INHIBITION OF THIOL-ACTIVATED AMINOPEPTIDASE BY PUROMYCIN

Stanley Ellis and Manuel Perry
Ames Research Center, NASA, Moffett Field, California

Received March 26, 1964

Pituitary extracts have been shown to contain a thiol-activated aminopeptidase(s) which preferentially hydrolyzes dibasic amino acyl derivatives of p-nitroaniline and β-naphthylamine (Ellis, 1963). In the course of recent inhibition studies, it was found that the thiol-activated aminopeptidase was strongly inhibited by puromycin. Leucine aminopeptidase, on the other hand, was not affected by puromycin either in the hydrolysis of p-nitroanilides or dipeptides.

The extracts employed in these studies were prepared by mincing bovine anterior pituitary lobes, homogenizing for 10 minutes in distilled water, adjusting the 20 per cent homogenate to pH 7.5 and centrifuging for 20 minutes at 6000 xg. The rate of formation of p-nitroaniline was measured by the absorbancy at 410 mμ as previously described (Ellis, 1963). The amino acyl-p-nitroanilides were purchased from Cyclo Chemical Corp., Los Angeles, and puromycin and the aminonucleoside from Nutritional Biochemicals Corp., Cleveland.

The effect of the concentration of puromycin on the hydrolysis of L-lysine-p-nitroanilide by pituitary extract is shown in Fig. 1. At concentrations of 10^{-4} to 10^{-3}M, the inhibition by puromycin ranged from 75 to 85 per cent. In contrast to the severe inhibition by puromycin, the aminonucleoside inhibited the hydrolysis by only 15 per cent. Similarly, N-L-phenylalanyl-D-glucosamine (10^{-3}M) inhibited by 20 per cent. The unrelated antibiotics chloramphenicol, dihydrostreptomycin, and penicillin G inhibited by 15, 5 and 36 per cent, respectively, at 10^{-3}M. In view of the relatively small effects of puromycin aminonucleoside and N-L-phenylalanyl-D-glucosamine, it appears that the inhibitory action of puromycin is due to the peptide linkage between the amino acyl and 3-amino-ribose moieties of this antibiotic.

The hydrolysis of L-Arg-, L-Met-, L-Phe-, L-Leu- and L-Ala-p-nitroanilides was also inhibited by puromycin to extent shown in Table 1. It is note-worthy that the hydrolysis of L-Arg-p-nitroanilide is much less inhibited by puromycin.

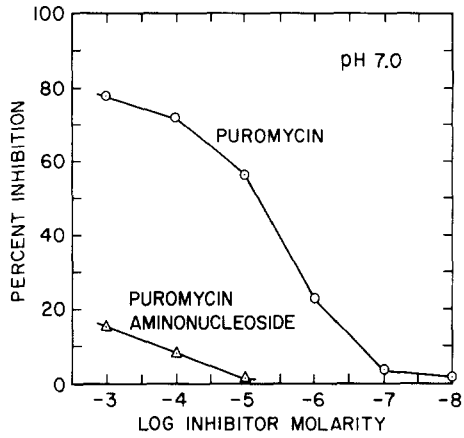

Fig. 1. Effect of Puromycin and Aminonucleoside on Lys-p-nitroanilide Hydrolysis. Reaction mixture contained 0.1 M Tris-HCl (pH 7.5), 0.01 M 2-mercaptoethanol, 10^{-3}M substrate, the indicated concentrations of puromycin or the aminonucleoside, and 0.02 ml. of the centrifuged extract; final volume 2.5 ml.; temp. 37°.

Table 1. Inhibition of Amino Acyl-p-nitroanilide Hydrolysis by Puromycin (10^{-3}M)

Amino Acyl-p-nitroanilide	Hydrolysis Rate, ΔA_{410}/min./ml.		Per cent Inhibition
	Control	Puromycin	
Lys	0.900	0.263	71
Arg	0.800	0.450	44
Met	0.550	0.068	88
Leu	0.425	0.100	81
Phe	0.375	0.050	87
Ala	0.150	0.010	93

Reaction volume 2.5 ml.: 10^{-3}M substrate, 0.1 M Tris-HCl (pH 7.05), 0.01 M 2-mercaptoethanol, 0.02 ml extract; ΔA_{410} in 1 cm. cuvette 37°. Hydrolysis rate expressed per ml. of extract.

