THE EFFECT OF L-1-TOSYLAMIDO-2-PHENYLETHYL CHLOROMETHYL KETONE ON THE ACTIVITY OF PROCARYOTE AND EUCARYOTE tRNA BINDING FACTORS

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1. Introduction

TPCK has been reported to be an inhibitor of protein synthesis in *E. coli* and *Bacillus stearothermophilus* [1–3]. Recently, it has been shown to inhibit specifically the EF-T mediated binding of Phe-tRNA to the ribosome by irreversibly destroying the ability of EF-Tu · GTP to combine with Phe-tRNA and form the ternary complex [4]. The studies reported here confirm the earlier findings that TPCK inhibits only EF-Tu function in a homologous bacterial system and extend the observation by demonstrating that: TPCK also inhibits the function of EF-Tu in a heterologous bacterial–avian system but does not inhibit EF-1 function in either a homologous avian or mammalian system.

2. Materials and methods

Bacterial ribosomes were prepared as previously described [5]. EF-Tu, EF-Ts and EF-G were prepared according to the method of Arai et al. [6], and purified through the G-100 step. The units of EF-Ts, EF-Tu and EF-G are as defined by Arai et al. [6]. Labelled Phe-tRNA was made by the method of Conway [7]. Avian ribosomes were prepared from chicken livers by a preparative adaptation of the method of Adelman et al. [8] for direct isolation of ribosomal subunits from the microsomal fraction [9]. EF-1 was prepared from the S-100 of the same cells by successive ammonium sulfate precipitation, Sephadex G-200 and hydroxyapatite steps, based on McKeehan and Hardesty [10].

Rabbit reticulocyte ribosomes and S-100 were prepared as described previously by Bulova and Burka [11]. TPCK was purchased from Serva, poly U from Miles, GTP from Boehringer, [^{3}H]Phe (specific activity of 12 Ci/mmole), [^{14}C]Phe (specific activity of 513 mCi/mmole) from Amersham and [^{14}C]Val (specific activity 207 mCi/mmole) from New England Nuclear.

3. Results and discussion

As shown in table 1, TPCK treatment of a bacterial S-100 completely inactivates it for polyphenylalanine synthesis. To determine the specificity of this inhibition, three separate reactions were carried out in which an S-100, treated with TPCK, was supplemented with either EF-Tu, EF-Ts or EF-G and the extent of polyphenylalanine synthesis measured. Only the addition of EF-Tu, table 1, restored activity. Addition of excess EF-Ts or EF-G was without effect.

Since TPCK specifically inactivated EF-Tu, we sought to determine if the analogous enzyme in a eucaryotic system would also be inhibited. We measured, therefore, EF-1 dependent Phe-tRNA binding, in the presence and absence of TPCK, in a system derived from chicken liver. In a parallel control experiment, EF-Tu dependent Phe-tRNA binding to *E. coli* ribosomes was measured under the identical conditions. The results of both experiments are shown in table 2. EF-Tu dependent binding is essentially completely inhibited, while EF-1 dependent binding is unaffected.

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Table 1
The effect of TPCK on polyphenylalanine synthesis in an *E. coli* synthesizing system.

Treatment	pmoles [^{14}C]Phenylalanine incorporated
Control S-100	2.6
TPCK treated S-100	0.0
TPCK treated S-100 + EF-Tu	4.5
TPCK treated S-100 + EF-Ts	0.2
TPCK treated S-100 + EF-G	0.1

Reaction mixtures contained in 50 μl, 50 mM Tris–HCl (pH 7.4), 0.16 M NH_4Cl, 10 mM $MgCl_2$, 1 mM DTT, 50 nmoles GTP, 2 μg [^{14}C]Phe-tRNA (containing 7.1 pmoles Phe), 2 μg S-100 protein, 1.4 pmoles ribosomes, and 1 unit of EF-Tu, 20 units of EF-Ts or 250 units of EF-G, where indicated. Incubation was for 10 min at 30°C. The S-100 was treated with a 1:20 dilution of 10 mM TPCK in methanol in 20 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$, 1 mM DTT, and 10 μM GDP. It was treated 10 min at ambient temperature and then 2 μl was added to the reaction mixture. The control S-100 was treated similarly with methanol only.

