MAGNESIUM-INDUCED ERRORS OF TRANSLATION IN A CELL-FREE SYSTEM FROM KREBS-II ASCITES CARCINOMA CELLS

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Received 27 August 1969

1. Introduction

It has been shown that the fidelity of translation of synthetic templates in cell-free protein synthesizing systems from *Escherichia coli* can be impaired by high concentrations of Mg^{2+} as well as in the presence of streptomycin and related aminoglycoside antibiotics, organic solvents and by some other factors [1–3]. Similar results have also been obtained in some other bacterial systems [4, 5].

The fidelity of translation in cell-free systems of animal origin has been studied less thoroughly so far but the coding ambiguity induced by the above-mentioned conditions, was reported to be expressed to a lesser extent in some animal systems than in bacterial ones [6–8]. As reported recently, the fidelity of translation may vary significantly in the protein-synthesizing systems derived from cells of different organs of the same animal [8].

In the present note the effect of increasing Mg^{2+} concentration on the phenylalanine-leucine ambiguity in a cell-free system from Krebs-II ascites carcinoma cells is described.

2. Methods

Preparation of ribosomes of Krebs-II cells were obtained as described by Kerr et al. [9]. The complete incubation mixture contained, in a volume of 1 ml, the following components: tris-HCl buffer pH 7.8 70 μmoles, KCl 25 μmoles, Mg acetate as indicated in the text, NH_4Cl 80 μmoles, ATP 3 μmoles, GTP 0.05 μmoles, phosphoenol pyruvate 3 μmoles, pyruvate kinase 50 μg, 2-mercaptoethanol 15 μmoles, C^{14}-labelled amino acid 0.05 μC, ribosomes 1 mg, supernatant fraction 1 mg of protein (the 105,000 g supernatant was subjected to gel-filtration through a column of Sephadex G-25). Polyuridylic acid (poly U) was added to some samples at a concentration of 100 μg/ml. Samples where protein synthesis directed by endogenous templates was studied contained a mixture of unlabelled amino acids (excluding the labelled ones) at a concentration of 10^{-4} M of each individual L-amino acid. The samples were incubated for 30 min at 37°C and the reaction was terminated by the addition of an equal volume of 10% trichloroacetic acid (TCA). The precipitates were collected on membrane filters, washed by cold and hot TCA, and radioactivity was determined in a Nuclear Chicago liquid scintillation counter. The specific activities (mC/mmole) of C^{14}-amino acids were as follows: phenylalanine 240, leucine 141, lysine 68; the preparation of C^{14}-leucine contained about 30% of isoleucine, as determined by paper chromatography.

3. Results and discussion

The addition of poly U to non-preincubated ribosome preparations resulted in 2- to 7-fold stimulation of phenylalanine incorporation. In order to lower amino acid incorporation directed by endogenous templates, ribosomes were preincubated in the complete medium without labelled amino acid for 30 min at 37°C. This procedure decreased the endogenous incorporation more than 50-fold. The

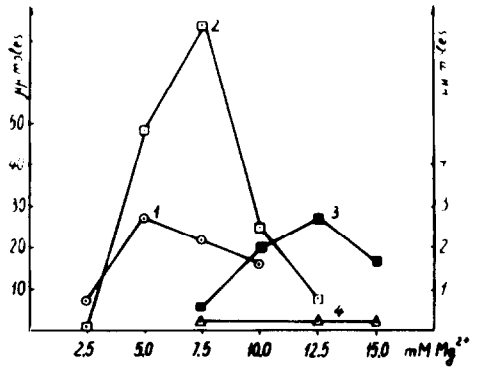

Fig. 1. Effect of Mg^{2+} concentration on endogenous and poly U-stimulated incorporation of amino acids. Left ordinate: endogenous (1) and poly U-stimulated (2) incorporation of C^{14}-phenylalanine. Right ordinate: poly U-stimulated incorporation of C^{14}-leucine (3) and lysine (4). Endogenous incorporation was tested in non-preincubated preparations of ribosomes; poly U-stimulated incorporation: in preincubated preparations. Poly U-stimulated incorporation: incorporation of a given amino acid in the presence of poly U minus incorporation in the absence of poly U.

addition of poly U to the preincubated ribosomes resulted in a marked (40- to 250-fold) stimulation of phenylalanine incorporation. The incorporation of phenylalanine in the presence of poly U was fairly constant (60–80 $\mu\mu$moles) and the different extent of poly U-induced stimulation was largely due to the different levels of endogenous incorporation, that is to the efficiency of preincubation.

In this system the effect of increased Mg^{2+} concentration on the fidelity of translation of poly U was studied. As seen in fig. 1, the endogenous phenylalanine incorporation was optimal at 5×10^{-3} M Mg^{2+}. The optimum of Mg^{2+} concentration for poly U-stimulated incorporation was, in accord with previous data [9], somewhat higher (7.5×10^{-3} M). Further increase in Mg^{2+} concentration resulted in a considerable inhibition on phenylalanine incorporation and in a marked stimulation of leucine incorporation. Maximal stimulation of leucine incorporation was at $12.5 - 15 \times 10^{-3}$ M Mg^{2+}. Accordingly, the increase in Mg^{2+} concentration was accompanied by the increase in the leucine to phenylalanine incorporation ratios, that is by the increase in the percentage of the translation errors. At 12.5×10^{-3} M Mg^{2+} the percent of errors varied from 5.6 to 48.8. It may be noted that at high Mg^{2+} concentration the percentage of errors appeared to depend more on the extent of inhibition of phenylalanine incorporation than on the extent of stimulation of leucine incorporation.

The increase in Mg^{2+} concentration resulted in some increase in leucine incorporation by preincubated ribosomes in the absence of poly U (not shown in the figure). Nevertheless, the poly U-stimulated incorporation of leucine was a specific reaction as evidences by the absence of any detectable stimulation of lysine incorporation by poly U at all the Mg^{2+} concentrations tested (fig. 1), although endogenous lysine incorporation was somewhat higher at higher Mg^{2+} concentrations.

Thus it may be concluded that the increase in Mg^{2+} concentrations in a cell-free protein-synthesizing system from ascites carcinoma cells led to the appearance of errors in translation of the same order as in bacterial systems. The reasons for the reported high fidelity of translation in some other mammalian systems [6–8] remain to be determined. In particular, it is interesting to compare, in different systems, properties of a special ribosomal protein which controls the ambiguity of translation [10].

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