INTERACTION OF HOMOLOGOUS TRANSFER RNA WITH YEAST AMINOACYL-RNA SYNTHETASES

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The studies reported herein show the effect of normal and periodate-oxidized tRNA on the activation of L-tyrosine, L-leucine, L-histidine and L-valine by synthetases preincubated at selected temperatures. Also shown is a comparison pertaining to the stability of the activation and transfer activities of each enzyme at the selected preincubation temperatures.

The investigation indicates that the ATP-$^{32}PP_i$ exchange activity catalyzed by these synthetases is stimulated by tRNA. Either normal or periodate treated tRNA produces this effect. With the exception of the tyrosyl-RNA synthetase, the transfer activity of these enzymes is relatively less stable to heat than the activation activity. The loss of transfer function due to heat denaturation does not prevent stimulation by tRNA of the exchange reaction catalyzed by these synthetases.

The aminoacyl-RNA synthetases catalyze both the carboxyl activation of specific amino acids and the formation of aminoacyl-tRNA (11, 12). These synthetases must possess specificity not only for an amino acid, but also for a transfer RNA (tRNA) which will accept the amino acid. The latter implies a specific mode of interaction between a particular tRNA and the corresponding

aminoacyl-RNA synthetases. It has been proposed that the tRNA molecule has an "enzyme recognition site" which may be composed of a specific arrangement of bases that "recognize" a complementary site on the enzyme molecule (5, 20, 26). Recent reports indicate that tRNA not only accepts the activated amino acid, but may also modify the capacity of the aminoacyl-RNA synthetase in formation of the activated amino acid complex. In certain studies tRNA has been shown to have a stimulatory effect on amino acid activation (6, 10, 15, 16, 19, 22, 23, 25); whereas, an inhibitory effect produced by tRNA was observed for the activation of other amino acids (8, 9). Considerable evidence has been accumulated in support of the hypothesis that tRNA does effect the catalytic activity of aminoacyl-RNA synthetases; however, the precise manner in which this effect is exerted and its significance are not entirely clear.

MATERIALS AND METHODS

Preparation of crude enzyme extract. - A crude extract of Bakers' yeast (Federal Yeast Corp.) was prepared by freezing three pounds of yeast cells in an ether-CO_2 mixture. The ether layer was decanted, and residual ether was removed by vacuum. The yeast homogenate was extracted overnight by stirring after the addition of 10 g KCl/1000 g of yeast. The extraction and all subsequent steps in the preparation were carried out at 0-4°. The supernatant liquid (300 ml) recovered by centrifugation (7,700 x g) from the KCl-extracted homogenate was passed through several layers of cheesecloth giving the crude extract.

Ammonium sulfate fractionation. - Solid ammonium sulfate (5.12 g for each 100 ml of crude extract) was added with stirring to the crude extract. The mixture was stored for 3 days, and

then centrifuged to remove the precipitate. The supernatant liquid was brought to 60% saturation by addition of 0.33 g of ammonium sulfate per ml of the protein solution. After 2 hours the precipitate was collected by centrifugation at 7,700 x g for 20 minutes, and dissolved in 180 ml of distilled water. The dissolved precipitate was made 42% with respect to saturation with ammonium sulfate. The precipitate was collected by centrifugation and dissolved in 150 ml of 0.1 M Tris-HCl, pH 7.25. After dissolving the precipitate, the volume was measured, and ammonium sulfate was added to a saturation of 31%. The precipitate obtained after centrifugation was discarded, and the supernatant liquid was retained. To the supernatant liquid ammonium sulfate was added to 52% saturation, and after 30 min the precipitate was collected by centrifugation at 7,700 x g for 30 min. The precipitate was dissolved in 100 ml of 0.1 M Tris-HCl, pH 7.25. Finally, to the redissolved precipitate, ammonium sulfate was added to a saturation of 48%. After centrifugation, the supernatant liquid was dialyzed against 0.1 M Tris-HCl, pH 7.25.

