SMALL CHANGES IN ENERGY CHARGE AFFECT PROTEIN SYNTHESIS IN RETICULOCYTE LYSATES

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1. Introduction

Diminished protein synthesis accompanied by polyribosome disaggregation is a common response of cells subjected to adverse changes of environment [1–3]. Under such conditions, the transport of nutrients may be depressed [4]. Uncharged tRNA may accumulate and cause polyribosome disaggregation [5]. Similarly, the energy charge [6] of adenine and guanine nucleotides might diminish because transport of glucose is inhibited.

The possibility that the rate of protein synthesis might be affected by physiologically-induced changes in the adenylate energy charge has been investigated by Live and Kaminskas (1975) [7], who find that complementary changes in the adenylate energy charge and the rate of protein synthesis occur in ascites cells deprived of glucose or amino acids. However, serum deprivation reduced protein synthesis and caused a slight increase in the adenylate energy charge. Thus, changes in protein synthesis may be secondary to changes in the adenylate energy charge in some instances, although these experiments do not prove a causal relationship and other factors are certainly involved.

To investigate possible effects of energy charge on protein synthesis we have studied the effects of omission of creatine kinase on protein synthesis occurring in lysates of rabbit reticulocytes, and find that small changes in the adenylate and guanylate energy charges have significant effects on the rate of protein synthesis.

2. Materials and methods

Reticulocytes were obtained on the fourth day after female rabbits had received the last of 5 daily intramuscular injections of 2.5% phenylhydrazine–HCl in saline (0.3 ml/kg body weight). Reticulocytes were isolated by the method of Lingrel and Borsook (1963) [8] and lysed by adding an equal volume of ice-cold distilled water. After removing cell debris, the lysate was stored in liquid nitrogen. [^{14}C] Leucine incorporation into protein was measured at 37°C in a reaction vol of 0.15 ml that contained 0.0875 ml of lysate, 5.0 nmol hemin, 0.15 μmol ATP, 0.34 μmol $MgCl_2$, 11.2 μmol KCl, 2.13 μmol Tris–HCl buffer (pH 7.8 at 20°C), 0.35 mg creatine phosphate, 0.15 μCi [^{14}C] leucine (spec. act. 348 mCi/mmol) and 2.5 μl of a mixture of amino acids [9]. Creatine kinase (0.15 mg) was added where indicated. Aliquots of 10 μl of the reaction mixture were removed at intervals to determine the incorporation of [^{14}C] leucine into protein.

To analyse changes in polyribosome profiles during protein synthesis, the reaction was stopped by chilling and diluting with 0.275 ml of ice-cold gradient buffer (100 mM KCl, 5.0 mM $MgCl_2$, 10 mM Tris–HCl buffer pH 7.8 at 20°C). 0.3 ml of this diluted reaction mixture was layered over an 8.5 ml exponential 10–40% sucrose gradient, with a 0.4 ml 40% sucrose cushion and centrifuged at 98 000 *g* (r_{av} 3.84 in) for 2 h at 4°C in a 3 × 10 ml swing-out rotor. Absorbance at 260 nm was measured by pumping the contents through a flow cell fitted to a Gilford model 2000 recording spectrophotometer.

The fate of ATP and GTP during the incubation was investigated by adding either 0.75 nmol [^{14}C] GTP (spec. act. 512 mCi/mmol), or 0.75 nmol [^{14}C] ATP (spec. act. 592 mCi/mmol) to the reaction mixture described above. At intervals, 30 μl aliquots of the reaction mixture were removed and added to 4 μl of 40% trichloroacetic acid to precipitate protein. After spinning, the nucleoside mono-, di-, and triphosphates in 5 μl of the supernatant were separated by chromatography on polyethyleneimine-cellulose (Machery-Nagel, Polygram cel 300 PEI) using 1.0 M LiCl, and the radioactivity associated with each was determined. Since nucleoside mono-, and diphosphates equilibrated rapidly with the nucleoside triphosphates, the energy charge could be calculated directly from the radioactivity measurements. The adenylate energy charge is defined as [ATP + 0.5 ADP] / [ATP + ADP + AMP] [6]; the definition of the guanylate energy change is exactly analogous.

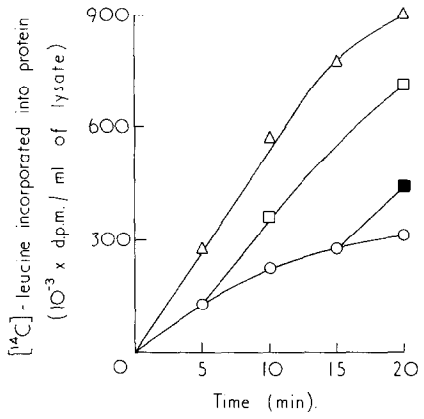

Fig.1. The effect of omission of creatine kinase on protein synthesis by a reticulocyte lysate. 0.15 mg of creatine kinase was added before (△), or 5 min (□) and 15 min (■) after protein synthesis had started in the absence of creatine kinase (○).

3. Results and discussion

In the presence of creatine kinase, (fig.1) the rate of protein synthesis was similar to that observed by Hunt et al. (1972) [9], who calculated that the transit time of ribosomes on globin mRNA was 0.6 min in lysates incubated at 35°C. Thus, under these conditions a steady-state distribution of ribosomes between monoribosomes and polyribosomes was maintained for nearly 15 min by the recycling of ribosomes before the rate of protein synthesis decreased, possibly as a result of the accumulation of the translational repressor of protein synthesis [10]. The initial adenylate and guanylate energy charges of 0.98 and 0.99 were close to their maximum values, and declined slightly after 20 min to values of 0.98 and 0.94 respectively.

