MEMBRANE MODIFICATION BY ARGINYL tRNA

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1. Introduction

Eukaryotic systems transferring arginine and prokaryotic systems transferring phenylalanine, leucine, and tryptophan from their corresponding aminoacyl tRNAs to the amino-terminal residue of an acceptor protein have been previously described [1–9]. These cell free systems, referred to as soluble systems in earlier publications, consist of three components – aminoacyl tRNA, the acceptor proteins which are being modified, and aminoacyl tRNA transferase. Although the functional significance of this system is not yet clear, it is known that a mutant of *E. coli* lacking this enzyme exhibited abnormal growth characteristics [10] suggesting that the soluble system may play an important physiological role in cells as a means of modifying preformed proteins.

It has been reported that the major proteins of human erythrocyte membranes are α and β lipoproteins whose amino terminal amino acids are mostly aspartic and glutamic acids [11]. Since these amino acids are found to be the NH_2-terminal residues of albumin which can serve as an exogenously added acceptor protein to which arginine can be transferred from arginyl tRNA [8], it seemed likely that the α and β lipoproteins might also function as suitable acceptors. In this communication we report that erythrocyte ghost membrane proteins can accept arginine from arginyl tRNA at their NH_2-terminal end in vitro.

2. Materials and methods

Arginyl tRNA transferase was prepared essentially as described [12] except that 70% ammonium sulfate saturated solution of postmicrosomal supernatant of beef liver was used for the enzyme preparation.

Transfer RNA and aminoacyl tRNA synthetase were prepared from established cell lines of normal rat kidney cells (NRK) transformed either by Rous Sarcoma virus (RSV) Prague strain of subgroup A or by a temperature-sensitive mutant of this RSVtsLA24 subgroup A [13] cultured in Dulbecco Modified Eagle's Medium supplemented with 10% calf serum. Cultures were washed twice with 5 ml phosphate buffered saline and the cells were scraped with a rubber policeman after addition of 2 ml solution containing 0.25 M sucrose, 0.15 M Tris-HCl pH 7.8, 0.03 M KCl, and 0.006 M β-mercaptoethanol. Cells were homogenized and aminoacyl tRNA synthetase was prepared as described [14]. For tRNA and arginyl tRNA preparation the cells were harvested by trypsinization, homogenized in 0.01 M Tris-HCl, pH 7.5, 0.001 M ethylenediamine tetraacetic acid (EDTA), 0.15 M NaCl, and 0.4 mg/ml bentonite, extracted with an equal volume of phenol. RNA was precipitated with two volumes of ethanol and 0.1 volume of 20% potassium acetate, pH 5.0 and the tRNA extracted with 1 M NaCl in the buffer (0.01 M $MgCl_2$ + 0.001 M EDTA, pH 4.5) containing bentonite 0.4 mg/ml. The NaCl concentration of the extract was brought to 0.25 M and the solution applied to a DEAE column (1 × 3 cm) equilibrated with the buffer containing 0.25 M NaCl at 4°C. After washing with 30 ml of the same buffer, tRNA was eluted with 30 ml of this buffer containing 0.7 M NaCl, and tRNA was precipitated with 2 volumes of ethanol and 0.1 volume of 20% potassium acetate, pH 5.0.

The reaction mixture (0.5 ml) for preparing arginyl-tRNA contained the following (in μmoles/ml): cacodylate (50), pH 6.9; KCl (10); $MgCl_2$ (10);

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ATP (2.0); and 1 μmole each of 19 of the amino acids except arginine. In addition it contained 100 μg of tRNA, 150 μg of synthetase and 200 μCi of [^{3}H] arginine (27.3 Ci/mmole) and incubated at 37°C for 30 min. Aminoacylated tRNA was obtained as above by DEAE column chromatography.

