aspartate 4-carboxy-lyase (E.C. 4.1.1.12), which catalyses the formation of α-alanine and CO_2 by the β-decarboxylation of L-aspartate, has been previously demonstrated to be present in a number of micro-organisms, including *Desulfovibrio desulfuricans*[1], *Nocardia globerula*[2] and *Clostridium perfringens*[3,4]. The enzyme has been partially purified from *Cl. perfringens* by NISHIMURA, MANNING AND MEISTER[4] and has been shown to contain firmly-bound pyridoxal 5'-phosphate; aspartate decarboxylation was stimulated by the further addition of pyridoxal 5'-phosphate and α-keto acids[1–4]. This communication describes the crystallization, and some of the properties, of L-aspartate 4-carboxy-lyase from *Achromobacter* sp.

Cultures of *Achromobacter* d-15 (ref. 5) were grown at 30° in 80 l of basal salts medium containing 25 mM-ammonium *d*(+)tartrate as sole source of carbon and nitrogen and harvested several hours after cessation of logarithmic growth. *Step I. Cell extract.* Cells (35 g dry wt.) were suspended in water to 580 ml and were disrupted by passage through a French Pressure Cell at 12 000 lb/in², followed by treatment for 5 min in an "M.S.E." ultrasonic disintegrator operating at 1.4 A. The extract thus obtained was centrifuged at 25 000 × *g* for 30 min, the precipitated material was discarded and the supernatant solution was diluted to 16 mg of protein/ml with potassium maleate (pH 5.0), L-aspartate and pyridoxal 5'-phosphate to final concentrations, respectively, of 50 mM, 10 mM and 0.1 mM; the pH was adjusted to 5.0 with 1 N acetic acid. *Step II. Heat treatment.* The solution was kept at 50° for 1 h, cooled, mixed with protamine sulphate ("ex-herring"; L. Light & Co.; 1 g/20 g of protein) and ammonium sulphate (to 30% saturation), and was centrifuged at 77 000 × *g* until the supernatant solution was clear (3–12 h). The solution was adjusted to pH 7.0 with 15 N NH_4OH. *Step III. Ammonium sulphate fractionation.* Ammonium sulphate was added to 50% saturation; the resultant precipitate, collected by centrifugation for 30 min at 15 000 × *g*, was discarded. Further addition of ammonium sulphate, to 68% saturation, yielded a precipitate which was collected by centrifugation and dissolved in 0.1 M sodium acetate (pH 5.0). *Step IV. pH Fractionation.* The dissolved precipitate was dialysed against 0.1 M sodium acetate (pH 7.0). Material precipitated in the dialysis tube was collected by centri-