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[1] P. HOFFMAN, A. LINKER AND K. MEYER, *Arch. Biochem. Biophys.*, (in the press).
[2] A. N. PAYZA AND E. D. KORN, *J. Biol. Chem.*, 223 (1956) 853.
[3] A. LINKER, K. MEYER AND P. HOFFMAN, *J. Biol. Chem.*, 219 (1956) 13.
[4] K. S. DODGSON AND A. G. LLOYD, *Biochem. J.*, 65 (1957) 4P.

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The possible role of the ribonucleic acid (RNA) of the pH 5 enzyme in amino acid activation

Recently HOAGLAND[1], and HOAGLAND, KELLER AND ZAMECNIK[2] discovered an enzyme (the pH 5 enzyme) in the soluble fraction of rat liver, which catalysed the activation of several amino acids. They[3] further succeeded in preparing a cell-free system from rat liver, which contained microsomes and the pH 5 enzyme and gave active incorporation of ^{14}C-amino acids into the microsome protein. In these reports[3,4], they emphasized the role of the microsome RNA in the protein synthesis by this enzyme system, but they made no reference to that of the RNA in the soluble fraction. The present communication deals with the possible role of the RNA of the pH 5 enzyme in amino acid activation, and the possibility of complex formation between this RNA and amino acid is also discussed.

The pH 5 enzyme, prepared from young rabbit liver according to the method of HOAGLAND *et al.*[2], was incubated with 100 μg crystalline RNase (Worthington Biochem. Corp.) per ml at 37° for 30 min (pH — 7.8). Its pH was then adjusted to 5.1 with dilute acetic acid, the precipitate was washed with acetate buffer and then dissolved in 0.1 *M* tris-buffer, pH 7.8. As a control, the same amount of the pH 5 enzyme was used, which was treated in the similar manner but without RNase. The amino-hydroxamate formation was measured by the procedure of HOAGLAND *et al.*[2], using acetohydroxamate as a standard.

As shown in Table I, when the pH 5 enzyme pretreated with RNase was incubated with ATP and the amino acid mixture, the amount of the hydroxamate formed by the amino acids was decreased, as compared with that in the control experiment. The results suggested that the RNA of the pH 5 enzyme might participate in the mechanism of amino acid activation. Recently HOLLEY[6] reported that the pH 5 enzyme brought about the conversion of radioactive AMP into ATP, which occurred only in the presence of L-alanine and further that this conversion was inhibited by RNase.

TABLE I

THE EFFECT OF RNASE ON AMINO HYDROXAMATE FORMATION BY THE pH 5 ENZYME

Expt. No.	*Enzyme pre-treated without RNase* — *Hydroxamate formed (μmoles per flask)*			*Enzyme pre-treated with RNase* — *Hydroxamate formed (μmoles per flask)*			*Relative RNA content of the enzyme (% of control value)*
	With amino acid mixture	*Without amino acid mixture*	*Difference*	*With amino acid mixture*	*Without amino acid mixture*	*Difference*	
31	0.16	0.05	0.11	0.06	0.01	0.05	50
32	0.21	0.07	0.14	0.07	0.04	0.03	52
33	0.15	0.01	0.14	0.07	0.03	0.04	53
Mean value	0.17	0.04	0.13	0.07	0.03	0.04	52

Incubation at 37° for 1 h. The same reaction mixture was used as that of HOAGLAND *et al.*[2].

TABLE II

^{14}C-ACTIVITY OF THE VARIOUS FRACTIONS OF THE pH 5 ENZYME AFTER THE INCUBATION OF THE ENZYME WITH ^{14}C-ALANINE*

Expt. No.		^{14}C-activity (division/min/mg dry weight)					
		Pr-RNA**	Pr_2**	Pr_1**	RNA_1**	RNA_2**	RNA_3**
42	with ATP	0.079	0.010		0.013	0.015	0.013
	without ATP	0.009	0.010				
43	with ATP	0.070	0.007	0.012	0.013	0.014	0.012
	without ATP	0.016	0.005	0.010			

Incubation at 37° for 1 h.

* DL-alanine-1-^{14}C (0.75 μcurie/ml = 367 division/min with the Lauritsen electroscope).

** See the text.

After the incubation of the pH 5 enzyme with ^{14}C-alanine, ATP, and the amino acid mixture (excluding alanine from the complete twelve amino acids mixture), the enzyme was precipitated at pH 5, washed 8–10 times with dilute acetate buffer (pH 5) until the washings contained no radioactivity, and then the radioactivity of the precipitate was measured. It was found that ^{14}C-activity remained behind in this washed protein fraction containing RNA (Pr-RNA fraction) and it remained unchanged even after dialysis against cold ^{12}C-alanine solution. The ^{14}C-activity of the Pr-RNA fraction in the zero time control experiment was negligible and the incorporation of ^{14}C-alanine into the protein (Pr_1) of the pH 5 enzyme was very slight. Pr_1 was prepared by washing the pH 5 enzyme repeatedly with cold and hot trichloroacetic acids, ethanol, ethanol-ether mixture, and chloroform-methanol mixture. The omission of ATP from the complete system resulted in a marked depression of the specific activity of Pr-RNA fraction, as shown in Table II. It is suggested, therefore, that ^{14}C-alanine may be bound to the pH 5 enzyme without incorporation into peptide bond after the incubation with the amino acid mixture and ATP.

Further fractionation of the ^{14}C-labeled pH 5 enzyme was undertaken after the addition of carrier yeast nucleic acid according to the modified method of VOLKIN AND CARTER[5].

It was found that ^{14}C-activity was bound to the RNA fraction (RNA_1), and the specific activity of RNA_1 did not change after the purification with ethanol was twice repeated (RNA_2, RNA_3). On the contrary, the specific activity of the protein fraction (Pr_2) after extraction of the RNA from the Pr-RNA fraction was very low, compared with that of the Pr-RNA fraction. So it may be reasonable to conclude that the RNA of the pH 5 enzyme is the site of the complex formation with amino acid.

Recently by a large-scale experiment using the same system without the addition of carrier RNA, it has been demonstrated that ^{14}C-activity/mg RNA is markedly higher than that of the Pr-RNA fraction.

These results were presented at the Symposium on "Biosynthesis of Protein and Enzymes" at the 29th annual meeting of the Japanese Biochemical Society held in Fukuoka on October 31, 1956.

Addendum. When this note was submitted, the similar results (using leucine) reported by HOAGLAND *et al.* in the April issue of this journal (*Biochim. Biophys. Acta*, 24 (1957) 215) were not available to the authors.

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[1] M. B. HOAGLAND, *Biochim. Biophys. Acta*, 16 (1955) 288.
[2] M. B. HOAGLAND, E. B. KELLER AND P. C. ZAMECNIK, *J. Biol. Chem.*, 218 (1956) 345.
[3] E. B. KELLER AND P. C. ZAMECNIK, *J. Biol. Chem.*, 221 (1956) 45.
[4] P. C. ZAMECNIK, E. B. KELLER, J. W. LITTLEFIELD, M. B. HOAGLAND AND F. B. LOFTFIELD, *J. Cellular Comp. Physiol.*, 47, Suppl. 1 (1956) 81.
[5] E. VOLKIN AND C. E. CARTER, *J. Am. Chem. Soc.*, 73 (1951) 1916.
[6] R. W. HOLLEY, *J. Am. Chem. Soc.*, 79 (1957) 658.

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