EFFECT OF ASSAY CONDITIONS ON THE MAGNESIUM REQUIREMENT OF THE TRANSFER REACTION CATALYZED BY PHENYLALANYL-tRNA SYNTHETASE FROM BAKERS' YEAST

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Summary: Phenylalanyl-tRNA synthetase exhibits an absolute requirement for magnesium ion in its transfer reaction when assayed in 10 mM Tris-acetate buffer at pH 7.2. This magnesium requirement can be largely eliminated by the use of 50 mM sodium cacodylate, citrate or succinate buffers at pH 6. It is thus demonstrated that, depending upon the assay conditions which are employed, an aminoacyl-tRNA synthetase can exhibit ambivalence with respect to the magnesium requirement of its transfer reaction.

Most aminoacyl-tRNA synthetases are able to catalyze the formation of an enzyme-bound aminoacyladenylate complex which can subsequently transfer its aminoacyl moiety to the appropriate tRNA (1). Previous studies of the transfer reactions catalyzed by different aminoacyl-tRNA synthetases have indicated a lack of uniformity concerning the cationic requirement of this class of reactions. A number of investigators have reported that various aminoacyl-tRNA synthetases catalyze this transfer reaction efficiently in the absence of Mg^{2+} (2-14), while other investigators have reported an absolute requirement for Mg^{2+} or another suitable oligovalent cation in the transfer reactions catalyzed by other aminoacyl-tRNA synthetases (11,15-22). However, a wide range of assay conditions were employed in these various studies and the effect of assay variables on the Mg^{2+} requirement of these transfer reactions is poorly understood.

In a previous investigation of the transfer reaction catalyzed by phenyl-

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alanyl-tRNA synthetase from bakers' yeast, we found an absolute requirement for Mg^{2+} when the reaction was carried out in 10 mM Tris-acetate buffer at pH 7.2 (20). We now report that this Mg^{2+} requirement can be largely eliminated by the use of 50 mM sodium cacodylate, citrate or succinate buffers at pH 6.

Experimental

Uniformly-labeled L-[^{14}C]phenylalanine, 460 μCi/μmole, was purchased from Schwarz/Mann. Yeast $tRNA^{phe}$ (73% pure) was from the preparation described previously (20) and was freed of metal ions by passage through a Sephadex G-25 column equilibrated and eluted with 0.1 mM EDTA (pH 7.0). The phenylalanyl-tRNA synthetase used in these experiments was assayed and partially purified from bakers' yeast as before (20). This enzyme preparation catalyzed the formation of 2.3 nmoles of phenylalanyl-tRNA/min/mg of protein and exhibited an A_{280}:A_{260} ratio of 1.85. Most of the materials employed in these experiments were obtained from sources identified previously (20). All other chemicals were of the highest purity available commercially.

The [^{14}C]phenylalanyladenylate-enzyme complex was formed in the standard reaction mixture and the resulting complex was purified on a Sephadex G-50 column as before (20), although for the transfer reactions conducted in sodium cacodylate buffer (Figures 1 and 2) the column was equilibrated and eluted with 10 mM sodium cacodylate (pH 7.0), 0.1 mM EDTA, 0.3 mM dithiothreitol. The complex isolated in these experiments contained 62,000-82,000 cpm of [^{14}C]-phenylalanine/mg of protein and 0.17-0.22 mg of protein/ml.

The transfer of [^{14}C]phenylalanine from the [^{14}C]phenylalanyladenylate-enzyme complex to purified yeast $tRNA^{phe}$ was measured at 21° in an assay mixture containing per 1.0 ml: 0.50 ml (6150-8530 cpm) of [^{14}C]phenylalanyladenylate-enzyme complex (in 0.1 mM EDTA, 0.3 mM dithiothreitol, plus either 10 mM Tris-acetate (pH 7.2) or 10 mM sodium cacodylate (pH 7.0)); 0.130 A_{260} unit of metal ion-free $tRNA^{phe}$; plus whatever other components were required to achieve the final assay conditions specified in the figure legends. Aliquots (100 μℓ) of the incubation mixtures were withdrawn at the appropriate times and processed to determine the amount of [^{14}C]phenylalanyl-tRNA formed as described previously (20). The pH values of transfer reaction mixtures were measured with a Radiometer model 22 pH meter equipped with a GK2321 C combination electrode.

