SC 2270

Inhibition in synthesis of β-alanine by D-serine

D-Serine inhibits growth and cell division in a species of Erwinia in a defined medium resulting in formation of filaments 100 μ or more in length[1]. Previously, it was shown that D-serine strongly inhibits pantothenate and coenzyme A synthesis through inhibition in synthesis of pantoic acid; this block is related to cell-division inhibition[2]. Two other amino acids which inhibit cell division (D-phenylalanine or D-histidine) also block pantothenate synthesis, but to a lesser extent than D-serine. D-Serine differs from the other two amino acids in that added pantoyllactone results in a limited (approx. 25 %) restoration of pantothenate synthesis, whereas with D-phenylalanine or D-histidine, restoration is over 100%[2].

Addition of β-alanine to D-serine-containing media results in a several-fold increase in pantothenate synthesis[2]. These data suggested that D-serine blocks β-alanine, as well as pantoic acid, synthesis. DURHAM AND MILLIGAN[3] reported that β-alanine or pantothenic acid overcomes growth inhibition in a Flavobacterium sp. by D-serine and inferred that D-serine blocked β-alanine synthesis. MAAS AND DAVIS[4] reported that D-serine inhibited the condensation of β-alanine and pantoic acid in *Escherichia coli*. Studies providing direct evidence for inhibition of β-alanine synthesis (via α-decarboxylation of aspartic acid) by D-serine are reported in this paper. Synthesis of β-alanine through α-decarboxylation of aspartic acid is well documented[5–7].

Initially, tests were done on the effects of the three D-amino acids upon utilization of aspartic acid during growth. Without glucose in the defined medium, aspartic acid is completely utilized in 16 h in the absence of D-amino acids. On a molar basis D-serine inhibits utilization much more effectively than D-phenylalanine or D-histidine (Table I).

Since inhibition of utilization of aspartic acid (in this case, the sole source of carbon, energy, and nitrogen) did not necessarily mean that α-decarboxylation was blocked, the effect of D-serine on CO_2 evolution from aspartic acid by washed cells was tested manometrically. Erwinia cells grown on nutrient agar at 28° for 16–17 h evolve an excess of CO_2 over O_2 from aspartic acid. After 21 h growth, although active in O_2 uptake, cells showed no net CO_2 evolution. Apparently, older cells lose α-decarboxylation activity. This is in accord with the observation of BILLEN AND

TABLE I

EFFECT OF D-SERINE, D-HISTIDINE, AND D-PHENYLALANINE ON UTILIZATION OF ASPARTIC ACID

Initial aspartic acid concentration was 2.8 mg/ml of medium. Supernatants were directly chromatographed in the two-dimensional system of REDFIELD[8]; amino acids were eluted and the amount present determined according to the technique of SALTON[9].

D-Amino acid	*Residual aspartic acid mg/mg dry wt. cells*
None	0
D-Serine (0.002 M)	0
D-Serine (0.004 M)	0.18
D-Serine (0.008 M)	1.17
D-Serine (0.004 M) plus β-alanine (0.0033 M)	0
D-Histidine (0.012 M)	0
D-Phenylalanine (0.008 M)	0.115

LICHSTEIN[7], who found that *Rhizobium trifolii* cells showed a marked decrease in ability to synthesize β-alanine from aspartic acid after a culture age of 15 h.

D-Serine did not inhibit O_2 uptake with L-aspartic acid by older cells (over 20 h), but with 16-h cells approx. a 20% inhibition was observed. Net CO_2 evolution was stopped by 0.057 M D-serine (Fig. 1). Since the increase in pH, resulting from metabolism of aspartic acid, was slightly greater in the absence of D-serine, cessation of

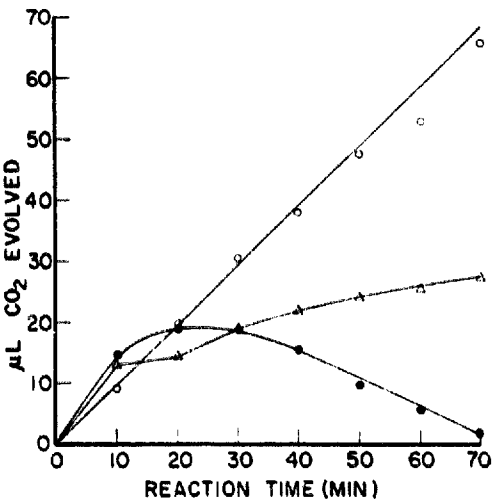

Fig. 1. Inhibition of net CO_2 evolution from L-aspartic acid by D-serine. D-Serine added at 10 min. Initial pH 6.2; final pH 7.0–7.2. Reactions were carried out at 30° with air as the gas phase using the direct method of Warburg[10]. 0.14 M phosphate buffer (pH 6.2) and 0.05 mM of L-aspartic acid were used. O—O, Control; Δ—Δ, D-serine 0.028 M; ●—●, D-serine 0.057 M.

