POLYAMINES AND YELLOW LUPIN AMINOACYL-tRNA SYNTHETASES

Spermine and spermidine help to maintain the active structures of aminoacyl-tRNA synthetases

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1. Introduction

The polyamines occur in essentially all biological systems [1,2]. Although the significance of polyamines in physiological and biochemical processes is recognized, their precise role in living systems is only beginning to be understood. Because of their cationic nature polyamines bind strongly to nucleic acids, the binding being considerably reduced by Mg^{2+} and Na^{+} [3]. Most remarkable is the stabilization of a tertiary structure of transfer RNA by spermine: the best crystals of transfer RNA grow in the presence of spermine [4]. The polyamines affect the biosynthesis, maturation and stability of ribosomes [5] and stimulate the translation of natural and synthetic mRNAs [6–9]. Spermidine increases the fidelity of poly(U) translation in *Escherichia coli* [8] and wheat germ [9] systems. The rate of many processes involving tRNA is significantly enhanced by polyamines: methylation [10], aminoacylation [11], and incorporation of 3′-terminal nucleotides into tRNA [12]. This effects can be explained as modification of the tRNA reactivity after direct polyamine binding [2,13,14]; however, the polyamine binding to an enzyme can not be excluded. For example, it has been shown, using fluorimetric titration, that spermine binds to an effector site of isoleucyl-tRNA synthetase with K_d 0.25 mM [15], but the significance of this binding in tRNA aminoacylation has not been elucidated. In another study of spermine–protein interaction it has been shown that the polyamine binds to an uridine-specific ribonuclease and changes the specificity of the enzyme [16]. The effects of polyamines on processes involving DNA are also well documented [17–20].

I have shown the role for protein–protein interactions in the maintenance of active forms of aminoacyl-tRNA synthetases [21]. Here data are presented which indicate that the polyamines spermine and spermidine maintain the active structure of aminoacyl-tRNA synthetases either themselves or in a synergistic cooperation with other proteins. This function is likely to occur in vivo.

2. Materials and methods

The sources of enzymes and other proteins were specified in [21]. Spermine · 4 HCl and spermidine 3 HCl were from Serva, putrescine from Merck and edeine from Calbiochem.

Assays of valyl-tRNA synthetase activity in the tRNA aminoacylation reaction were carried out at 25°C in 75 μl total vol. in a medium containing 100 mM Hepes (pH 8.0), 10 mM $MgCl_2$, 8 mM 2-mercaptoethanol, 1 mM ATP, 0.2 mM EDTA, 4 mg/ml unfractionated lupin tRNA, 22.5 μM [^{14}C]valine (175 Ci/mol) and 1 nM lupin valyl-tRNA synthetase [22,23]. The other components of the reaction mixture are specified in the legend to figures. The reactions were initiated by the addition of the enzyme which had been pre-diluted in 20 mM Hepes (pH 8.0), 40 mM 2-mercaptoethanol. After appropriate time intervals 15 μl aliquots of the reaction mixture were analysed for [^{14}C]valyl-tRNA using the standard trichloroacetic acid precipitation procedure [23].

The molar concentrations of aminoayl-tRNA synthetases were determined by titration with either [^{14}C]aminoacyl adenylate or [^{14}C]aminoacyl-tRNA using the nitrocellulose filter assay [23,24].

Protein concentrations were determined using the tanin method [25] with bovine serum albumin as standard.

3. Results and discussion

High purified yellow lupin aminoacyl-tRNA synthetases are reversibly inactivated by dilution. The enzymes regain their activity when assayed in the presence of ribosomes or some proteins, e.g., other homologous aminoacyl-tRNA synthetases, high concentrations of bovine serum albumin, hemoglobin, myoglobin and cytochrome *c* [21]. The reactivation of valyl-, seryl-, phenylalanyl- and tyrosyl-tRNA can also be achieved in the presence of polyamines. An example of this behaviour is presented in fig.1 for valyl-tRNA synthetase. The enzyme regains its activity in the presence of spermine and spermidine but not putrescine. This effect is apparently different from the usually observed stimulation of tRNA aminoacylation by polyamines at low magnesium concentrations [11]. As the experiments described here were carried out at 10 mM Mg^{2+} and the enzyme exhibited no activity in the absence of polyamines, the observed activity is likely to be due to polyamine-enzyme interactions. The polyamine effects shown in fig.1 resemble the protein and ribosome effects described for valyl-tRNA synthetase [21]. The polyamine–synthetase interactions have already been observed either directly as binding of spermine to *E. coli* isoleucyl-tRNA synthetase [15] or indirectly as modification, by spermine, of the ATP–PP_i exchange activity of rat liver threonyl-tRNA synthetase [26] and lupin valyl-tRNA synthetase

