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THE MODE OF ACTION OF ZALUZANIN C, AN INHIBITOR OF TRANSLATION IN EUKARYOTES

F. Santamaría, E. Zaera, D. Vázquez and A. Jiménez

Instituto de Biología Molecular C.S.I.C. and Departamento de Microbiología. Universidad Autónoma de Madrid. Canto Blanco. Madrid-34. Spain

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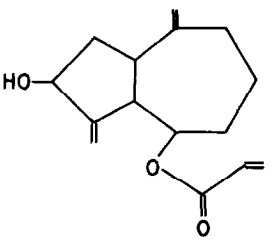
Zaluzanin C, a substance extracted from several species of the genus Zaluzania (Compositae), has been shown to inhibit protein synthesis in intact HeLa cells preferentially to DNA and RNA synthesis. "In vitro" protein synthesis was also blocked by zaluzanin C and the study of the effects of the drug on resolved model systems indicates that it inhibits enzymic translocation of peptidyl-tRNA specifically.

Zaluzanin C has a rather unusual structure (Figure 1) and is obtained in extracts from different species of compositae plants of the genus Zaluzania (1,2,3). Since the drug has been reported to have a certain inhibitory activity towards the lymphocytic leukemia (3), we have studied its inhibitory activity on (a) synthesis of macromolecules by HeLa cells and (b) the resolved steps of protein synthesis in rabbit reticulocyte and yeast cell-free systems. In these systems we have observed that zaluzanin C specifically blocks enzymic translocation of peptidyl-tRNA.

MATERIALS AND METHODS

High salt washed ribosomes and polyribosomes from yeast, partially purified yeast supernatant fraction containing elongation factors EF1 and EF2, (^{3}H)Phe-tRNA and rabbit reticulocyte ribosomes and purified elongation factors EF1 and EF2 were prepared as described (4,5). The assay systems for poly(U)- and endogenous mRNA-directed polypeptide synthesis, enzymic binding of (^{3}H)Phe-tRNA to reticulocyte ribosomes and the enzymic and non enzymic translocation of peptidyl-tRNA by yeast polyribosomes were as described (4,6,7). Saccharomyces cerevisiae Y166 (4) was used unless otherwise indicated. Zaluzanin C was provided by J.J. Hoffmann, College of Pharmacy, University of Arizona, Tucson (USA).

0006-291X/84 $1.50



① Zaluzanin-C

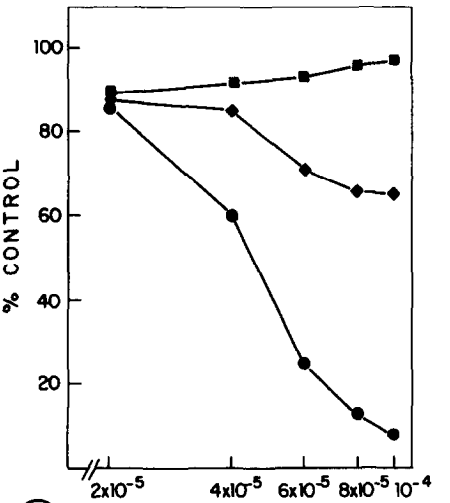


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Figure 1. Chemical structure of zaluzanin C.

Figure 2. The incorporation of ^{35}S-methionine (1450 Ci/mmol) (●-●), ^{3}H-uridine (50 Ci/mmol) (■-■) and ^{3}H-thymidine (52 Ci/mmol) (◆-◆) into the hot trichloroacetic acid-insoluble fraction was determined as described (13), using HeLa cell tissue cultures, as a measure of protein, RNA and DNA synthesis respectively. The uptake of radioactive compounds took place for three hours. The results are presented as percentage of incorporation of control radioactive compounds in the absence of the inhibitor. Essentially similar results were obtained when the uptake was studied at 1,2,4 and 5 hours of incorporation.

RESULTS AND DISCUSSION

When the effects of zaluzanin C on the synthesis of macromolecules by whole HeLa cells were studied it was observed that the drug at growth inhibitory concentrations blocked protein synthesis and accordingly also inhibited synthesis of DNA and RNA, to a lesser extent, as expected (Figure 2). Indeed, zaluzanin C was very effective in blocking both endogenous mRNA-directed polypeptide synthesis and poly(U)-directed polyphenylalanine synthesis in yeast cell-free systems (Figure 3). However, the drug does not affect either (^{3}H)Phe-tRNA binding to ribosomes or peptide bond formation (Table 1). On the other hand zaluzanin C inhibits the enzymic translocation of peptidyl-tRNA in yeast polyribosomes at concentrations of the drug similar to those required to block

