OCCURRENCE OF ARGINYL-tRNA:PROTEIN ARGINYL TRANSFERASE IN BAKER'S YEAST

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Summary

A soluble fraction from baker's yeast was found to incorporate arginine into 95°C CCl_3COOH-insoluble fraction. The incorporation of arginine required arginyl-tRNA synthetase, tRNA, ATP, Mg^{2+}, 2-mercaptoethanol and bovine serum albumin. A mixture of nineteen amino acids other than arginine had little effect on the degree of incorporation. Incorporation of the arginyl moiety of arginyl-tRNA required only KCl, 2-mercaptoethanol and bovine serum albumin. RNase A and trypsin inhibited the incorporation while DNase I and puromycin did not. It was possible to replace bovine serum albumin in the incorporation of the arginyl moiety with thyroglobulin but not with the other proteins tested here.

Introduction

Kaji et al first described the incorporation of arginine into the hot CCl_3COOH-insoluble fraction by a soluble system from rat liver (1). Since then the occurrence of similar soluble systems in various mammalian tissues has been reported (2-6), and attributed to the presence of a soluble enzyme, arginyl-tRNA:protein arginyl transferase (4), which catalyzed the transfer of the arginyl moiety from arginyl-tRNA to the α-amino groups at amino terminals of proteins having either glutamic or aspartic acid residues at the terminals (4, 7-9). The arginyl-tRNA:protein arginyl transferase is currently believed to be a mammalian enzyme (10) since arginine incorporation by the transferase has thus far been reported only in mammalian tissues.

During the preparation of arginyl-tRNA synthetase from baker's yeast, we observed the incorporation of arginine into the 95°C CCl_3COOH-insoluble fraction catalyzed by the 105,000 x g supernate. Some characteristics of

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this incorporation described below suggest that the arginyl-tRNA:protein arginyl transferase occurs in a single celled eukaryote, baker's yeast.

Materials and Methods

Bovine DNase I, horse heart cytochrome c, chicken ovalbumin, chicken ovomucoid trypsin inhibitor, rabbit muscle aldolase, bovine α-casein, bovine thyroglobulin, bovine serum albumin and Escherichia coli B tRNA were commercially available samples and practically free from RNase and proteinase activities at the concentrations used here. Baker's yeast, Escherichia coli B, bovine RNase A, bovine trypsin, L-[U-^{14}C]arginine and other chemicals were obtained from commercial sources. Arginyl-tRNA synthetase was purified from Escherichia coli B according to Zubay (11). The specific activity of the synthetase thus purified was 7.6 nmol/min/mg. Arginyl-tRNA was prepared by the method of Mitra and Mehler (12) and stored frozen until use. One mg of tRNA was charged with 1.8 nmol of arginine carrying 7.92×10^5 cpm. Baker's yeast was cultured for 18 h according to Wickerham (13) and harvested by centrifugation at 3,000 x g, followed by one washing with a large excess volume of 20 mM Tris-HCl buffer, pH 7.8, containing 0.9% NaCl and 5 mM magnesium acetate. After the washing the wash was centrifuged off and the wet cell was stored at -20°C until homogenization.

All procedures for preparation of supernates from baker's yeast were performed at 5°C. The wet cells (45 g) were mixed with 90 g of Al_2O_3, homogenized in a MRK-RETSCH mortar grinder for 20 min and suspended for 30 min in 126 ml of 20 mM Tris-HCl buffer, pH 7.8, containing 5 mM magnesium acetate, 0.1 mM EDTA, 5 mM 2-mercaptoethanol, 30 mM KCl and 250 mM sucrose. The suspension was centrifuged for 20 min at 3,000 x g and then the turbid supernate was further centrifuged for the same period at 20,000 x g. The still turbid supernate (20,000 x g supernate) was centrifuged for 90 min at 105,000 x g and the upper one-third portion of the resultant clear supernate was taken as 105,000 x g supernate I. The centrifugation at 105,000 x g was repeated twice to yield the 105,000 x g supernates II and III described below. For preparation of the 78,000 x g supernate I, the starting material for purification of arginyl-tRNA synthetase from baker's yeast, the initial suspension was first centrifuged for 15 min at 17,000 x g and then the 17,000 x g supernate was centrifuged for 90 min at 78,000 x g. The upper one-third portion of the clear supernate was taken as 78,000 x g supernate I. Similarly, the upper portion of the clear supernate obtained by centrifugation of the 78,000 x g supernate I at the same g value was taken as 78,000 x g supernate II. All supernates were dialyzed overnight against a large excess of 20 mM Tris-HCl buffer, pH 7.8, containing 5 mM 2-mercaptoethanol before use.