Table 2
The effect of TPCK treatment on enzymatic Phe-tRNA binding.

System employed	Percent of untreated control
Homologous *E. coli*	8
Homologous avian	100
Heterologous *E. coli*–avian	4

Each reaction mixture contained in 50 μl, 50 mM Tris–HCl (pH 7.4), 60 mM NH_4Cl, 8 mM $MgCl_2$, 10 mM DTT, 2 nmoles GTP, 10 μg poly U and 20 μg [^{3}H]Phe-tRNA (containing 7.1 pmole Phe). In the homologous bacterial system 8 pmoles of ribosomes, 1 unit of EF-Tu and 20 units of EF-Ts were also present. In the homologous avian system 5 pmoles of ribosomes and 0.6 units of EF-1 were present. One unit of EF-1 is defined as the amount of enzyme which catalyzes the binding of 1 pmole of Phe-tRNA in 5 min at 0°C. In the heterologous bacterial–avian system 5 pmoles of avian ribosomes, 1 unit of EF-Tu and 20 units of EF-Ts were present. TPCK treatment of EF-Tu or EF-1 was as described in table 1. The amount of Phe-tRNA bound was measured after an incubation of 5 min at 0°C. The values reported are percentages of control reactions treated with methanol only. The binding in the control systems were: homologous bacterial 0.1 pmole Phe-tRNA bound/pmole ribosome, homologous avian 0.04 pmole Phe-tRNA bound/pmole ribosome and heterologous avian–bacterial 0.03 pmole Phe-tRNA bound/pmole ribosome.

Table 3
The effect of TPCK on protein synthesis in a reticulocyte cell-free system.

Treatment	pmoles [^{14}C]Valine incorporated
Control system	5.8
TPCK treated S-100	6.7
TPCK treated ribosomes	6.0

The incorporation of [^{14}C]Val into protein was assayed in the reticulocyte cell-free system previously described by Bulova and Burka [10]. Incubation was for 45 min at 37°C. Each 200 μl reaction contained 3 A_{260} units of ribosomes and 10 μl of ribosome-free supernatant (S-100) as a source of soluble factors and tRNA. TPCK treatment of ribosomes or S-100 was as described in table 1.

Since it had been shown by Krisko et al. [12] that EF-Tu could function in a heterologous procaryote–eucaryote system, we thought it of interest to measure the effect of TPCK on this activity of EF-Tu. To do this, we prepared a heterologous bacterial–avian system composed of EF-Tu, EF-Ts and chicken liver ribosomes and measured the effect of TPCK on Phe-tRNA binding. The results of such an experiment are also shown in table 2. EF-Tu function in this system is completely inhibited as in the homologous bacterial system.

Finally, we sought to determine the effect of TPCK in a mammalian system. We employed a reticulocyte cell-free synthesizing system and compared the level of synthesis in a control reaction with that in which either the S-100 or ribosomes were treated with TPCK prior to polymerization. As can be seen in table 3, TPCK treatment is without effect, consistent with our observations in the avian system.

The fact that EF-Tu can substitute for EF-1 in either polyphenylalanine synthesis or Phe-tRNA binding, suggests that some homology exists between the two factors [2]. It therefore seemed of interest to determine whether TPCK, a reported inhibitor of EF-Tu function, would also inhibit EF-1. The results reported here show that although TPCK does inhibit EF-Tu activity in a heterologous bacterial–avian system, it does not inhibit EF-1 in either an avian or mammalian bacterial–avian system. TPCK is thus non-toxic for eucaryote protein synthesis. This is of interest in relation to the current use of protease in-

hibitors as tools in the elucidation of the mechanism of the expression of malignancy [13–15].

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