STIMULATION AND INHIBITION BY PUROMYCIN OF CELL-FREE AMINO ACID INCORPORATION

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SUMMARY

Puromycin led either to stimulation or to inhibition of phenylalanine incorporation in cell-free systems from Bacillus licheniformis and Bacillus stearothermophilus 10. Stimulation was observed at low puromycin concentrations, at high puromycin concentrations in the presence of excess transfer RNA, and over a narrow range of temperatures. Inhibition was observed at high puromycin concentrations and at temperatures below and above the range where stimulation occurred. Freshly prepared subcellular fractions were inhibited to a greater extent than stored fractions.

INTRODUCTION

Puromycin is used routinely to inhibit cell-free amino acid incorporating systems (1). In this process puromycin mimics the action of transfer RNA (2) and binds to the growing polypeptide chain which is then released as peptidyl puromycin (3). A secondary effect associated with puromycin consists of a breakdown of polysomes due to an acceleration of the ribosomes along the messenger RNA and subsequent release of the ribosomes from the polysome (4-6). While the above has been well established, we have found that under certain conditions puromycin can actually lead to a stimulation of cell-free amino acid incorporation. Whether stimulation or inhibition is observed depends on the concentration of puromycin, the temperature of incubation, the concentration of transfer RNA, and the age of the subcellular fractions.

MATERIALS AND METHODS

Growth of the organisms (B. licheniformis, NRS 243, and B. stearothermophilus 10), isolation of the subcellular fractions, and the assay for phenylalanine incorporation were as described previously (7). Incubation mixtures

were set up under the following standard conditions unless otherwise specified: Incubation time, 30 minutes; incubation temperature, 37°; puromycin concentration (if present), 0.001 M. Control incubation mixtures (without polyuridylic acid) gave incorporation values of the order of 1% of the regular incubation mixtures. Puromycin dihydrochloride was purchased from Nutritional Biochemicals Corporation.

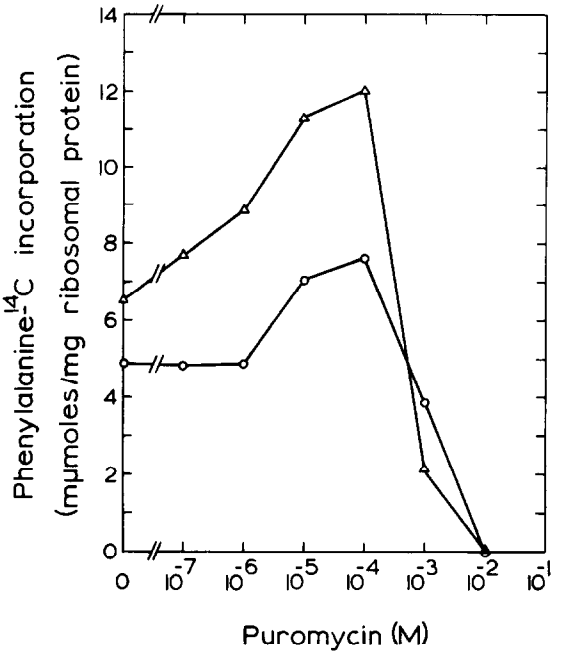

Fig. 1 Effect of puromycin concentration. (O), B. licheniformis; (Δ), B. stearothermophilus 10.

RESULTS AND DISCUSSION

The effect of puromycin concentration on phenylalanine incorporation is shown in Fig. 1. Low concentrations of puromycin stimulated the incorporation while high concentrations of the antibiotic were inhibitory. This effect may have escaped other workers because the range of concentrations covered by them was generally much smaller than that depicted in Fig. 1 (3,8,9). A similar stimulation can be observed even at high concentrations of puromycin if the effective concentration of the antibiotic is lowered by the addition of an excess of transfer RNA (Table I).

Table I

Effect of transfer RNA on inhibition by puromycin

Transfer RNA (μg)	Puromycin	Phenylalanine incorporation (mμmoles/mg ribosomal protein)	
		B. licheniformis	B. stearothermophilus 10
0	-	1.61	3.79
0	+	1.33	2.06
400	-	3.97	4.05
400	+	10.03	5.55
700	-	4.55	4.02
700	+	11.52	5.89
1000	-	4.60	4.10
1000	+	11.84	5.98

