ACTIVITY OF RAT-LIVER ENZYMES IN THE TRANSFER OF s-RNA-BOUND AMINO ACIDS TO PROTEIN BY RIBONUCLEOPROTEIN PARTICLES

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SUMMARY

1. The transfer of labeled amino acids from s-RNA to protein by purified RNP-particles has been studied by use of enzyme preparations from the soluble fraction of rat liver, and some characteristic properties of this reaction have been described.

2. By a subfractionation procedure this transfer activity has been partially separated from the activity of the preparations in amino acid dependent PP–ATP exchange (amino acid activation).

3. Evidence has been obtained that different enzymes may be involved in the transfer of different s-RNA-bound amino acids.

INTRODUCTION

A transfer of s-RNA-bound, labeled amino acids to protein by incorporation systems containing rat-liver microsomes has been reported by HOAGLAND *et al.*[1]. The reaction was markedly enhanced in the presence of a "pH 5 enzyme fraction". A similar enhancement has more recently been observed by GROSSI AND MOLDAVE[2] with an enzyme preparation from the soluble liver fraction, non-precipitable at pH 5. Since both the pH 5 enzyme fraction and the pH 5 supernatant contain amino acid activating enzymes[3,4] it may be asked whether or not the activating enzymes are involved also in the transfer reactions[5].

In attempts to elucidate this problem it seemed particularly desirable to employ systems of increased purity. It has previously been shown that purified RNP-particles isolated from rat-liver microsomes can be used with considerable advantage in different kinds of transfer systems[6]. By use of such purified particles the enzymic activities of amino acid transfer and amino acid activation were studied in some detail in subfractions of soluble liver fractions, and characteristic differences in their distribution and general properties were observed.

Abbreviations: RNP-particles, ribonucleoprotein particles; RNA, ribonucleic acid; s-RNA, soluble RNA, precipitable at pH 5; PP–ATP exchange, isotope exchange between [^{32}P]pyrophosphate and ATP; ATP, adenosine triphosphate; GTP, guanosine triphosphate; CTP, cytidine triphosphate; PEP, phosphoenolpyruvate; Tris, tris(hydroxymethyl)aminomethane buffer, pH 7.8; GSH, gluthathione, reduced form; MEA, 2-mercaptoethylamine; TCA, trichloroacetic acid.

EXPERIMENTAL

The livers of Sprague Dawley rats were used. The animals were kept without food for 15–20 h[7] before being killed by decapitation. All preparations were carried out at 0–4°.

Preparation of RNP-particles

RNP-particles were prepared by the method of RENDI AND HULTIN[8] and purified as described previously[6].

Preparation and fractionation of S_{50}-protein

S_{50}-protein was prepared as described by RENDI AND HULTIN[8] (see ref. 9).

The dialyzed solution of S_{50}-protein from 3 rats (approximately 8 mg protein/ml) was treated with 2 volumes of a hydroxylapatite suspension[10], pH 7.2, (150 mg dry weight/ml). A series of fractions was eluted from the apatite with increasing concentrations of potassium phosphate buffer, pH 7.2 (*cf.* Tables V and VI). The suspensions were allowed to equilibrate with the different buffers for at least 20 min, with constant stirring. After each elution the apatite was washed in a larger volume of the same buffer before being treated with the buffer of next higher concentration.

Preparation and fractionation of ammonium sulfate precipitates

Rats with a weight of 100–150 g were used. The livers were minced and homogenized in 2.5 volumes of 0.01 *M* $MgCl_2$ in 0.25 *M* sucrose and were centrifuged in a Spinco preparative ultracentrifuge for 55 min at 105,000 × *g*. The middle part of the supernatant was collected.

Ammonium sulfate (0.32 g/ml) was added to the soluble liver fraction to give 55 % saturation[11]. The suspension was centrifuged after 1 h and the supernatant was brought to 85 % saturation by addition of 0.24 g ammonium sulfate/ml. Both precipitates, Am 0–55 and Am 55–85, were dissolved in 4 ml of 0.2 *M* Tris, centrifuged for 10 min at 12,000 × *g* and dialyzed against 0.002 *M* Tris for 2 h. The fractions were adsorbed on 2 volumes of calcium phosphate gel, pH 6.6 and eluted with potassium phosphate buffers of successively increasing concentrations, as was described for the fractionations of S_{50}-protein (*cf.* Tables II and III).

