COMPARATIVE STUDIES ON THE ISOLEUCYL-tRNA SYNTHETASES FROM *BACILLUS STEAROTHERMOPHILUS* AND *ESCHERICHIA COLI*: II. THE EFFECT OF MAGNESIUM IONS IN THE TRANSACYLATION REACTION

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1. Introduction

Each aminoacyl-tRNA synthetase (ARS) catalyzes two distinct and very specific reactions: the formation of an enzyme-bound aminoacyl-adenylate (activation step) and the transfer of this aminoacyl-group to the appropriate tRNA's (transacylation step).

It is now well established that the first reaction, as measured by the amino acid-dependent ATP-PPi exchange is completely dependent on magnesium ions. This requirement most probably corresponds to the formation of ATP-Mg^{2+} and PPi-Mg^{2+} complexes, which are essential for effective interactions of these substrates with the amino acid in the active site of the synthetase [1, 2].

The participation of magnesium ions in the second (transacylation) step seems, however, not to be a general rule. Numerous reports have shown that the transacylation reaction catalyzed by different aminoacyl-tRNA synthetases from *E. coli* and yeast, does not require magnesium ions and even proceeds in the presence of EDTA [3–6]. On the other hand, the same reaction catalyzed by other synthetases is completely dependent on magnesium ions and requires a relatively high level of these ions (1–5 mM) for maximum transfer of the aminoacyl group to tRNA [7–9]. In general, the authors postulated that this requirement for metal ions is related to the stabilization of the conformation of the nucleic acids by these ions.

In a previous paper, we have reported such a magnesium requirement in the transacylation reaction catalyzed by the IRS from *Bacillus stearothermophilus* [10]. These results contrast with those of Norris and Berg [3] in the case of IRS from *E. coli*.

In order to determine the origin of this difference, we have compared the effect of magnesium ions in cross reactions catalyzed by each of the Ile-AMP-IRS complexes with the heterologous tRNA. The present paper shows that the strict magnesium requirement is a property of the synthetase itself and does not depend on the origin of the tRNA used. The results we obtained with IRS of *E. coli* confirm those of Norris and Berg [3]: however, despite the fact that the *extent* of the transacylation is not dependent on the presence of magnesium ions, the *rate* of this reaction is greater in their presence.

Abbreviations and enzymes:

IRS	: isoleucyl-tRNA synthetase or L-isoleucine: sRNA ligase (AMP) (EC 6.1.1.5).
Ile-AMP-IRS	: a complex including isoleucyl-adenylate and the IRS.
tRNA	: transfer ribonucleic acid.

2. Materials and methods

The source of materials and the preparation of pure IRS from *B. stearothermophilus* or unfractionated tRNA from *E. coli* (strain 112-12: ref. [11]) or *B. stearothermophilus* (strain NCA 1518) have been described or referred to elsewhere [10, 12, 13]. tRNA

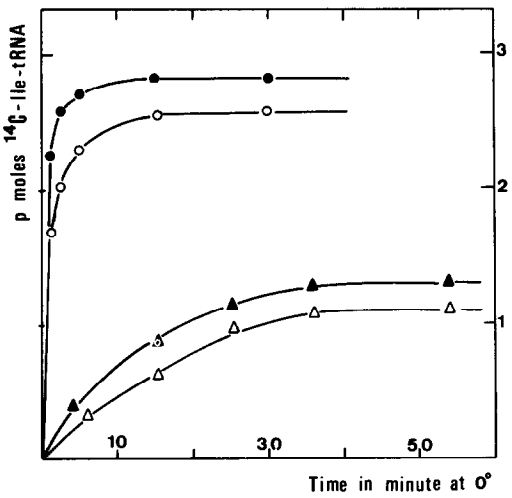

Fig. 1. Comparison of the transfer kinetics obtained with IRS of *B. stearothermophilus* (▲ ; △) and of *E. coli* (● ; ○) in the presence of their homologous (● ; ▲) and heterologous tRNA (○ ; △).

The [^{14}C]-Ile-AMP-IRS complexes were isolated and incubated at 0° in the presence of tRNA (1 mg/ml) as described in Methods. The concentration of Mg^{2+} was 5 mM in both cases.

