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A possible role of D-valine and related D-amino acids in repression of enzyme and actinomycin synthesis

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Summary. The addition of D-valine and related D-amino acids to the production medium blocks kynurenine formamidase II and actinomycin D synthesis by *Streptomyces parvulus*. This effect may be due to a direct inhibition of the actinomycin synthetase-catalyzed racemization of L-valine to its peptide bound D-form during antibiotic formation. In addition, the D-amino acid(s) may influence enzyme and actinomycin synthesis through carbon and/or nitrogen catabolite regulation. The relatively slow uptake and metabolism of the D-amino acid would provide a continuous supply of catabolite(s) that repress transcription of actinomycin biosynthetic genes over a long period of time.

Introduction

D-Amino acids, often present as constituents of peptide antibiotics, can inhibit synthesis of the relevant antibiotic when provided in the culture medium. For example, D-valine inhibits synthesis of actinomycin (Katz 1960; Katz et al. 1961; Yajima et al. 1972) and penicillin (Demain 1956) and D-leucine blocks formation of etamycin (Kamal and Katz 1976) and polymyxin (DiGirolamo et al. 1964). Moreover, radioisotope experiments have demonstrated that the L-form rather than its D-isomer is the precursor of the D-amino acid in the antibiotic peptide (Arnstein and Margreiter 1958; Katz and Weissbach 1963; DiGirolamo et al. 1964). The mechanism of D-amino acid inhibition of antibiotic synthesis has not been elucidated; it has been suggested, however, that the exogenous D-amino acid may block the antibiotic synthetase-catalyzed reaction concerned with the racemization of the L-amino acid to its peptide-bound D-form (Katz et al. 1961; DiGirolamo et al. 1964). D-Glutamic acid and D-aspartic acid as well as a number of L-amino acids markedly repress the synthesis of kynurenine formamidase II, the second enzyme in the pathway from L-tryptophan to the actinomycin chromophore, by *Streptomyces parvulus* during short term experiments (Katz 1980). In light of these findings a reexamination of the effect of D-valine upon actinomycin formation and the synthesis of kynurenine formamidase II by *S. parvulus* was carried out. The results of these experiments are presented in the present communication.

Materials and methods

The procedures for cultivation of *S. parvulus* in NZ-amine medium (vegetative growth) and in L-glutamic acid-L-histidine-D-fructose-mineral salts medium (GHF, production medium) have been described previously (Katz and Goss 1959; Williams and Katz 1977). Actinomycin titers were determined spectrophotometrically at 443 nm by the method of Katz and Weissbach (1963).

Preparation of washed mycelium, disruption of mycelium by French pressure cell treatment and the assay procedure for measurement of kynurenine formamidase activity in cell-free extracts have been presented elsewhere (Brown et al. 1980, 1986). A unit of enzyme activity and the specific activity of kynurenine formamidase have been described previously (Brown et al. 1980, 1986). *S. parvulus* synthesizes two kynurenine formamidase activities (Brown et al. 1980, 1986). Kynurenine formamidase I (MW = 42000) appears to be constitutive and is formed prior to antibiotic production during growth in NZ-amine medium. No significant synthesis of this enzyme occurs during cultivation in production medium. By contrast, kynurenine formamidase II (MW = 25000) is inducible (derepressible) and its synthesis is initiated just prior to actinomy-

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cin formation at 21 h. The increase of kynurenine formamidase II activity is actually 20 to 22-fold during actinomycin production (Brown et al. 1980). Both enzyme activities are present throughout the fermentation, however, only formamidase II activity increases during this time. Therefore, we measure the increase (Δ) of kynurenine formamidase II. Protein was measured by the method of Lowry et al. (1951).

Amino acid concentration in GHF medium was measured by the procedure of Naftalin (1948) as modified by Katz and Weissbach (1963) after desalting an aliquot (5 ml) of GHF medium on a Dowex 50 ion-exchange chromatographic column (Katz and Weissbach 1963).