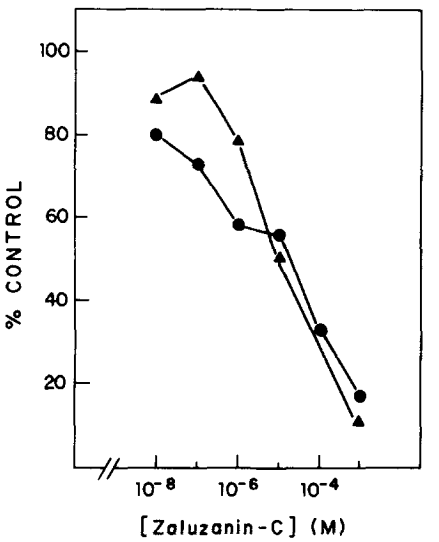

Figure 3. Effects of zaluzanin C on poly(U)- and endogenous mRNA-directed polypeptide synthesis in yeast cell-free systems. Reaction mixtures for poly(U)-directed polyphenylalanine synthesis (100 ul) contained 50 mM Tris-HCl buffer, pH 7.4; 60 mM KCl; 11 mM $MgCl_2$; 9 mM 2-mercaptoethanol; 0.25 mM GTP; 2.5 ug poly(U); 0.8 OD_{260} yeast ribosomes, about 2 mg/ml yeast supernatant fraction containing EF1 and EF2 and (^{3}H)Phenylalanine 30 (uCi/umol). In the controls in the absence of drug 230 pmol (^{3}H)Phe were incorporated. Endogenous mRNA-directed polypeptide synthesis by yeast polyribosomes was performed by following the incorporation of (^{14}C)Leu (140 uCi/umol) as previously described (4). In the control in the absence of the drug, 12 pmol (^{14}C)Leu were incorporated (●-●) Poly(U) and (▲—▲) endogenous mRNA-directed synthesis.

Table 1

Effects of zaluzanin C on the binding of aminoacyl-tRNA to ribosomes, and peptide bond formation

Zaluzanin C concentration (M)	Percent of control		
		Peptide bond formation	
	(^{3}H)Phe-tRNA binding	Fragment reaction	Puromycin reaction
2×10^{-5}	84	100	101
2×10^{-4}	82	100	107
2×10^{-3}	81	100	110

Reaction mixtures for aminoacyl-tRNA binding (100 µl) contained 50 mM Tris-HCl buffer, pH 7.4; 60 mM KCl; 6 mM $MgCl_2$; 0.1 mM GTP; 5 µg poly(U); 10 pmol (^{3}H)Phe-tRNA (470 uCi/µmol); 1 OD_{260} rabbit reticulocyte ribosomes, and 1.8 ug EF1. Reactions took place for 20 min at 37°C; 2.9 pmol (^{3}H)Phe-tRNA were bound to ribosomes in the control reactions in the absence of the drug. The fragment and the puromycin reactions to study peptide bond formation were performed with yeast ribosomes as described (7).

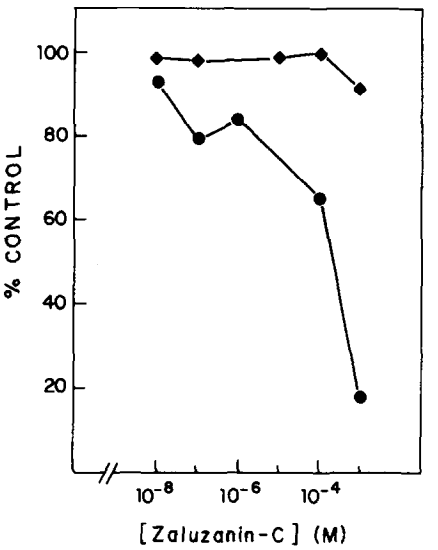

Figure 4. The effects of zaluzanin C on the enzymic and non-enzymic translocation of peptidyl-tRNA. In the enzymic translocation by yeast polyribosomes (4) 7.1 and 3.5 pmol peptidyl-(^{3}H)puromycin were synthesized in the controls in the presence and in the absence of added yeast supernatant factor respectively. In the non-enzymic translocation by yeast polyribosomes (6) 16.1 and 3.4 pmol peptidyl-(^{3}H)puromycin were synthesized in the controls (plus KCl and minus KCl) in the absence of the drug (●–●) Enzymic and (◆–◆) non-enzymic translocation.

polypeptide synthesis (Figure 4). Since the drug does not affect the non-enzymic translocation that takes place in the presence of 1 M KCl without the requirement of EF2 and GTP, it appears that zaluzanin C specifically prevent the action of EF2 at the ribosomal level. Zaluzanin C is also an active inhibitor of translocation in polyribosomes from yeast resistant mutants to some other inhibitors of translocation such as cycloheximide (strain SR_{17}) (4) and cryptopleurine (strain CRY 6) (8). It is remarkable indeed that, unlike the case of prokaryotic ribosomes, translocation in eukaryotic ribosomes appears to be affected by an increased number of inhibitors acting possibly in independent sites including cycloheximide, emetine, pederine, hygromycin B, cryptopleurine (9, review), bouvardin (10), cyclopiazonic acid (11) 6,6'-dihydroxythiobinuphuridine and PR toxin (12). Hence, these inhibitors should be important tools to elucidate the different ribosomal sites involved in the complex step of translocation.

ACKNOWLEDGEMENT

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