Results

Cacodylate has been employed in several studies as a buffer for the transfer reaction catalyzed by different aminoacyl-tRNA synthetases (2-5). In all of these cases the transfer reactions were reported to proceed efficiently in the absence of added Mg^{2+}. The effect of this buffer on the transfer reaction catalyzed by phenylalanyl-tRNA synthetase from bakers' yeast was therefore examined in some detail.

As shown in Figure 1, at pH 7.0 and 21°, the transfer reaction failed to

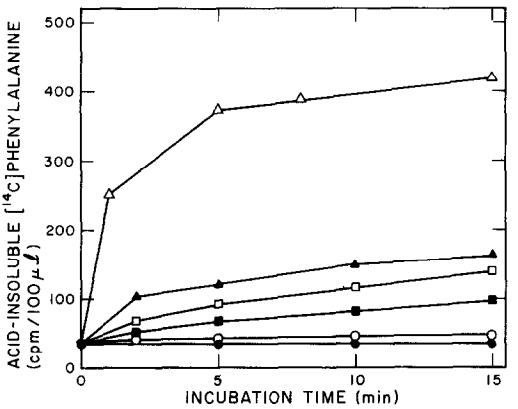

Figure 1. Effect of sodium cacodylate concentration on the kinetics of the transfer reaction. The assay incubation mixtures contained per 1.0 ml: 0.1 μmole of EDTA; 0.15 μmole of dithiothreitol; 0.130 A_{260} unit of $tRNA^{phe}$; [^{14}C]phenylalanyladenylate-enzyme complex (7255 cpm); plus the following amounts of sodium cacodylate: 10 μmoles (●—●); 25 μmoles (○—○); 50 μmoles (■—■); 75 μmoles (□—□); 100 μmoles (▲—▲); 10 μmoles of sodium cacodylate plus 0.2 μmole of magnesium acetate (△—△). The assays were conducted at pH 7.0 and 21°.

proceed in 10 mM sodium cacodylate but was increasingly stimulated as the concentration of sodium cacodylate was raised to 100 mM, the highest concentration which was investigated. However, both the rate and the extent of the transfer reaction carried out in 100 mM sodium cacodylate (pH 7.0) were substantially less than those observed in the presence of 10 mM sodium cacodylate (pH 7.0) plus 0.2 mM magnesium acetate (Figure 1).

At 21° and 50 mM sodium cacodylate, the transfer reaction was stimulated markedly (530%) by lowering the pH of the assay from 7.7 to 6.2 (Figure 2). A further reduction in pH from 6.2 to 5.5 resulted in a substantial inhibition of the transfer reaction. It is thus apparent that this transfer reaction is extremely sensitive to the pH of the assay, the optimum pH being ca. 6. At this optimum pH, the rate of the transfer reaction in the absence of Mg^{2+} approached to within 49% of that observed in the Mg^{2+}-containing control at pH 7.0. However, since 50 mM sodium cacodylate was a suboptimum concentration at pH 7.0 (Figure 1), a higher concentration of this buffer at pH 6 may well bring the transfer rate much closer to that observed in the presence of Mg^{2+}.

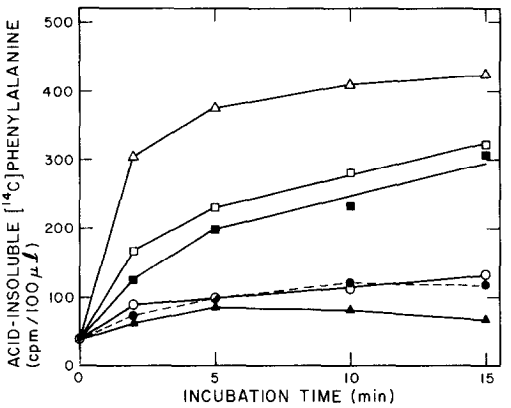

Figure 2. Effect of pH on the transfer reaction stimulated by 50 mM sodium cacodylate. The assay incubation mixtures contained per 1.0 ml: 50 μmoles of sodium cacodylate; 0.1 μmole of EDTA; 0.15 μmole of dithiothreitol; 0.130 A_{260} unit of $tRNA^{Phe}$; and [^{14}C]phenylalanyladenylate-enzyme complex (6150 cpm). The assays were conducted at 21° and pH 7.7 (▲—▲), pH 7.0 (○—○), pH 6.5 (■—■), pH 6.2 (□—□) or pH 5.5 (●····●). The magnesium acetate (0.2 mM) control assay (△—△) contained 10 mM sodium cacodylate (pH 7.0) and was conducted at 21°.