Incorporation of arginine into the 95°C CCl_3COOH-insoluble fraction was assayed as described previously (4) and the specific activity was expressed in pmol of arginine or arginyl moiety incorporated per min per mg of supernate protein. Protein was assayed by the method of Lowry et al (14).

Results

Incorporation of arginine into the hot acid-insoluble fraction was examined on various supernates from commercial baker's yeast (Table I). All the supernates specified as I, II and III in the table were upper one-

Table I

Incorporation of arginine with fractions from baker's yeast

	Activity pmol/min/ml supernate	Specific activity pmol/min/mg
Commercial yeast		
17,000 x g Supernate	60.0	3.4
78,000 x g Supernate I	41.7	4.3
78,000 x g Supernate II	30.2	3.8
20,000 x g Supernate	77.7	4.0
105,000 x g Supernate I	66.5	6.0
105,000 x g Supernate II	47.7	5.7
105,000 x g Supernate III	42.9	6.0
Cultured yeast		
105,000 x g Supernate I	76.5	7.4

The incubation mixture contained 5 μmol of Tris-HCl buffer (pH 8.0), 5 μmol 2-mercaptoethanol, 2 nmol [^{14}C] arginine (diluted to 202 nCi per nmol with [^{12}C]arginine), 9 μmol KCl, 500 μg bovine serum albumin, 24 μg arginyl-tRNA synthetase, 280 μg tRNA, 0.3 μmol ATP, 1 μmol magnesium chloride and 5 μl of each dialyzed supernate in a total volume of 100 μl, and was incubated for 20 min at 37°C. Activity was expressed in pmol per min per ml of each supernate before the dialysis. The change of each supernate volume during the dialysis was within 10%.

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third portions of clear supernates from the 78,000 x g and 105,000 x g centrifugations and only those portions were taken in order to avoid the ribosomal contamination even when nothing was sedimented during centrifugation. The specific activities of the 78,000 and 105,000 x g supernates remained constant through the successive centrifugations. These results exclude contamination with ribosomal particulates. The decreases in activity with repeated centrifugation may be attributed to taking of only the upper one-third portions of the supernates. The 105,000 x g supernate I from baker's yeast cultured here also incorporated arginine.

Requirements for the incorporation of arginine by the 105,000 x g supernate III and effects of various additions on the incorporation are summarized in Table II. The incorporation required arginyl-tRNA synthetase,

Table II

Requirements for the incorporation of free arginine

	Relative activity %
Complete	100
- arginine	0
- 105,000 x g supernate III	0
+ the supernate III exposed to 90°C for 1 min, 15 μl	1
- KCl	68
- bovine serum albumin	13
- 2-mercaptoethanol	30
- arginyl-tRNA synthetase	16
- tRNA	0
- ATP	0
- Mg^{2+}	18
- above four components	0
+ arginyl-tRNA, 47 μg	45
+ cold arginine, 4 nmol	32
+ 19 amino acids other than arginine, 2 nmol each	117
+ DNase I, 20 μg	92
+ RNase A, 20 μg	0
+ trypsin, 4 μg	0
+ puromycin, 22 μg	96

The complete mixture contained the same components as the incubation mixture of Table I except that 15 μl (81 μg) of dialyzed 105,000 x g supernate III was used as the supernate. Incubation was performed under the same condition as of Table I.

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tRNA, ATP and Mg^{2+}, which are required for the arginyl-tRNA synthesizing system (12). These four components were successfully replaced by arginyl-tRNA, as expected. The incorporation also required bovine serum albumin and 2-mercaptoethanol. Addition of a mixture of nineteen other amino acids enhanced the incorporation slightly. RNase A and trypsin inhibited the incorporation but DNase I and puromycin did not.

