# $^1$H NMR STUDIES ON DEUTERIUM - HYDROGEN EXCHANGE AT C-5 IN URIDINES

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During an investigation of the association of nucleic acid derivatives with amino thiols it was observed that the C-5 hydrogen atom in uridine was readily exchangeable in aqueous base (1). In basic $D_2O$, deuterium was exchanged for the C-5 hydrogen atom and in basic $H_2O$, hydrogen was exchanged for the C-5 deuterium atom.

The exchange occurred when an equimolar mixture of uridine and various bases in $D_2O$ was held at room temperature (ca. 19°), while no exchange occurred in a 0.4 M uridine/$D_2O$ solution ( pH = 5.3 ) left standing for three months. Using a typical base, 2-mercaptoethylamine (MEA), a new peak appeared after 24 hours in the $^1$H NMR spectrum (Fig. 1b), midway between the peaks of the C-6 proton doublet (2). Raising the temperature to ca. 60° for 4 days, or allowing the solution to remain at room temperature for 21 days, resulted in the complete disappearance of the C-6 doublet peaks and the new single peak increased in intensity (Fig. 1c). Simultaneously one of the two sets of doublets ($H_5$,$H_1'$) at ca. $\delta$ = 5.9 ppm, also disappeared. The addition of MEA also produced a slight shift of 1-3 Hz in the chemical shifts of $H_6$ and $H_1'$, while the remainder of the $^1$H NMR spectrum was unchanged (not shown). Other bases(NaOH, ethylamine and 2-aminoethanol) also produced shifts in the positions of $H_6$ and $H_1'$.

Uridine-$d_5$ was isolated in 95% yield. A mass spectrum of uridine-$d_5$ gave a parent peak at m/e 249*. Uridine-$d_4$ and uridine gave parent peaks

* Mass spectra were obtained on an AEI MS 12 mass spectrometer at 100°.

METHODS.

The enzyme fraction containing isoleucyl-RNA synthetase (EC.6.1.1.5) was prepared from Bacillus stearothermophilus by a procedure similar to that described by ZUBAY (1962) for the E. coli enzyme.

Transfer ribonucleic acids of B. stearothermophilus was prepared according to the procedure described by BRUBAKER and Mc CORQUODALE (1963) for E. coli tRNA. The resulting crude tRNA preparation was further purified on DEAE-cellulose according to STEPHENSON and ZAMECNIK (1961).

The isoleucyl-adenylate enzyme complex was made and isolated as described by NORRIS and BERG (1964) for the E.coli enzyme.

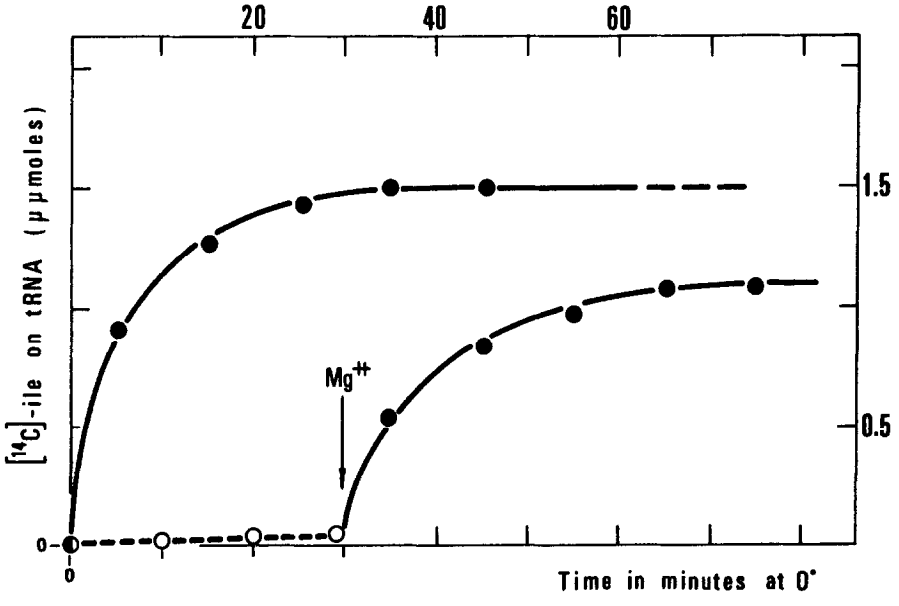


Figure 1. Kinetics of transfer of the activated isoleucine from the ile-AMP-enzyme complex to tRNA of B. stearothermophilus. The incubation system contained per ml : 50 μmoles of sodium succinate buffer pH 6.0, 25 μmoles KCl, 0.8 mg tRNA (20 D.O. 260 mμ) and 7.5 μmoles $Mg^{++}$ions (●) or 2 μmoles EDTA (○). The reaction was started by the addition of 16 μmoles of C-labelled ile-AMP-enzyme (specific activity : 174 mC/mM) isolated from gel filtration experiment. The incubation was carried out at 0°. Aliquotes of 0.2 ml were withdrawn at different intervals of time and the amount of $^{14}C$-ile transferred to tRNA was measured by the radioactivity associated to the nucleic acids precipited by cold trichloracetic acid and collected on Millipore filters. In the experiment performed with EDTA, $Mg^{++}$ (11 μ moles/ml) was added after 30 minutes of incubation.