Results

As noted in earlier experiments with *S. antibioticus*, (Katz 1960) D-valine, at 1–2 mM, completely inhibited the synthesis of actinomycin D by *S. parvulus* (Table 1). An examination of kynurenine formamidase II synthesis revealed that as the level of D-valine in the medium increased there was increased repression of enzyme synthesis (Table 1). A study of the time course of kynurenine formamidase II and actinomycin D synthesis in the presence and absence of D-valine (2 mM) is shown in Fig. 1. The data reveal a partial repression of enzyme synthesis initially by the D-amino acid; however, after an additional 27 h incubation the repression was complete. The biphasic nature of this effect may be attributable to the time required for uptake of the D-amino acid intacellularly (Table 2). D-Valine, in comparison with L-valine or other L-amino acids such as L-glutamic acid, is taken up slowly from the culture medium and accumulates intracellularly (Katz and Weissbach 1963). The decrease in enzyme activity during the continued incubation may be a consequence of the degradation or inactivation of the enzyme previously synthesized. By comparison, L-valine, at 2 mM, severely repressed enzyme synthesis after a 6 h incubation (Fig. 1); thereafter, the rate of enzyme synthesis was similar to that seen in the untreated culture.

We have previously reported that actinomycin synthesis is also inhibited by D-amino acids such as D-alloisoleucine, D-isoleucine and D-leucine (Katz et al. 1961; Yajima et al. 1972). Their in-

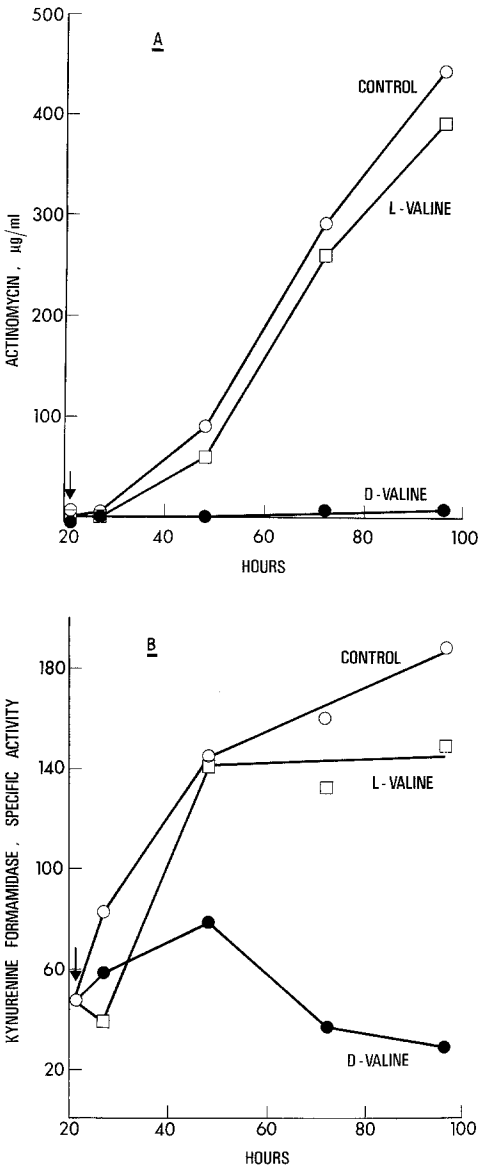

Fig. 1. Influence of D-valine (●) or L-valine (□) on actinomycin **(A)** and kynurenine formamidase II **(B)** synthesis by *S. parvulus* in GHF medium. The amino acid (2 mM, final concentration) was added at 21 h; the incubation was resumed for an additional 75 h period

Table 1. Effect of D-valine concentration on kynurenine formamidase II and actinomycin D synthesis by *S. parvulus*

D-Valine mM	Kynurenine formamidase II		Actinomycin Titer Inhibition	
	Δ Specific activity Units/mg	Repression %	µg/ml	%
0	81.6	0	121	0
0.05	74.0	9.3	93	23.1
0.1	38.3	53.1	85	29.8
0.25	21.2	73.9	52	57.0
0.5	4.5	94.5	6	95.0
1.0	− 7.2	100	2	98.3
2.0	−40.8	100	1	>99

D-Valine was added at 21 h; incubation was resumed for 27 h. Enzyme activity and actinomycin titers were determined as described previously (Brown et al. 1980, 1986; Katz and Weissbach 1963)

Table 2. Utilization of amino acids by *S. parvulus* grown in glutamic acid-histidine-fructose medium

Amino acid	Time h	Concentration mM
D-Valine	0	2.13
	6	2.13
	24	1.38
	48	0.58
	72	0.054
L-Valine	0	2.21
	6	1.06
	24	0.02
L-Glutamic acid	0	2.62
	6	0.08
	24	n.d.

