Amino Acid Activation and Formation of Amino Acyl s-RNA in Bovine Parotid Gland

by

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Introduction

Our previous report described the general characteristics of cell-free amino acid incorporating system consisting of bovine parotid gland microsome and cell sap [4]. The requirement of adenosine triphosphate (ATP), guanosine triphosphate (GTP) and cell sap for the maximal incorporation in the cell-free system may indicate that the general pathway of the protein biosynthesis, amino acid activation, amino acyl soluble ribonucleic acid (amino acyl s-RNA) formation and transfer of amino acid to microsomes, may operate in this system.

However, there has been no study on each step by which this protein biosynthesis takes place in parotid gland.

In an attempt to elucidate in detail the each step in protein biosynthesis of parotid gland, the following experiments were carried out; Experiments on the amino acid activation and amino acyl s-RNA formation in parotid gland cell-free system.

P³²-labeled pyrophosphate (PP³²) was prepared by the method of KORNBERG and PRICER[7] and purified by the modified procedure of COHN and CARTER[1]. The preparation of microsome and soluble fraction from parotid gland was carried out as described in the previous paper[4].

Assay Method of Amino Acid Activating Activity

One ml. of incubation mixture for the PP³²-ATP exchange assay contained 3 μ moles of ATP, 3 μ moles of PP³²(about 100,000 counts/minute by Geiger-Müller counter), 10 μ moles of MgCl₂, 100 μ moles of tris-HCl buffer (pH 7.8), each 3 μ moles of amino acid and the enzyme fraction (1 mg. of protein). The cell sap, pH 5.2 fraction, pH 4.5 fraction and pH 4.5 supernatant fraction prepared by the method of SATAKE *et al.* [9], were used respectively as the enzyme fraction. After incubation at 37°C for 15 minutes, the reaction was stopped by addition of 2.0 ml. of 7.5%-trichloroacetic acid and PP³² and ATP were separated by adsorption on charcoal according to the method of CRANE and LIPMANN[2].

The concentration and radioactivity of ATP and pyrophosphate were determined as inorganic orthophosphate after hydrolysing these compounds in 0.7 ml. of 1 N HCl at 100°C for 10 minutes. Inorganic phosphate was determined by the method of FISKE and SUBBAROW[3].

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The radioactivity was measured with a Geiger-Müller counter. The data were expressed as per cent exchange according to HOAGLAND et al.[5].

Assay Method for Incorporation of Labeled Amino acid into s-RNA

Two ml. of the reaction mixture contained pH 4.5 fraction (about 12 mg. of protein), 5 μ moles of ATP, 7.5 μ moles of $MgCl_2$, 37.5 μ moles of KCl, 375 μ moles of sucrose, 75 μ moles of tris-HCl buffer (pH 7.6) and 20 μ moles of uniformly labeled C^{14} -L-valine (66 mcuries/mmmole). After incubation at 37°C for 15 minutes, the reaction mixture was diluted with 4 ml. of cold distilled water and made to pH 4.5 with 0.1 N acetic acid at 0°C. The precipitate formed was separated by centrifugation and washed with 8 ml. of cold water of pH 4.5 (adjusted with acetic acid) by homogenization followed by centrifugation, then dissolved in 7 ml. of medium A which was prepared according to the method of LITTLEFIELD and KELLER[8], and s-RNA was isolated from the solution with carrier RNA (5 mg. of yeast RNA) by the phenol treatment followed by ethanol precipitation as described by HOAGLAND et al.[6].

The RNA fraction obtained was transferred into an aluminum dish, dried under infrared light, and its radioactivity was measured by a gas flow counter.

The amino acid activation by the cell sap of bovine parotid gland was studied by PP^{32} -ATP exchange reaction in the presence of 10 mM of NaF to inhibit adenosine triphosphatase [EC 3.6.1.4.] and pyrophosphatase (EC 3.6.1.1.) activity completely. In the cell sap, high PP^{32} -ATP exchange was found in the presence of amino acid mixture, and some activity was determined in the absence of amino acid mixture as shown Table 1.

