Growth of *Pseudomonas reptilivora* on *N*-Methylglycines

By D. E. HALL,* I. A. SIMPSON and G. W. CROSBIE.
(Department of Biochemistry, University of Hull,
Hull HU6 7RX, U.K.)

Pseudomonas reptilivora (N.C. 8906) grows equally well aerobically on a simple inorganic salts medium containing glycine, sarcosine, *NN*-dimethylglycine or glycine betaine as sole carbon and energy source. Cell yields are linear with respect to substrate concentration in the suboptimum range 0–6 mM, the molar growth yields being 17, 35, 40 and 47 g dry wt. of cells/mol respectively.

Washed-cell suspensions rapidly oxidized the *N*-methylglycines without lag provided that the cells had been grown on the test substrate or on a higher methylated homologue. Low rates of O₂ uptake were observed under the following conditions: (a) when washed-cell suspensions of succinate-grown cells were exposed to glycine or its *N*-methyl derivatives; (b) when glycine-grown cells were exposed to the *N*-methyl compounds; (c) when cells grown on an *N*-methyl derivative were exposed to glycine.

Cell-free extracts of betaine- or dimethylglycine-grown cells possess high activities of very labile particulate dehydrogenases capable of yielding formaldehyde by an oxidative demethylation of dimethylglycine or sarcosine linked via phenazine methosulphate to 2,6-dichlorophenol-indophenol reduction. Extracts of sarcosine-grown cells possess only the sarcosine dehydrogenase activity. Glycine-grown cells and succinate-grown cells have no significant amounts of the dehydrogenases, the activities of which rise sharply during the lag period (1–3 h) that ensues when cells are transferred from succinate to betaine medium. Extracts of cells grown on betaine, dimethylglycine or sarcosine have serine dehydratase activities similar to those reported for glycine-grown cells, but possess no 'glycine enzyme' activity.

It is concluded that *Ps. reptilivora* utilizes the *N*-methylglycines by a pathway similar to that operating during growth on glycine (Hall & Crosbie, 1971), except that the 'glycine enzyme', which yields *N*⁵*N*¹⁰-methylenetetrahydropteroylglutamate from glycine, is completely repressed and that glycine and C₁ units at the oxidation level of formaldehyde are provided by (successive) demethylation of the growth substrate. These conclusions are supported by the observation that during growth on [*Me*-¹⁴C]sarcosine the *N*-methyl group provides upwards of 90% of the C₁ residues in thymine (methyl group), serine (C-3) and purines (C-2).

* Present address: Fisons Pharmaceuticals Ltd.,
Bakewell Road, Loughborough, Leics., U.K.

We thank Miss Joan Farr for skilled technical assistance.

Hall, D. E. & Crosbie, G. W. (1971). *Biochem. J.* **124**, 30P.

Specificity of 4-Aminoimidazole-5-carboxamide Ribotide Transformylase of *Escherichia coli*

By T. W. PATRICK and G. W. CROSBIE. (Department of Biochemistry, University of Hull, Hull HU6 7RX, U.K.)

Cheeseman & Crosbie (1966) have reported that, when *Escherichia coli* 5928 (a wild-type strain) and PA 15 (a glycine/serine auxotroph) are grown in a simple glucose-inorganic salts medium containing [^{3-¹⁴C}]serine (2.9 mM), C-2 of the polynucleotide purine ring system is quantitatively derived from the β-carbon atom of the serine supplement. Little or no incorporation into the C-8 position occurs. Further, evidence was adduced that formate, which accumulates during growth through the thioclastic fission of pyruvate, was utilized extensively in the biogenesis of the C-8 position but not of the C-2 position. These observations, which appeared not to be consistent with the roles of *N*¹⁰-formyltetrahydropteroylglutamate and *N*⁵*N*¹⁰-methenyltetrahydropteroylglutamate in purine biosynthesis as proposed for avian systems by Hartman & Buchanan (1959), have been reinvestigated and confirmed.

One possible explanation of the above observations is that the specificities of glycine amide ribotide transformylase and 4-aminoimidazole-5-carboxamide ribotide transformylase with respect to the formyl donor are the inverse of those reported for the avian enzymes and that there is additionally some control over *N*⁵*N*¹⁰-methenyltetrahydropteroylglutamate cyclohydrolase activities dependent on formate concentrations.

The specificity of 4-aminoimidazole-5-carboxamide ribotide transformylase of *E. coli* 5928 has now been investigated with a preparation purified by ammonium sulphate fractionation and ion-exchange chromatography on DEAE-cellulose to the stage of separation from cyclohydrolase activity. By using the assay method of Flaks, Erwin & Buchanan (1957) to follow the progress of the formylation reaction, activity could be demonstrated only when *N*¹⁰-formyltetrahydropteroylglutamate was used as formyl donor, no activity being demonstrable with the *N*⁵*N*¹⁰-methenyl analogue except when purified cyclohydrolase was added to the system. No control over cyclohydrolase activity other than product inhibition by

N^{10} -formyltetrahydropteroylglutamate could be demonstrated.

The specificity of glycine amide ribotide trans-formylase is under investigation.

