POLYDEOXYTHYMIDYLATE INHIBITION OF GLOBIN SYNTHESIS IN A RABBIT RETICULOCYTE LYSATE SYSTEM

Effect of concentration of potassium salts

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1. Introduction

A large number of synthetic polynucleotides inhibit globin synthesis in a rabbit reticulocyte lysate system [1]. Miller et al. [2,3] reported the inhibition of globin synthesis by polydeoxythymidylate (poly(dT)) in an ascites cell-free system and suggested that hybridization of poly(dT) to poly(rA) sequences in globin mRNA does not account for the inhibition. It was reported that the sensitivity of globin synthesis to the inhibition by $m^7G^{5'}{}_p$ [4,5] and by double-stranded RNA [6] depended on the concentration of potassium salts. To investigate the mechanism of inhibition of globin synthesis by poly(dT), we studied the effect of concentration of potassium salts on the inhibition in a rabbit reticulocyte cell-free system.

The data showed that the inhibition by poly(dT) was greater with increasing concentrations of potassium salts and that the inhibition appeared to be at the step of initiation of globin synthesis.

2. Materials and methods

2.1. *Materials*

Rabbit reticulocyte lysate was prepared as in [7] and kept frozen in small vials at −70°C. Hemin was added to the lysate at 60 μM final conc. just after thawing the frozen lysate. The addition of hemin decreased the loss of globin synthesis activity. L-[U-^{14}C]Leucine (308 mCi/mmol or 355 mCi/mmol), L-[^{35}S]methionine (424.8 mCi/mmol) and Aquasol-2 were purchased from New England Nuclear. Poly(dT) was from P-L Biochem. Ca-Leucovorin for the preparation of f[^{35}S]Met-tRNA was from Nippon Lederle Co. L-f[^{35}S]Met-tRNA was prepared from unfractionated tRNA as in [9]. *Escherichia coli* aminoacyl-tRNA synthetase and transformylase for the preparation of f[^{35}S]Met-tRNA were the same preparations as in [9]. The analysis of L-f[^{35}S]Met-tRNA showed that >90% of the ^{35}S-radioactivity was fMet [10].

2.2. *Amino acid incorporation experiments*

Each 50 μl incubation mixture contained 15 or 20 μl lysate, 2.7 μg creatine kinase, 0.062 μCi L-[^{14}C]Leu in 30 mM Tris–HCl (pH 7.4), 2.5 mM magnesium acetate, 5 mM creatine phosphate, 3 mM mercaptoethanol, 0.86 mM GTP, 0.36 mM ATP, 40 μM each of 19 L-amino acids minus Leu, 30 μM hemin and various concentrations of poly(dT) and KCl or KOAc. After incubation for given times at 30°C, the incorporation of [^{14}C]Leu into hot trichloroacetic acid-insoluble material was determined for 5 μl incubation mixture as in [7].

2.3. *Effect of concentration of poly(dT) and potassium salts on the elongation or release of nascent chains*

Experimental conditions were detailed in [9,11]. Labeled nascent chains in the supernatant of the original lysate were released with various concentrations of poly(dT) and potassium salts for given times at 30°C in the globin synthesis system as above. After incubation, the radioactivities retained on the ribosomes were measured as in [9,11].

3. Results

3.1. *Effect of various concentrations of poly(dT) and potassium salts on rabbit globin synthesis*

Rabbit reticulocyte lysate was incubated with various concentrations of poly(dT) and KCl or KOAc as in section 2. Aliquots (5 μl) of the incubation mixture were taken at given times and analyzed for the incorporation of [^{14}C]Leu into hot trichloroacetic acid-insoluble material. Figure 1 shows the time course of incorporation of [^{14}C]Leu at 50 mM and 150 mM KOAc in the presence of 0.13 A_{260}/ml poly(dT). As shown in fig.1, poly(dT) inhibited globin synthesis and the inhibition was greater at 150 mM than at 50 mM KOAc. Figure 2 shows the effect of concentration of KCl and KOAc on the inhibition of globin synthesis by poly(dT). Greater inhibition of globin synthesis by poly(dT) was observed with higher concentrations of potassium salts. As the above data were obtained at 0.13 or 0.16 A_{260}/ml poly(dT) the effect of concentration of poly(dT) on globin synthesis was studied at various concentrations of the potassium salts. As shown in fig.3, the concentration of poly(dT) for the 50% inhibition of globin synthesis was lowered with higher concentrations of potassium salts.

