INHIBITION OF POLYPEPTIDE SYNTHESIS BY A FACTOR ISOLATED FROM RIBOSOMES OF RESTING HUMAN LYMPHOCYTES

Studies on the mechanism of action

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1. Introduction

Peripheral blood lymphocytes are non-dividing cells which synthesize small amounts of RNA and protein. These resting cells are stimulated when incubated in the presence of mitogens, and after a variety of metabolic changes which include a marked increase of RNA and protein synthesis, they start DNA replication and reach a state of active proliferation [1,2].

The enhancement of protein synthesis occurring during the first hours after the addition of PHA to a lymphocyte culture does not require the synthesis of RNA [3–5], indicating that during this period preexisting mRNA molecules and ribosomal particles are being utilized. Thus lymphocyte activation may involve regulation at the translational level besides other controls which might be occurring on transcription and DNA replication.

Experiments with cell-free systems have indicated that the low rate of protein synthesis observed in unstimulated lymphocytes is probably due to the absence of initiation factors [6] and/or to the presence of cytoplasmic inhibitors which block the formation of initiation complexes [7,8]. The possibility of a more complex control of protein synthesis in lymphocytes has been reinforced by the isolation of a new ribosome-bound translational inhibitor (TI) obtained from resting human lymphocytes [9].

Abbreviations: PHA, phytohaemagglutinin; TI, translational inhibitor; ATA, aurintricarboxylic acid

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Here we show that TI, which blocks protein synthesis in cell-free extracts from stimulated lymphocytes, also inhibits translation in wheat germ and rat liver systems, and that the inhibitory effect occurs at the level of polypeptide chain elongation rather than on protein synthesis initiation.

2. Materials and methods

Lymphocyte cell-free systems, ribosomes, cell sap and ribosomal wash fraction containing TI were prepared as in [9,10].

Wheat germ cell-free extracts and the in vitro polyphenylalanine synthesis were performed either in one- or two-step reactions as in [11].

Rat liver free polysomes were obtained according to [12], but adding 10–20% of high speed supernatant fraction (S_{150}) from rat liver to all the solutions in order to inhibit ribonuclease activity [13]. S_{150} rat liver supernatant fraction was purified as in [14].

The incubation mixture for polypeptide synthesis with rat liver polysomes was carried out in 0.075 ml total vol. which contained: 20 mM Hepes–KOH buffer (pH 7.6); 90 mM potassium acetate; 3.5 mM magnesium acetate; 1 mM ATP; 0.02 mM GTP; 8 mM creatine phosphate; 3 μg creatine phosphokinase; 2 mM dithiothreitol; 7.5 μg rat liver tRNA; 19 unlabeled amino acids excluding methionine, 0.03 mM each; 5 μCi [^{35}S]methionine (1000 Ci/mmol); 20–40 μg supernatant fraction protein and 0.5 A_{260} unit of polyribosomal suspension. Aurintricar-

boxylic acid, sparsomycin or ribosomal wash fraction from lymphocytes were added as indicated in each experiment. The incubation was performed at 27°C and 10 μl aliquots were taken from the reaction mixture at different times. After treatment with 0.3 N NaOH at 37°C for 15 min the incorporation of radioactivity into trichloroacetic acid-insoluble material was determined.

Peptidyl-puromycin synthesis was measured with rat liver polysomes essentially as in [15] in 50 mM Tris–acetate buffer (pH 7.9); 5 mM $MgCl_2$ and 10 μM [^{3}H]puromycin (5.7 Ci/mmol). KCl was added as indicated in each case.

3. Results and discussion

An inhibitory factor (TI) of polypeptide synthesis can be released by washing ribosomes of resting human lymphocytes with solutions containing KCl at high concentration [9]. Ribosomal particles of PHA-stimulated lymphocytes treated in a similar way liberate only very little or no inhibitory activity [9].

The inhibitor was able to block translation directed either by poly(U) or endogenous mRNA in homologous cell-free systems prepared from activated lymphocytes, and the kinetics of this inhibition strongly suggested that polypeptide synthesis was affected at the step of elongation [9]. To confirm this possibility we have measured the effect of TI on different eucaryotic cell-free systems, such as wheat germ and rat liver extracts. Fig.1 shows that the ribosome-bound factor from resting lymphocytes also inhibited markedly the translation programmed by poly(U) and endogenous mRNA in wheat germ and rat liver systems, respectively. However, the ribosomal wash fraction from stimulated cells provoked only a slight inhibition in both cases. These results, almost identical to those reported with lymphocyte extracts [9], allowed us to start a more detailed study of the mechanism of TI action without using human lymphocytes for the preparation of cell-free systems required to test the factor.