Transfer of arginine from arginyl tRNA to membrane was carried out in the reaction mixture (100 μl) containing the following (in μmoles/ml): Tris-HCl, pH 7.6, (100); KCl (45); $MgCl_2$ (5); β-mercaptoethanol (50). In addition, it contained 5 μg of arginyl tRNA transferase, various amounts of membranes, and 20 000 cpm of arginyl tRNA unless otherwise noted. An aliquot of 15 ∿ 20 μl was treated with hot trichloroacetic acid as described [15].

Hemoglobin free erythrocyte ghosts were prepared as described [16]. Ghosts were homogenized in 0.01 M Tris pH 7.8 using Dounce homogenizer A, and centrifuged for 30 min at 15 000 *g* at 4°C to obtain membrane pellets.

3. Results and discussion

The stimulatory effect of membrane on the transfer of arginine from arginyl tRNA is shown in fig.1. The incorporation of arginine increased as the increased amount of membrane was added to the reaction mixture. The stimulation of arginine incorporation into protein by the membrane fraction was observed both at the initial rate of arginine

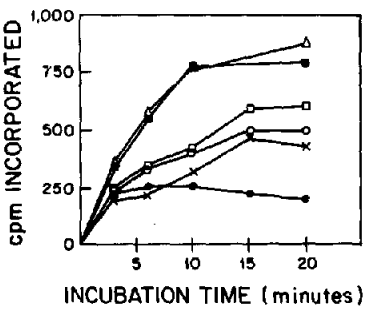

Fig.1. The transfer of arginine from arginyl tRNA to membrane. Experimental conditions are as in text except 15 μl aliquots were taken from 100 μl reaction mixture at various time intervals. The amounts of membrane in the reaction mixture are: 39 μg (X–X); 64 μg (o–o); 90 μg (□–□); 130 μg (■–■); 194 μg (△–△); and none (●–●).

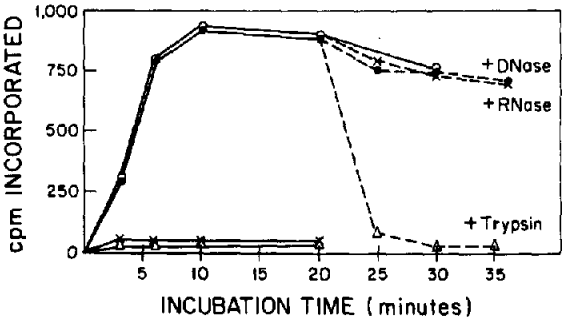

Fig.2. Effect of DNase, RNase, and trypsin on the transfer of arginine from arginyl tRNA to membrane. Aliquots 20 μl were taken at various time intervals from 400 μl of the following incubation mixtures: (A) transferase 20 μg, 624 μg membrane, 80 000 cpm of [^{3}H]arginyl tRNA (o–o). (B) To 90 μl of (A) after 20 min incubation at 37°C was added 25 μg RNase (X—X). (C) Same as (B), except that 25 μg DNase was added (●—●). (D) Same as (B), except that 5 μg trypsin was added (△- -△). (E) Transferase 5 μg, 156 μg membrane, 20 000 cpm [^{3}H]arginyl tRNA in 100 μl reaction mixture were incubated with 25 μg RNase (X – X). (F) Same as (E) except that 5 μg of trypsin were added at the onset of the reaction (△–△). (G) Same as (E) but the incubation was with 25 μg DNase (●–●).

incorporation as well as the final level. Approximately a 4-fold stimulation over the base line was observed. As reported earlier [5] a crude postmicrosomal supernatant of eukaryotic cells contains acceptor proteins which accept arginine at the NH_2-terminal end. Although the enzyme was purified it still retained acceptor capacity since arginine incorporation took place even in the absence of added membrane.

