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INHIBITION BY FUSIDIC ACID OF THE REACTION BETWEEN PUROMYCIN AND DONOR SITE BOUND *N*-ACETYL-PHENYLALANYL-tRNA ON YEAST RIBOSOMES

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1. Introduction

Fusidic acid is a well known inhibitor of both prokaryotic and eukaryotic protein synthesis [1]. It interferes with the elongation of the peptide chain by interacting with the translocation factor, forming an EF-G-GDP-ribosome–fusidic acid complex [2–4]. The working mechanism has been under discussion continuously. Several modes of action have been proposed: inhibition of translocation of peptidyl-tRNA and subsequent release of deacylated tRNA from the donor site [5–9], inhibition of the translocation after one additional translocation step [4,10], and on the other hand the inhibition of binding of aminoacyl-tRNA to the ribosomal acceptor site [10–13]. These at first sight contradictory findings were reconciliated by the discovery that there is only one binding site for aminoacyl-tRNA (bound enzymatically or non-enzymatically) and EF-G. There is now a vast amount of evidence both for prokaryotic ribosomes [14–19] and eukaryotic ones [20–22] that EF-Tu(EF-1) and EF-G(EF-2) interact in a mutually exclusive fashion with ribosomes. Thus fusidic acid stabilized binding of the translocation factor to ribosomes inhibits the subsequent binding of aminoacyl-tRNA.

However, another complication in the use of fusidic acid seems to be the dependence on the relative amounts of fusidic acid, of EF-G(EF-2) and of ribosomes present in the incubation mixture [20,23,24]. If too less fusidic acid or free ribosomes are present to trap all EF-G (or EF-2) in an EF-G-GDP-ribosome–fusidic acid complex, the not sequestered EF-G can continue to catalyze translocation reactions.

In this paper we present evidence, that fusidic acid inhibits the reaction between puromycin and donor site-bound *N*-Ac-[^{14}C] Phe-tRNA too. This effect of fusidic acid has not been reported untill now.

2. Methods

Sources of reagents and methods for preparing yeast ribosomal subunits, *N*-Ac-[^{14}C] Phe-tRNA, enzyme fractions, treatment of EF-2 with diphtheria toxin and incubations were essentially as described previously [25,26].

3. Results

When *N*-Ac-[^{14}C] Phe-tRNA is bound non-enzymatically to yeast ribosomes, addition of EF-2 leads to an additional binding of *N*-Ac-[^{14}C] Phe-tRNA, which is inhibited by fusidic acid or pretreatment of EF-2 with diphtheria toxin (table 1). We have explained this finding by assuming that, after translocation, the acceptor sites of the ribosomes had become available for the additional binding of *N*-Ac[^{14}C] Phe-tRNA [25].

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Abbreviations:
EF-Tu, EF-G, EF-1, EF-2: elongation factors Tu, G, 1 and 2; *N*-Ac-Phe-tRNA: *N*-acetyl-phenylalanyl-tRNA

Table 1
EF-2 mediated binding of *N*-Ac-[^{14}C]Phe-tRNA in a pyromycin-reactive state and inhibition by fusidic acid of *N*-Ac-[^{14}C]Phe-puromycin formation

Exp.	Additions		Molecules bound per ribosome	Molecules released by puromycin per ribosome
	1st step	2nd step		
1.	none	none	0.27	0.06
2.	EF-2	none	0.50	0.19
3.	EF-2(inactivated by diphtheria toxin)	none	0.28	0.06
4.	EF-2+2 mM fusidic acid	none	0.27	0.03
5.	EF-2	2 mM fusidic acid	0.50	0.03

N-Ac-[^{14}C]Phe-tRNA was bound to ribosomes in an incubation mixture containing 40S and 60S ribosomal subunits, poly(U), an energy generating system and *N*-Ac-[^{14}C]Phe-tRNA at 24 mM $MgCl_2$. Where indicated fusidic acid, EF-2 or EF-2, inactivated by NAD and diphtheria toxin were added. This is called the first step of the incubation. After 45 min puromycin and KCl (final concentration 1 mM and 0,2 M respectively) were added and fusidic acid only in Exp. 5, and the incubation was continued for another 15 min. This is called the second step of the incubation. Binding and formation of *N*-Ac-[^{14}C]Phe-puromycin were measured as described. Also for further details see references [25] and [26].

Both fusidic acid and pretreatment of EF-2 with diphtheria toxin inhibited the reaction of *N*-Ac-[^{14}C] Phe-tRNA and puromycin. An explanation for this observation is that both inhibitors restrain *N*-Ac-[^{14}C] Phe-tRNA on the ribosomal acceptor site. This would mean that fusidic acid does not allow under these circumstances one additional round of translocation. In order to subject this assumption to a further proof, the influence of fusidic acid on the peptidyl-transferase was tested.

It appeared that, when fusidic acid was added to an incubation mixture containing ribosomes which had bound *N*-Ac-[^{14}C] Phe-tRNA in a puromycin reactive site, it completely inhibited the reaction of this *N*-Ac-[^{14}C] Phe-tRNA with puromycin (table 2, exp. 4).

The rather high fusidic acid concentration of 2 mM was suspected to have had a non-specific inhibitory effect on the peptidyl-transferase mediated reaction of *N*-Ac-[^{14}C] Phe-tRNA with puromycin. Therefore lower fusidic acid concentrations were tested. Table 2 demonstrates that the inhibitions of the peptidyl-transferase reaction is also found at lower concentrations. At 0.5 mM this reaction is even more sensitive to fusidic acid than the EF-2 mediated binding of *N*-Ac-[^{14}C] Phe-tRNA.

Table 2
Effect of fusidic acid concentration on binding and release by puromycin of *N*-Ac-[^{14}C]Phe-tRNA

EF-2	Fusidic acid concentration (mM)	Molecules bound per ribosome	Molecules released per ribosome
–	0	0.155	not determined
+	0	0.250	0.090
+	0.1	0.235(15%)	0.070(20%)
+	0.5	0.230(20%)	0.038(60%)
+	1	0.190(65%)	0.033(65%)
+	2	0.155(100%)	0.018(80%)

N-Ac-[^{14}C]Phe-tRNA was bound to ribosomes, fusidic acid was added during the binding reaction and in parallel incubations only during the puromycin reaction. The formation of *N*-Ac-[^{14}C]Phe-puromycin was measured [25,26]. In parentheses the percentage inhibition of the EF-2 mediated additional binding and of the reaction with puromycin are given.

4. Discussion

From experiments designed to answer questions about the mode of action of fusidic acid a hitherto not found inhibition of the peptidyl-transferase reaction by fusidic acid emerged. It appeared that 80S ribosomes from yeast are inhibited with respect to the reaction of donor site bound *N*-Ac-[^{14}C] Phe-tRNA and puromycin.

This inhibition is probably not a direct inhibition of the peptidyl-transferase reaction. At least if we assume that this reaction can be measured by the so called fragment reaction in the presence of 30% alcohol. It has been found [27, and our own unpublished results] that under these circumstances fusidic acid has no inhibitory activity on eukaryotic ribosomes.

Our results are in contradiction with these obtained for 70S ribosomes [12]. It seems that there is a difference in fusidic acid sensitivity between prokaryotic and eukaryotic ribosomes in such a way that the presence of the EF-2-fusidic acid–GDP complex impairs the peptidyl-transferase activity.

Thus caution is needed in interpreting effects of fusidic acid for the elucidation of ribosomal functioning.

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