Sephadex G-200 chromatography. - The buffered protein solution was passed through a column of Sephadex G-200 (4.7 x 148 cm) previously equilibrated with 0.1 M Tris-HCl, pH 7.25. The fractions (10 ml each) collected, containing the first one-third of the total protein eluted from the column, were pooled, dialyzed against distilled water and lyophilized to dryness. The residue obtained after lyophilization was dissolved in 20 ml of 0.1 M Tris, pH 7.25, and the solution containing 2 mg of protein per ml was referred to as Fraction G-200. Fraction G-200, which was free of tRNA, exhibited a high level of activity for L-tyrosine, L-leucine, L-histidine and L-valine and relatively little activity for any other amino acid. Aliquots of Fraction

G-200 were preincubated for 10 minutes at 8°, 25°, 30°, 37°, 41° and 55°, and then equilibrated at 37° for 3 minutes prior to being used as the source of enzyme in the assay procedures. Alterations in the time of preincubation or equilibration produced significant changes in the data obtained.

ATP-$^{32}PP_i$ exchange assay for amino acid activation. - The components of the assay were as follows: 0.2 mg of protein of Fraction G-200, after preincubation at the indicated temperature described in the text; 200 mM Tris-HCl, pH 7.25; 5.0 mM disodium ATP; 6 mM L-isomer of the amino acid; 5 mM $MgCl_2$; 5 mM $K_4{}^{32}P_2O_7$, pH 7.25; and 100 mM KF. As indicated under Figure 1, certain tubes contained 0.67 mg of tRNA in addition to the other components. The total volume was adjusted to 1 ml with water. The tubes were incubated for 30 minutes at 37° after which the reaction was stopped by the addition of 10% trichloracetic acid and the coagulated protein was removed by centrifugation. The ATP-^{32}P was absorbed onto Norit and treated according to the method of Crane and Lipmann (3) as modified by DeMoss and Novelli (4). Activity was expressed as total cpm incorporated into ATP per 30 minutes.

Assay for formation of aminoacyl-RNA. - The reaction mixture contained the following components: 1.6 mg of yeast tRNA (General Biochemicals); 100 mM Tris-HCl, pH 7.25; 25 mM $MgCl_2$; 2 mM disodium-ATP; 0.5 mM disodium-EDTA; 0.01 mM L-isomer of ^{14}C-labeled amino acid; 0.2 mg of protein of Fraction G-200 (precinubated at the prescribed temperatures), and water added to a total volume of 1.0 ml. The reaction mixtures were incubated for 10 minutes at 37°. The reaction was stopped by submersing the tubes in a NaCl-ice water bath, and 0.1 ml aliquots of the mixture were pipeted onto paper discs, dried and analyzed

similar to the methods described by Holley, et al., (13) and Nishimura and Novelli (21). Activity was expressed as total cpm transferred to tRNA per 10 minutes incubation at 37°.

Other methods. - The $K_4{}^{32}P_2O_7$ was prepared from carrier-free ^{32}P-orthophosphate (New England Nuclear Corp.) by pyrolysis (14). Protein concentrations were determined by the method of Lowry, et al., (17). Radioactivity was measured in a Packard Tri-Carb liquid scintillation counter using the scintillation liquid described by Bray (1). Orthophosphate was determined by the method of Fiske and SubbaRow (7).