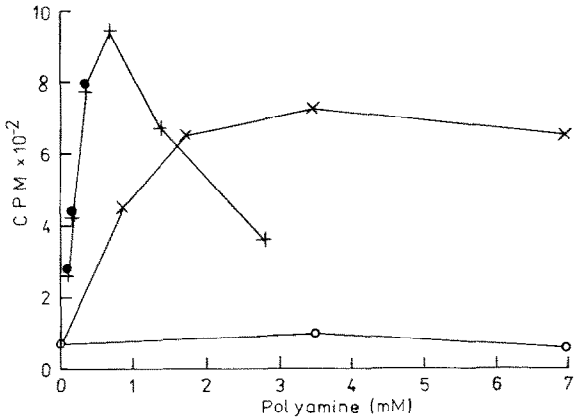

Fig.1. Reactivation of lupin valyl-tRNA synthetase by polyamines. Stock solution (1.7 μM) of lupin valyl-tRNA synthetase was diluted 333-times and assayed as in section 2. Diluted enzyme (15 μl) was used to initiate the reactions. In addition to the components specified in section 2 the reaction mixtures contained indicated concentrations of spermine (+), spermidine (X), edeine (•) or putrescine (○). The 50–75 cpm blank values are not subtracted from the results. No reaction is observed in the absence of polyamines. The reactivation effects are not due to the well-known synergism in the action of polyamines and Mg^{2+} [11].

(Jakubowski, unpublished). The optimal concentrations of spermine and spermidine needed for reactivation of valyl-tRNA synthetase are ~0.7 mM and ~3.5 mM, respectively. Thus, like in many other systems, the action of spermine is superior to that of

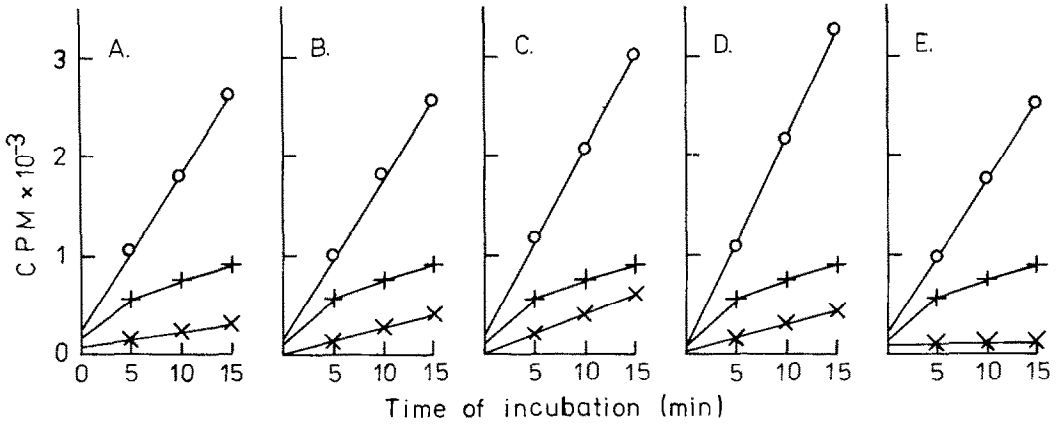

Fig.2. Reactivation of lupin valyl-tRNA synthetase: synergistic action of spermine and proteins. The enzymatic activity of valyl-tRNA synthetase was measured as in section 2 in the presence of either 0.28 mM spermine (+), protein (X), or 0.28 mM spermine and protein (○). The following proteins were used: (A) 0.14 μM phenylalanyl; (B) 0.1 μM tyrosyl- and (C) 0.01 μM arginyl-tRNA synthetase; (D) 43 μM bovine serum albumin; (E) 6 μM cytochrome *c*. No activity was observed in the absence of spermine and protein.

spermidine. It should be noted that a peptide antibiotic edeine which may be regarded as the derivative of spermidine, can also reactivate valyl-tRNA synthetase with the concentration dependence of the effect resembling that for spermine (fig.1). It may be that for optimal stabilization of the enzyme in its active conformation some features of a polypeptide chain are required in addition to the polyamine requirement. This point is illustrated in fig.2 which shows that the reactivating effect of spermine or protein is greatly enhanced by protein plus spermine. The effects are not simply cumulative but rather synergistic. The proteins used in this experiment are known to reactivate valyl-tRNA synthetase when used at higher concentrations [21]. At the low concentrations used in the experiment of fig.2 the proteins probably undergo inactivation by dilution, just like valyl-tRNA synthetase. Apparent synergism of the action of spermine and the reactivating proteins allows one to conclude that spermine interacts with and maintains the active structure of phenylalanyl-, tyrosyl- and arginyl-tRNA synthetases, bovine serum albumin and cytochrome *c*, which in turn become more effective in restoration of the most active structure of valyl-tRNA synthetase. Thus the mixed protein–spermine–protein interactions may be implicated in the stabilization of the active forms of the aminoacyl-tRNA synthetases.