RESULTS.

The isoleucyl-adenylate enzyme complex of B.stearothermophilus is very stable and can transfer its activated aminoacid to tRNA of B. stearothermophilus. The results shown in Fig. 1 demonstrate that this transfer occurs only in the presence of magnesium ions. In the presence of EDTA, the reaction is completely inhibited; addition of an excess of magnesium ions to the reaction medium immediately releases the inhibition.

The maximum amount of isoleucine transferred to tRNA depends upon the concentration of magnesium ions. Fig. 2 shows that in our assay conditions, the optimal concentration is 5 mM $Mg^{++}$. Under optimal conditions the maximum yield of the transfer reaction never exceeds 50 % of the radioactivity added with the $^{14}C$-isoleucyl-adenylate enzyme complex.

A DEAE-cellulose chromatography of the incubation mixture shows that the $^{14}C$-aminoacid not transferred is recovered as free isoleucine. In the absence of tRNA or in

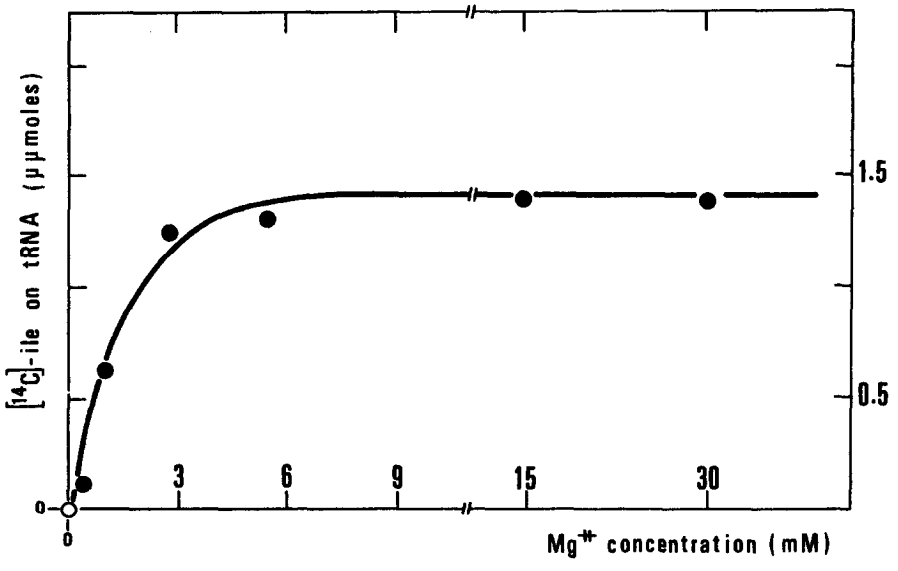


Figure 2. Effect of magnesium ions concentration on the maximum yield of the transfer reaction.
The composition of the reaction mixture was the same as in Fig. 1, but the concentration of $Mg^{++}$ ions in each reaction medium was as indicated on the figure (●). The zero concentration of $Mg^{++}$ ions was in fact 2 mM EDTA (○). Incubation : 45 minutes at 0°. The amount of $^{14}C$-ile transferred totRNA was measured as in Fig. 1.