Preparation of the pH 5 enzyme fraction

The pH 5 enzyme fraction was prepared by the method of HOAGLAND *et al.*[3] with the modification that 0.008–0.01 *M* HCl was used for the precipitation.

Prelabeling of s-RNA with amino acids

s-RNA was prelabeled by the method of HOAGLAND *et al.*[1] with L-[^{14}C]valine, L-[^{14}C]tyrosine or a hydrolysate of ^{14}C-labeled algal proteins. (The hydrolysate had been treated for 10 h at 120° with 10 % HCl and chromatographed twice on Dowex 50 columns[12]. In control experiments no radioactivity was observed in proteins which had been incubated with s-RNA, prelabeled with this purified hydrolysate.)

In order to get a higher content of labeled amino acid in the s-RNA preparations (and higher radioactivities in the final proteins[13]) the pH 5 enzyme fractions in some cases were treated with repeated preincubation–reprecipitation cycles, as described by HECHT *et al.*[14] (preincubated s-RNA).

Measurement of amino acid dependent PP–ATP exchange

The activity of PP–ATP exchange in the different fractions was measured as described previously[15,16]. In many cases an amino acid mixture, containing 3 μmoles of each of 12 amino acids[3] was used. In more detailed studies of the enzyme distribution, however, the amino acids were added separately to the incubation mixture (3 μmoles per tube). The amount of enzyme preparation added is indicated in the tables. Since the activity of amino acid activation was low in most of the enzyme preparations investigated, the incubation time was usually increased to 60 min at 35°. The percentual exchange values were calculated by the equation of DAVIE *et al.*[17].

Measurement of transfer activity

Unless otherwise indicated, the incubation tubes contained in 1.8 ml of 0.025 *M* KCl, 0.01 *M* $MgCl_2$, 0.035 *M* Tris, 0.25 *M* sucrose and 0.01 *M* GSH or MEA: 1 μmole of ATP, 10 μmoles of PEP, 15 μg of pyruvate kinase, 0.2 μmoles of GTP, about 100 μg of prelabeled s-RNA, 0.2 μmoles of the corresponding non-labeled amino acid (or 3.75 μmoles of an amino acid mixture analogous to the amino acid composition of rat-liver proteins[18]), RNP-particles (4–6 mg of protein), and the indicated amount of the protein fractions to be studied. The tubes were incubated for 30 min at 35°. The proteins were precipitated in 5 % TCA, extracted twice with hot TCA, once with warm ethanol, twice with a mixture of ethanol–ether–chloroform (2:2:1), dried with ether and plated on alumina planchets. The radioactivity was measured by a Tracerlab thin mica window counter and the number of total counts per minute was calculated.

Determination of RNA

The content of RNA was determined by the method of CERIOTTI[19], yeast RNA being used as a reference standard.

Determination of protein

The protein content was determined by the method of LOWRY *et al.*[20], crystallized bovine plasma albumin being used as a reference standard.

MATERIALS

ATP, GTP, CTP, Tris, calcium phosphate gel and protamin sulfate were purchased from Sigma Chemical Co. St. Louis, Miss.; PEP and pyruvate kinase from C. R. Boehringer und Soehne, Mannheim; GSH, MEA and non-labeled amino acids from California Foundation, Los Angeles, Cal.; L-[^{14}C]valine from Institut Pasteur, Paris and the Radiochemical Centre, Amersham (74mC/mmole, and 8 mC/mmole, respectively); L-[^{14}C]tyrosine (11.6 mC/mmole), ^{14}C-algal-protein hydrolysate (0.088 mC/mg) and [^{32}P]phosphate from the Radiochemical Centre, Amersham; yeast RNA from Hopkins and Williams Ltd., Ilford; bovine plasma albumin from Armour Lab., Chicago Ill.; puromycin from Lederle Laboratory Division, New York; chloramphenicol from Parke Davis, Hounslow. Hydroxylapatite was prepared by the method of TISELIUS *et al.*[10], PEP was recrystallized according to BAER[21].