The concentration of phenylalanyl-tRNA synthetase required for maximal reactivation of valyl-tRNA synthetase is evidently >280 nM and it is lowered to 70 nM in the presence of 0.28 mM spermine (fig.3A). Also the concentration of spermine required for maximal reactivation of valyl-tRNA synthetase is lowered from 0.7–0.17 mM in the presence of 140 nM phenylalanyl-tRNA synthetase (fig.3B). It is apparent from fig.3B that the greatest reactivation is brought about exclusively by the protein–polyamine mixture. Again the data indicate that spermine enhances the reactivation of valyl-tRNA synthetase by phenylalanyl-tRNA synthetase. Both enzymes apparently interact with spermine and themselves.

Since the in vivo polyamine concentrations are of the order of 1 mM [28] it is possible that the active structure-stabilizing synthetase–synthetase and synthetase–ribosome interactions [21] are enhanced by polyamines in the cell. In particular the labile structure of the supramolecular complexes of aminoacyl-tRNA synthetases [30] may possibly be stabilized by polyamines in vivo. The general function of polyamines in vivo may be the stabilization of the active structures of various macromolecular components of the cell. In addition to ribosomes [5], membranes [29] and transfer RNA [4], polyamines also maintain the active structures of aminoacyl-tRNA synthetases. It has been suggested that the protein biosynthetic apparatus (aminoacyl-tRNA synthetases, tRNA, initiation and termination factors and ribosomes) is integrated into a superstructure associated with, and organized by, membranes [30]. As most components of the putative superstructure are stabilized by poly-

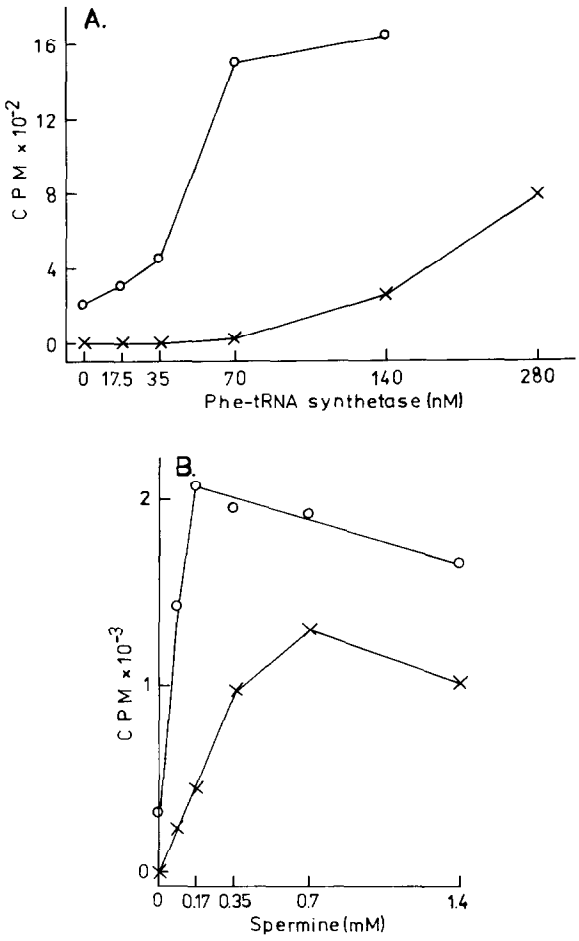

Fig.3. Reactivation of lupin valyl-tRNA synthetase: synergistic effect of spermine and lupin phenylalanyl-tRNA synthetase. The enzymatic activity of valyl-tRNA synthetase was measured as a function: (A) of phenylalanyl-tRNA synthetase concentration in the presence of 0.28 mM spermine (○) and in its absence (X); (B) of spermine concentration in the presence of 140 nM phenylalanyl-tRNA synthetase (○) and in its absence (X).

amines it is reasonable to speculate that the whole superstructure may be stabilized by polyamines. Only within that structure can protein biosynthesis proceed at adequate rates and with high fidelity.

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