FIDELITY OF PROTEIN SYNTHESIS IN VITRO IS INCREASED IN THE PRESENCE OF SPERMIDINE

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1. Introduction

There is now considerable evidence that polyamines can enhance protein synthesis both in prokaryotic and eukaryotic cell-free systems [1,2]. Spermidine was shown [2] to reduce the amount of short polypeptide products normally appearing during in vitro translation of natural messengers. In a study [3] of tobacco mosaic virus (TMV) translation in the wheat germ system it was shown that when Mg^{2+} was partially replaced by spermidine, elongation rather than initiation was increased and also that the presence of polyamines increased the yield of full-length translation products with a corresponding reduction in the short polypeptides formed. The observed accumulation of incomplete chains was suggested due to endonucleolytic cleavage of mRNA.

The possibility occurred to us that the accumulation of incomplete polypeptide products under in vitro translation might be partially due to premature termination and that if spermidine increases the fidelity of translation this might account for its ability to enhance protein synthesis. To test this hypothesis we have studied in the wheat germ system the effect of spermidine on the fidelity of translation of the synthetic messenger poly(U) and its effect on translation of TMV RNA.

2. Materials and methods

Poly(U)-directed synthesis of polyphenylalanine and polyleucine was measured in incubation mixtures (200 μl) containing 6 A_{260} units wheat germ extract, prepared as in [4], 40 mM Hepes (pH 7.6), 100 mM KCl, 1 mM ATP, 0.2 mM GTP, 10 mM creatine phosphate, 50 units creatine phosphokinase, 40 μg poly(U) and 10 μM [^{14}C]phenylalanine or [^{3}H]leucine. Incubations were carried out at 25°C. Samples (10 μl) were withdrawn at different times for estimation of trichloroacetic acid-insoluble radioactivity.

Poly(U)-directed synthesis of polyphenylalanine and polyleucine from the corresponding aminoacyl-tRNA was measured using 1 μM [^{14}C]phenylalanyl-tRNA or 2.3 μM [^{14}C]leucyl-tRNA instead of the amino acids.

TMV RNA-directed synthesis of polypeptides was measured in the wheat germ system. The reaction mixture (200 μl) contained 40 mM Hepes (pH 7.6), 66 mM KCl, 1 mM ATP, 0.2 mM GTP, 10 mM creatine phosphate, 50 units creatine phosphokinase, 100 μg/ml TMV RNA, 10 μM each of 19 amino acids, 6 μM [^{35}S]methionine (350 Ci/mmol), or 5 μM [^{3}H]leucine (5 Ci/mmol), 3 mM Mg^{2+}, or 1.5 mM Mg^{2+} and 0.66 mM spermidine. In the pulse-chase experiment using [^{35}S]methionine the reaction mixtures were incubated at 30°C for 5 min and chased with 200 mM cold methionine. Samples (30 μl) were withdrawn at 0.20, 40 and 60 min after the chase, treated with RNase (50 μg/ml) and EDTA (10 mM) for 15 min at 37°C and dissolved in sample buffer, boiled for 2 min and run on 12.5% sodium dodecyl sulfate–polyacrylamide gels by Laemmli method [5]. The gels were fixed in methanol/acetic acid/H_2O (30/10/60, by vol.) overnight, dried and exposed to Kodak RP/R 54 X-ray film for autoradiography.

3. Results and discussion

The most likely misreading of the poly(U)-messenger involves a misreading of the third base of the codon, UUU, leading to the incorporation of leucine which is coded for by UUG and UUA. We therefore first measured the effect of spermidine on the incorporation of phenylalanine and leucine. The results in fig.1A show that, at the temperature used (25°C), the rate of synthesis of polyphenylalanine was significantly enhanced when Mg^{2+} was partially replaced by spermidine. On incubation with labelled leucine a considerable formation of polyleucine took place (fig.1B), in agreement with the finding [6,7]. The significant fact emerging is that in the presence of spermidine the formation of polyleucine was reduced 10-times. Negligible incorporation was found with labelled serine and tyrosine (data not shown). Such incorporations would require misreading of one of the two first bases of the codon which is less likely to occur [8,9].

To study whether the infidelity of translation, as measured by poly(U)-directed incorporation of leucine could be due to lack of specificity of aminoacyl-tRNA synthetases, the labelled amino acids phenylalanine and leucine were replaced by the corresponding aminoacyl-tRNAs. The results in table 1 demonstrate that also under these conditions the misreading was significantly reduced by spermidine. In the presence of cold phenylalanine the incorporation of leucine was reduced.