RESULTS

Participation of soluble liver fractions in the transfer of s-RNA-bound amino acids to protein by RNP-particles

The enhancing effects of soluble liver fractions on the transfer of s-RNA-bound amino acids to protein in systems containing purified RNP-particles was studied in a series of introductory experiments. The s-RNA preparations used in these experiments had been prelabeled with L-[^{14}C]valine or a mixture of L-[^{14}C]amino acids. They were incubated with purified particles in the presence of a nucleoside triphosphate generating system and GTP or with only GTP (5 μmoles)[22]. Since there possibly might be some release of labeled amino acid from s-RNA under these conditions, the corresponding L-[^{12}C]amino acids were included in the systems in at least 100-fold excess to prevent any incorporation of isotope by way of the free amino acid pool.

In the absence of soluble liver enzymes only a limited isotope incorporation was observed and the addition of the SH-compounds MEA or GSH had no appreciable effect (Fig. 1).

When small amounts of a pH 5 enzyme fraction were included in the incubation systems, the labeled amino acids were readily transferred from s-RNA and incorporated into protein. Enzyme preparations, which had been stored for some time at —20° were significantly stimulated by SH-compounds. A similar stimulation was observed also with freshly prepared enzyme, especially at higher concentrations.

A rapid isotope incorporation was obtained also when S_{50}-protein was added to

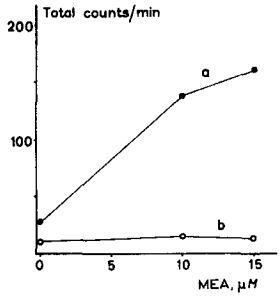

Fig. 1. Effect of MEA on the transfer of s-RNA-bound L-[^{14}C]amino acids to protein by purified RNP-particles. Curve a, 1.8 mg S_{50}-protein present; curve b without soluble liver enzymes. The incubation tubes contained in 1.8 ml of 0.025 *M* KCl, 0.01 *M* $MgCl_2$, 0.035 *M* Tris and 0.25 *M* sucrose: 1 μmole of ATP, 10 μmoles of PEP, 15 μg of pyruvate kinase, 0.2 μmole of GTP, 3.75 μmoles of an amino acid mixture, RNP-particles (5 mg of protein), 50 μg of prelabeled s-RNA (1600 counts/min) and the amount of MEA indicated. Incubation for 30 min at 35°.

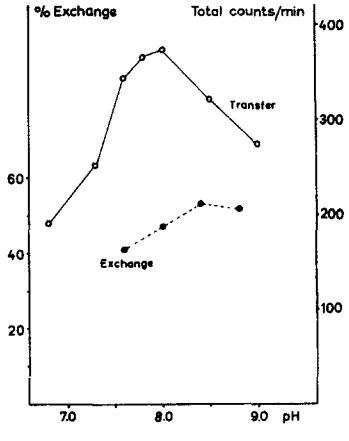

Fig. 2. Effect of pH on transfer activity and amino acid dependent PP–ATP exchange activity of liver enzyme preparations. Transfer experiment: Incubation system as in Fig. 1, but with 10^{-2} *M* GSH, 6 mg S_{50}-protein, RNP-particles corresponding to 4 mg of protein and Tris buffer at different pH. PP–ATP exchange experiment: The tubes contained in a final volume of 1.3 ml: 4 μmoles of [^{32}P]pyrophosphate, 6 μmoles of ATP, 6 μmoles of $MgCl_2$, 60 μmoles of KF, 3 μmoles of each of 12 amino acids[3], 50 μmoles of Tris buffer at different pH and 0.7 mg of pH 5 enzyme protein. Incubation for 12 min at 35°.

the incubation system in combination with MEA or GSH. The stimulation by the SH-compounds was very marked in experiments of this kind (Fig. 1).