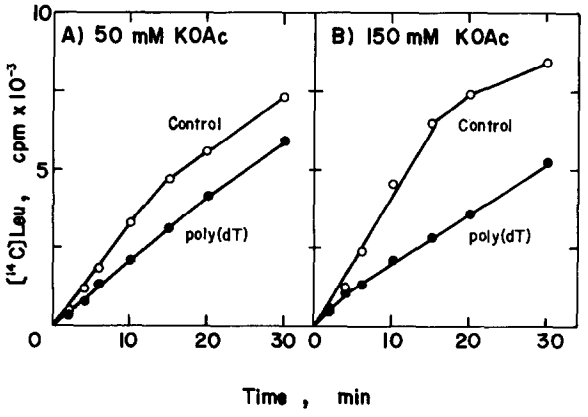

Fig.1. Effect of time on the incorporation of [^{14}C]Leu into hot trichloroacetic acid-insoluble material at 50 mM (A) and 150 mM KOAc (B). Aliquots (15 μl) of lysate were incubated with 0 (○) and 0.13 A_{260}/ml poly(dT) (●) in the presence of 50 mM (A) and 150 mM KOAc (B) in 50 μl incubation mixture as in section 2. At given times, 5 μl incubation mixture was taken to measure the incorporation of [^{14}C]Leu into hot trichloroacetic acid-insobluble material.

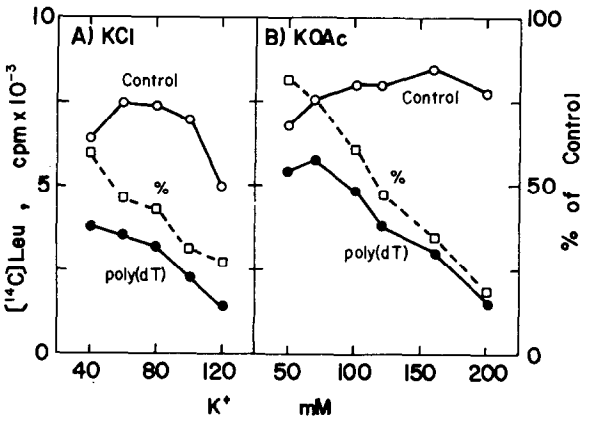

Fig.2. Effect of concentration of KCl (A) and KOAc (B) on the incoporation of [^{14}C]Leu into hot trichloroacetic acid-insoluble material at 0 (○) and 0.16 A_{260}/ml poly (dT) (●). Lysates were incubated with poly(dT) at various concentrations of potassium salts as described in the text. After incubation for 30 min at 30°C, 5 μl the mixture was taken to measure the incorporation of [^{14}C]Leu into hot trichloroacetic acid-insoluble material. (□–□) % control.

3.2. *Effect of various concentrations of poly(dT) and potassium salts on the elongation or release of nascent chains*

To explain the increase in the inhibition of globin synthesis by poly(dT) at higher concentrations of potassium salts, effects of various concentrations of poly(dT) and potassium salts on the elongation or

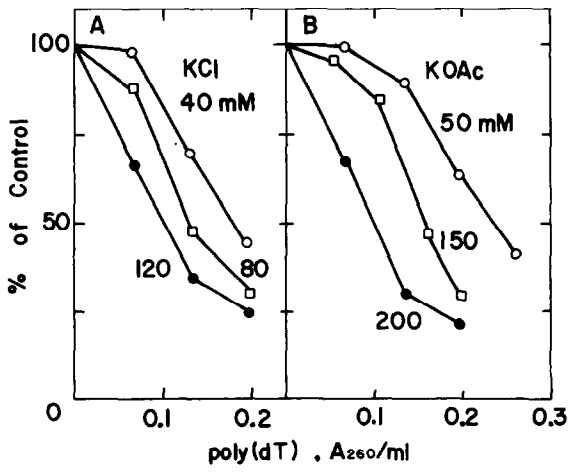

Fig.3. Effect of various concentrations of poly(dT) on globin synthesis at various concentrations of KCl(A) and KOAc(B). Experiments were done as in fig.2. KCl was 40 (○), 80 (□) and 120 mM (●) and KOAc was 50 (○), 150 (□) and 200 mM (●).