Dialysis of cell sap against redistilled water resulted in reduction of PP^{32} -ATP exchange activity in the absence of amino acid mixture (Table 1). After heating of the cell sap at 60°C for 5 minutes, PP^{32} -ATP exchange activity was decreased considerably and even after heating at 90°C for 5 minutes the activity was negligible (Table 1).

Table 1. Amino acid dependent PP^{32} ATP exchange by cell sap

	Exp. 1		Exp. 2	
	Amino acid mixture		Amino acid mixture	
	+	-	+	-
Non-dialyzed	17.5	12.0	20.9	15.3
Dialyzed	14.9	2.4	15.0	2.5
Dialyzed and heated				
at 60° C for 5 min.	1.9		2.1	
at 90° C for 5 min.	0		0	

(%/mg. of protein)

It was found that the cell sap and its subfraction had a high PP^{32} -ATP exchange activity, and among three subfraction of cell sap, the pH 4.5 fraction had the highest activity and the pH 4.5 supernatant fraction was almost inactive (Table 2).

The results of the experiments, in which individual amino acid dependent PP^{32} -ATP exchange activity was determined, indicate that the PP^{32} -ATP exchange activity by dialysed cell sap was increased significantly by isoleucine, valine, threonine and

tryptophan and moderately increased by histidine, lysine and phenylalanine, while hydroxyproline, arginine, glutamic acid, glycine, aspartic acid, methionine, alanine, proline and leucine had little or no effect as shown Table 3.

In the experimental conditions described above, incorporation of C^{14} -L-valine into s-RNA in the pH 4.5 fraction was observed (Table 4).

Table 2. PP^{32} -ATP exchange activity of subfraction of cell sap

	Exp. 1		Exp. 2	
	Amino acid mixture		Amino acid mixture	
	+	-	+	-
cell sap	17.6	4.2	19.8	2.9
pH 5.2 fraction	25.7	3.4	25.0	2.8
pH 4.5 fraction	41.0	12.3	41.2	10.5
pH 4.5 supernatant fraction	10.6	2.8	8.9	2.5

(%/mg. of protein)

In this experiment, the cell sap used was dialyzed against redistilled water.

Table 3.
Efficiency of individual amino acid on PP^{32} -ATP exchange by cell sap

	Exp. 1	Exp. 2	Exp. 3
Amino acid mixture			
(+)	16.9	45.9	31.5
(-)	2.2	7.2	5.2
Hydroxyproline	2.3	6.6	6.3
Arginine	2.2	7.0	6.2
Histidine	5.2	8.4	8.6
Glutamic acid	2.3	7.0	6.1
Glycine	3.4	5.0	5.9
Isoleucine	4.7	13.5	8.8
Valine	6.4	10.1	9.0
Lysine	4.9	9.7	6.8
Aspartic acid	3.8	9.0	7.3
Methionine	4.2	4.9	5.5
Alanine	3.7	7.3	6.9
Threonine	8.8	20.9	15.9
Phenylalanine	3.7	10.1	7.5
Tryptophan	6.8	15.1	10.1
Proline	2.4	10.9	6.1
Leucine	3.5	7.2	6.2

(%/mg. of protein)

In this experiment, the cell sap used was dialyzed against redistilled water.

Table 4.
Incorporation of C^{14} -L-valine into s-RNA
by pH 4.5 fraction

Exp. No.	Total c.p.m. of s-RNA
1	1096
2	1141
3	1447

Conclusion

The present experiments suggest that the protein biosynthesis by the cell-free system of bovine parotid gland may consist of a sequence of the following steps: amino acid activation, formation of amino acyl s-RNA, and transfer of amino acid from s-RNA to microsomes. But, in this experiment, we did not demonstrate the last step, which is, transfer of amino acid from s-RNA to microsomes.

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