Characteristic properties of transfer systems containing S_{50}-protein

On the basis of equal protein content the activity of the pH 5 enzyme fraction in the transfer systems was considerably higher than that of the 50-S-protein[6]. In spite of this fact a particular attention was paid to the properties of the transfer systems with S_{50}-protein. This preparation had a relatively low activity of amino acid activating enzymes (except for tyrosine, proline and cysteine activating enzymes) and no soluble RNA could be observed even by use of radioactivity measurements after pre-labeling with large doses of [^{32}P]phosphate *in vivo*[6].

The effect of pH on the rate of amino acid transfer by a system with S_{50}-protein is illustrated by Fig. 2. A fairly sharp activity optimum was found near pH 8. For comparison, the pH curve for amino acid dependent PP–ATP exchange by a pH 5 fraction, incubated with a mixture of 12 amino acids[3], is included in the figure. The optimum activity of this system was at about pH 8.4 with only a slight decrease on either side.

Under optimum conditions 30–40 % of the s-RNA-bound L-[^{14}C]amino acids were transferred to protein by the system. With S_{50}-protein added in some excess this transfer was completed in about 5 min. Approximately the same incorporation level was reached, however, with lower concentrations of S_{50}-protein, if the incubation periods were increased to 30 min or more. A preincubation of the particles for 20 min (35°) before the additon of L-[^{14}C]valine-s-RNA, S_{50}-protein, GTP and the nucleoside triphosphate generating system did not significantly decrease the rate of L-[^{14}C]valine-transfer.

As is shown by Fig. 3 the amount of L-[^{14}C]valine transferred by the system was a linear function of the amount of L-[^{14}C]valine-s-RNA added. Within certain limits, the reaction proceeded at a rate proportional to the amount of S_{50}-protein added to the system (Fig. 4).

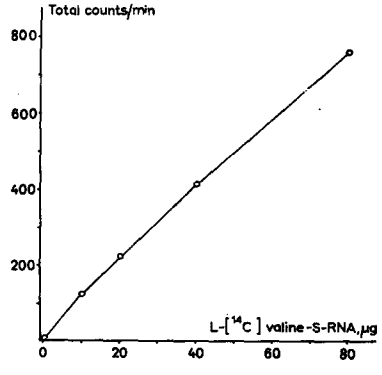

Fig. 3. Dependence of transfer rate on substrate concentration. Incubation system as in Fig. 1, but with 10^{-2} *M* GSH, 0.2 µmole of L-valine instead of the amino mixture, 4 mg of S_{50}-protein and 5.7 mg of RNP-particle protein and the indicated amounts of preincubated (*cf.* EXPERIMENTAL) L-[^{14}C]valine-s-RNA (10 µg corresponded to 250 counts/min).

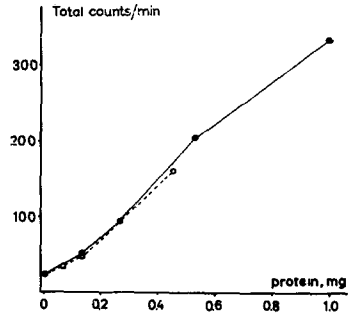

Fig. 4. Dependence of transfer rate on enzyme concentrations. ●—●, whole S_{50}-protein; ○—○, 0.15 *M* subfraction of S_{50}-protein. Incubation system as in Fig. 3, but with RNP-particles, corresponding to 6.5 mg of protein, 30 µg of preincubated L-[^{14}C]valine-s-RNA (750 counts/min), and the indicated amounts of enzyme.

The transfer reaction was inhibited by puromycin[23] and to a minor extent by chloramphenicol (Table I).

Amino acid transfer and amino acid activation by subfractions of soluble liver fractions

On the basis of the information obtained about the transfer systems the possible relationship between amino acid transfer and amino acid activation was further studied in a series of subfractionation experiments. Experiments of this kind were carried out with the ammonium sulfate fractions Am 0–55 and Am 55–85 of the whole soluble liver fraction, and with S_{50}-protein preparations.

