PURIFICATION OF SOLUBLE RNA FROM SHEEP THYROID GLAND. ABSENCE OF ACCEPTOR ACTIVITY FOR THE IODOTYROSINES

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Received January 18, 1964

It has been shown recently that free iodotyrosines of rat thyroid glands had a relative specific activity higher than that of thyroglobulin-bound iodotyrosines at early time intervals following the injection to rats of a single dose of I^{131} (Pitt-Rivers and Cavalieri, 1963), (Haney and Lissitzky, 1963). Two interpretations of these results have been proposed 1/ free iodotyrosines are direct precursors of thyroglobulin-bound iodotyrosines 2/ thyroglobulin is heterogeneous with regard to turn over. Although many arguments favour the latter interpretation, the former cannot be eliminated without direct investigation. If free iodotyrosines are incorporated in peptide linkages, the thyroid gland must contain soluble RNA's specific for them together with the correlated activating enzymes. Purification of soluble RNA's from sheep thyroid gland has been carried out. The purified material is active towards non iodinated protein amino acids but is unable to accept 3-iodo-L-tyrosine (MIT) and 3,5-di-iodo-L-tyrosine (DIT) in the presence of sheep thyroid or rat liver activating enzymes.

METHODS AND MATERIAL

Sheep thyroid glands were collected immediately after death at the local abattoir, carefully dissected and frozen in solid CO_2. Extraction of soluble RNA was carried out by the method of Kirby (1962) modified by using bentonite as a molecular inhibitor of ribonuclease (Brownhill et al, 1959) (Fraenkel-Conrat et al, 1961). The viscous solution obtained was treated by DNAase [Worthington 1 × cryst., repurified by DEAE-cellulose chromatography (Keller et al, 1958)] at the

concentration of 0.5 µg/ml and in the presence of 0.01 M $MgCl_2$ for 10 min at 5° C.

RNA was precipitated twice with ethanol-acetate (Kirby, 1962) and dissolved in 0.05 M NaCl. Further purification was carried out by chromatography on DEAE-cellulose at + 2° C according to Monier et al (1960) : 66 mg of DEAE-cellulose (Schleicher and Schüll, 0.9 meq/g) were used for 1 mg RNA assuming that 1 mg/ml was equivalent to an OD of 24 at 260 mµ). After extensive washing with 0.35 M NaCl, the RNA was eluted with 1 M NaCl, precipitated with cold ethanol-acetate and dissolved in 0.05 M NaCl (12 mg/ml). The yield was 140 mg for 200 g thyroid glands and the RNA obtained had a ratio A 260 mµ/A 280 mµ = 1.87.

Ultracentrifugation in a 5-20 % sucrose gradient in TRIS-HCl 0.05 M pH 7.5 (Martin and Ames, 1961) showed a single peak with a sedimentation constant of about 4. The activating enzymes of sheep thyroid or rat liver were obtained as the 105.000 g supernatant (S_{105}) according to Keller and Zamecnik (1956). Assays of amino acid-acceptor activity were carried out according to Nathans and Lipmann (1961). The incubation mixture contained for a final volume of 0.30 ml : 0.5 to 1.0 mg s-RNA, 2.5×10^{-5} M C^{14}- amino acid or 0.16×10^{-5} M I^{131}- (MIT or DIT), 0.003 M ATP, 0.006 M PEP, 0.010 mg PEP kinase, 0.006 M GSH, 0.005 M $MgCl_2$, 0.1 M TRIS-HCl pH 7.4 and 0.05 ml S_{105}.

It has been verified that, in these conditions, the activating enzymes were in excess and the RNA limiting. The mixture was incubated for 30 min at 37° C, C^{12}- or I^{127}- amino acids and carrier RNA were added and the RNA precipitated by 2.5 ml of cold 0.6 N perchloric acid itself containing 0.5 mg/ml of the convenient carrier amino acid. The precipitate was washed four times with perchloric acid, once with ethanol-ether (1:1) and dissolved in 0.5 ml 4M NH_4OH.

Blanks were obtained by omitting the S_{105} or by adding the C^{14}- or I^{131}- amino acid after incubation and precipitation of the mixture with perchloric acid.