In the experiments shown in fig.2, the effects of various degradative enzymes on the incorporation of [^{3}H]arginine into membrane were studied. When RNase or trypsin was added to the reaction mixture, prior to the addition of [^{3}H]arginyl tRNA, the incorporation of arginine was completely inhibited presumably because RNase hydrolyzed arginyl tRNA and trypsin digested acceptor proteins in membrane as well as arginyl tRNA transferase. On the other hand, when these inhibitors were added to the radioactive product after the reaction was completed, very little effect of RNase or DNase was observed. However, trypsin almost completely solubilized the radioactivity indicating that radioactivity incorporated into the membrane is incorporated into protein.

The presence of β-mercaptoethanol is essential for

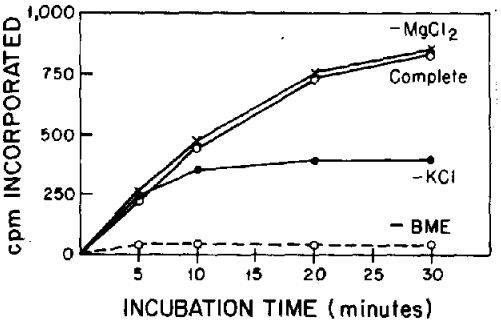

Fig.3. Dependence of arginylation of membrane proteins on the presence of K ion and β-mercaptoethanol. (1) Complete reaction mixture with 130 μg membrane (○—○). (2) No $MgCl_2$ was added (×—×). (3) No β-mercaptoethanol (BME) was added (○—○). (4) No KCl was added (●—●).

arginylation of membrane proteins as shown in fig.3. In addition, potassium ion but not magnesium ion appears to be required for maximum incorporation of arginine by membrane fraction. Although data are not shown here the transfer of arginine from arginyl tRNA was optimum at pH 7.6 ∿ 7.8. However, the stimulation of arginine incorporation by membrane was observed at all pHs tested (pH 7.0 ∿ 8.5).

The incorporation of arginine by the soluble amino acid incorporating system previously described [5] involves transfer of arginine to the amino-terminal of acceptor proteins. In order to establish that this is also true with arginine incorporated into membrane, membrane labeled with arginine was analyzed for aminoterminal radioactivity. As shown in table 1, incubation of membrane by itself in the absence of arginyl tRNA transferase showed very little arginine incorporation at NH_2-terminal end, whereas a major portion of incorporated arginine in the reaction containing both membrane and transferase reacted with dinitrofluorobenzene indicating that it had a free amino group. When membrane pellets and supernatant were separately analyzed for NH_2-terminal amino acids the major portion of radioactivity was also found at NH_2-terminal end. The fact that the supernatant itself had a significant amount of radioactivity shows that arginine incorporated into acceptor proteins present in the enzyme preparation is also at the NH_2-terminal and that considerable radioactivity is released from membrane after the transfer reaction. It is clear from these data that arginine is incorporated into NH_2-terminal of membrane proteins by arginyl tRNA transferase.

Acknowledgements

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Table 1
Amino terminal analysis of incorporated product

	(1) Complete	(2) Membrane without transferase	(3) Isolated membrane	(4) Isolated supernatant
Arginine (internal)	85	61	131	160
DNP Arginine (NH_2-terminal)	2307	252	680	1332

(1) Complete contained membrane fraction 1.296 mg, 25 μg arginyl tRNA transferase and other reaction components in 0.5 ml as described in text. After 20 min incubation the total reaction mixture was treated with 50 μg each of RNase and DNase for 20 min at 24°C, and was brought with solid $(NH_4)_2CO_3$ to 0.6 M together with 0.5 ml, 5% DFNB in 95% ethanol.

(2) Membrane without transferase – same as (1) except no transferase was added.

(3) Isolated membrane – same as (1) except mixture was centrifuged for 30 min at 15 000 *g* to separate membrane fraction from supernatant (4) after the transfer reaction. Amino terminal analysis of the dinitrophenylated product was carried out as described previously [5]. Approximately 40% of the hydrolyzed materials were chromatographed and values here represent cpm recovered on paper.

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