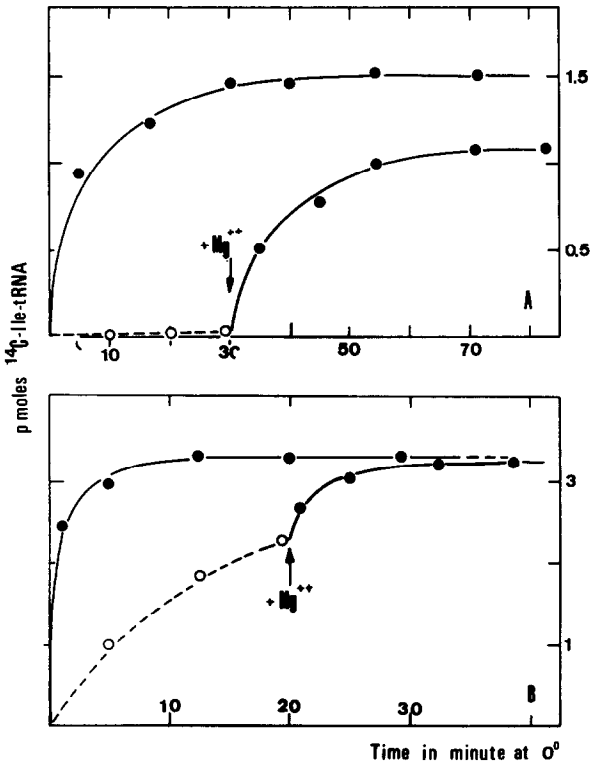

Fig. 2. Kinetics of the transfer reaction catalyzed by the IRS of *B. stearothermophilus* (part a) and of *E. coli* (part b), in the presence of Mg^{2+} (●——●) or EDTA (○——○).

The experimental conditions are identical as described in Methods, except for the experiments performed in the presence of 2.2 mM EDTA (○ - - - ○), where addition of Mg^{2+} was made after 20 or 30 min of incubation at 0°. The amount of Mg^{2+} added was calculated to reach the same final concentration of free Mg^{2+} as in the control experiments (●——●), i.e. 7.5 mM with the *B. stearothermophilus* enzyme and 3 mM with the *E. coli* enzyme. The same tRNA from *E. coli* was used in each cases (0.8 mg/ml).

from *E. coli* purchased from Schwarz Bio-Research gave identical results; it was further purified by chromatography on DEAE-cellulose as described by Stephenson and Zamecnik [15]. The IRS from *E. coli* (AS_3-Ile fraction) was prepared according to Bergmann et al. [14].

The [^{14}C]-labelled Ile-AMP-IRS complexes formed with either the synthetase from *E. coli* or *B. stearothermophilus* were isolated from the reaction mixture by gel filtration on Sephadex G-50, according to the method of Norris and Berg [3], except that 2-mercaptoethanol was omitted from the eluting buffer.

The enzymatic transfer of the activated isoleucine from the Ile-AMP-IRS complex to tRNA was achieved by mixing 0.1 ml aliquots of the isolated complex (about 3–4 pmoles) with 0.15 ml of tRNA (0.2 mg) in 50 mM sodium succinate buffer pH 6.0 containing EDTA or an excess of magnesium ions adequate to get the concentration of free metal ions indicated in the captions. Before the reaction was started, the mixture containing the tRNA was preincubated for 3 min at 37° and then cooled in ice before adding the complex. A few experiments performed in 0.1 M Tris-HCl buffer, pH 7.4, instead of succinate buffer, pH 6.0, gave similar results. The amount of [^{14}C]-isoleucine transferred to tRNA was determined by measuring the radioactivity associated with tRNA, after precipitation by cold trichloroacetic acid (10% w/v final) and filtration on a Millipore membrane.

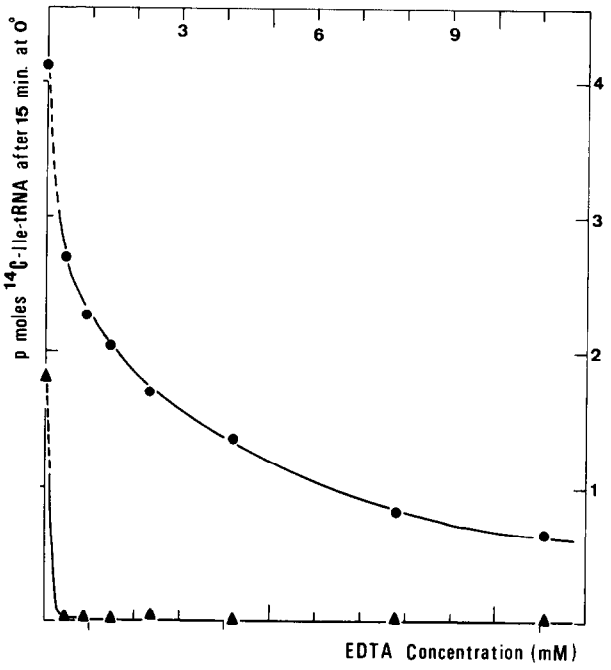

Fig. 3. Effects of increasing concentration of EDTA on the amount of [^{14}C]-isoleucine transferred from the Ile-AMP-IRS complex to tRNA of *E. coli*, after 15 min incubation at 0°. *E. coli* tRNA (0.3 mg/ml) and EDTA were first incubated one min at 37° and then cooled to 0° before the addition of the [^{14}C]-Ile-AMP-IRS of *B. stearothermophilus* (▲——▲) or of *E. coli* (●——●). The zero concentration of EDTA concerns in fact the control experiments performed in the presence of 6 mM Mg^{2+} in the case of *B. stearothermophilus* enzyme (▲) and 1 mM of these ions with the *E. coli* enzyme (●). Other details were as described in Methods.

3. Results

The Ile-AMP-IRS complex formed with either the synthetase from *B. stearothermophilus* and *E. coli* have been isolated by gel filtration, and then incubated at 0° with an excess of tRNA from *E. coli* or *B. stearothermophilus*.

Fig. 1 shows the kinetics of the transfer reactions obtained in the presence of Mg^{2+} ions. Under these conditions each of the two enzyme-substrate complexes are able to amino-acylate both tRNA species, but the rate of the reaction catalyzed by the two synthetases is quite different, the reaction being much slower in the case of the *B. stearothermophilus* enzyme complex. This difference is observed with tRNA from both species; it thus reflects a characteristic of the synthetase itself.

When the reaction is performed in the presence of EDTA, no transfer whatsoever occurs with Ile-AMP-IRS complex of *B. stearothermophilus*; the addition of an excess of Mg^{2+} ions to the incubation system is enough to start the transacylation reaction, indicating a strong dependence on magnesium ions. This is observed as well with tRNA from *E. coli* (fig. 2a) as with tRNA from *B. stearothermophilus* [10].

Conversely, as already shown by Norris and Berg [3], the corresponding Ile-AMP-IRS complex from *E. coli* does transfer its aminoacyl group to *E. coli* tRNA, even in the presence of EDTA (fig. 2b). However, the *rate* of the transfer reaction is considerably reduced and a longer incubation (60 min at 0°) is necessary for reaching approximately the same yield of isoleucyl tRNA as in the presence of Mg^{2+} ions. The addition of magnesium ions to such a mixture results in an acceleration of the reaction, showing that in this case also the bivalent cation participates in the reaction.

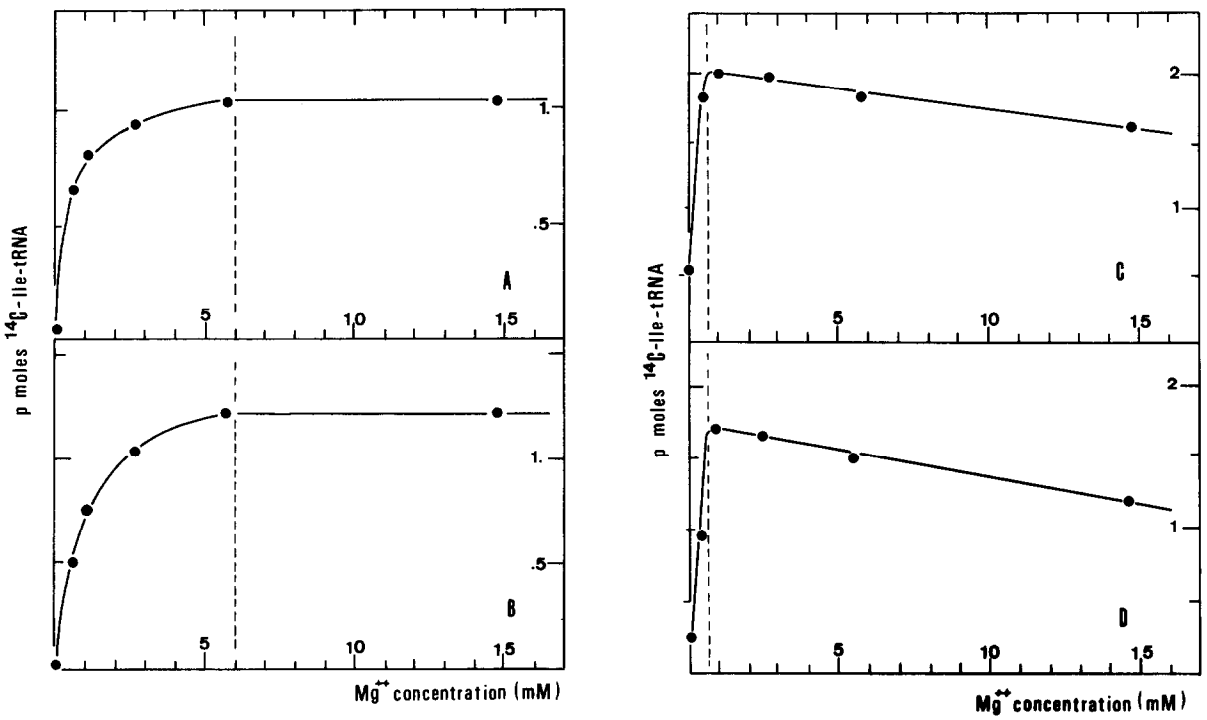

Fig. 4. Effect of increased Mg^{2+} concentration on the maximum yield of the transfer reaction in the homologous and heterologous systems between the Ile-AMP-IRS and tRNA of *E. coli* and *B. stearothermophilus*. The experimental conditions were the same as described in Methods, except that 100 mM Tris-HCl buffer pH 7.4 was used and the concentration of Mg^{2+} was as indicated on the captions. The zero concentration of these ions correspond in fact to 2 mM EDTA. Incubation was performed 45 min at 0°. Part A of the figure concerns the transfer reaction obtained with the IRS and tRNA both of *B. stearothermophilus*; part B, with IRS of *B. stearothermophilus* and tRNA of *E. coli*; part C, with IRS and tRNA both of *E. coli*; part D, with IRS of *E. coli* and tRNA of *B. stearothermophilus*.

As the amount of Ile-tRNA which is formed in the presence of EDTA is nearly proportional to time in the first few minutes of incubation at 0° (see fig. 2b), it has been possible to analyze the effect of increased EDTA concentration on the rate of the transfer reaction catalyzed by the IRS from *E. coli*. Fig. 3 shows that the rate of this reaction is considerably reduced but that complete inhibition is not achieved, even in 10 mM EDTA. This contrasts with the synthetase of *B. stearothermophilus* which is completely inhibited in the presence of as low a concentration of EDTA as 0.5 mM. Essentially the same results were obtained with tRNA from either *E. coli* or *B. stearothermophilus*, indicating that these effects of EDTA are independent on the origin of tRNA; they are characteristic of the synthetase used.

The effect of increased Mg^{2+} concentration on the extent of the reaction was also analyzed.

With the IRS of *B. stearothermophilus*, the maximum amount of isoleucine which is transferred to the tRNA increases with the concentration of magnesium ions, reaching a plateau at 5 to 6 mM Mg^{2+}. Exactly the same dependence was obtained whether the tRNA used originates from *B. stearothermophilus* (fig. 4A) or of *E. coli* (fig. 4B).

When the synthetase from *E. coli* is used, the maximum yield of the transfer reaction is reached at a very low Mg^{2+} concentration (fig. 4C and D); increasing the concentration of the Mg^{2+} is slightly inhibitory. This latter effect, which was also observed by Norris and Berg [3], again does not depend on the origin of the nucleic acid. (fig. 4C and D).

4. Discussion

The results we obtained clearly demonstrate that the IRS of *E. coli* and *B. stearothermophilus* differ in their magnesium requirements for the transacylation reaction. The IRS from *B. stearothermophilus* shows a clear cut dependence on the bivalent cation and requires a relatively high concentration of this metal for maximum transfer of the activated isoleucine onto tRNA. In a preceding paper [13] we have established that the Mg^{2+} ions are required for strong binding of the nucleic acid to the synthetase. It is, however, improbable that this requirement is related to stabilization of the nucleic acid conformation, since the same tRNA that cannot react with *B. stearothermophilus* IRS is able to react properly with the *E. coli* IRS under identical conditions.

The exact role of Mg^{2+} ions in the transacylation reaction is not understood, but our results suggest that these ions must either act on the synthetase to insure efficient interaction of the enzyme with the nucleic acids, or be involved directly in the binding of tRNA with the enzyme.

It is conceivable that, in the case of *B. stearothermophilus* enzyme a transconformation essential for binding the nucleic acid and/or for effective transfer of the activated isoleucine to tRNA is not possible in the absence of Mg^{2+} ions; whereas with the *E. coli* IRS such transconformation can occur in the absence of Mg^{2+}, but is accelerated by the divalent cation. Such transconformation of the *E. coli* IRS during the catalysis cycle has in fact been postulated by Yarus and Berg [17] and would fit also with results we have obtained more recently in the case of *B. stearothermophilus* [13, 18].

Another possibility is that Mg^{2+} ions are required for the transacylation catalyzed by both synthetases, but that the affinity for Mg^{2+} is so great in the case of *E. coli* IRS that traces of this metal, present in the incubation mixture or tightly bound to the enzyme-substrate complex, are sufficient to promote the reaction, no external addition of Mg^{2+} ions being necessary. Further experiments are necessary to elucidate this point.

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