REVERSAL OF NETROPSIN INHIBITION OF GROWTH IN ESCHERICHIA COLI

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Netropsin, an antibiotic produced by the actinomycete Streptomyces netropsis, was first described by Finlay, et al. (1951). Sinanomycin (Watanabe, 1956), congocidine (Julia and Joseph, 1956) and T-1384 (Waller, et al., 1956) appear to be identical with netropsin whose composition and structure is $C_{18}H_{26}N_{10}O_3$, β-[4-(4-guanidinoacetamidino-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]propionamide, as determined by Waller, et al. (1956). The antibiotic has a broad antibacterial spectrum; but its toxicity precludes use as a chemotherapeutic agent.

There has been little investigation of the mode of action of netropsin. Preliminary studies, undertaken to determine if it exerts an effect on synthesis or utilization of amino acids in bacteria, revealed a reversal of in vitro antibacterial activity for Escherichia coli strain B by synthetic amino acid mixtures.

The minimal medium of Davis and Mingioli (1950) was used. Ten ml. of medium in 22 x 175 mm. culture tubes of both aerated and non-aerated cultures were observed at 35 C. after 18 or 90 hours incubation using a Coleman Junior spectrophotometer at a wave length of 620 mμ; the growth was recorded as optical density.

Netropsin as the hydrochloride was dissolved in a 0.1M pH 8 phosphate buffer and sterilized by Seitz filtration. Equivalent volumes of phosphate buffer were added to all control tubes.

Growth of E. coli was completely inhibited, in both aerated and non-aerated cultures, by 5 mcg. of netropsin hydrochloride per ml. This antibacterial activity is reversed by yeast or beef extracts and by enzyme and acid hydrolyzed proteins (Table 1).

Table 1

REVERSAL OF NETROPSIN INHIBITION OF GROWTH OF E. COLI BY NATURAL MATERIALS [a]

TEST MATERIALS [b]	CONTROL	WITH NETROPSIN.HCl 5 mcg./ml.
	O.D.	O.D.
None	0.690	0.010
Beef Extract (Difco)	0.710	0.720
Yeast Extract (Difco)	0.840	0.840
Amigen® Powder [c] (Mead Johnson)	0.990	0.930
Casamino Acids [d] (Difco)	0.720	0.700

[a] Medium of Davis and Mingioli; 18 hour incubation; cultures aerated with sterile air.
[b] All additions were at 2 mg./ml.
[c] Pancreatic digest casein (pretreated with carbon).
[d] Acid-hydrolyzed casein (vitamin free).

A mixture of 18 amino acids was also found to be highly effective (Table 2). Deletion of individual amino acids from the mixture showed an effect of the following on reversal of netropsin activity: serine, valine, lysine, methionine, phenylalanine, leucine, isoleucine and tryptophan. A mixture of these eight amino acids caused a 40 to 50% reversal of the growth inhibition of E. coli. Single additions of lysine or phenylalanine reversed the inhibition of netropsin, but the data were sporadic. Furthermore, an incubation

Table 2

REVERSAL OF NETROPSIN INHIBITION OF GROWTH IN *E. COLI* BY AMINO ACIDS[a]

ADDITION	Mg./ml.	CONTROL	WITH NETROPSIN 5 mcg./ml.	% REVERSAL OF GROWTH INHIBITION
		O.D.		
		18 Hours, 35 C.		
None		0.232	0.000	0
Mixture of 18 Amino Acids[b]	1.0	0.250	0.270	100
	2.0	0.280	0.220	79
		90 Hours, 35 C.		
None		0.320	0.000	0
Mixture of 8 Amino Acids[c]	1.0	0.330	0.140	42
	2.0	0.340	0.168	49
L-Lysine.HCl	0.1	0.345	0.116	34
	0.2	0.375	0.048	13
DL-Phenylalanine	0.1	0.315	0.140	44
	0.2	0.320	0.055	17

[a] Medium of Davis and Mingioli; static cultural conditions.

[b] The synthetic amino acid mixture was previously prepared for another purpose and consisted of 10 gm. each of DL-alanine, DL-aspartic acid, and L-glutamic acid; 2 gm. each of L-arginine.HCl, DL-isoleucine, L-lysine.HCl, DL-phenylalanine, DL-serine, DL-threonine, DL-tryptophan, DL-valine, and DL-methionine; and 1 gm. each of L-cystine, glycine, L-histidine.HCl.H_2O, L-leucine, L-proline, and L-tyrosine (total weight = 54 gm.).

[c] A mixture of DL-serine, DL-valine, L-lysine.HCl, DL-methionine, DL-phenylalanine, L-leucine, DL-isoleucine, and DL-tryptophan at the concentrations indicated above.

period of 18 hours is sufficient to observe reversal of netropsin inhibition with the substances in Table 1 and with the 18 amino acids; whereas the single amino acids or the mixture of eight amino acids required 72 to 90 hours.

In summary, the *in vitro* antibacterial activity of the antibiotic netropsin toward *E. coli* strain B is reversed by several amino acids; a mixture of 18 amino acids was a fully effective reversal agent; whereas, a mixture of eight amino acids was only partially effective. It is, therefore, suggested that the

antibacterial activity of netropsin is related to the inhibition of synthesis or utilization of amino acids.

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