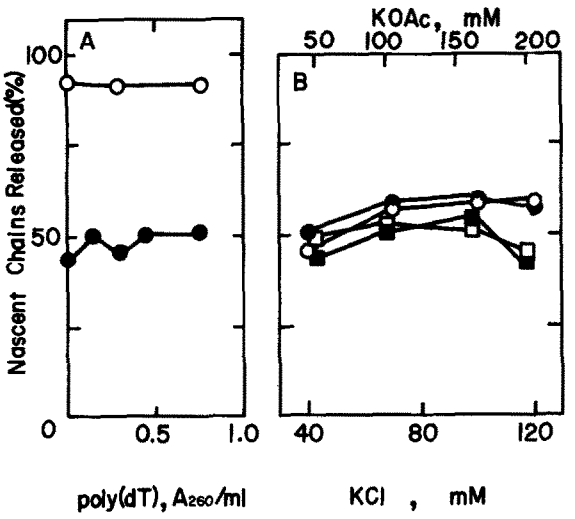

Fig.4. Effect of various concentrations of poly(dT) (A) and potassium salts (B) on the release of nascent chains. (A) Nascent chains labeled with [^{14}C]Leu or f[^{35}S]Met in 40 or 20 μl postribosomal supernatant, respectively, were released at various concentrations of poly(dT). The total volume of the incubation mixture was 100 μl for ^{14}C-labeled nascent chains and 50 μl for ^{35}S-labeled nascent chains. The radioactivities of the postribosomal supernatants without incubation were 13 800 cpm/40 μl for ^{14}C-labeled nascent chains and 3360 cpm/20 μl for ^{35}S-labeled nascent chains. The time of incubation at 30°C was 4 min (○) for ^{14}C-labeled nascent chains and 2 min (●) for ^{35}S-labeled nascent chains. (B) Nascent chains labeled with f[^{35}S]Met in 15 μl postribosomal supernatant were released at 0 (○,□) and 0.12 A_{260}/ml poly(dT) (●,■) and at various concentrations of KCl (○,●) and KOAc (□,■) in 50 μl incubation mixture. The mixture was incubated for 2 min at 30°C. The radioactivities of f[^{35}S]Met in 15 μl postribosomal supernatant was 1290 cpm.

release of nascent chains were studied. As fig.4A shows, ~50% and 90% of nascent chains were released with the incubation of lysate for 2 min and 4 min, respectively, and poly(dT) did not inhibit the release of nascent chains at 70 mM KCl. When labeled nascent chains on ribosomes were released at various concentrations of potassium salts and 0.13 A_{260}/ml poly(dT), the release of nascent chains was not affected by poly(dT) at various concentrations of KCl and KOAc (fig.4B).

4. Discussion

Greater inhibition of globin synthesis by poly(dT) was observed with increasing concentrations of potassium salts (fig.1–3). And poly(dT) did not inhibit the elongation or release of nascent chains at various concentrations of potassium salts (fig.4). Therefore, increased inhibition of globin synthesis by poly(dT) with increasing concentrations of potassium salts was at the level of peptide chain initiation.

The reason for the increased inhibition of globin synthesis by poly(dT) at higher concentrations of potassium salts is not clear. However, there are several possibilities to explain the present results. One possibility is that the affinity of initiation factor for poly(dT) might increase with the increase of concentrations of potassium salts. This is supported in part by the fact [12] that the poly(dT) inhibition was prevented by the 40–50% ammonium sulfate fraction of KCl-wash of rabbit reticulocyte ribosomes [13]. Another possibility is that affinities of ribosomes and unknown factors for poly(dT) might increase with increasing concentrations of potassium salts, since ascites ribosomes or 100 000 × g supernatant fraction partially reversed the inhibition [3]. Work is in progress in our laboratory to clarify these possibilities.

Acknowledgements

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