The ammonium sulfate fractions Am 0–55 and Am 55–85 were adsorbed on calcium phosphate gel and eluted with potassium phosphate buffers (pH 7.2) of stepwise increasing concentrations (0–0.6 *M*). The activities of amino acid transfer and amino acid dependent PP–ATP exchange observed in the different subfractions are shown in Tables II and III.

With a mixture of 12 amino acids[3], PP–ATP exchange activity was found in both ammonium sulfate fractions, although with very different patterns of distribution.

TABLE I

EFFECTS OF CHLORAMPHENICOL AND PUROMYCIN ON THE TRANSFER OF LABELED AMINO ACIDS FROM S-RNA TO PROTEIN

Incubation system as in Fig. 1, but with 10^{-2} *M* GSH and 4 mg S_{50}-protein. The prelabeled s-RNA (100 μg, 1000 counts/min) was added after a preincubation of 10 min at 23°. Further incubation 30 min at 35°.

	Total counts/min
Complete system	117
Complete system, plus 10^{-3} *M* chloramphenicol	124
Complete system, plus $5 \cdot 10^{-3}$ *M* chloramphenicol	103
Complete system, plus $2 \cdot 10^{-6}$ *M* puromycin	100
Complete system, plus $2 \cdot 10^{-3}$ *M* puromycin	18

TABLE II

ACTIVITIES OF AM 0–55 SUBFRACTIONS IN TRANSFER AND AMINO ACID ACTIVATION

The PP–ATP exchange activity was measured as described in Fig. 2 (pH 7.8), with the indicated amounts of protein. In the case of valine specific PP–ATP exchange, the amino acid mixture was replaced by 3 μmoles of L-valine. Incubation for 60 min at 35°. Measurements of transfer activity as in Fig. 1, but with 10^{-2} *M* GSH and the amounts of protein indicated. When s-RNA, prelabeled with L[^{14}C]amino acids was used, 50 μg (1,600 counts/min) were added to the system. In the tubes with L-[^{14}C]valine-s-RNA (150 μg, 300 counts/min), the mixture of unlabeled amino acids was replaced by 0.2 μmole of L-valine.

Buffer for elution	*Protein in enzyme assay (mg)*	*% exchange*		*Isotope transfer (counts/min) from s-RNA, prelabeled with*		*RNA/protein* $\times 10^{-3}$
		Valine	*Amino acid mixture*	L-[^{14}C]valine	L-[^{14}C]amino acids	
0	1.66	0	0	3	23	<0.7
0.001 *M*	0.09	0	0.6	1	6	0
0.05 *M*	1.39	0	10.2	1	14	1.6
0.1 *M*	0.49	0	4.7	1	13	<0.7
0.2 *M*	0.50	0.2	17.0	72	156	—
0.3 *M*	0.36	17.0	48.0	51	128	1.2
0.6 *M*	0.22	5.3	18.0	5	20	<0.7

With valine alone, exchange activity was observed only in Am 0–55, and more exactly in two consecutive subfractions eluted with 0.3 and 0.6 *M* phosphate.

The transfer activity, as measured both with a mixture of s-RNA-bound L-[^{14}C]-amino acids and with L-[^{14}C]valine-s-RNA showed a distribution which was characteristically different from that of the PP–ATP exchange activities. As might be anticipated, the difference was most distinct in experiments involving only one amino acid, *e.g.* valine. The subfraction of the Am 0–55 fraction eluted with 0.2 *M* phosphate was of a particular interest, since the L-[^{14}C]valine transfer by this subfraction was relatively high, while the valine dependent PP–ATP exchange was almost insignificant. A more detailed study of this subfraction was therefore undertaken (Table IV). Not only with L-[^{14}C]valine-s-RNA but also with L-[^{14}C]tyrosine-s-RNA a relatively high transfer was obtained, in spite of the very low PP–ATP exchange activities with these amino acids.