Polyphenylalanine synthesis can be carried out in wheat germ extracts either in a single step at 6 mM Mg^{2+} or in a two-step incubation including a first step at 6 mM Mg^{2+} during which a poly(U)–ribosome complex is formed in the absence of labeled amino acids, followed by a second incubation after adjusting the final Mg^{2+} levels to 2 mM with the simultaneous addition of radioactive phenylalanine. During this second step no new poly(U)–ribosome complexes can be formed due to the low [Mg^{2+}] present, and only the elongation of polyphenylalanine chains corresponding to the complexes already formed during the first incubation can occur [11]. Fig.2

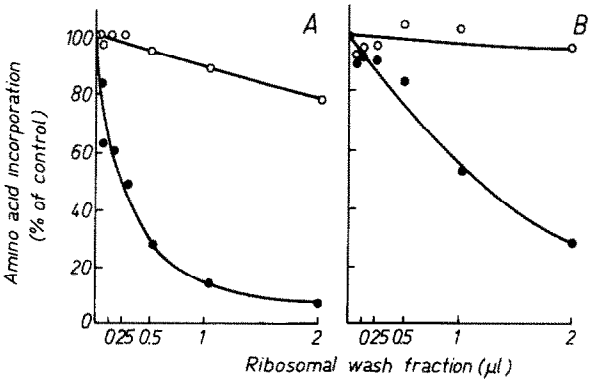

Fig.1. Effect of ribosomal wash fraction obtained from resting (•) and stimulated lymphocytes (○) on polypeptide synthesis: (A) polyphenylalanine synthesis in a wheat germ extract carried out in a one-step reaction; (B) polypeptide synthesis directed by endogenous mRNA in the rat liver system. Assays were performed as in section 2. Control reaction mixtures without ribosomal wash fractions (100% values) were 51 530 cpm for [^{14}C]phenylalanine incorporation (A) and 103 945 cpm for [^{35}S]methionine incorporation (B). Both ribosomal wash fractions contained 5 mg protein ml.

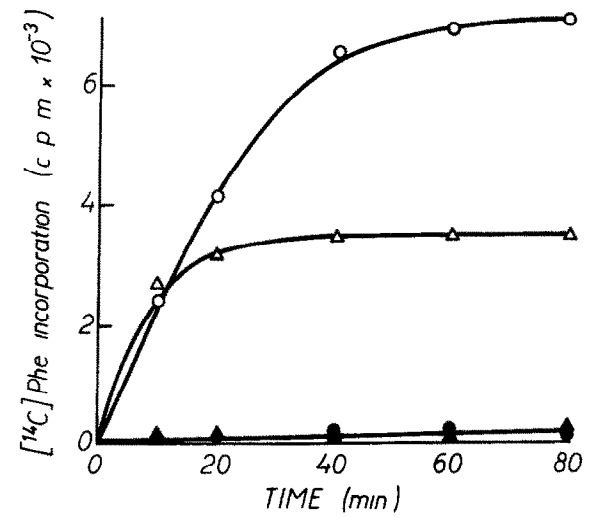

Fig.2. Effect of TI on the kinetics of polyphenylalanine synthesis. Assays were carried out in a one-step reaction at 6 mM Mg^{2+} (○,•) or in a two-step reaction (△,▲) as in the text. The incorporation was measured in the absence (○,△) or in the presence (•,▲) of TI (10 μg protein). Radioactivity in the absence of poly(U) (220 cpm) was subtracted in each case.

shows that TI blocked completely the poly(U) translation performed either in one- or two-step reactions.

The kinetics of polyphenylalanine synthesis was also followed in the absence and presence of sparsomycin or ATA added after 10 min incubation. Sparsomycin is an inhibitor of polypeptide elongation whereas ATA, under these conditions, blocks all the initiations due to the late binding of poly(U) to ribosomes. Therefore, sparsomycin caused an immediate cessation of translation while ATA decreased the polypeptide formation only after a long delay (fig.3). The addition of TI to the reaction inhibited polyphenylalanine synthesis with the same kinetics as sparsomycin, supporting the idea that TI acts at the level of elongation. It is worth mentioning that TI does not inhibit aminoacyl-tRNA formation (not shown).

