SPECIFICITY OF FACTORS REQUIRED FOR PEPTIDE ELONGATION IN MAMMALIAN CELLS

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Received 30 July 1970

1. Introduction

Peptide elongation on mammalian ribosomes requires two different enzymes present in the soluble fractions of the cell. Transferase I binds the aminoacyl-tRNA to the acceptor site [1] while the translocation of peptidyl-tRNA from the donor to the acceptor site is catalyzed by transferase II [2]. Transfer factors from different mammalian species are apparently interchangeable [3]. Even bacterial T factor can replace transferase I in mammalian systems [4]. Similarly, algal transfer factors are active with ribosomes from *E. coli* [5]. On the other hand, *E. coli* G factor cannot replace mammalian TF II [4]. A similar result was found with yeast transfer enzymes tested with *E. coli* ribosomes [6]. These results seem to indicate that peptide elongation reactions are limited by species- or perhaps even organ-specificity. Moreover, no systematic experiments were reported as yet on the specificity of the other tissue components used in peptide elongation experiments, i.e. ribosomes and tRNA.

This paper reports results obtained with different combinations of tissue preparations from rat liver and human tonsils that indicate a considerable species-specificity of some of these components.

2. Materials and methods

Peptide elongation factors from rat liver and human tonsils were prepared by the method of Bermek and Matthaei [7, 8]. Ribosomes from both tissues were purified as described by the same authors [9]. Methods used for the assay of polyU-directed polyphenylalanine synthesis were described elsewhere [10, 11]. All incubation mixtures contained saturating quantities of tRNA and both elongation enzymes while the amount of ribosomes was kept limiting.

3. Results and discussion

A summary of results obtained with 3 different batches of elongation factors and ribosomes and with 2 preparations of tRNA from both sources are given in tables 1 and 2.

Apparently a rather low species-specificity is exhibited by tRNA in peptide elongation reactions. This explains why even bacterial tRNA may be succsessfully used with mammalian ribosomes and transfer factors [3, 7–9]. Addition of heterologous ribosomes

Table 1
Polyphenylalanine synthesis in combined systems containing ^{14}C-tRNA from rat liver.

Transferase I	Transferase II	Ribosomes	
		Rat	Human
rat	rat	29.0	8.7
rat	human	48.0	48.9
human	rat	10.4	4.2
human	human	32.5	34.0

Incubation mixtures contained 0.8 mg of ribosomes/ml, saturating amounts of tRNA and both elongation factors as well as all other components of the standard polymerization assay [10]. All values are pmoles of ^{14}C-Phe polymerized/ml of reaction mixture (30 min, 37°).

Table 2
Polyphenylalanine synthesis in combined systems containing ^{14}C-Phe-tRNA from human tonsils.

Transferase I	Transferase II	Ribosomes	
		Rat	Human
rat	rat	22.2	6.6
rat	human	44.6	38.0
human	rat	5.5	7.8
human	human	41.3	41.0

Incubation mixtures contained 0.8 mg of ribosomal/ml, saturating amounts of tRNA and both elongation factors as well as all other components of the standard polymerization assay [10]. All values are pmoles of ^{14}C-Phe polymerized/ml of reaction mixture (30 min, 37°).

to rat liver systems significantly inhibits peptide elongation. However, no such effect is seen with human tonsil systems. Also transferase I shows a high species-specificity in rat liver systems but not in incubation mixtures containing components of human tonsils. Surprisingly, the phenylalanine polymerization in all combinations tested is limited by the origin of transferase II. In general, systems containing human tonsil transferase II show a much higher polyphenylalanine synthesis than corresponding mixtures with rat liver translocase (fig. 1). The phenylalanine polymerization in homologous rat liver systems

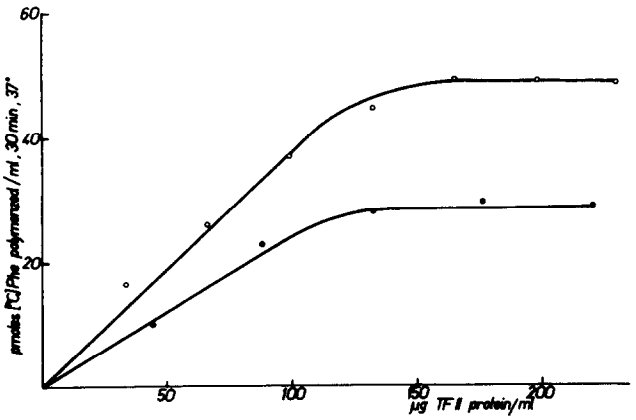

Fig. 1. Effect of increasing amounts of rat liver (●——●) or human tonsil (○——○) peptidyl-tRNA translocase on the polyU-directed polyphenylalanine synthesis in a homologous rat liver system.

containing human tonsil TF II is even more intensive than in homologous human tonsil mixtures. Moreover, mixtures containing human tonsil translocase are much less sensitive to the addition of the other heterologous components than systems with rat liver TF II.

It is scarcely possible to explain at the moment this rather strange activity of human tonsil transferase II. Since saturating amounts of transfer enzymes were used in our experiments the possibility of a higher specific activity of human translocase when compared with the corresponding enzyme of rat liver can be excluded. Besides that, comparable results in this respect were obtained with different batches of both elongation factors and ribosomes. The higher activity of human tonsil translocase is apparently a property of this enzyme itself since it is not limited by the origin of ribosomes. Thus it would seem that the mechanism of transferase II action in different species may be different. This could explain the specificity of reticulocyte translocase which, unlike transferase I, cannot be replaced by the corresponding bacterial enzyme [4].

Acknowledgements

The carefull technical assistance of Mrs. M.Čechová and Miss J.Maierová is gratefully acknowledged.

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