The kinetics of Lys-p-nitroanilide hydrolysis by pituitary extract in the presence of 10^{-3}M puromycin are shown in Fig. 2. These results indicate that puromycin acts as a competitive inhibitor. Moreover, no significant hydrolysis of puromycin by the extract could be detected by ninhydrin analysis, chromatography, or high voltage electrophoresis (pyridine-acetic acid, pH 6.15) even after 6 hours of incubation with

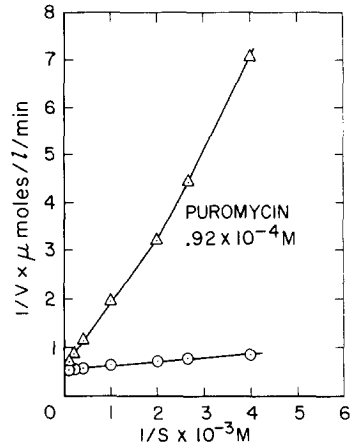

Fig. 2. Kinetics of Inhibition of Thiol-activated Aminopeptidase by Puromycin. Reaction conditions were the same as in Fig. 1 except that Lys-p-nitroanilide concentration was varied as indicated. Upper curve: puromycin; lower curve, control.

up to 5 times the concentration of pituitary extract given in Fig. 1. Hence, puromycin appears to function as a true competitive inhibitor rather than as a substrate for the aminopeptidase.

Leucine aminopeptidase (Worthington) was also tested for inhibition by puromycin employing the p-nitroanilides shown in Table 2 as substrates. The rate of hydrolysis of these substrates was not affected by 10^{-3}M puromycin. Moreover, puromycin was also without effect on the hydrolysis of dipeptides (Leu·Ala, Arg·Val, and Lys·Val) by leucine aminopeptidase or by pituitary extract. The latter observation suggests, therefore,

Table 2. Hydrolysis of Amino Acyl-p-nitroanilides by Leucine Aminopeptidase

Amino Acyl-p-nitroanilide	Hydrolysis Rate, ΔA_{410}/min./mg.
Leu	0.300
Phe	0.165
Met	0.147
Arg	0.022
Lys	0.016

Reaction volume 2.5 ml.: 10^{-3}M substrate, 0.1 M Tris-HCl (pH 7.6, 10^{-3}M Mn^{++}, 0.083 mg. leucine aminopeptidase. ΔA_{410} in 1 cm. cuvettes, 37°.

that the thiol-activated aminopeptidase does not participate significantly in the hydrolysis of dipeptides by pituitary extracts (Adams and Smith, 1951).

These results provide a basis for distinguishing the action of the thiol-activated aminopeptidase on p-nitroanilide substrates from that of leucine aminopeptidase, in addition to known differential effects of -SH on these two enzymes (Smith, 1955; Ellis, 1963). The properties of the thiol activated aminopeptidases when considered in relation to the inhibition by puromycin are suggestive of involvement in amino acyl s-RNA metabolism (Yarmolinsky and de la Haba, 1959; Fessenden and Moldave, 1962). Experiments concerned with this aspect will be reported separately.

REFERENCES

Adams, E. and Smith, E.L., J. Biol. Chem., 191, 651 (1951).
Ellis, S., Biochem. Biophys. Res. Comm., 12, 452 (1963).
Smith, E.L., and Spackman, D.H., J. Biol. Chem., 212, 271 (1955).
Yarmolinsky, M.B., and de la Haba, G.L., Proc. Natl. Acad. Sci. U.S. 45, 1721 (1959).
Fessenden, J.M., and Moldave, K., Biochemistry 1, 485 (1962).