RESULTS AND DISCUSSION

The effect of preincubation at selected temperatures on the activation and transfer activities of the L-tyrosyl-, L-leucyl-, L-histidyl- and L-valyl-RNA synthetases, and the effect of normal and periodate-oxidized tRNA on the ATP-$^{32}PP_i$ exchange reaction catalyzed by these preincubated synthetases are shown in Figure 1. With the exception of the tyrosyl-RNA synthetase, the transfer activity of each of the enzymes displayed a greater lability toward heat than did the capacity for activation of the amino acid. As illustrated in Figure 1, activation activity, as measured by the ATP-$^{32}PP_i$ exchange reaction, was relatively stable at preincubation temperatures between 8° and 41°. Of the four enzymes studied, the tyrosyl-RNA synthetase was the least stable at temperatures above 41°, with no detectable ATP-$^{32}PP_i$ exchange activity remaining at 48°. In contrast, the histidyl-RNA synthetase was the most stable at the various preincubation temperatures with measurable ATP-$^{32}PP_i$ exchange activity remaining after preincubation of the enzyme at 55° prior to assay. With regards to transfer activity, the L-leucyl- and valyl-RNA synthetases

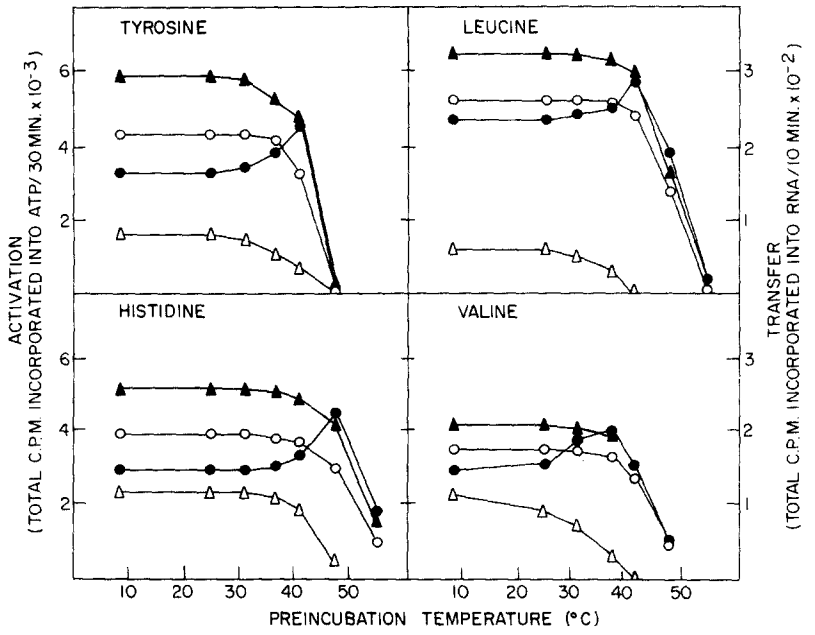

Fig. 1. Effect of preincubation and tRNA on L-tyrosyl-, L-leucyl-, L-histidyl- and L-valyl-RNA synthetase activity. The ATP-$^{32}PP_i$ exchange assay was conducted in the absence of tRNA (O) and in the presence of 0.67 mg of normal (●) or periodate-oxidized tRNA (▲). Transfer of ^{14}C-amino acids to tRNA (Δ) was measured using 1.6 mg of normal tRNA. Both methods of assay are described under "Materials and Methods." Prior to the addition of the enzyme (Fraction G-200) to the incubation mixtures, aliquots of the enzyme fraction were preincubated at the indicated temperatures for 10 minutes and then equilibrated at 37° for 3 minutes. Following this treatment, 0.1 ml (0.2 mg of protein) of the appropriate preincubated enzyme samples was added to the respective assay mixture.

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were inactive after preincubation at 41°; whereas, transfer activity of the L-histidyl- and L-tyrosyl-RNA synthetases, although decreased, still remained active after preincubation at 41°. The differences in stability between the exchange and transfer activities of these synthetases to heat become more apparent whenever normal tRNA is added to the ATP-$^{32}PP_i$ exchange assay medium. In the presence of normal tRNA (Figure 1), there is a progressive increase in the stimulation of the exchange reaction with enzyme preincubation at 25° through 41° for tyrosine

and leucine, from 25° through 37° with valine and from 30° through 48° with histidine. The observed increase in ATP-$^{32}PP_i$ exchange activity with tRNA added to the reaction mixture was coincident with the decrease in transfer activity of the enzyme at that particular preincubation temperature.