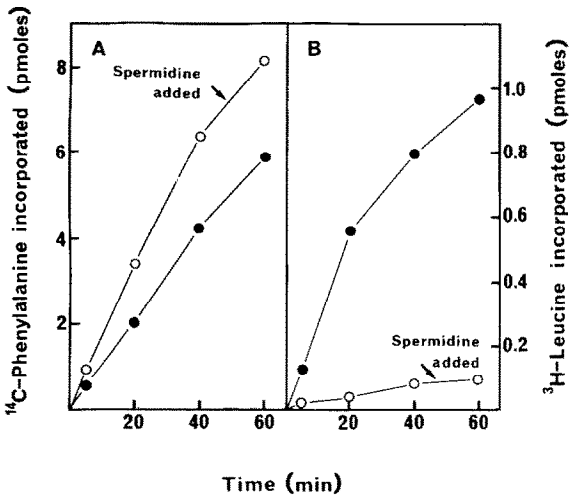

Fig.1. Poly(U)-directed synthesis of polyphenylalanine and polyleucine in a wheat germ system. Incubations were at 25°C as in section 2. Samples (10 μl) were withdrawn at different times for estimation of trichloroacetic acid-insoluble radioactivity. (•) 10 mM Mg^{2+}; (○) 2 mM Mg^{2+} and 1.6 mM spermidine.

In the case of natural messengers, ambiguity in the reading of merely the third base of certain codons could result in the misreading of codons specifying amino acids as termination codons. This possibility was investigated in experiments using TMV RNA as messenger. The peptide patterns were studied after different periods of incubation, following pulse

Table 1
Ability of spermidine to reduce poly(U)-directed incorporation of leucine from [^{14}C]leucine-tRNA as well as from [^{14}C]leucine

Conditions	Poly(U)	Incorporation of radioactivity from			
		[^{14}C]leucyl-tRNA (pmol)	[^{14}C]leucine (pmol)	[^{14}C]phenylalanyl-tRNA (pmol)	[^{14}C]phenylalanine (pmol)
10 mM Mg^{2+}	+	0.49 (0.22)[a]	0.98	4.02	6.02
	–	0.05 (0.03)	0.07	0.15	0.28
2 mM Mg^{2+} and	+	0.08 (0.05)	0.10	4.97	8.12
1.6 mM spermidine	–	0.05 (0.03)	0.07	0.14	0.31

[a] Incorporation of [^{14}C]leucine observed in the presence of 1 μM cold phenylalanine

Incubations (50 μl) were carried out as in section 2, except that 1 μM [^{14}C]phenylalanyl-tRNA or 2.3 μM [^{14}C]leucyl-tRNA were used when indicated instead of the corresponding free labelled amino acids. Incubation time 60 min

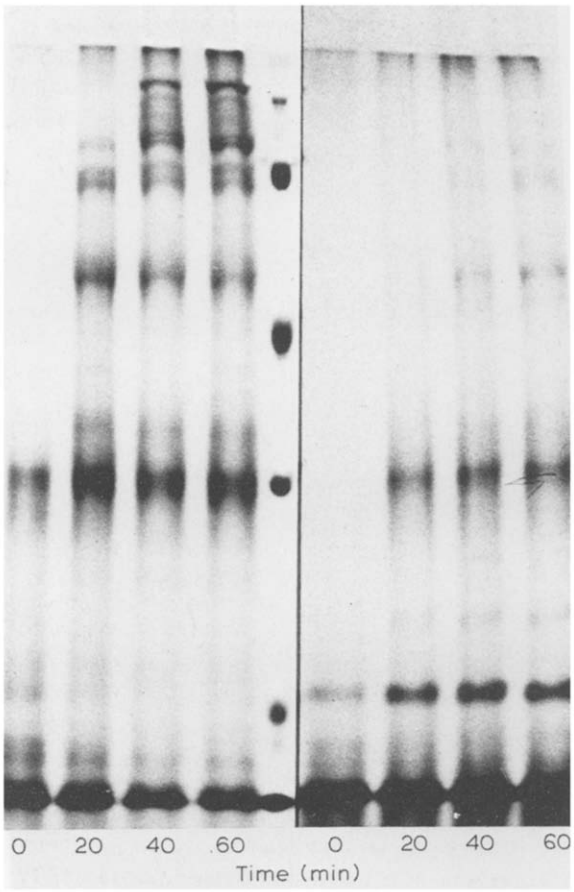

Fig.2. Effect of spermidine on growth rate of TMV RNA-directed polypeptides. Autoradiographic patterns of polypeptides separated by SDS–polyacrylamide electrophoresis at the times indicated after pulse labelling with [^{35}S]-methionine. Left panel, spermidine added, right panel, no spermidine. Lane 5 indicates the position of the marker [^{14}C]proteins; viz. phosphorylase *b* (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100) and α-lactalbumin (14 400).