In a separate experiment not shown here it was found that, at pH 6.2 and 50 mM sodium cacodylate, the transfer rate was increased upon raising the temperature of the assay from 0 to 21° but exhibited a slight reduction upon further increasing the temperature to 30°.

Citrate (6) and succinate (7-11) have also been employed as buffers in studies where Mg^{2+} was reported to be a nonessential cofactor for such transfer reactions. In all of these cases the transfer assays were carried out at pH 6. The effect of these two buffers on the transfer reaction catalyzed by phenylalanyl-tRNA synthetase was examined briefly. At 21° and 50 mM sodium citrate (pH 6.2) or succinate (pH 6.1), the transfer reaction proceeded efficiently in the absence of Mg^{2+} (Figure 3). Also confirmed in Figure 3 is the previously reported (20) inability of this transfer reaction to proceed in 10 mM Tris-acetate (pH 7.2) in the absence of Mg^{2+}. Furthermore, we have found higher concentrations (50 and 100 mM) of Tris-acetate (pH 7.2) to be equally ineffective in activating this transfer reaction in the absence of Mg^{2+}.

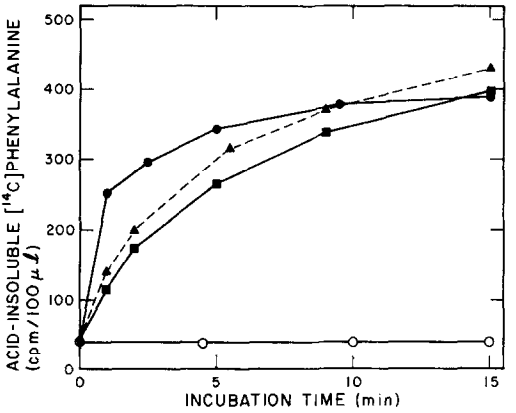

Figure 3. Effect of 50 mM sodium citrate (pH 6.2) or succinate (pH 6.1) on the transfer reaction. The assay incubation mixtures contained per 1.0 ml: 10 µmoles of Tris-acetate (pH 7.2); 0.1 µmole of EDTA; 0.15 µmole of dithiothreitol; 0.130 A_{260} unit of $tRNA^{phe}$; [^{14}C]phenylalanyladenylate-enzyme complex (8530 cpm); plus the following: no further addition (O—O); 50 µmoles of sodium citrate (pH 6.2) (■—■); 50 µmoles of sodium succinate (pH 6.1) (▲--▲); 0.2 µmole of magnesium acetate (●—●). The assays were conducted at 21°. The pH values included in parentheses for the assays containing citrate and succinate are the actual measured values for these incubation mixtures; they resulted from the addition of a concentrated solution of either sodium citrate or succinate (pH 6.0) to the remainder of the assay mixture containing dilute Tris-acetate buffer (pH 7.2).

Discussion

The results presented above demonstrate that the requirement for Mg^{2+} exhibited by phenylalanyl-tRNA synthetase in its transfer reaction can be largely eliminated by a change in assay conditions. Whereas Tris-acetate buffer was inert, sodium cacodylate, citrate and succinate buffers were all highly active in supporting this transfer reaction in the absence of Mg^{2+}. When examined in sodium cacodylate buffer, the transfer reaction was stimulated both by increasing the concentration of buffer and by lowering the pH to 6.2, the optimum temperature being ca. 20° at pH 6.2.

These findings suggest the possibility that other aminoacyl-tRNA synthetases may behave in a similar manner with respect to the Mg^{2+} requirement of their transfer reactions. It may perhaps prove possible to reconcile seemingly opposing conclusions concerning the Mg^{2+} requirement of different transfer reactions through a greater appreciation of the effect of assay conditions upon this matter.

Acknowledgments

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