INCORPORATION OF ARGININE BY A SOLUBLE SYSTEM FROM SHEEP THYROID

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During an investigation of protein synthesis by cell-free extracts from thyroid, it became evident that incorporation of arginine differed from that of other amino acids and could proceed by two separate mechanisms. One depended on the presence of both ribosomes and a supernatant fraction, whereas the other required only the supernatant fraction. This report describes the properties of the soluble system for incorporation of arginine. The reaction is dependent on an energy source and supplementary S-RNA. Arginyl S-RNA is an intermediate. The transfer of arginine from S-RNA into hot TCA-insoluble material does not require magnesium ions and is not inhibited by puromycin. The product of the reaction is made acid-soluble by trypsin but not by RNAase.

Materials and Methods: Thyroid glands of sheep from a local abbatoir were dissected, immersed in liquid nitrogen, and stored frozen till use. They were suspended in 3 vol of buffer A [tris HCl (pH 7.8) 50 μmoles/ml; $MgAc_2$, 1; EDTA, 0.5; 2-mercapto-ethanol, 5; sucrose, 440; KCl, 30] and homogenized first in a chilled Waring blendor for 3 min, then by a stroke of a Teflon tissue grinder. The suspension was centrifuged once at 20,000 g for 20 min, then twice for 90 min at 150,000 g. The upper half of the final supernate (S 150) was dialyzed overnight against 200 vol of buffer B [tris HCl (pH 7.8) 20 μmoles/ml; $MgAc_2$, 5; EDTA, 0.1; 2-mercapto-

ethanol, 5; sucrose, 250; KCl, 30] or modified buffer B ($MgAc_2$, sucrose, KCl omitted). The microsomal pellet was rinsed five times with buffer B and frozen in samples. Alternatively, ribosomes were prepared by treatment of the 20,000-g supernate with sodium deoxycholate (Acs et al., 1961). The ratio of RNA to protein for the microsomes and ribosomes was 0.12-0.26 and 0.36-0.60.

E. coli B C^{14} leucyl S-RNA was obtained from the New England Nuclear Corporation; it contained 1.46 mμmoles L-leucine (specific activity 222 μc/μmole)/mg of RNA. Stripped E. coli B S-RNA (General Biochemicals) was charged with C^{14} arginine or C^{14} phenylalanine (specific activities 250 and 375 μc/μmole, New England Nuclear Corp.) essentially as described by Von Ehrenstein and Lipmann (1961), save that a precipitation step in 0.25 N perchloric acid was employed. The final products contained 1.04 mμmoles of arginine and 0.70 mμmoles of phenylalanine/mg of S-RNA.

Reaction mixtures for amino acid incorporation (150 μliters) contained (in μmoles/ml): tris HCl (pH 7.8) 50; NH_4Ac, 30; $MgAc_2$, 10; 2-mercaptoethanol, 10; ATP, 3; GTP, 0.2; PEP, 10; pyruvate kinase, 30 mg; 19 C^{12} L-amino acids, 0.1; and uniformly labeled C^{14} arginine or leucine (specific activity 250 μc/μmole) 0.02. Ribosomes or microsomes and S 150 were added as indicated. Reactions were carried out at 37° C, and 25- or 50-μliter samples were removed at various times and processed batchwise on filter paper discs with hot 5% TCA, ethanol-ether, and ether (Bennett et al., 1965). In some experiments, duplicate samples were treated with cold vs. hot TCA to determine the "hot TCA labile" fraction which is considered to represent a measure of amino acyl S-RNA (Bennett et al., 1965). Radioactivity was determined in a scintillation counter with an efficiency of 40%.

TABLE I

Comparison of Incorporation of Leucine and Arginine into TCA-Insoluble Material

	L-leucine (μμmoles/ml)		L-arginine (μμmoles/ml)	
	Heat Labile	Heat Stable	Heat Labile	Heat Stable
A				
S 150		3		6
Ribosomes		< 2		25
S 150 plus ribosomes		51		74
B				
S 150	3	< 2	13	8
Plus S-RNA (300 μg/ml)	155	< 2	683	316
Plus S-RNA (300 μg/ml) plus ribosomes	110	80	656	416
Plus S-RNA (1200 μg/ml)		3		696
Plus S-RNA (2400 μg/ml)		4		844

Reactions were run for 40 min as described in "Materials and Methods." Concentrations of S 150 protein were 10.8 and 11.9 mg/ml, respectively, for series A and B. Where indicated, ribosomes were used at the concentration of 500 μg of RNA/ml in A, and 1000 μg of RNA/ml in B. Different preparations of supernates and ribosomes were used in the two series. *E. coli* B S-RNA was used throughout.

Results: Incorporation of arginine by the cell-free system

Table I indicates that arginine can be incorporated into hot TCA-insoluble material in a process requiring both ribosomes and S 150. It also shows, however, that when supplementary S-RNA is supplied, arginine is incorporated by the soluble system alone, whereas under these conditions the activation of leucine is stimulated, but its transfer into protein remains strictly dependent on the presence of ribosomes. At sufficient concentrations of S-RNA, at least 200 times as much arginine as leucine is incorporated into hot TCA-insoluble material by the supernatant fraction.

Table II presents a comparison of leucine incorporation by

TABLE II

Requirements and Inhibitors of Incorporation of Amino Acid into Hot TCA-Insoluble Material. Comparison of the Ribosomal System for Leucine and the Soluble System for Arginine.