CO_2 evolution cannot be attributed to a rise in pH. With D-serine alone (no aspartic acid), cells took up O_2 at a rate about one-fourth that with aspartic acid. No net CO_2 evolution occurred.

To confirm that inhibition of α-decarboxylation occurred, samples of flask contents were assayed microbiologically for β-alanine. Basal medium of KING AND CHELDELIN[11] and *Saccharomyces cerevisiae* ATCC 9371 were used. Under conditions of our testing, it was determined that neither pantothenate nor D-serine interfered with results of the assay. Without D-serine in the reaction mixture, 10 µg/ml β-alanine was present. With 0.057 M D-serine, the concentration of β-alanine was 4.5 µg/ml.

TABLE II

INHIBITION BY D-SERINE OF SYNTHESIS OF EXTRACTABLE INTRACELLULAR β-ALANINE

Reaction run for 90 min at 30°. Concentrations: 0.014 M L-aspartic acid, 0.03 M phosphate buffer (pH 7.0), 20 mg dry wt. cells per flask.

D-Serine (M)	*µg β-alanine per mg cells*
0	1.06
0.0013	0.92
0.003	0.64
0.006	0.37
0.011	0.17
0.017	0.14
0.028	0.11

Further tests of inhibition of β-alanine synthesis by D-serine were done by incubation of washed 16-h cells in a Dubnoff shaker at 30°. Analysis for β-alanine was accomplished using cell supernatants and a boiled extract of cells (10 min using 3 ml water per 20 mg cells). Under these conditions, D-serine strongly decreases the level of intracellular β-alanine (Table II).

Although most β-alanine was found to be intracellular, the same pattern of inhibition by D-serine was observed when testing for extracellular β-alanine. Surprisingly, when the pH of the incubation medium was varied, 100 % more β-alanine was excreted at pH 6.0 as compared to pH 7.0 (0.26 *vs.* 0.13 μg β-alanine per ml supernatant per mg cells) in control flasks (no D-amino acid present). Thus it could be postulated that cellular retention of β-alanine and subsequent synthesis of pantothenic acid is, in part, pH dependent. This has been confirmed using 16-h growing cultures of Erwinia sp. Cells from a medium wherein pH at harvest time was 5.6 contained 0.66 μg pantothenic acid per unit cell mass whereas cells from a medium wherein pH at harvest was 6.9 contained 1.4 μg pantothenic acid per unit cell mass.

In conclusion, our data provide direct evidence for inhibition of synthesis of β-alanine from aspartic acid by D-serine in this species of Erwinia. Further, we have demonstrated that excretion of β-alanine and subsequent synthesis of pantothenic acid is, in part, pH dependent.

This investigation was supported in part by research Grants No. G-9848 and GB-259 from the National Science Foundation and a Public Health Service research career program award GM-K3-13,968 from the Division of General Medical Sciences.

Department of Microbiology, Oklahoma State University, Stillwater, Okla. (U.S.A.)

E. A. GRULA
MARY M. GRULA

1 E. A. GRULA, *J. Bacteriol.*, 80 (1960) 375
2 M. M. GRULA AND E. A. GRULA, *J. Bacteriol.*, 83 (1962) 989.
3 N. N. DURHAM AND R. MILLIGAN, *Biochem. Biophys. Res. Commun.*, 7 (1962) 342.
4 W. K. MAAS AND B. D. DAVIS, *J. Bacteriol.*, 60 (1950) 733.
5 J. M. RAVEL AND W. SHIVE, *J. Biol. Chem.*, 166 (1946) 407.
6 W. SHIVE AND J. MACOW, *J. Biol. Chem.*, 162 (1946) 451.
7 D. H. BILLEN AND H. E. LICHSTEIN, *J. Bacteriol.*, 58 (1949) 215.
8 R. R. REDFIELD, *Biochim. Biophys. Acta*, 10 (1953) 344.
9 M. R. J. SALTON, *Biochim. Biophys. Acta*, 39 (1960) 398.
10 W. W. UMBREIT, R. H. BURRIS AND J. F. STAUFFER, *Manometric Techniques and Tissue Metabolism*, Burgess Pub. Co., Minneapolis, Minn., 1949, p. 17.
11 T. E. KING AND V. H. CHELDELIN, *J. Biol. Chem.*, 174 (1948) 273.

Received February 11th, 1963

Biochim. Biophys. Acta, 74 (1963) 776–778

SC 2254

Alterations of rat-tissue cytochrome c levels by a chronic cold exposure

Recent studies have indicated that one of the factors responsible for the improved thermogenic capacity of the cold-acclimatized animals is an alteration in the pattern and magnitude of electron transport. Thus, within the electron-transport system an increased activity or concentration of succinate dehydrogenase (EC 1.3.99.1) and