Amino acid was added at 21 h; culture filtrates were collected and desalted on a Dowex 50 ion-exchange column. Determination of amino acid concentration was carried out by the method of Naftalin, 1948. n.d. = not detected

Table 3. Repression of kynurenine formamidase II and actinomycin formation by D-amino acids

Compound	Kynurenine formamidase II		Actinomycin	
	Δ Spec. activity Units/mg	Repression %	Titer μg/ml	Inhibition %
Control	101.9	0	264	0
D-Valine	3.5	97	2	99
D-Alloisoleucine	19.7	81	5	98
D-Isoleucine	49.9	51	29	89
D-Leucine	62.7	39	89	66
D-Glutamic acid	84.8	17	276	0
D-Threonine	123.0	0	274	0

The D-amino acid (2 mM, final concentration) was added at 21 h; incubation was resumed for 50 h. Enzyme activity and actinomycin titers were determined as in *Materials and methods*

fluence upon kynurenine formamidase II and actinomycin D synthesis by *S. parvulus* is shown in Table 3. The order of their effectiveness was: D-valine > D-alloisoleucine > D-isoleucine > D-leucine. Although D-glutamate repressed enzyme synthesis severely (60%) after a 6 h incubation (data not shown), the influence of this amino acid was quite limited (Table 3) after a longer incubation period possibly as a result of metabolism of the amino acid.

Discussion

The repression of kynurenine formamidase II synthesis in addition to an effect upon actinomycin D production by D-valine was an unexpected finding as we had considered it likely that the site of action of the D-amino acid involved the conversion of the L-amino acid to its D-form (Katz et al. 1961; DiGirolamo et al. 1964). The present data suggest alternative possibilities which may influence the biosynthetic process directly or indirectly. Thus, D-valine (or certain related D-amino acids) may function as a specific effector which acts upon a regulatory determinant of the actinomycin biosynthetic gene cluster thereby preventing expression. On the other hand, D-valine may block a specific step in the formation of actinomycin biosynthetic proteins (e.g., activation of valyltRNA synthetase). This action appears unlikely in light of previous observations (Katz 1960; Katz et al. 1961) that the D-amino acid does not inhibit growth of actinomycin-producing cultures. Conceivably, the D-amino acid may influence actinomycin production through carbon and/or nitrogen catabolite regulation (Martin and Demain 1980) — its slow uptake and metabolism ensuring a continuous supply of catabolite(s) that block specifically transcription of actinomycin biosynthetic genes over a long period of time.

It is noteworthy that the repression of enzyme synthesis as well as actinomycin formation appears to be specific for certain related D-amino acids. However, only a limited number of D-amino acids have been examined thus far. The long term repression seen with D-valine (or D-alloisoleucine, D-isoleucine, D-leucine) in contrast to L-amino acids (or D-glutamic acid or D-threonine) may be a function of the rate of metabolism of the compound rather than the relationship of chemical structure. Additional experiments are needed to establish the specificity of the effect described. Moreover, it will be of interest to determine whether other enzymes in the actinomycin biosynthetic pathway (*S. parvulus*, *S. antibioticus*) are also repressed by D-valine. Preliminary experiments indicate that formation of hydroxykynureninase (*S. parvulus*) is also diminished by addition of D-valine (unpublished results). The question also remains whether the repression of enzyme synthesis observed with D-valine (and related amino acids) is limited to actinomycin production or whether the D-amino acids affect formation of synthetases that participate in the synthesis of other peptide antibiotics.

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