AMINOACYL-tRNA-DEPENDENT INTERACTION OF A TRANSFER ENZYME FROM YEAST WITH A COMPLEX FORMED BY TWO OTHER TRANSFER FACTORS WITH GUANOSINE TRIPHOSPHATE

D.RICHTER and F.KLINK

Institute of Physiological Chemistry, University of Kiel, Kiel, Germany

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1. Introduction

From yeast cell supernatant three amino acid transfer-active protein fractions (1-P; 2-P; 3-P) were isolated, two of which (1-P and 2-P) were complementary in ribosomal peptide synthesis [1]. The two complementary fractions were purified and tested for functional behaviour [2]: 1-P was able to bind ^{3}H-GTP; further purification led to a decrease of this activity. From 2-P a factor T_2 was obtained which caused ribosome-dependent GTP hydrolysis only in combination with 1-P. The purified factors no longer complemented each other in peptide synthesis [2].

In the present paper we want to show that – similar to the results from *E. coli* [3–5] and *Bac. stearothermophilos* [6] – the GTP-binding yeast fraction can be separated into two factors (T_1 and T_3) which are both necessary for the binding function. Addition of T_2 and phenylalanyl-tRNA causes the removal not only of the γ-phosphate but also of the nucleotide moiety from the GTP-binding enzymes. The binding of Phe-tRNA to ribosomes from liver depends on T_1, T_3 and GTP. Supplementation with T_2 increases the Phe-tRNA binding but does not result in peptide synthesis.

2. Methods and materials

For materials, for isolation and further purification of 1-P and 2-P and for preparation of poly U-dependent ribosomes from liver see ref. [2]. For assay methods for GTPase activity and amino acid polymerization see ref. [2] except that all incubation mixtures additionally contained 0.06 M $NH_4(CH_3COO)$. For assay methods for GTP-enzyme complex formation see table 1, for ^{14}C-tRNA binding to ribosomes see table 2.

3. Results and discussion

Separation of the GTP-binding fraction 1-P into two complementary factors was achieved with gel filtration on Sephadex G-200 (fig. 1). In the two peaks only little activity was detected, but a combination of the two peak fractions proved highly active. We called the lighter fraction T_1, the heavier one T_3. The amount of the complex formed with GTP increased with the concentration of either factor (fig. 2). In contrast to Ravel et al. [4] we have not been able to demonstrate a dependence on Phe-tRNA (see table 1), and the amounts of the complex recovered from Sephadex G-50 were twice as high in relation to μg enzyme, as those retained by millipore filters. It remains to be investigated how far aminoacyl-tRNA still contained in the enzyme, or the relatively crude Phe-tRNA preparations used in this test, are responsible for this.

As shown in a preceding paper, one of the GTP-binding factors (T_1), when combined with T_2, had a Phe-tRNA-dependent GTPase effect which was enhanced by the addition of ribosomes and poly U [2]. Binding tests with ^{3}H-GTP suggested that the fraction T_2 which was complementary to T_1 in GTP hydrolysis, contained certain amounts of T_3. Some T_2 preparations which may have been less contaminated by T_3, only split GTP in the presence of T_1 and T_3. Hence one function of T_2 is to remove the

Table 1
Influence of T_2 on the ^{3}H-GTP-binding reaction.

	^{3}H-GTP-binding in $\mu\mu$moles tested with		(γ-^{32}P) GTP hydrolysis in $\mu\mu$moles
	Sephadex G-50	Millipore filter	
$T_1 + T_3$	103	74	35.8
+ ^{12}C-Phe-tRNA	105	70	53.9
+ T_2	95	88	230.0
+ ^{12}C-Phe-tRNA + T_2	29	28	690.0

Millipore filter and GTPase tests: 0.5 ml of buffer 1 (0.05 M Tris-HCl, pH 7.6; 0.1 M KCl; 0.06 M $NH_4(CH_3COO)$; 0.012 M $Mg(CH_3COO)_2$; 0.001 M dithiothreitol) contained 120 μg of T_1; 50 μg of T_3, 2.5 mμmoles of ^{3}H-GTP, 2.5 mμmoles of (γ-^{32}P) GTP, and, as indicated, 80 μg of ^{12}C-Phe-tRNA and 70 μg of T_2. After 10 min at 30°, 1.5 ml cold buffer 1 was added; 0.5 ml of the dilution was tested for hydrolysis, 1.5 ml for binding. The values were calculated for the total of 0.5 ml.
Gel filtration tests: reaction conditions as above. The chilled solutions were filtered through columns (1 X 13 cm). The GTP-binding values represent the peak fractions of the excluded material.

Table 2
Transfer of ^{14}C-phenylalanyl-tRNA to liver ribosomes.