Subfractionation experiments of a similar kind were carried out with the S_{50}-protein preparations. The fractionations, however, in this case were made with hydroxylapatite[10] instead of calcium phosphate gel. An experiment of this kind is shown in Table V. With the mixture of 12 amino acids the PP–ATP exchange activities were low and concentrated in the 0.3 *M* and 0.6 *M* subfractions. On the other hand, the transfer activity, as determined with the mixture of s-RNA-bound [^{14}C]amino acids was highest in the 0.2 *M* subfraction. A definite transfer was obtained already

TABLE III

ACTIVITIES OF AM 55–85 SUBFRACTIONS IN TRANSFER AND AMINO ACID ACTIVATION

For details see Table II.

Buffer for elution	*Protein in enzyme assay (mg)*	*% exchange*		*Isotope transfer (counts/min) from s-RNA, prelabeled with*		*RNA/protein* $\times 10^{-3}$
		valine	*amino acid mixture*	L-[^{14}C]*valine*	L-[^{14}C]*amino acids*	
0	2.43	0	25.2	214	294	2.3
0.001 *M*	0.18	0.5	2.6	5	30	18.0
0.05 *M*	0.30	0	0	0.4	31	4.3
0.1 *M*	0.31	0	6.0	2	9	19.4
0.2 *M*	0.44	0.5	11.5	10	26	7.3
0.3 *M*	0.17	0.2	43.8	14	36	0
0.6 *M*	0.11	0	2.7	1	18	0

TABLE IV

ACTIVITIES OF AM 0–55 SUBFRACTIONS IN TRANSFER AND AMINO ACID ACTIVATION

For details see Table II. Isotope transfer system, with 150 μg of L-[^{14}C]valine-s-RNA (500 counts/min), 180 μg L-[^{14}C]tyrosine-s-RNA (200 counts/min), or 100 μg s-RNA prelabeled with a mixture of [^{14}C]amino acids (1000 counts/min). L-valine (0.2 μmole), L-tyrosine (0.2 μmole) and an amino acid mixture (3.75 μmoles), respectively, were added to prevent isotope incorporation from free amino acids.

Buffer for elution	*Protein in enzyme assay (mg)*	*% exchange*			*Isotope transfer (counts/min) from s-RNA, prelabeled with*		L-[^{14}C]*amino acids*
		Valine	*Tyrosine*	*Amino acid mixture*	L-[^{14}C]*valine*	L-[^{14}C]*tyrosine*	
0.15 *M*	1.18	0.9	0.6	5.6	5	2	11
0.19 *M*	0.76	1.5	0	10.3	54	23	85
0.23 *M*	0.52	1.4	1.2	13.2	67	19	107

with the 0.1 *M* subfraction by which only an insignificant amino acid dependent PP–ATP exchange was catalyzed.

The activity of valine dependent PP–ATP exchange was concentrated in the 0.4 *M* subfraction, although some activity was also found in the 0.2 *M* fraction (Table VI). The same was true of the tyrosine dependent exchange. In the 0.4 *M* subfraction this latter exchange activity was relatively high (it should be noted that the exchange experiments with the mixture of 12 amino acids[3] did not include tyrosine).

The transfer ratio of [^{14}C]valine to [^{14}C]tyrosine was not constant in the different subfractions (Table VI). The activity of [^{14}C]tyrosine transfer was highest in the 0.2 *M* fraction, while the maximum activity of [^{14}C]valine transfer was not reached until later. As in the case of the [^{14}C]amino acid mixture (Table V) an activity of [^{14}C]-tyrosine transfer was observed already in the 0.1 *M* fraction, in which there was no evidence of valine or tyrosine activating enzymes.

The PP–ATP exchange ratios of valine to tyrosine were different from the corresponding transfer ratios. In the case of the 0.4 *M* subfraction the tyrosine activation was about 10 times higher than the valine activation, while, on the other hand, the transfer of tyrosine was considerably less active than the transfer of valine (Table VI).

TABLE V

ACTIVITIES OF S_{50}-PROTEIN SUBFRACTIONS IN TRANSFER AND AMINO ACID ACTIVATION

See Table II for details.