We are indebted to the Medical Research Council for the award of a Research Studentship to T.W.P. and to Dr N. Belcher (Pfizer and Co., Groton, Conn., U.S.A.) and Dr R. R. Widman (Lederle Laboratories, Cyanamid International, Pearl River, N.Y., U.S.A.) for generous gifts of 4-aminoimidazole-5-carboxamide ribotide and leucovorin respectively.

Cheeseman, P. & Crosbie, G. W. (1966). *Biochem. J.* **99**, 24P.

Flaks, J. G., Erwin, M. J. & Buchanan, J. M. (1957). *J. biol. Chem.* **229**, 603.

Hartman, S. C. & Buchanan, J. M. (1959). *J. biol. Chem.* **234**, 1812.

Plastocyanin from *Cucurbita pepo* L.

By M. D. SCAWEN and E. J. HEWITT. (*Long Ashton Research Station, University of Bristol, Bristol BS18 9AF, U.K.*)

Plastocyanins of higher plants have been purified from spinach (Katoh, 1962) and French bean (Wells, 1966). We obtain homogeneous plastocyanin easily in good yield from plantation-grown vegetable marrow and the protein has been crystallized.

Extraction from washed frozen leaves and initial fractionation with acetone, DEAE-cellulose and ammonium sulphate are followed to the second DEAE-cellulose stage (Hewitt, Hucklesby & Betts, 1968). Repeated adsorption is wasteful and is replaced by chromatography on Sephadex G-100 and final concentration on a Diaflo UM2 membrane. The yield, estimated spectrophotometrically (Plesničar & Bendall, 1970), was 10mg/kg, i.e. 15% recovery. Initial concentrations varied with copper concentration in the nutrient.

Electrophoresis on cellulose acetate (pH 4.5, 6.5, 7.7 and 8.6) and in polyacrylamide gel (pH 6.6 and 9.6) and ultracentrifugation indicated homogeneity. From $s_{20,w}^0$ 1.69×10^{-13} S, $D_{20,w}^0$ 14.6×10^{-7} cm²·s⁻¹ and $\bar{v}$ 0.73 (by amino acid analysis) and 0.74 (by pycnometry), a molecular weight between 10300 and 10900 was derived.

Hydrolysis (6M-hydrochloric acid at 110°C for 16–100h) yielded 99 amino acid residues and molecular weight 10580 for 1 copper atom/molecule (0.58% Cu). Chromatography on Sephadex G-100 or G-75 in 0.1M-potassium chloride, pH 7.5, indicated molecular weight 12500 for five determinations. Tryptophan, determined spectrophotometrically (Beaven & Holiday, 1952) or by ninhy-

drin (Gaitonde & Dovey, 1970) and arginine were absent. Aspartate (15), glycine (11), valine (11) and glutamate (10) were most abundant. Phenylalanine (6), tyrosine (3), proline (5), histidine (2), amide (6), methionine (2) and cysteine (1) were found. The thiol group is protected and reacts slowly with 2-chloromercuri-4-nitrophenol (McMurray & Trentham, 1969) unless denatured by 6M-guanidinium chloride, pH 7, or trichloroacetic acid.

Light-absorption maxima were at 775, 597 (ϵ_{mM} 4.7) and 278nm, with subsidiary maxima at 269, 265, 259 and 253nm due to phenylalanine and at 284nm due to tyrosine. The maximum E_{597}/E_{278} ratio was 0.85. This was low compared with spinach plastocyanin, which has 2 tyrosine residues/molecule (Katoh *et al.* 1962), but similar to bean plastocyanin, with 3 tyrosine residues/molecule (Milne & Wells, 1970). CO had no effect on the u.v.-visible spectrum.

The redox potential was +0.35V (pH 5.6–9.5) for le^- against ferricyanide (E_0' standardized as +0.41V). Copper was removed by Chelex 100 resin in 6M-guanidinium chloride, but did not recombine readily, and fine structure in the u.v. spectrum was lost irreversibly. Mercuric acetate treatment allowed reversible copper removal. The protein crystallized as deep-blue, apparently hexagonal, rods in clusters from 65%-saturated ammonium sulphate after prolonged standing at 1°C.

We thank Dr C. F. Phelps for making available and assisting with the analytical ultracentrifuge and synthetic-boundary cell, and Dr B. T. Pickering for operating and assisting with the amino acid analyser.

Beaven, G. H. & Holiday, E. R. (1952). *Adv. Protein Chem.* **7**, 319.

Gaitonde, M. K. & Dovey, T. (1970). *Biochem. J.* **117**, 907.

Hewitt, E. J., Hucklesby, D. P. & Betts, G. F. (1968). In *Recent Aspects of Nitrogen Metabolism in Plants*, p. 47. Ed. by Hewitt, E. J. & Cutting, C. V. London: Academic Press Inc. (London) Ltd.

Katoh, S., Shiratori, I. & Takamiya, A. (1962). *J. Biochem., Tokyo*, **51**, 1.

McMurray, C. H. & Trentham, D. R. (1969). *Biochem. J.* **115**, 913.

Milne, P. R. & Wells, J. R. E. (1970). *J. biol. Chem.* **245**, 1566.

Plesničar, M. & Bendall, D. S. (1970). *Biochim. biophys. Acta*, **216**, 192.

Wells, J. R. E. (1966). *Nature, Lond.*, **212**, 895.