A MECHANISM OF GROWTH INHIBITION BY D-SERINE IN A *FLAVOBACTERIUM*[1]

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Received January 15, 1962

Certain D-amino acids have been reported to produce aberrant morphological forms in microorganisms (Lark and Lark, 1959; Tuttle and Gest, 1960; Grula, 1960) and it has been suggested that these compounds interfere with biosynthesis of the microbial cell wall. D-Serine influences both growth and cell division in a *Flavobacterium* sp. capable of oxidizing *p*-aminobenzoic acid as the source of carbon and energy for aerobic growth (Durham and Milligan, 1961). Pantothenic acid reverses the inhibition of growth but is unable to reverse the inhibition of division while *p*-aminobenzoic acid can overcome inhibition of both growth and division. This paper reports additional studies on the mechanism by which pantothenic acid reverses the inhibition of growth by D-serine.

Growth, as measured by optical density (O.D.) at 540 mμ, was followed by culturing the *Flavobacterium* sp. in an inorganic salts medium containing 0.2% succinic acid as the carbon and energy source (Durham, 1956). All additives were dissolved in 0.01 M phosphate buffer and sterilized by filtration. The pH of the complete medium was 7.2 prior to inoculation with *p*-aminobenzoic acid-grown cells. All cultures were incubated at 37° C with constant shaking. Dry cell mass and O.D. were closely correlated during the first 10 to 12 hours in the present of D-serine. However, some lysis was evident after 12-15 hours.

[1]This work was supported in part by Contract NR 103-504 between the Office of Naval Research, Department of the Navy, and the Oklahoma State University, and in part by the Oklahoma Agricultural Experiment Station (Project No. 976).

Addition of D-serine (8.7 μmoles/ml) to the succinate-salts medium inhibited growth of the microbial cell as evidenced by a significant decrease in O.D. (Table 1) and microscopic examination revealed the formation of "chain-like" filaments which did not develop in the succinate control. A higher D-serine concentration (11.1 μmoles/ml) showed a more pronounced effect. The incorporation of pantothenic acid (0.015 μmoles/ml) in the medium reversed the inhibition of growth as evidenced by the O.D. readings (Table 1) but did not overcome inhibition of division since "chain-like" filaments were still observed. Likewise, an approximately equimolar concentration of β-alanine (0.018 μmoles/ml), a precursor of pantothenic acid, reversed the inhibition of growth but was unable to overcome "chain" formation. Increasing the concentration of these metabolites by 100-fold did not enhance reversal. Maas and Davis (1950) reported that low levels of D-serine interfered with the enzymatic conversion of β-alanine to pantothenic acid and high levels of D-serine affected processes other than pantothenic acid synthesis. Similar reversal trends with equimolar concentrations of β-alanine and pantothenic acid would suggest that, in this biological system, D-serine interferes with the biosynthesis of β-alanine.

Since β-alanine reversed the inhibition of growth by D-serine, metabolites closely associated with β-alanine in various biochemical sequences were studied for reversing ability. Results presented in Table 1 show that L-alanine and L-aspartic acid also reversed D-serine inhibition of growth. Reversal by L-alanine was somewhat less than the reversal observed with β-alanine and a concentration effect was apparent in both D-serine concentrations. L-Aspartic acid also showed a concentration effect and good reversal was observed in 8.7 μmoles/ml D-serine when the aspartic acid concentration was 3.07 μmoles/ml. A specific cellular requirement for these amino acids and the fulfillment of this requirement prior to functioning in the reversal of growth inhibition by D-serine could explain the need for larger quantities of these reversing metabolites in the inhibited system. Also, the failure of L-alanine to show reversal equivalent to that obtained with β-alanine might suggest

TABLE 1

INFLUENCE OF METABOLITES ON D-SERINE INHIBITION OF GROWTH IN A FLAVOBACTERIUM.

Medium Components	Metabolite Concentration (μmoles/ml)	O.D. 540 mμ (10 hours) D-serine concentration (μmoles/ml) 0.0	8.7	11.1
Succinate*		.70	.22	.07
Succinate* + β-alanine	0.018	.76	.63	.51
+ β-alanine	1.83	.74	.65	.55
+ L-alanine	0.19	.75	.31	.19
+ L-alanine	4.58	.81	.40	.29
+ D-alanine	0.018	.68	.22	.12
+ D-alanine	4.58	.67	.20	.14
+ L-aspartic acid	0.125	.76	.26	.22
+ L-aspartic acid	3.07	.82	.58	.41
+ pyruvic acid	0.188	.70	.25	.06
+ pyruvic acid	4.63	.73	.19	.10
+ pantothenic acid	0.015	.64	.71	.49
+ pantothenic acid	1.50	.72	.74	.48
+ threonine	3.43	.80	.30	.14
+ glycine	5.43	.76	.24	.10

*16.66 μmoles per ml

another site of D-serine action. Since β-alanine appears to be the limiting metabolite, these findings suggest that L-aspartic acid and L-alanine are readily converted to β-alanine. The decarboxylation of aspartic acid readily yields β-alanine (Virtanen and Laine, 1937; Shive and Macow, 1946). The conversion of L-alanine to β-alanine might be carried out by a mechanism similar to the new transaminase system in *Pseudomonas fluorescens* which catalyzes the reversible transamination of L-alanine to β-alanine (Hayaishi *et al.*, 1961).

The inability of D-alanine to reverse the inhibition of growth would suggest that this organism does not possess an active alanine racemase or that D-serine does not interfere with the metabolism or incorporation of D-alanine into components of the microbial cell. D-Alanine did not influence the growth or division of the *Flavobacterium* sp. in the concentrations used in this investigation (0.018 and 4.58 umoles/ml). Likewise, pyruvic acid, threonine, and glycine

in concentrations as high as 4.63, 3.43, and 5.43 μmoles/ml respectively were unable to reverse the inhibition of growth.

None of the compounds, as listed in Table 1, reversed the formation of "chain-like" filaments by D-serine. Increasing the concentration of D- or L-alanine to 18.7 μmoles/ml did not effect reversal. This observation is in contrast to the report that D- or L-alanine reversed filament formation in an Erwinia sp. due to 0.0335 M DL-serine (Grula, 1960). Thus emphasizing the difference in susceptibility of microorganisms to this D-amino acid.

These findings establish that pantothenic acid and β-alanine, a precursor of pantothenic acid, reverse the inhibition of growth by D-serine in a Flavobacterium sp. The results suggest that one mechanism by which D-serine inhibits growth of this microorganism is by interfering with the biosynthesis of β-alanine. L-Aspartic acid and L-alanine also reverse the inhibition suggesting that, since β-alanine apparently is one of the limiting growth components, these compounds may be enzymically converted to this metabolite.

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