Buffer for elution	*Protein in enzyme assay (mg)*	*% exchange amino acid mixture*	*Isotope transfer (counts/min) from s-RNA, prelabeled with [^{14}C]amino acids*
0	1.32	0	124
0.001 *M*	0.40	0	13
0.05 *M*	1.0	0.43	11
0.1 *M*	1.70	0.45	54
0.2 *M*	1.08	2.2	248
0.3 *M*	1.28	7.40	179
0.6 *M*	0.40	3.3	28

TABLE VI

TRANSFER AND ACTIVATION OF VALINE AND TYROSINE BY SUBFRACTIONS OF S_{50}-PROTEIN

See Table II for details. Measurement of transfer activity as in Table II, but with 70 μg (400 counts/min) of L-[^{14}C]valine-s-RNA or 150 μg of L-[^{14}C]tyrosine-s-RNA (220 counts/min), 0.2 μmole of L-valine or L-tyrosine instead of the amino acid mixture and the amounts of protein indicated.

Buffer for elution	*Protein in enzyme assay (mg)*	*% exchange*		*Isotope transfer (counts/min) from s-RNA. prelabeled with*	
		Valine	*Tyrosine*	L-[^{14}C]valine	L-[^{14}C]tyrosine
0.05 *M*	1.0	1.4	1.3	15	9
0.1 *M*	1.0	0.3	0	41	13
0.2 *M*	1.0	0.3	0.3	65	26
0.4 *M*	0.5	5.5	53.4	113	13
—					
Original S_{50}-protein	1.0	0.4	14.4	143	27

Like the 0.1 *M* fraction the eluate with 0.15 *M* phosphate had no measurable activity of amino acid dependent pyrophosphate exchange. Some properties of this fraction were studied for comparison with the S_{50}-protein. The transfer activity of the 0.15 *M* fraction was stimulated by GSH (10^{-2} *M*). The enzyme concentration curve is included in Fig. 4.

DISCUSSION

The experiments reported here suggest that the amino acid activation and the transfer of s-RNA-bound amino acids to RNP-particles are catalyzed by different enzymes, and the following evidence may be presented in favor of this view. (a) The transfer activity was stimulated by MEA and GSH, while the activation of amino acids, as measured by amino acid dependent PP–ATP exchange, does not require the SH-compounds[24]. (b) The activation and transfer reactions have slightly different pH optima. The possibility should be kept in mind, however, that the lower pH optimum of the transfer reaction may be related to an increased instability of amino acid-s-RNA at higher pH (see ref.[25]). (c) By fractionation of liver enzyme preparations on calcium phosphate gel or hydroxylapatite, transfer activity was obtained in subfractions, which did not show any corresponding amino acid dependent PP–ATP exchange activity. (d) In overlapping subfractions there was no direct correlation between the activities of amino acid activation and amino acid transfer. (e) The S_{50}-protein had a relatively high content of tyrosine activating enzyme, which mainly appeared in the 0.4 *M* subfraction (Table VI). The isotope transfer from L-[^{14}C]tyrosine-s-RNA was rather low, however, and less transfer activity was shown by this fraction than by that eluted with 0.2 *M* phosphate.

The different ratios of valine to tyrosine transfer in the individual subfractions of S_{50}-protein (Table VI) may possibly indicate that different enzymes are involved in the transfer of different amino acids.

The non-amino acid dependent PP–ATP exchange is little understood at present. As a rule, this activity was relatively low in the S_{50}-protein. A high activity, however, was shown by some subfractions of the ammonium sulfate precipitates (particularly in Am 55-85). No correlation was observed between this non-amino acid dependent exchange and the RNA content. As a matter of fact, by this subfractionation method RNA was largely separated also from the amino acid dependent exchange activities[26].