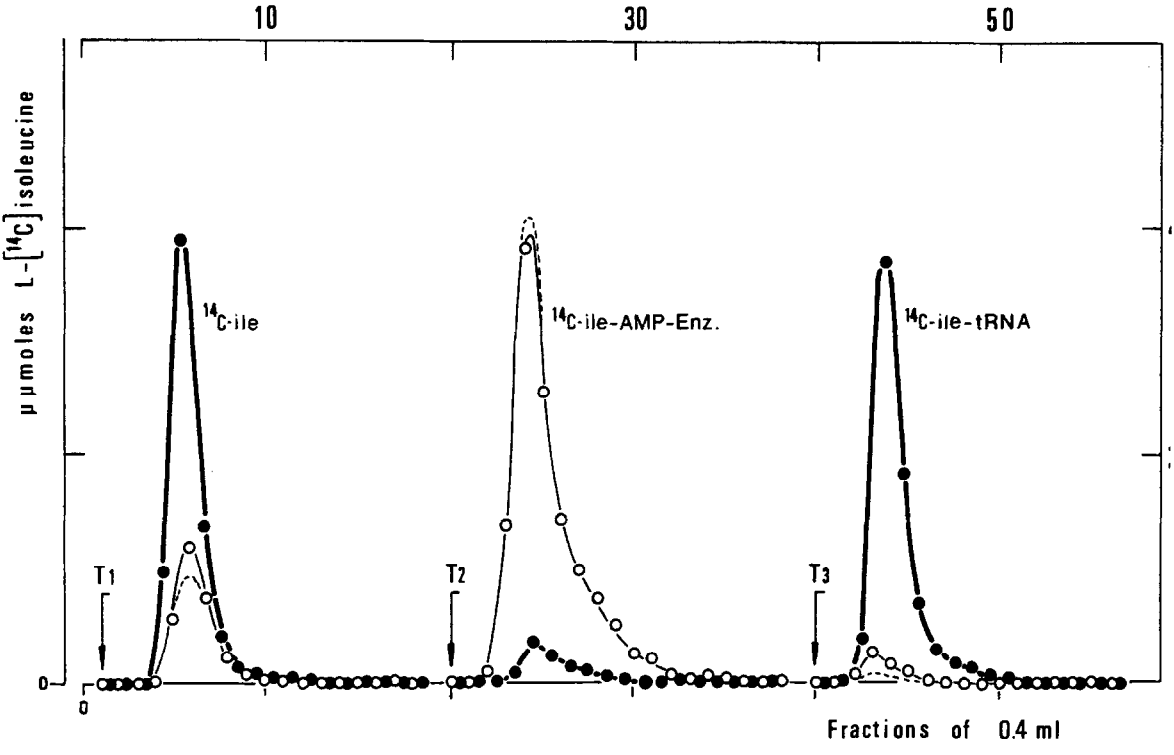


Figure 3. Stepwise fractionation of the transfer reaction mixture on DEAE-cellulose.

Three series of experiments were performed. In the first experiment, 15 µmoles of $^{14}$C-ile-AMP-enzyme complex were incubated in the presence of 0.35 mg tRNA of B.stearothermophilus (8.5 D.O.260 mµ) and 7.0 µmoles $Mg^{++}$ in a final volume of 0.8 ml of 25 mM sodium succinate buffer pH 6.0 containing 12.5 mM KCl. After 45 min. incubation at 0°, the reaction mixture was diluted 2 fold with water and adsorbed on the top of a short column (0.6 x 3 cm) packed with DEAE-cellulose (Biorad-Cellex D of 0.88 m.eq./gr pf capacity). Elution was performed at 4° successively with 0.01 M sodium succinate buffer pH 6.0 containing 0.01 M KCl and 0.001 M EDTA (T-1), then with 0.01 M tris-HCl buffer pH 7.4 containing 0.25 M NaCl and 1 mM EDTA (T-2) and finally with 0.01 M tris-HCl buffer pH 7.4 - NaCl 1 M-EDTA 1 mM (T-3). In these conditions, free isoleucine is not adsorbed on the resine and eluted with T-1 buffer, while the ile-AMP-enzyme complex and ile-tRNA were eluted respectively with T-2 and T-3 buffers. Fractions of 0.4 ml were collected and the radioactivity was determined by liquid scintillation counting (●—●).

The second experiment was identical to the first one except that 3.5 mM EDTA replaced the $Mg^{++}$ ions in the reaction mixture (o—o).

In the third experiment, the complex in presence of magnesium ions but without tRNA was incubated and subjected to chromatography under the same conditions as above (- - -). This experiment serves as the control of the stability of the ile-AMP-enzyme complex in the conditions of the enzymic assay.

Total recovery of the radioactivities from the columns was 95-100 %.

presence of EDTA, the amount of isoleucine released is less important (Fig. 3 O — O and ---). These experiments suggest that the low efficiency of the transfer reaction is caused by the release of isoleucine occuring simultaneously to the aminoacylation of tRNA. Further investigations are necessary to establish the exact nature of this reaction, but it is clear from Fig. 3 that it is not due to spontaneous breakdown of the aminoacyl-AMP-enzyme complex.

The most striking feature of these results is that the transfer reaction as well as the release of isoleucine occur only when tRNA and $Mg^{++}$ are present simultaneously. This indicates that a certain type of interaction between the isoleucyl-adenylate enzyme complex and tRNA chains of B. stearothermophilus occurs only in the presence of magnesium ions.

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