When creatine kinase was omitted, the initial rate of protein synthesis was 2–3-fold less, and diminished continuously throughout 20 min of incubation (fig.1). This diminished rate of protein synthesis was associated with reduced adenylate and guanylate energy charges that initially were 0.92, and declined to 0.88 and 0.83

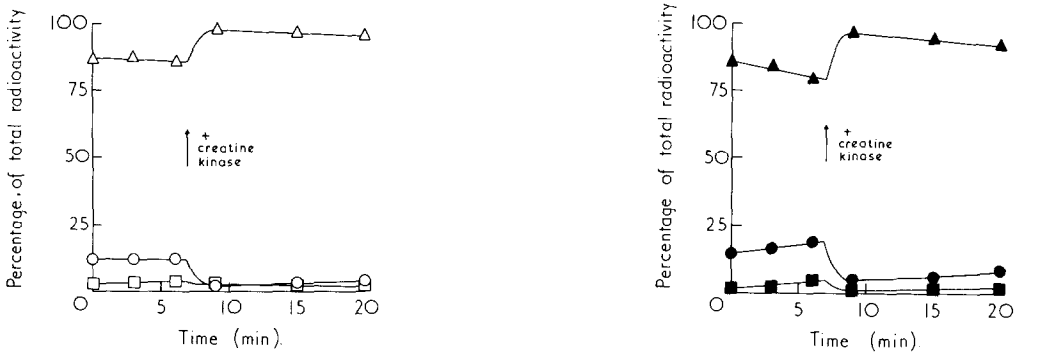

Fig.2. Effect of addition of creatine kinase on the fate of (a) [^{14}C] ATP and (b) [^{14}C] GTP during protein synthesis by a reticulocyte lysate. Creatine kinase was added 7 min after protein synthesis had started. The radioactivity of chromatogram segments containing ATP (△), ADP (○), AMP (□), GTP (▲), GDP (●) and GMP (■) was measured, and the results expressed as a percentage of the total radioactivity on the chromatogram.

respectively after 20 min of incubation. If creatine kinase was added subsequently, the rate of protein synthesis increased, as did the adenylate and guanylate energy charges (figs.1 and 2). Thus, protein synthesis in reticulocyte lysate is sensitive to quite small changes in the adenylate and guanylate energy charges.

To discriminate between effects of omission of creatine kinase on the initiation and elongation reactions of protein synthesis, the proportion of ribosomes in the form of polyribosomes was determined under the various incubation conditions. In the presence of creatine kinase, 50–60% of ribosomes were in the form of polyribosomes during the initial linear phase of protein synthesis (fig.3). In the absence of creatine kinase, the proportion of ribosomes in the form of polyribosomes diminished from 55% after 5 min of incubation to 35% after 10 min (fig.3). This decrease in the amount of polyribosomes was retarded if creatine kinase was added 5 min after protein synthesis had started (result not shown).

These results indicate that both the initiation and elongation reactions of protein synthesis were inhibited when the energy charge was reduced by the omission of creatine kinase. After 5 min the rate of protein synthesis was diminished 3-fold by omission of creatin' kinase, yet the proportion of ribosomes in the form of polyribosomes was unchanged. Thus, the velocity of the ribosome cycle was diminished 3-fold with all steps being affected equally. After 10 min of incubation in the absence of creatine kinase, the rate of protein synthesis was further diminished and a preferential inhibition of the initiation reaction was indicated by the decrease in the number of polyribosomes.

Previous studies using purified components have shown that GDP inhibits the formation of the ternary initiation complex, EIF2-GTP-met-tRNA$_f$ [11] and that several nucleoside diphosphates inhibit the elongation reaction of protein synthesis [12]. Thus, an effect of energy charge in a fully-constituted system might be expected. The present work shows that protein synthesis in lysates of reticulocytes is sensitive to quite small changes in the energy charge, and that the effects on the initiation and elongation reactions are of similar magnitude. The aminoacylation of tRNA could also be affected by the energy charge, although this reaction is very insensitive to AMP [13], and in the present instance the concentration of ATP remained high even in the absence of creatine kinase.

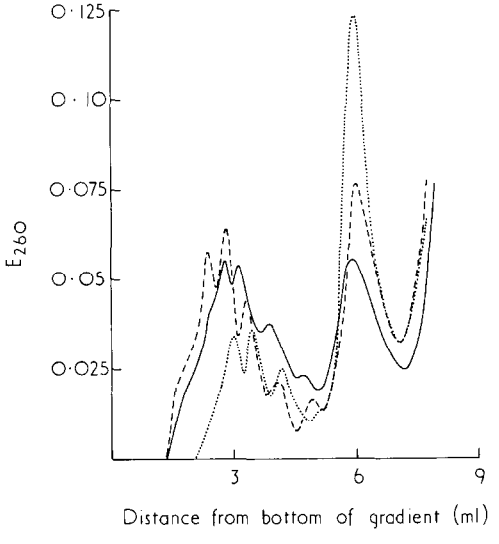

Fig.3. Polyribosome profiles during protein synthesis. Polyribosomes of reticulocyte lysates in which protein synthesis had proceeded for 7 min in the presence of creatine kinase (——), or for 5 min (- - -) or 10 min (. . . .) in the absence of creatine kinase.

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