	L-leucine (μμmoles/ml)		L-arginine (μμmoles/ml)	
	25 min	50 min	25 min	50 min
Complete system	44	65	222	367
Minus ATP, PEP	< 2	2	2	11
Minus amino acid mixture	27	48	170	333
Plus RNAase (20 μg/ml) at t=0	< 2	< 2	2	4
Plus RNAase (20 μg/ml) at t=25	44	47	206	204
Plus DNAase (20 μg/ml)	41	69	234	360
Plus Trypsin (30 μg/ml) at t=25	44	30	211	49
Plus Puromycin ($3X10^{-4}$M)	6	7	218	387

All reaction mixtures contained 12 mg/ml of S 150 protein and 300 μg/ml of *E. coli* B S-RNA. The complete system for incorporation of leucine also contained ribosomes at a concentration of 668 μg RNA/ml of reaction mixture.

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a complete system containing ribosomes and incorporation of arginine by the supernatant fraction alone. In many respects the two reactions are similar; thus, both are virtually completely inhibited by omission of ATP and an ATP-generating system, or by treatment with RNAase, and both are slightly dependent on an amino acid mixture. Neither is sensitive to DNAase, and the products of neither process are degraded by RNAase. Trypsin renders both products acid-soluble, but has a greater effect on incorporated arginine. The reactions differ completely in one important respect: Arginine incorporation is uninhibited by puromycin, whereas that of leucine is reduced by about 90% at the same concentration of this reagent.

TABLE III

Experiments on the Transfer of Amino Acids from S-RNA into Hot TCA-Insoluble Material

A

	Requirements for the Transfer of Different Amino Acids		
	L-arginine (μμmoles/ml)	L-phenylalanine (μμmoles/ml)	L-leucine (μμmoles/ml)
Complete system	52.6	153	60.8
Plus puromycin (3×10^{-4}M)	46.2	40.4	8.1
Minus ribosomes	36.3	< 1	< 1
Minus ribosomes plus C^{12} arginine (6×10^{-4}M)	39.8		
Minus ribosomes plus puromycin (3×10^{-4}M)	37.2		
Minus ribosomes and S 150 boiled	< 1		

B

	Effect of Magnesium Deprivation on the Transfer of Arginine and Phenylalanine	
	L-arginine (μμmoles/ml)	L-phenylalanine (μμmoles/ml)
Complete system minus Mg^{++}	40.0	< 1
Plus $MgCl_2$ (5×10^{-3}M)	41.5	19.4
Plus $MgCl_2$ (1×10^{-2}M)	39.8	94

All reaction mixtures contained 12 mg/ml of S 150 protein. The complete system for series A contained ribosomes at a concentration of 1000 μg of RNA/ml. For series B the complete system for phenylalanine incorporation included microsomes (476 μg of RNA/ml) whereas that for arginine did not. Polyuridylic acid (300 μg/ml) was added to all reaction mixtures with phenylalanyl S-RNA. The concentrations of S-RNA were, respectively, 450 μg/ml (containing 469 μμmoles of L-arginine), 440 μg/ml (containing 308 μμmoles of L-phenylalanine) and 230 μg/ml (containing 320 μμmoles of L-leucine). Reactions were carried out for 20 min.

The transfer of arginine from S-RNA into hot TCA-insoluble material by the cell-free system. The results of these experiments

are shown in Table III and may be summarized here:

1. Incorporation of leucine from S-RNA and the polyuridylic acid-directed transfer of phenylalanine from S-RNA exhibit an absolute dependence on the presence of ribosomes and are strongly inhibited by puromycin. Arginine is transferred in the absence of ribosomes, and this reaction is unaffected by puromycin. Arginine incorporation is, however, somewhat stimulated by the addition of ribosomes, and puromycin does appear to inhibit this increase, although quantitation of this effect is difficult because the increment dependent upon ribosomes is small relative to the backgroun activity of the soluble system.

2. The transfer of arginine by the supernatant fraction does not require magnesium ions, whereas their omission quantitatively inhibits the transfer of phenylalanine in the presence of polyuridylic acid and microsomes.

Discussion: The arginine-incorporating system described in this report is unusual because it requires neither ribosomes nor magnesium ions and is unaffected by puromycin. Kaji et al. (1963) have described the incorporation of several amino acids by soluble liver fractions, one of which was specific for arginine. Incorporation of arginine by that system was, however, strongly inhibited by puromycin. These authors have also studied a soluble system from E. coli (Kaji et al., 1965) which is concerned with the incorporation of leucine, phenylalanine, and tryptophane and which is also inhibited by puromycin. They noted that leucyl S-RNA was an intermediate and that most of the leucine in their product reacted with dinitrofluorobenzene, which suggested that it was located at the NH_2-terminal position. Most of the arginine incorporated by this system has also been found, in preliminary experiments, to react with dinitrofluorobenzene.

It is not yet clear whether there is a requirement for a nucleic acid template or a special substrate in the reaction; nor is it clear that the arginine-transferring enzyme(s) differs from the enzyme which transfers other amino acids in the presence of ribosomes. It is well known that certain amino acyl S-RNAs are heterogeneous (Sueoka et al., 1962), and it is possible that a specific species of arginyl S-RNA is involved in the soluble system. Finally, the biological significance of this reaction is unknown. The answers to some of these questions should be forthcoming after purification of the enzyme and precise characterization of the product.

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