	Binding of ^{14}C-phenylalanyl-tRNA to ribosomes (cpm)		Polyphenylalanine synthesis * in the presence of ribosomes (cpm)	
	+ Poly **	– Poly U	+ Poly U	– Poly U
Complete ***	173	34	35	15
– T_1, T_3, GTP	83	25	15	0
– Ribosomes	9	10	10	0
– GTP	76	38	3	0
– T_1	81	15	2	0
– T_3	95	35	5	0
– T_1, T_3	75	35	9	0
Complete + T_2 †	291	30	40	0
Complete †	198	–	38	–

* In this system a crude enzyme mixture incorporated 600 cpm into polyphenylalanine.
** 100 μg.
*** Complete mixture contained in 0.5 ml of buffer 1 : 200 μl of ribosome solution (2.3 Å units (260 mμ, 1%, 1 cm)); 80 μg of ^{14}C-Phe-tRNA = 4500 cpm; 20 mμmoles of GTP; 80 μg of T_1 and 50 μg of T_3. After 15 min at 30°, 3 ml of cold buffer 1 were added; the ribosome-bound Phe-tRNA was determined according to Nirenberg and Leder [7].
† The complete mixture was incubated as above, chilled and again incubated for 10 min at 30° in the presence or absence of 70 μg of T_2.

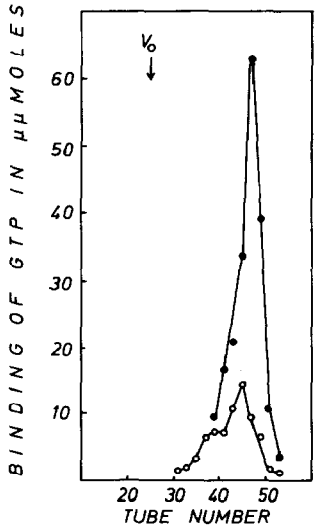

Fig. 1. Gel filtration of 1-P (1 ml = 2.1 mg protein) on Sephadex G-200. Column size : 2.5 × 45 cm. Elution ascendent at the rate of 5 ml/hr. Binding tests with filter technique, (see table 1, except incubation for 2 min at 2°). ^{3}H-GTP binding by: o——o, 200 μl of the fractions alone; •——•, 200 μl of the fractions in the presence of the combined fractions 36–38.

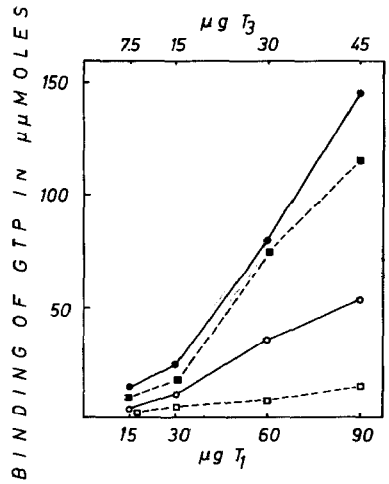

Fig. 2. ^{3}H-GTP binding by T_1 and T_3. T_1 and T_3 were isolated as shown in fig. 1. Incubation 10 min at 2°. Binding assay with filter technique (see table 1). Binding of ^{3}H-GTP by: □——□, T_3; ■——■, T_3 plus 90 μg of T_1 (the blank of 90 μg T_1 = 55 μμmoles GTP was already subtracted); o——o, T_1; •——•, T_1 + 45 μg T_3 (the blank of 45 μg T_3 = 15 μμmoles was subtracted).

γ-phosphate from the GTP-enzyme complex. For this, Phe-tRNA is required.

Table 1 shows another function of T_2: The ^{3}H-GTP binding effects obtained by millipore or gel filtration techniques were three times lower when T_2 and Phe-tRNA were both present. Apart from releasing the γ-phosphate, T_2 obviously also causes a separation of the nucleotide moiety from the nucleotide-enzyme complex. Both effects were observed in the absence of ribosomes and required higher temperatures (30°) than the complex formation which rapidly occurred at 2°.

A participation of the T factors in ribosomal processes is shown in table 2. The enzymatic binding of Phe-tRNA to poly U-linked ribosomes depends upon T_1, T_3 and GTP. No formation of TCA-insoluble peptides happened. The combination (T_1 + T_3) thus corresponds to the T factors from bacteria [4–6], to "Transferase I" from liver [8], to "factor TF 1" from reticulocytes [9] and to "fraction A" from yeast [10]. Having performed Phe-tRNA binding with T_1, T_3 and GTP we were able additionally to attach about an equal amount of Phe-tRNA to the ribosomes by supplying T_2 (table 2). Polymerization tests proved clearly that even now no polyphenylalanine synthesis had taken place. The catalysis of a tRNA translocation on the ribosome with release of energy required and simultaneous removal of the nucleotide moiety and of the γ-phosphate from the aminoacyl-tRNA-enzyme-ribosome complex could be discussed as a specific role of T_2. Possibly yeast T_2 corresponds to a part of the factor G from *E. coli*; in that case the yeast enzyme must have lost a partial function closely connected with peptide synthesis itself. Experiments in progress will show whether more evidence can be found for the translocation function of T_2, and whether the system can be completed for polyphenylalanine synthesis by addition of a fourth transfer factor.

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