C^{14}- was counted in a dry state on 2.5 × 7.5 Whatman n°3 paper pieces in a Tri-Carb liquid scintillation spectrometer. The solutions were counted for I^{131}- in a well-type crystal of Tl activated NaI.

Incorporation is expressed in mμmole amino acid per μmole of s-RNA.

Chromatographically pure I^{131}- MIT and I^{131}- DIT (S.A. : 2.1 and 2.7 μc/μg) were prepared by paper chromatographic separation of the enzymic digest (Pronase) of a rat I^{131}- thyroid extract.

C^{14}- amino acids were products of New England Nuclear, Boston, USA . Specific activities of final solutions were 26.4 μc/μmole.

RESULTS

Table 1 shows the acceptor activity of the thyroid s-RNA preparation for nine amino acids, using rat liver S_{105} as a source for the amino acyl RNA synthetases.

TABLE I

Spectrum of amino acid acceptor activity of sheep thyroid soluble RNA

amino acid	mμmoles amino acid incorporated per 1 μmole of s-RNA*
Isoleucine	20.5
Leucine	7.6
Serine	6.3
Phenylalanine	3.2
Tyrosine	2.9
Histidine	2.3
Arginine	2.2
Glutamic acid	1.8
Aspartic acid	1.4

* calculated assuming an average molecular weight of 25.000 for s-RNA.

Similar experiments with sheep thyroid S_{105} have given the same spectrum of amino acid acceptor activity but with half the values obtained with rat liver S_{105}.

With both enzymes no incorporation of MIT or DIT was noticed. Table 2 shows the figures obtained with tyrosine, MIT and DIT for a typical experiment.

TABLE II

Compared acceptor activity of sheep thyroid soluble RNA for tyrosine, MIT and DIT

S_{105}	Counts per minute in the precipitates for		
	I^{131}-MIT	I^{131}-DIT	C^{14}-tyrosine
Assay { sheep thyroid	402	2.041	780
Assay { rat liver	457	1.530	1.240
Blank { sheep thyroid or rat liver	425	1.977	180

The incubation mixtures (see methods) contained for a volume of 0.30 ml : I^{131}- MIT (1.6×10^{-6} M and 1.37×10^5 c.p.m.), I^{131}- DIT (1.2×10^{-6} M and 1.81×10^5 c.p.m.) or C^{14}- tyrosine (2.5×10^{-5} M and 1.12×10^5 c.p.m.). Each experiment in duplicate.

Other preparations of sheep thyroid s-RNA have given the same negative results.

The addition of tyrosine (or iodotyrosines) to the incubation mixture containing I^{131}- MIT or I^{131}- DIT (or C^{14}- tyrosine) does not modified the results.

Same experiments carried out with a s-RNA preparation from E. Coli also failed to show any incorporating activity for the iodotyrosines.

DISCUSSION

The preparations of sheep thyroid s-RNA obtained appear 1/ to be reasonably active to accept the common protein amino acids 2/ to contain convincingly no acceptor activity for MIT or DIT.

Although the direct iodination of tyrosyl-s-RNA by thyroid enzymes seems to be highly improbable, this possibility is nevertheless under investigation.

ACKNOWLEDGMENTS

We are indebted to Prof. R. Monier and Dr. R. Rosset for counsels and advices.

REFERENCES

Brownhill, T.S., Jones, A.S., and Stacey, M., Biochem. J., 73, 434 (1959).
Fraenkel-Conrat, H., Singer, B., and Tsugita, A., Virology, 14, 54 (1961).
Haney, J.B., and Lissitzky, S., Gen. Comp. Endocrinol., 3, 139 (1963).
Keller, E.B., and Zamecnik, P.C., J. Biol. Chem., 221, 45 (1956).
Keller, P.J., Cohen, E., and Neurath, H., J. Biol. Chem., 233, 344 (1958).
Kirby, K.S., Biochim. Biophys. Acta, 55, 545 (1962).
Martin, R.G., and Ames, B.N., J. Biol. Chem., 236, 1372 (1961).
Monier, R., Stephenson, M.L., and Zamecnik, P.C., Biochim. Biophys. Acta, 43, 1 (1960).
Nathans, D., and Lipmann, F., Proc. Natl. Acad. Sci. US, 47, 497, (1961).
Pitt-Rivers, R., and Cavalieri, R.R., Biochem. J., 86, 86 (1963).