One interpretation of these observations is that tRNAs interact with the synthetases producing a stimulatory effect on the ATP-$^{32}PP_i$ exchange reaction at all temperatures, but at the lower preincubation temperatures, the stimulation was not observed presumably because the enzymes possessed both activation and transfer activities which were in competition for the aminoacyl-AMP-enzyme complex. On the other hand, preincubation of the enzyme at temperatures destructive to the transfer activity (Figure 1) would decrease or eliminate the competition of tRNA with PP_i for the aminoacyl-AMP-enzyme complex permitting the exchange reaction to proceed unaffected. Under these conditions, the stimulation by tRNA of the enzyme-catalyzed ATP-$^{32}PP_i$ exchange reaction can be observed. In order to examine whether the amino acid-acceptor portion of the tRNA molecule was involved in stimulation of the exchange activity of the enzymes, periodate-oxidized tRNA (27) was employed. Assay of the periodate-oxidized tRNA indicated that it was incapable of accepting activated amino acids from the aminoacyl-AMP-enzyme intermediates. As shown in Figure 1, the level of exchange activity in the presence of periodate-oxidized tRNA was as high at lower preincubation temperatures as the maximal level of activity observed for that particular synthetase with tRNA which had not been exposed to periodate oxidation. Thus, it would appear that the amino acid acceptor site of tRNA need not be intact in order for the stimulatory effect by tRNA to be exerted. The difference in the level of

stimulation of the exchange reaction observed at lower preincubation temperatures with untreated tRNA as compared to periodate-oxidized tRNA is most likely due to the transfer of the activated amino acid to the untreated tRNA. As preincubation temperatures are increased, however, transfer activity of the enzyme is decreased. As a consequence, the competition between normal tRNA and PP_i for tha aminoacyl-AMP-enzyme complex is decreased, and the observed level of ATP-$^{32}PP_i$ exchange stimulated by normal tRNA would approach that with periodate-oxidized tRNA. It seems likely that the same absolute stimulatory effect is exerted by both the periodate-oxidized and the untreated tRNA, and that the stimulation may be due to an interaction of the tRNA with the synthetase altering the conformation of the enzyme molecule to a more reactive form. Our observations together with those of other laboratories (6, 9, 10, 15, 16, 18, 22, 25) suggest that tRNA has a decided stimulatory effect on certain aminoacyl-RNA synthetase and that the amino acid binding site of the tRNA need not be intact for the effect to take place.

The loss of transfer activity without a significant decrease in exchange activity in the absence of tRNA demonstrates differences in stability of the two activities of the enzymes to heat. Apparently this characteristic is not limited to these four synthetases of Bakers' yeast. Charlier and Grosjean (2) reported that the transfer activity of the isoleucyl-RNA synthetase of Bacillus stearothermophilus was denatured at lower temperatures than the ATP-$^{32}PP_i$ exchange activity of the enzyme. More recently Papas and Mehler (24) reported that transfer activity of the prolyl-RNA synthetase of E. coli, but not the ATP-$^{32}PP_i$ exchange activity, was impaired by storage of the enzyme in the cold. It seems reasonable to assume that other activating enzymes may

show similar characteristics.

Apparently some significant irreversible change in conformation of the enzymes takes place at specific preincubation temperatures which prevents the amino acid binding site of tRNA from accepting the aminoacyl-adenylate complex being formed. Conversely, the active centers of the enzyme concerned with formation of the aminoacyl-adenylate and with the recognition of tRNA do not appear to be effected at this temperature. This is supported by the observed stimulatory effect of the exchange reaction by normal and periodate-oxidized tRNA in the absence of transfer activity and by the constant level of ATP-$^{32}PP_i$ exchange activity observed with enzyme preincubated at the lower temperatures.

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