POLYAMINES CAN REPLACE THE DIALYZABLE COMPONENT FROM CRUDE RETICULOCYTE INITIATION FACTORS

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SUMMARY

The polyamines spermidine and spermine can replace the dialyzable component, previously indicated as "iRNA", in restoring the activity of dialyzed initiation factors on messenger RNA translation *in vitro*. These results further sustain our earlier suggestions (1, 2) that the RNA nature of the dialyzable component (3) is questionable.

INTRODUCTION

Several reports describe the effect of a dialyzable component isolated from the 0.5 M KCl wash of reticulocyte ribosomes, which restores the activity of dialyzed crude initiation factors (1, 2, 3). While we were able to show that this factor was apparently unspecific in its action with regard to messenger translation *in vitro* (1, 2), Heywood et al (4) described a dialyzable component (called translational control RNA (tcRNA)) from an initiation factor preparation (IF_3) of embryonic chick muscle cells. According to the latter authors their factor exhibited specificity in that it inhibits the translation of heterologous mRNA. In connexion with reports in the literature (5, 6, 7) dealing with the aspecific stimulatory activity of polyamines in cell-free protein biosynthesis, we investigated whether or not spermine and spermidine could replace our dialyzable component. In the present report it is shown

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"iRNA" = dialyzable factor
tcRNA = translational control RNA
IF_3 = initiation factor 3

that these polyamines indeed have the capacity to restore the activity of dialyzed initiation factors.

MATERIALS AND METHODS

The isolation of the dialyzable component from rabbit reticulocytes, and the preparation of RNase treated ribosomes and Krebs II pH5 enzymes has been described previously (1, 2). Cell-free protein synthesis was performed at 30°C for 1 h.
The reaction mixture contained per ml: 10 A_{260} units of RNase treated ribosomes from rabbit reticulocytes, 0.3 mg ^{260} of Krebs II pH 5 enzymes, 40 µg of lens 10-14S mRNA, 100 mM KCl, 1 mM DTT, 10 mM Tris-HCl, pH 7.4, 40 µCi [^{35}S]-methionine (specific activity ± 200 Ci/mmol), and 3 mg of crude initiation factors either without or supplemented with 0.25 mM spermidine or spermine. The dialyzed initiation factors in contrast to the crude factors required 1 mM spermidine or spermine for optimal stimulation. The optimal $MgAc_2$ concentration was 2 mM in the presence of polyamines or 3 mM without polyamines. The ATP generating system and amino acid mixture were as described earlier (2). The final reaction mixture was 25 µl.
The polypeptides synthesized *de novo* in the cell-free system were analyzed by electrophoresis on 7-18% polyacrylamide gradient slab gels in the Tris-glycine buffer system (8). After the electrophoretic run the gels were treated with Me_2SO-PPO (9) and dried down on filter paper before scintillation autoradiography.

RESULTS AND DISCUSSION

Although Bogdanovsky et al (3) suggested that the dialyzable component from the 0.5 M KCl wash of reticulocyte ribosomes was a small RNA species, our previous results (1, 2) were not in favor of this assumption. Firstly: The dialyzable component could not be precipitated with ethanol even in the presence of carrier RNA; its activity was completely recovered from the ethanol layer (2). Secondly: Treatment with alkali or pancreatic RNase did not destroy the activity of the dialyzable component. Likewise the factor resisted heating for 5 min at 100°C (1, 2). Thirdly: A polystyrene column, which normally binds nucleotides, did not retain the dialyzable activity (1).
Another discrepancy between our results and the first observation of Bogdanovsky et al (3) concerned the molecular weight of the component. Whereas a molecular weight of approximately 10,000 was reported

DTT = 1,4-dithioerythreitol
PPO = 2,5-diphenyloxazol

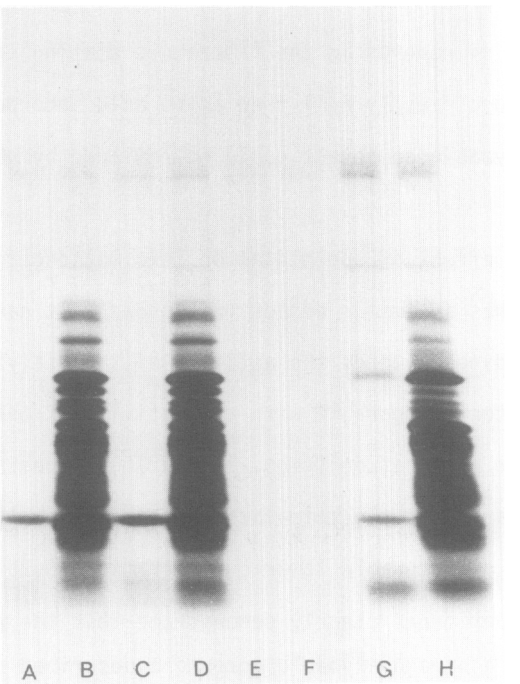

Figure 1

Autoradiography of the SDS polyacrylamide gel electrophoresis of [^{35}S]-methionine-labeled products of cell-free protein synthesis. Two microliters of the incubation mixtures described in the Materials and Methods section were used for each sample. The X-ray film was exposed in contact with the dried gel for 2 days.

