THE EFFECT OF FUSIDIC ACID ON PROTEIN SYNTHESIS IN A MAMMALIAN SYSTEM

Sir:

Fusidic acid and related steroidal antibiotics inhibit protein synthesis in the *in vivo* and *in vitro* bacterial systems[1,2,3]. In an *E. coli* system, they inhibit ribosome-dependent GTPase activity of G factor, and the grade of inhibition is parallel to that of polypeptide synthesis[4]. The antibiotic seems to be a specific inhibitor of G factor[4,5], and to inhibit translocation of peptidyl-tRNA on the ribosomes[4,8].

For the purpose of elucidating the biochemical basis of the selective toxicity, the sensitivity of mammalian protein-synthesizing systems to fusidic acid has been investigated. It has been observed that fusidic acid inhibits ribosome-dependent GTPase activity of TF-II, using a purified reticulocyte system. The results are presented in this communication.

Reticulocytes were collected from rabbits, which had been injected with phenylhydrazine[6]. Washed ribosomes and transferases (TF-I and TF-II) were prepared by the method of ARLINGHAUS *et al.*[6] or by that of FELICETTI and LIPMANN[7]. GTPase assay was performed, following FELLICETTI and LIPMANN[7]. ^{14}C-amino acid mixture of *Chlorella* protein hydrolysate was labelled to reticulocyte sRNA.

Protein synthesis was inhibited by fusidic acid, phenomycin, and blasticidin S in the highly fractionated reticulocyte system with endogenous mRNA. Ribosome-dependent GTPase activity of TF-II was significantly inhibited by fusidic acid, but not by phenomycin and blasticidin S. The grade of inhibition by fusidic acid of both reactions was parallel and it was comparable to what was observed with the bacterial system. The results summarized in Tables 1 and 2 show that fusidic acid inhibits TF-II GTPase activity and hence protein synthesis in the mammalian system.

The present study suggests that the basic mechanism of amino acid polymerization or the function of polymerization factors is similar in the bacterial and mammalian systems. TF-II is the mammalian equivalent of G-factor. However, less grade of inhibition by fusidic acid of protein synthesis was

Table 1. Inhibition by antibiotics of protein synthesis in a reticulocyte system.

Series		Incorporation of ^{14}C-amino acids	
		cpm/mg protein	% inhibition
Complete		1,100	
− TF-I		234	
− TF-II		51	
− TF-I, TF-II		24	
− ribosomes		0	
− GTP		34	
+ Fusidic acid	370 μM	187	83
//	37	439	60
+ Phenomycin	10	262	76
//	1	338	69
+ Blasticidin S	23.7	287	74

The assay for protein synthesis was performed in the reaction mixture, containing (per ml): 0.1 M KCl-washed ribosomes 500 μg, TF-I 100 μg, TF-II 130 μg, GTP 0.05 μmoles, $MgCl_2$ 6.7 μmoles, KCl 67 μmoles, Tris-HCl, pH 7.5, 33 μmoles, GSH 10 μmoles, aminoacyl-tRNA of ^{14}C-amimo acid mixture of chlorella protein (83,000 cpm/mg RNA) 4,340 cpm, in a total volume of 0.5 ml. It was incubated for 10 minutes at 37°C. The radioactivity of the hot TCA-insoluble fraction was determined in a windowless gas flow counter.

Table 2. Effects of antibiotics on ribosome-dependent GTPase activity of TF-II.

Series		GTP hydrolyzed
Complete		100*
− ribosomes		16
− TF-II		6
+ Fusidic acid	370 μM	21
//	37	51
+ Phenomycin	10	121
//	1	107
+ Blasticidin S	237	112

* 100=7,420 cpm/0.2 ml

The ribosome-dependent GTPase activity of TF-II was assayed by measuring liberation of radioactive inorganic phosphate from GTP-γ-^{32}P[7]. The reaction mixture contained (per ml): 0.5 M NH_4Cl-washed ribosomes 585 μg, TF-II 146 μg, GTP-γ-^{32}P (2×10^6 cpm/μmole) 0.1 μmole, DTT 16 μmoles, KCl 80 μmoles, $MgCl_2$ 10 μmoles, and Tris-HCl, pH 7.4, 50 μmoles. It was incubated for 15 minutes at 37°C. The radioactivity was determined in a GM counter.

observed in a crude extract of reticulocytes. The mechanism of selective toxicity of fusidic acid, including certain barriers, remains to be determined.

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