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PRELIMINARY REPORT

# ABSENCE OF AMINO ACID HYDROXAMATE FORMATION IN AMINOACYL TRANSFER RNA FORMATION STIMULATED BY SPERMINE

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It has been reported that the aminoacyl-tRNA synthetase catalyzes the following two reactions.

Amino acid+ATP+enzyme ⇄ aminoacyl-AMP-enzyme+PPi (a)

Aminoacyl-AMP-enzyme+tRNA ⇄ aminoacyl-tRNA+AMP+enzyme (b)

Hydroxylamine has frequently been used for assay of reaction (a), since Hoagland (1955) reported that it trapped the carboxyl-activated amino acid as amino acid hydroxamate.

Previously, we reported that polyamines, such as spermine, spermidine and puterescine, stimulate aminoacyl-tRNA formation (Takeda and Igarashi, 1969). We have found also that there was no PPi-ATP exchange in the presence of polyamines (Igarashi and Takeda, manuscript in preparation). In further studies on the mechanism of aminoacyl-tRNA formation stimulated by polyamines, we measured amino acid hydroxamate formation in this reaction and found that none was formed in the presence of sufficient spermine to stimulate aminoacyl-tRNA formation.

Aminoacyl-tRNA synthetase was prepared from *E. coli* B as described previously (Takeda and Igarashi, 1969). Salt-free hydroxylamine was prepared as described by Beinert et al. (1953). Hydroxylamine was measured colorimetrically (Frear and Burrell, 1955).

Amino acid hydroxamate formation requires magnesium ions (Hoagland, 1955), but the optimal concentration of magnesium ions varies with different enzyme preparations (Van De Ven et al., 1958; George and Mesiter, 1967). The effect of magnesium ions upon amino acid hydroxamate formation with the aminoacyl-tRNA synthetase preparation used in this study is shown in Fig. 1. Under the experimental conditions used, the amount of amino acid hydroxamate formed increased with increase in the magnesium ion concentration in the reaction mixture. Fig. 2 shows the effect of spermine upon amino acid hydroxamate formation. Both in the absence (curve 1) and presence of 1 mM (curve 2) or 2 mM (curve 3) magnesium ions, spermine had no effect upon amino acid hydroxamate formation. With the enzyme preparation used in this experiment, spermine stimulated several aminoacyl-tRNA formation, such as isoleucyl-, valyl- and leucyl-tRNA formation. Even with 5 mM spermine, no stimulation of amino acid hydroxamate formation was observed. Moreover, no stimulation of amino acid hydroxamate formation was detected in the presence of 2 mM spermine and various amounts of ATP (1 mM to 15 mM).

This suggests that the aminoacyl-AMP-enzyme complex is not formed in the presence

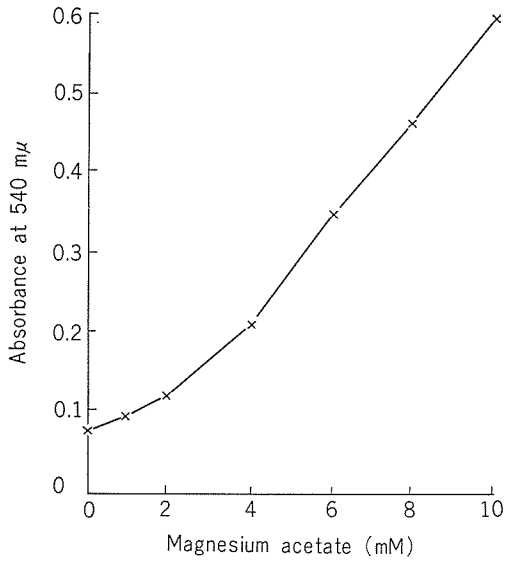


FIGURE 1. *Effect of magnesium ions on amino acid hydroxamate formation. The reaction mixture for assay of amino acid hydroxamate formation contained the following constituents in 0.5 ml: 0.08 M Tris-HCl, pH 7.8, 0.05 M $NH_4Cl$, 0.006 M 2-mercaptoethanol, 0.01 M ATP, 0.0005 M each of the 20 usual amino acids, 1 M hydroxylamine and 5 μl (0.04 mg) of enzyme solution. Magnesium acetate was added at the concentrations indicated. The reaction mixture was incubated at 37 C for 60 minutes and then 1.5 ml of solution containing 5% trichloroacetic acid, 0.67 N hydrochloric acid, and 10% ferric chloride was added. The precipitate was removed by centrifugation. The amino acid hydroxamate formed was determined from the absorbance at 540 mμ. Under these conditions the absorbance of 1 μmole of DL-phenylalanine hydroxamate (Sigma Chemical Co.) at 540 mμ was 0.09.*

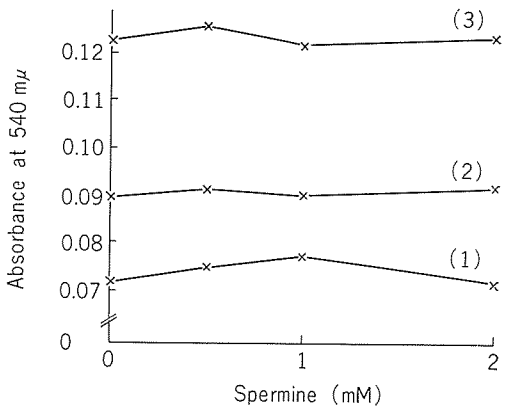


FIGURE 2. *Effect of sperimine on amino hydroxamate formation in the absence and presence of magnesium ions. The reaction mixture and assay procedure were as described for Fig. 1. Sperimine tetrahydrochloride (Sigma Chemical Co.) was added at the concentrations indicated; (1) magnesium acetate 0 mM, (2) magnesium acetate 1 mM, (3) magnesium acetate 2 mM.*

of spermine. There are several explanations for this finding. It is possible that the aminoacyl-AMP-enzyme complex is not sufficiently stable in the presence of spermine to form amino acid hydroxamate, while the complex is stable in the presence of magnesium ions. If this is a case, however, 2 mM spermine should stimulate amino acid hydroxamate formation on addition of 1 mM or 2 mM magnesium acetate (cf. Fig. 2). Another possibility is that reaction (b) proceeds much more rapidly than reaction (a), so that the formation of a small amount of the aminoacyl-AMP-enzyme compoex, which is insufficient to cause formation of amino acid hydroxamate, is sufficient to cause formation of aminoacyl-tRNA in the presence of spermine. Another possibility is that, in the presence of spermine, the amount of the aminoacyl-AMP-enzyme complex formed is not enough to allow formation of amino acid hydroxamate. In this case, the stimulation of aminoacyl-tRNA formation may be explained as follows. When tRNA exists in the reaction mixture, spermine causes a conformational changes of tRNA (Igarashi and Takeda, 1970), making it easily esterified. Under these conditions, reaction (b) proceeds with a small amount of aminoacyl-AMP-enzyme complex. In the presence of hydroxylamine, however, there is no interaction of spermine with hydroxylamine and no formation of amino acid hydroxyamate is observed. Further, the mechanism of aminoacyl-tRNA formation stimulated by spermine may differ from that stimulated by magnesium ions. Recently, we found that no aminoacyl-AMP-enzyme complex is formed in the presence of polyamines (Igarashi and Takeda, unpublished observation). A detailed study on the mechanism of aminoacyl-tRNA formation stimulated

by polyamines is now in progress and will be published elsewhere.

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