The reaction mixtures were supplemented with:
A) crude reticulocyte initiation factors, 3 mM $MgAc_2$; no added mRNA
B) crude initiation factors, 3 mM $MgAc_2$ and 10-14S lens mRNA
C) crude initiation factors, 2 mM $MgAc_2$, 0.25 mM spermidine; no added mRNA
D) crude initiation factors, 2 mM $MgAc_2$, 0.25 mM spermidine and 10-14S lens mRNA
E) dialyzed initiation factors, 3 mM $MgAc_2$; no added mRNA
F) dialyzed initiation factors, 3 mM $MgAc_2$ and 10-14S lens mRNA
G) dialyzed initiation factors, 2 mM $MgAc_2$, 1 mM spermidine; no added mRNA
H) dialyzed initiation factors, 2 mM $MgAc_2$, 1 mM spermidine and 10-14S lens mRNA

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by the former authors, in our experiments, using Sephadex G10 and G15 gel filtration columns, the activity was localized in the 300-600 daltons region.

SDS = $NaDodSO_4$

Since the molecular weight of the unidentified component was relatively low, and in view of reports in the literature stating that polyamines can stimulate aspecifically cell-free amino acid incorporation, we verified if the dialyzable component could be replaced by one or more of these polyamines.

Fig. 1 shows the effect of spermidine on lens messenger translation *in vitro*. Spermine has a similar effect (not shown). It can clearly be seen that 1 mM spermidine restores the activity of the dialyzed initiation factors to an extent comparable with the activity of the undialyzed crude initiation factor preparation (Figs. 1H and 1B). Albeit also crude initiation factors are slightly stimulated by spermidine (Figs. 1B and 1D), this occurs at a considerable lower concentration (0.25 mM) of the polyamines. These experiments clearly demonstrate that the polyamines have a similar effect as the dialyzable component described previously (1). Even in greater detail the similarity in effects of the dialyzable component and polyamines is striking. The high molecular weight polypeptides are less efficiently synthesized in the presence of the dialyzed initiation factors recombined with the dialyzable component than with crude initiation factors. The same holds true when the dialyzed initiation factors are "recombined" with the polyamines.

Since the yield of the dialyzable component is extremely low and batchwise preparation is not feasable due to decrease of activity upon storage, up till now a definite identification is seriously hampered. Nevertheless there are no obvious arguments against the assumption that the real nature of the aspecific dialyzable component, previously designated as "iRNA", is that of a polyamine. Even the observed loss of activity upon storage is in striking accordance with the strongly decreased activity of the polyamines due to oxidation.

The present studies add some new data to the already known biochemical effects of the polyamines spermidine and spermine in protein synthesis (10).

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REFERENCES

1. Salden, M., Bisseling, T., Berns, A. and Bloemendal, H., Biochem. Biophys. Res. Commun. 65, 317-322 (1975).
2. Berns, A., Salden, M., Bloemendal, H., Bogdanovsky, D., Raymondjean, M. and Schapira, G., Proc. Nat. Acad. Sci. USA 72, 714-718 (1975).
3. Bogdanovsky, D., Hermann, W. and Schapira, G., Biochem. Biophys. Res. Commun. 54, 25-32 (1973).
4. Heywood, S.M., Kennedy, D.S. and Bester, A.J., Proc. Nat. Acad. Sci. USA 71, 2428-2431 (1974).
5. Igaraski, K., Sugawara, K., Izumi, I., Nagajama, C. and Hirose, S., Eur. J. Biochem. 48, 495-502 (1974).
6. Fleischer-Lambropoulos, H., Sarkander, H.I. and Brade, W.P., Biochem. Biophys. Res. Commun. 63, 792-800 (1975).
7. Atkins, J.F., Lewis, J.B., Andersson, C.W. and Gesteland, R.F., J. Biol. Chem. 250, 5688-5695 (1975).
8. Laemmli, U.K., Nature 227, 680-685 (1970).
9. Bonner, W.J. and Laskey, R.A., Eur. J. Biochem. 46, 83-88 (1974).
10. Raina, A. and Jänne, J., Med. Biol. 53, 121-147 (1975).