Since translation with poly(U) does not initiate in the same way as with natural mRNAs we have also used a polysome-containing rat liver system to investigate further the level of TI action. The results

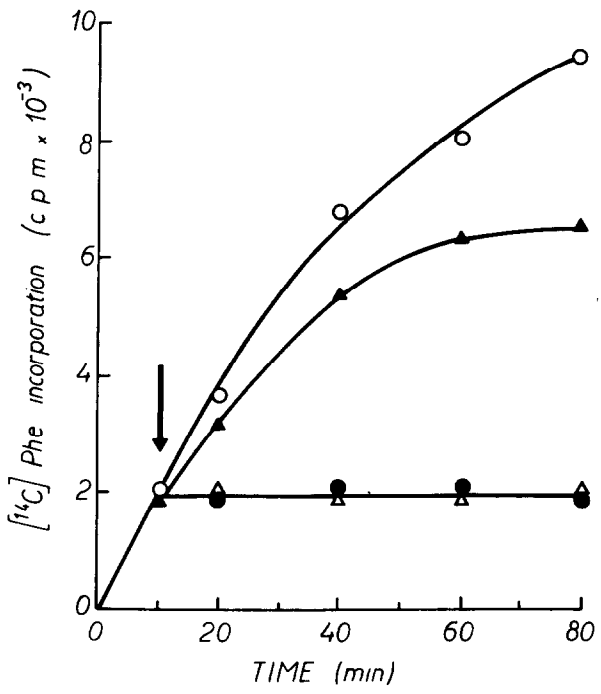

Fig.3. Comparative effects of ATA, sparsomycin and TI on the kinetics of poly(U) translation in wheat germ extracts. Polyphenylalaline synthesis was carried out in a one-step-reaction at 6 mM Mg^{2+} without any addition (○), or adding 7×10^{-5} M ATA (▲), 7×10^{-5} M sparsomycin (△) or 10 μg TI (●) after 10 min incubation, as indicated by the arrow.

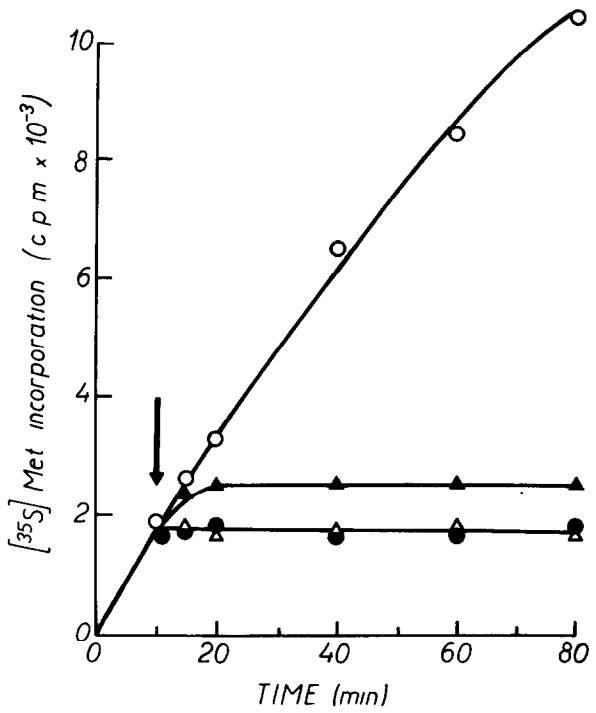

Fig.4. Effects of ATA, sparsomycin and TI on the kinetics of polypeptide synthesis in rat liver polysomes. Reaction mixtures were as in section 2. Polypeptide synthesis was performed without any addition (○), or with the addition of 7×10^{-5} M ATA (▲), 7×10^{-5} M sparsomycin (△) or 10 μg TI (●) after 10 min incubation.

in fig.4 show that ATA was able to block polypeptide synthesis of rat liver polysomes after a lag of 5–10 min during which all growing peptides had presumably been completed. This fact indicates that polysomes could reinitiate translation, and that ATA stopped completely the reinitiation process. However, the addition of sparsomycin inhibited polypeptide synthesis instantaneously as would be expected of an inhibitor of elongation. The effect of TI on the rat liver polysome system was again identical to that observed with sparsomycin.