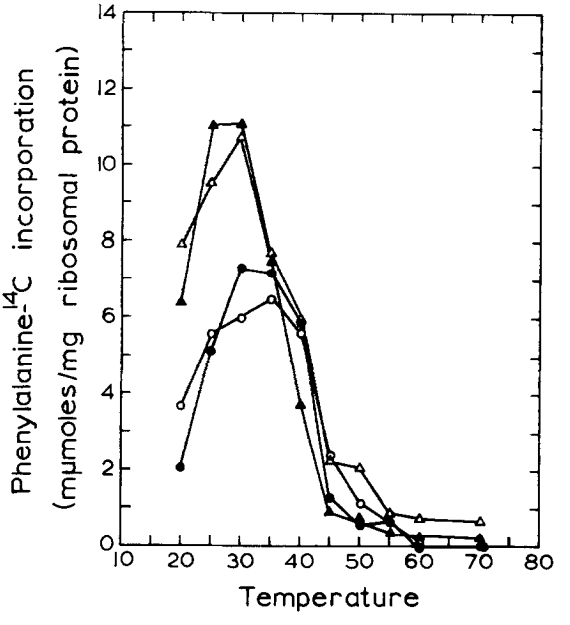

Fig. 2 Effect of incubation temperature. (○), B. licheniformis without puromycin; (●), B. licheniformis with puromycin; (△), B. stearothermophilus 10 without puromycin; (▲), B. stearothermophilus 10 with puromycin.

The stimulation by puromycin can be interpreted on the basis of the secondary effect mentioned above. This effect involves an acceleration of the ribosomes along the messenger RNA. Moreover, ribosomes which have lost the growing polypeptide chain in the form of peptidyl puromycin, continue along the messenger RNA and continue to function in amino acid incorporation (4-6). Because of these considerations, low concentrations of puromycin will lead to an increase in amino acid incorporation. At high concentrations, this secondary effect of puromycin is offset by the increased release of short, acid-soluble peptides so that the observed amino acid incorporation decreases.

The importance of temperature for the puromycin reaction has been noted before (8,10,11) but no study over an extended range of temperatures has been reported. We have carried out such a study and the results are given in Fig. 2. Puromycin was stimulatory over a narrow range of temperatures at which the systems showed maximal incorporation. On either side of this range puromycin caused inhibition. The temperature dependence of the reaction in the presence of puromycin was similar to that in the absence of puromycin. The conditions used in this experiment were such that both cell-free systems incorporated maximally at about 30° even though the B. stearothermophilus 10 system was derived from a thermophile. For such organisms, the temperature range of maximum incorporation can be shifted by changes in the magnesium ion, ammonium ion, and polyuridylic acid concentrations (12,13).

Since it was shown previously that the subcellular fractions lost some amino acid incorporating activity upon storage (7), it was of interest to determine whether the fractions would lose their activity to react with puromycin in a similar manner. Table II gives the results of an experiment performed with freshly prepared and stored fractions. In both cell-free systems the stored fractions were less inhibited by puromycin than the freshly prepared ones. This means that the subcellular fractions, upon storage, lost their activity to react with puromycin at a faster rate than they lost their activity to carry out amino acid incorporation.

Table II

Effect of storage of subcellular fractions

Subcellular fractions[A]	Puromycin (M)	Phenylalanine incorporation (mμmoles/mg ribosomal protein)
B. licheniformis		
fresh	none	4.29
	0.001	1.21
stored	none	3.59
	0.001	3.46
B. stearothermophilus 10		
fresh	none	5.06
	0.001	1.58
stored	none	3.62
	0.001	1.66

[A] fresh: used within 1 week
stored: used after 11 weeks

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REFERENCES

1. Nathans, D., in "Antibiotics", Gottlieb, D., and Shaw, P. D., eds., vol. 1, p. 259. Springer, New York (1967).
2. Yarmolinsky, M. B., and de la Haba, G. L., Proc. Natl. Acad. Sci. U. S. 45, 1721 (1959).
3. Nathans, D., Proc. Natl. Acad. Sci. U. S. 51, 585 (1964).
4. Villa-Trevino, S., Farber. E., Staehelin, T., Wettstein, F. O., and Noll, H., J. Biol. Chem. 239, 3826 (1964).
5. Williamson, A. R., and Schweet, R., J. Mol. Biol. 11, 358 (1965).
6. Noll, H., in "Developmental and Metabolic Control Mechanisms and Neoplasia". Symp. Fundamental Cancer Res. 19, 67 (1965).
7. Stenesh, J., and Schechter, N., J. Bacteriol. 98, 1258 (1969).
8. Morris, A., Arlinghaus, R., Favelukes, S., and Schweet, R., Biochemistry 2, 1084 (1963).
9. Traut, R. R., and Monro, R. E., J. Mol. Biol. 10, 63 (1964).
10. Morris, A., Favelukes, S., Arlinghaus, R., and Schweet, R., Biochem. Biophys. Res. Commun. 7, 326 (1962).
11. Rabinovitz, M., and Fisher, J. M., J. Biol. Chem. 237, 477 (1962).
12. Algranati, I. D., and Lengyel, P., J. Biol. Chem. 241, 1778 (1966).
13. Friedman, S. M., and Weinstein, I. B., Biochim. Biophys. Acta 114, 593 (1966).