SPECIFICITY OF ACCEPTOR RNA FOR ALANINE ACTIVATION*

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Two, apparently different, alanine-activating enzymes have been found in extracts of pig liver. The first, from pig liver cytoplasm, has been purified some 3800-fold, and exhibits a single component upon electrophoresis in three different buffer systems at pH 6.8, 7.5, and 8.6. The second enzyme has been purified about 30-fold from disrupted pig liver nuclei. Both enzymes appear to catalyze the same reaction, namely

$$\text{L-Alanine} + \text{ATP} + \text{SRNA} \rightleftharpoons \text{L-Alanine-SRNA} + \text{AMP} + \text{P-P}_i$$

as well as an alanine-dependent exchange of P-P_i with ATP. Both enzymes are specific for L-alanine and for ATP. Both require a divalent cation for activity. However, the cytoplasmic enzyme is most active in the presence of low concentrations of cobalt ions, while the nuclear enzyme is most active with magnesium ions.

The two enzymes are highly specific with respect to the employment of SRNA. Table I shows that the cytoplasmic enzyme is highly active at binding alanine only to the SRNA obtained from either pig liver or pig muscle cytoplasm. The SRNA from pig liver nuclei is poorly utilized. In contrast, the nuclear enzyme readily binds alanine to nuclear SRNA from pig liver, but is relatively inactive with SRNA from all other sources, including pig liver

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cytoplasm.

It is of interest to note not only that pig liver nuclei and cytoplasm apparently contain different alanine-activating enzymes, but also that the enzymes exhibit such a high degree of specificity for SRNA. This specificity appears to be similar to that reported by Berg and Ofengand (1958) for valine activation by an E. coli enzyme, and in contrast to the lack of specificity reported for amino acid-activation by guinea pig liver (Schweet, et al., 1958) and ascites tumor (Hecht, et al., 1959) extracts. In the absence of further evidence, it appears that amino acid-activating enzymes may differ considerably in their ability to utilize SRNA preparations from various sources.

TABLE I

SPECIFICITY OF SRNA ACCEPTOR FOR ALANINE ACTIVATION BY NUCLEAR AND CYTOPLASMIC ENZYMES

	mμ moles C^{14}-Alanine incorporated into SRNA	
SRNA	Nuclear enzyme	Cytoplasmic enzyme
Pig liver cytoplasm (2.0 mg.)	1.0	7.4
Pig liver nuclei (2.0 mg.)	10.2	1.8
Pig liver cytoplasm (2.0 mg.) plus pig liver nuclei (2.0 mg.)	11.0	8.5
Pig muscle cytoplasm (2.0 mg.)	1.8	8.4
Calf liver cytoplasm (2.0 mg.)	1.0	0.8
Yeast (2.0 mg.)	0.2	0.6
Pea seed (2.0 mg.)	0.4	0.8

Assay for incorporation was as described previously (Webster, 1959). Reaction mixture for the cytoplasmic enzyme consisted of: 0.05 M tris (hydroxymethyl) aminomethane-HCl buffer of pH 7.5, 0.01 M alanine-C^{14}

(3,000,000 cts/min.), 0.01 M potassium ATP, 0.005 M $CoCl_2$, 2.0 mg. SRNA, 0.1 mg. crystalline yeast pyrophosphatase, and 0.001 mg. cytoplasmic alanine-activating enzyme in a total volume of 1 ml.

Reaction mixture for the nuclear enzyme consisted of: 0.05 M tris (hydroxymethyl) aminomethane of pH 8.0, 0.01 M C^{14}-alanine (3,000,000 cts/min.), 0.01 M potassium ATP, 0.01 M $MgCl_2$, 2.0 mg. SRNA, 0.1 mg. crystalline yeast pyrophosphatase, and 0.1 mg. nuclear alanine-activating enzyme in a total volume of 1 ml. SRNA was prepared and purified from the non-sedimentable portion of disrupted cells or nuclei by the method of Kirby (1956). The reaction mixtures were shaken for 20 min. at 38° C. Longer incubation periods resulted in no further labeling of the SRNA.

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