Peptide bond formation is one of the reactions involved in the elongation process. Peptidyl-puromycin synthesis carried out with polysomes has been used to measure transpeptidation [15]. We have studied the effect of TI on this reaction using rat liver polysomes and radioactive puromycin. Fig.5 shows that TI decreased drastically the peptidyl transferase reaction in a wide range of salt concentration. This inhibition was somewhat variable with different TI preparations

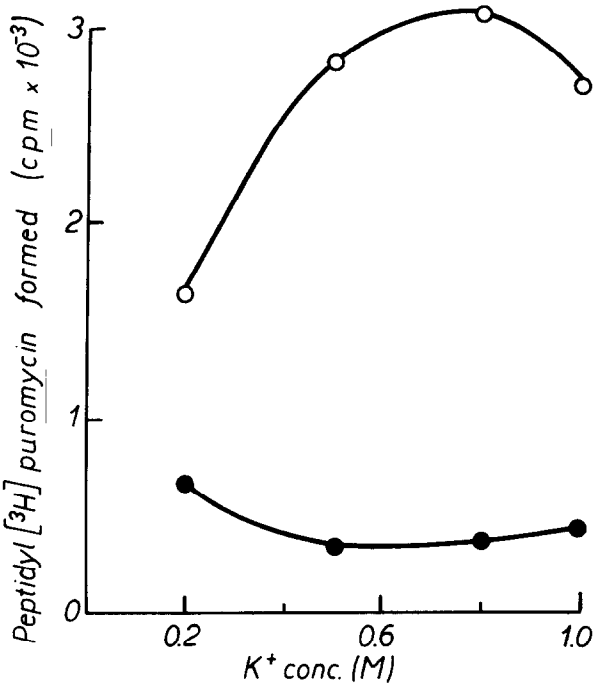

Fig.5. TI effect on peptidyl-puromycin formation. Transpeptidation reaction was carried out as in section 2 at the indicated [K^+], in the absense (○) or presence (●) of TI (10 μg protein.

even though all of them blocked polypeptide synthesis almost completely.

These data confirm our conclusion that an inhibitory factor affecting polypeptide elongation can be extracted from ribosomal particles of human resting lymphocytes. This factor, which disappears upon lymphocyte activation by PHA, might participate in a multiple translational control responsible for the low levels of polypeptide synthesis in unstimulated lymphocytes.

Acknowledgements

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References

[1] Nowell, P. C. (1960) Cancer Res. 20, 462–466.
[2] Ling, N. R. and Kay, J. E. (1975) in: Lymphocyte stimulation, 2nd edn, pp. 324–337, Elsevier/North-Holland, Amsterdam, New York.
[3] Jagus-Smith, R. and Kay, J. E. (1976) Biochem. Soc. Trans. 4, 783–785.
[4] Jagus, R. and Kay, J. E. (1979) Eur. J. Biochem. 100, 503–510.
[5] Wettenhall, R. E. H. and London, D. R. (1974) Biochim. Biophys. Acta 349, 214–225.
[6] Ahern, T., Sampson, J. and Kay, J. E. (1974) Nature 248, 519–520.
[7] Kay, J. E., Benzie, R., Dicker, P. and Lindahl-Kiessling, K. (1978) FEBS Lett. 91, 40–44.
[8] Wettenhall, R. E. H. and Slobbe, A. (1979) Biochem. Biophys. Acta 562, 400–412.
[9] Burrone, O. R. and Algranati, I. D. (1979) FEBS Lett. 102, 91–94.
[10] Burrone, O. R. and Algranati, I. D. (1977) Mol. Cell. Biochem. 16, 105–110.
[11] Algranati, I. D. (1980) Biochem. Biophys. Res. Commun. in press.
[12] Ramsey, J. C. and Steele, W. (1979) Anal. Biochem. 92, 305–313.
[13] Blobel, G. and Potter, V. R. (1967) J. Mol. Biol. 28, 539–542.
[14] Arlinghaus, R. B. and Ascione, R. (1972) in: Protein biosynthesis in non-bacterial systems (Last, J. A. and Laskin, A. I. eds) pp. 52–53, Marcel Dekker, New York.
[15] Pestka, S. (1974) Methods Enzymol. 30, 479–488.