In order to gain further insight into the incorporation of arginyl moiety from arginyl-tRNA, the requirements and the effects of some materials were examined with arginyl-tRNA (Table III). This incorporation required KCl and 2-mercaptoethanol and was inhibited by RNase A and trypsin. DNase

Table III

Requirements for the incorporation of the arginyl moiety of arginyl-tRNA

	Relative activity %
Complete	100
- arginyl-tRNA	0
- 105,000 x g supernate III	0
+ the supernate III exposed to 90°C for 1 min, 15 µl	1
- KCl	28
- bovine serum albumin	31
- 2-mercaptoethanol	19
+ Mg^{2+}, 1 µmol	80
+ DNase I, 20 µg	105
+ RNase A, 20 µg	5
+ trypsin, 4 µg	0
+ puromycin, 22 µg	118

The complete mixture contained 5 µmol of Tris-HCl buffer (pH 9.0), 5 µmol 2-mercaptoethanol, 9 µmol KCl, 500 µg bovine serum albumin, 47 µg [^{14}C]arginyl-tRNA (see text) and 15 µl (100 µg) of dialyzed 105,000 x g supernate III in a total volume of 100 µl. Incubation was performed for 10 min at 37°C.

I and puromycin did not inhibit. The incorporation was enhanced by more than 3-fold by the addition of bovine serum albumin as was the case for the incorporation of free arginine. The incorporation was also increased by the addition of thyroglobulin but not by cytochrome c, ovalbumin, trypsin inhibitor, aldolase and α-casein (Table IV).

Discussion

The incorporation of arginine or the arginyl moiety of arginyl-tRNA observed here is not due to the contaminants in the commercial baker's yeast because similar incorporation was also observed with the 105,000 x g supernate I from baker's yeast cultured here under controlled conditions. The 105,000 x g supernate III retained more than 60% activity of the 105,000 x g supernate I. Since 105,000 x g supernates were prepared carefully avoiding contamination of ribosomal particulates as already detailed,

Table IV

Effects of proteins on the incorporation of arginyl moiety of arginyl-tRNA

	Relative activity %
Complete	100
- bovine serum albumin	29
+ thyroglobulin, 500 μg	48
+ cytochrome c, 500 μg	28
+ ovalbumin, 500 μg	29
+ trypsin inhibitor, 500 μg	26
+ aldolase, 500 μg	24
+ α-casein, 500 μg	28

The composition of the reaction mixture and incubation conditions were as in Table III.

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the incorporation by the supernate III could not be ascribed to the participation of peptidyl transferase associated with the ribosomal particulates (15). This conclusion is supported by the facts that puromycin did not inhibit incorporation, and the addition of the amino acid mixture had little effect. It may be concluded that the incorporation of arginine was brought about by a soluble, heat labile and non-dialyzable factor(s) in the supernate from baker's yeast.

Incorporation was inhibited by RNase A and trypsin but not by DNase I. The results are consistent with the observations on the incorporation of arginine or the arginyl moiety of arginyl-tRNA by the soluble enzyme, arginyl-tRNA:protein arginyl transferase, from mammalian tissues (4). This transferase catalyzes the transfer of arginyl moiety of arginyl-tRNA to the amino terminals of proteins with aspartic or glutamic acid residues at those terminals (4, 7-9). The incorporation of arginyl moiety of arginyl-tRNA observed here was also stimulated by bovine serum albumin and thyroglobulin, which possess aspartic acid residues at the amino terminals (16, 17). From this fact, together with the similarity of the characteristics of

incorporation by the yeast supernates to those by the mammalian arginyl-tRNA:protein arginyl transferase, it may be reasonably assumed that the incorporation of arginine or the arginyl moiety observed here was due to the action of arginyl-tRNA:protein arginyl transferase.

Arginyl-tRNA:protein arginyl transferase is currently believed to be a mammalian enzyme (10). The present results suggest that it may occur as well in other eukaryotes, including microbial forms.

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