labelling with [^{35}S]methionine. The results in fig.2 demonstrate that even after 60 min a considerable proportion of the peptides, initiated during the first 5 min incubation, had not yet reached full size. When Mg^{2+} was partially replaced by spermidine (left panel) the proportion of incomplete polypeptides decreased. Significantly, in the absence of spermidine (right panel) two small discrete polypeptides were found that were not seen in the presence of spermidine. With increasing incubation time these peptides increased in amount rather than in length.

It is well known that in the wheat germ system a certain amount of nucleolytic cleavage of mRNA occurs. It might therefore be argued that the short polypeptides seen in the absence of spermidine arose by nucleolytic cleavage rather than by premature termination. However, in this event one would have to postulate that the nucleolytic cleavage occurs at specific sites of mRNA and that this process is decreased by spermidine. In separate experiments using labelled light chain immunoglobulin mRNA we found that spermidine did not reduce the breakdown of the mRNA (data not shown). Moreover, peptides arising by such a mechanism would be expected to be bound to tRNA. Evidence that this was not the case was obtained in experiments where we measured the fraction of tRNA-bound peptides [10]. If the short peptides arose largely by nucleolytic cleavage, one should expect to find relatively more radioactivity precipitable by cetyltrimethylammonium bromide in the absence than in the presence of spermidine, since more short peptides appear under the former conditions. However, the opposite was found to be the case (data not shown).

In the presence of excess ribosomes, as used here, the number of initiation sites is a rate limiting step in the incorporation of labelled leucine into peptides. If the increased amounts of short peptides in the absence of spermidine were due to unmasking on new initiation sites under such conditions [11], a higher initial incorporation of labelled leucine would be expected in the absence than in the presence of spermidine. However, the results in fig.3 demonstrate that at all times the incorporation of leucine was actually lower in the absence of spermidine. The data therefore do not support the view that multiple initiation sites are involved in the appearance of short peptides.

These results show that partial replacement of Mg^{2+} by spermidine decreases the misreading of the synthetic messenger poly(U), and they are consistent with the view that the accumulation of incomplete chains during translation of the TMV messenger is, at least partly, due to premature termination, a process which is decreased in the presence of spermidine. Obviously, the ability of spermidine to decrease such premature termination could explain the increase in

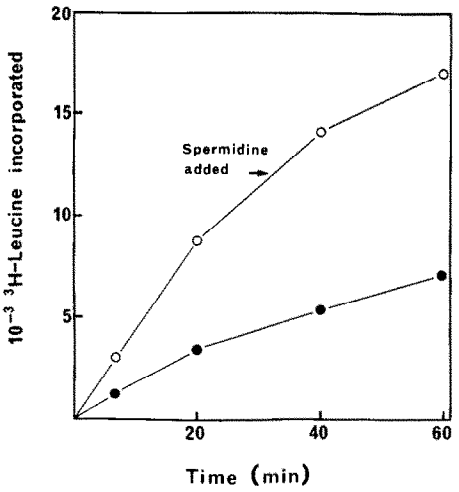

Fig.3. Time course of TMV RNA-directed incorporation of [^{3}H]leucine in the presence and absence of spermidine. The incubation mixtures were as in section 2. [^{3}H]Leucine (spec. act. 5 Ci/mmol) 5 μM was used as the radioactive amino acid. Samples (10 μl) were withdrawn at the times indicated and hot trichloroacetic acid-insoluble radioactivity was determined. (•) 3 mM Mg^{2+}; (○) 1.5 mM Mg^{2+} and 0.16 mM spermidine.

the overall rate of elongation observed [3].

Studies of protein synthesis in vitro have to a large extent overlooked the fidelity problem. Emphasis has been placed on finding conditions for maximum rate of translation. However, such conditions do not necessarily give the highest degree of fidelity. In fact, we have obtained evidence that the fidelity of translation decreases with increasing incubation temperature. Thus, in the case of the wheat germ system the highest fidelity in the presence of spermidine was found at 25°C. Considerably higher total amino acid incorporation is obtained if the temperature is raised to 37°C.

The mechanism whereby spermidine increases fidelity of translation is not clear. However, it is known that spermidine alters the conformation of tRNA [12] and also that tRNA conformation influences the codon–anti-codon recognition [13].

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