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REFERENCES

1 M. B. HOAGLAND, P. C. ZAMECNIK AND M. L. STEPHENSON, *Biochim. Biophys. Acta*, 24 (1957) 215.
2 L. G. GROSSI AND K. MOLDAVE, *Biochim. Biophys. Acta*, 35 (1959) 275.
3 M. B. HOAGLAND, E. B. KELLER AND P. C. ZAMECNIK, *J. Biol. Chem.*, 218 (1956) 345.
4 L. I. HECHT, P. L. ZAMECNIK, M. L. STEPHENSON AND J. F. SCOTT, *J. Biol. Chem.*, 233 (1958) 954.
5 P. C. ZAMECNIK, M. L. STEPHENSON AND L. I. HECHT, *Proc. Natl. Acad Sci. U.S.*, 44 (1958) 73.
6 A. VON DER DECKEN AND T. HULTIN, *Biochim. Biophys. Acta*, 40 (1960) 189.
7 T. HULTIN, *Exptl. Cell Research*, 12 (1957) 290.
8 R. RENDI AND T. HULTIN, *Exptl. Cell Rearch*, 19 (1960) 253.
9 H. SACHS, *J. Biol. Chem.*, 228 (1957) 23.
10 A. TISELIUS, S. HJERTÉN AND Ö. LEVIN, *Arch. Biochem. Biophys.*, 65 (1956) 132.

[11] H. M. RAUEN, *Biochemisches Taschenbuch*, Springer-Verlag, Berlin-Göttingen-Heidelberg 1956 p. 1151.
[12] J. L. SIMKIN, *Biochem. J.*, 70 (1958) 305.
[13] W. MORGAN, A. VON DER DECKEN AND T. HULTIN, *Exptl. Cell Research*, 20 (1960) 655.
[14] L. I. HECHT, M. L. STEPHENSON AND P. C. ZAMECNIK, *Biochim. Biophys. Acta*, 29 (1958) 460.
[15] T. HULTIN AND A. VON DER DECKEN, *Exptl. Cell Research*, 15 (1958) 581.
[16] A. VON DER DECKEN AND T. HULTIN, *Exptl. Cell Research*, 17 (1959) 188.
[17] E. W. DAVIE, V. V. KONINGSBERGER AND F. LIPMANN, *Arch Biochem. Biophys.*, 65 (1956) 21.
[18] D. MÜTING AND V. WORTMANN, *Biochem. Z.*, 325 (1954) 448.
[19] G. CERIOTTI, *J. Biol. Chem.*, 214 (1955) 59.
[20] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
[21] E. BAER, *Biochem. Preparations*, 2 (1952) 25.
[22] A. VON DER DECKEN, *Exptl. Cell Research*, in the press.
[23] M. B. YARMOLINSKY AND G. L. DE LE HABA, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1721.
[24] R. RENDI AND T. HULTIN, *Exptl. Cell Research*, 17 (1959) 540.
[25] M. B. HOAGLAND, M. L. STEPHENSON, J. F. SCOTT, L. I. HECHT AND P. C. ZAMECNIK, *J. Biol. Chem.*, 231 (1958) 241.
[26] E. HERBERT, *Ann. N. Y. Acad. Sci.*, 81 (1959) 679.

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A HYDROLYTIC PROCEDURE FOR RIBONUCLEOSIDES AND ITS POSSIBLE APPLICATION TO THE SEQUENTIAL DEGRADATION OF RNA

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SUMMARY

Ribonucleosides are converted quantitatively to the corresponding bases by reacting successively with periodate and cyclohexylamine. The hydrolysis of the glycosidic linkage of the nucleoside is presumably due to the formation of a double Schiff base between the periodate oxidized nucleoside and cyclohexylamine. This method has been also applied to the hydrolysis of the terminal nucleoside of s-RNA. The molar ratios of the free bases (uracil:adenine:cytosine, 1:2.8:6.1) thus obtained from the terminal nucleosides of RNA were found to be the same as those of the terminal nucleosides (uridine:adenosine:cytidine, 1:2.9:6.1) obtained by alkaline hydrolysis of RNA of the same preparation. The phosphoester linkage between the diesterphosphate and the 5′-hydroxyl group of the oxidized terminal nucleoside of RNA was also found to be hydrolyzed during the cyclohexylamine treatment, thus exposing a new terminal 3′-phosphate. It has been shown that, following prostatic phosphomonoesterase treatment, the periodate and cyclohexylamine reactions can be repeated and thus a possible method for the sequential degradation of RNA is provided. An application of this method to the sequential degradation of phenol extracted s-RNA and preliminary results thereof have been discussed.

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid.