INHIBITION BY CALCIUM OF tRNA AMINOACYLATION IN PREPARATIONS FROM RAT LIVER

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1. Introduction

For some time it has been recognized that the translation of messenger RNA into protein is a metabolic process particularly dependent for activity on added Mg^{2+}. In this set of reactions Mg^{2+} is an obligatory component of tRNA aminoacylation, polypeptide chain initiation and elongation [1] and also influences the three dimensional structures of ribosomes and tRNA molecules [2, 3]. Because the activating effects of Mg^{2+} in many cellular enzymic processes are often antagonized by Ca^{2+} [4, 5] the notion has arisen that the cellular Mg^{2+} to Ca^{2+} ratio could be a factor involved in metabolic control [6–9].

Recently Roy [10] reported that the in vitro aminoacylation of $tRNA^{Ala}$, $tRNA^{Cys}$ and $tRNA^{Phe}$ from *Escherichia coli* is inhibited by added Ca^{2+}. This author suggested that Ca^{2+} might have a regulatory role in protein and RNA synthesis. In the light of these observations and of our interest in the role of Ca^{2+} and Mg^{2+} in controlling cell metabolism in mammalian cells (see *ref.* [5–9, 11–14]) we undertook a study of the effect of Ca^{2+} on tRNA aminoacylation in preparations from rat liver. We report here that aminoacylation of $tRNA^{Ala}$ in these preparations is also sensitive to added Ca^{2+}.

2. Materials and methods

Livers were obtained from 200 g Wistar albino rats. Extraction and preparation of the tRNA from liver was carried out according to the procedure described by Kirby [15]. The final product was stored as a suspension in 70% ethanol at −20°C until required. It was free from rRNA and was at least 90% homogeneous as indicated by MAK chromatography [16].

The aminoacyl-tRNA synthetase fraction was prepared from rat liver by a modification of the procedure of Wevers et al. [17]. Livers were homogenized in three volumes of buffered medium (20 mM Tris–HCl, pH 7.6, 12 mM $MgCl_2$, 14 mM KCl, 6 mM 2-mercaptoethanol and 10% (w/v) glycerol), the homogenate was centrifuged and the final supernatant treated with streptomycin sulphate as described by Wevers et al. [17]. Endogenous amino acids were removed by Sephadex G25 chromatography, using homogenizing buffer to elute the sample. The fraction was stored in small aliquots at −20°C but was usually discarded after 3 days due to loss of activity.

Incubation mixtures were as described in the legends to figs. 1, 2. Controls were run in which tRNA was omitted from the reaction mixture. Reactions were stopped by the addition of 200 µl of 20% TCA and the mixture allowed to stand on ice for 30 min. BSA (1 mg) was added to each tube and the contents transferred to Eppendorf centrifuge tubes and centrifuged. The pellets were washed at least 4 times with cold 5% TCA. Finally the pellets were collected on glass fibre discs, placed in scintillation vials, dried and assayed for radioactivity in a Beckman scintillation counter.

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Analysis of the composition of aminoacyl-tRNA labelled with 10 different amino acids was carried out according to the procedure of Davey and Howells [18]. Incubations were exactly as for normal experiments except they were scaled up 12-fold.

3. Results

In initial experiments optimal conditions for aminoacylation in vitro were examined using a labelled amino acid mixture with tRNA and aminoacyl-tRNA synthetase fraction isolated from rat liver. Results of these experiments showed that the properties of the incorporating system does not differ significantly from those obtained in preparations from other species and tissues. Particularly relevant to the present work was the confirmatory observation that little aminoacylation occurs in the absence of added Mg^{2+} that optimal incorporation occurs at about 5 mM Mg^{2+} and that higher levels of Mg^{2+} produce decreased rates of incorporation.

Data in fig. 1 show the effect of varying Ca^{2+} concentrations on the aminoacylation of a mixture of amino acids in the presence of 5 mM Mg^{2+}. Amino acid acceptor activity falls off sharply as the concentration of Ca^{2+} is increased. Approximately 50% inhibition occurs at about 2 mM Ca^{2+} and maximal inhibition (some 70–75%) at approximately 5–10 mM Ca^{2+}.

The effect of Ca^{2+} on the aminoacylation of different tRNA species is shown in table 1. This ion was found to inhibit the aminoacylation in particular of $tRNA^{ala}$ (98%), $tRNA^{phe}$ (81%) and $tRNA^{gly}$ (76%) when present at a concentration of 5 mM. The remaining species, apart from $tRNA^{thr}$ were also inhibited by Ca^{2+} but not to the same degree.

The finding that $tRNA^{ala}$ was particularly sensitive to Ca^{2+} prompted experiments on the aminoacylation of this species of tRNA. Data collected in fig. 2 show how the rate of $tRNA^{ala}$ aminoacylation changes in the presence of Ca^{2+} (fig. 2a) and the concentration of Ca^{2+} required to bring about this change (fig. 2b). Finally the data also show (fig. 2c) that the inhibitory effect of Ca^{2+} can be overcome by increasing the Mg^{2+} concentration in the system.

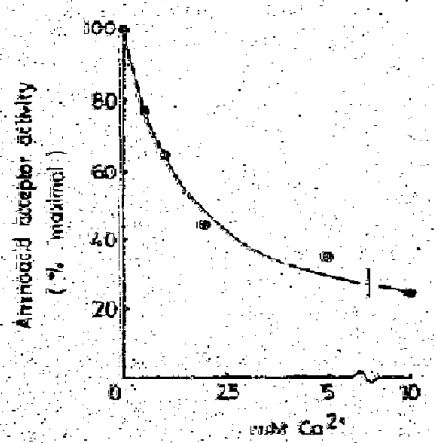

Fig. 1. Effect of Ca^{2+} on amino acid acceptor in preparations from rat liver. The basic reaction mixture contained 26 mM Tris–HCl (pH 8.0) and unless otherwise indicated 5 mM ATP, 0.3 mM CTP, 5 mM Mg^{2+}, 1 mM non-radioactive amino acid mixture, containing 2 μCi ^{14}C-amino acid mixture), 50 μg tRNA and 0.2 ml aminoacyl-tRNA synthetase fraction (1–1.5 mg protein). Ca^{2+} was varied as indicated. Final vol., 1.0 ml. Incubations were for 5 min at 37°C. Other procedures as in Methods.

Table 1
Effect of Ca^{2+} on aminoacylation of different tRNA's in preparations from rat liver

Amino acid	nmoles/mg tRNA		% Inhibition
Ala	0.473	0.011	98
Asp	0.082	0.057	30
Gly	0.092	0.022	76
Glu	0.092	0.052	43
Leu	0.755	0.498	34
Phe	0.297	0.057	81
Ser	0.550	0.312	43
Thr	0.745	0.902	21*
Tyr	0.120	0.094	22
Val	0.174	0.138	21

The reaction mixture was as described in fig. 1. Incubations were carried out for 5 min at 37°C and stopped by the addition of 8-hydroxyquinoline: phenol: water. The purification, separation and analysis of the tRNA species was carried out as described in Methods. Ca^{2+} was present where indicated at a concentration of 5 mM.

* Denotes activation.

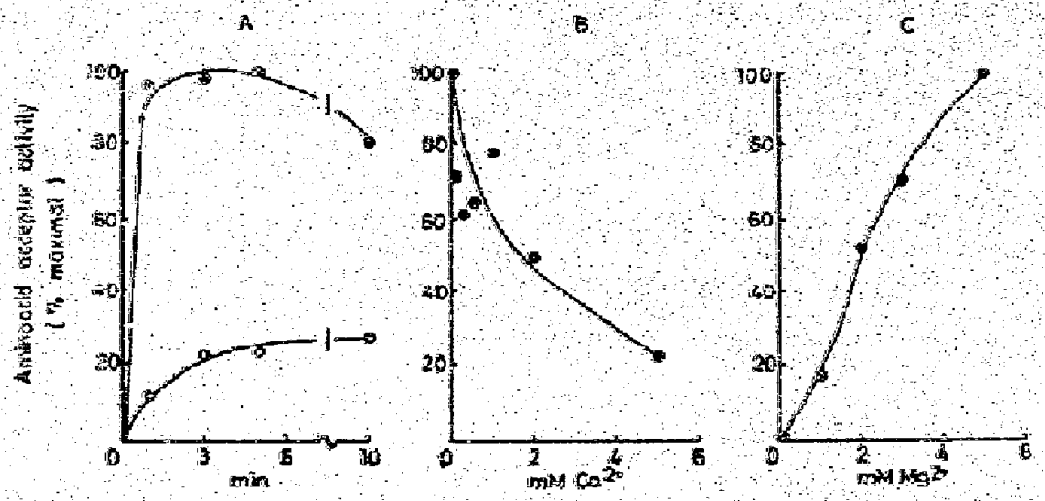

Fig. 2. Effect of Ca^{2+} on $tRNA^{ala}$ aminoacylation. Experimental procedures as in fig. 2 except that 0.5 mM L-alanine (containing 2 μCi [^{14}C] alanine) was present in place of the amine acid mixture. The times of incubation in the absence (●) and presence (○) of 5 mM Ca^{2+} was varied as shown (A); the concentration of Ca^{2+} was varied as shown in the presence of 5 mM Mg^{2+} (B); the concentration of Mg^{2+} was varied as shown in the presence of 3 mM Ca^{2+} (C).

4. Discussion

Data in this communication show that aminoacylation of $tRNA^{ala}$, $tRNA^{phe}$ and $tRNA^{gly}$ in preparations from rat liver are sensitive to low concentrations of added Ca^{2+}. Although amino acid acceptor activity of $tRNA^{cys}$ and $tRNA^{pro}$ was not examined in the present study it is of interest that $tRNA^{ala}$ in preparations from *Escherichia coli* was also the species most sensitive to added Ca^{2+} [10]. Clearly the acceptor activity of only one tRNA species need be inhibited by Ca^{2+} to produce a modified rate of protein synthesis. Studies with bacterial and yeast mutants having temperature-sensitive aminoacyl-tRNA synthetases have established that adequate levels of all species of aminoacyl tRNA are necessary for continued protein synthesis in vivo. Different mutants have different defective synthetases [1] but in all cases protein synthesis ceases rapidly when cultures are shifted to restrictive temperatures.

Although the rate of protein synthesis can be changed solely by altering the availability of a single species of aminoacyl-tRNA, aminoacylation of tRNA is not the only step of protein synthesis dependent on Mg^{2+} and therefore potentially susceptible to inhibition by Ca^{2+}. The possible influence of Ca^{2+} on the rate of protein synthesis in rat liver mediated by effects on the level of alanyl-tRNA, could be enhanced by simultaneous but independent effects on polypeptide chain initiation and elongation.

Elsewhere we have provided evidence that Ca^{2+} has the capacity to modify metabolic events such as glycolysis [7, 11] and phospholipid synthesis [8] and that this could form the basis of a metabolic control system involving movements of the ion into and out of mitochondria [6, 7, 9, 14]. We suggest that protein synthesis in eukaryotes might also be regulated, in part, by a similar form of control.

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