TRANSLATIONAL CONTROL OF GLOBIN SYNTHESIS BY LOW MOLECULAR WEIGHT RNA

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SUMMARY

A species of low molecular weight RNA implicated in the translational control of protein synthesis was analyzed for heterogeneity on Dowex-1 columns. Highest apparent purity was associated with preparations obtained from the high salt wash of ribosomes, and which were subsequently further purified by ethanol precipitation. Kinetic analyses indicated the RNA was not capable of blocking the formation of an inhibitor in the lysate system. Addition of the RNA preparation stimulated the synthesis of all three globin chains by a lysate prepared from cord blood reticulocytes.

INTRODUCTION

Since the initial report by Fuhr and Natta (1) that a novel species of low molecular RNA was involved in the apparently selective control of globin synthesis, a few apparently conflicting reports have appeared (2,3,4). Bogdanovsky, Hermann, and Schapira (2) confirmed the existence of the new RNA species and implied that its presence was required for activity of an initiation protein (IFE_3). In both the cell-free system prepared from human reticulocytes (1) and rabbit reticulocytes (2) the addition of the RNA factor exerted a preferential stimulatory effect upon the synthesis of one of the globin chains.

More recently, the RNA factor has been isolated from chick red muscle (3) and been named translational control RNA (tcRNA) (4). In this instance specificity was again postulated but the tcRNA was found to inhibit translation of heterologous messenger RNAs, while having no effect upon the translation of homologous mRNAs.

The present study reports some analyses of different low molecular weight RNA preparations from human reticulocytes. In order to avoid unnecessary proliferation of terms, the active RNA preparation discussed in this study will be called translational control (tc) RNA. In addition data are presented which show the effect of tcRNA prepared from human cord blood reticulocytes and may also show why some tcRNAs have no apparent effect upon the translation of homologous mRNAs.

MATERIALS AND METHODS

The preparation of human cord blood lysates and the conditions for the cell-free system assay of globin synthesis have already been described (1). Instead of a complete ^{3}H-amino acid mixture, however, ^{3}H-leucine (5 mc/ 0.013 mgm; NEN) was the isotopic precursor. Low molecular weight RNA was prepared from reticulocyte pH 5 preparations or from a high salt wash of reticulocyte ribosomes as already described. Total protein synthetic activity was determined by removing 5 µl aliquots from the reaction tube into 1.0 ml of standard buffer (0.01 M tris, pH 7.4; 0.01 M KCl, 0.0015 M $MgCl_2$) and precipitating the protein with an equal volume of 20% TCA. After heating at 90° C. for 20 min. the precipitates were collected on Millipore filters, washed with an excess of 5% TCA and counted in a Packard scintillation counter after the addition of Aquasol and 0.2 ml of 88% formic acid. Where indicated, the rate of synthesis of specific globin chains was determined by dripping the entire reaction mixture into acid acetone. Globin chains were separated on CM cellulose columns in 8.0 M urea with a linear gradient of 0.005 M phosphate to 0.03 M phosphate, pH 6.8, as described by Clegg et al. (5). The method of determining specific activity of the isolated globin chains has already been described.

Analysis of low molecular weight RNA fractions was performed on Dowex-1 ion exchange columns, chloride form (6).

RESULTS

The elution profiles shown in figure 1 indicate the degree of heterogeneity possible in the low molecular weight RNA prepared by different methods. In particular, these patterns would explain why a higher level of activity was associated with RNA prepared from the high salt wash of ribosomes (1). Activity in all runs shown was associated exclusively with the last peak eluted from the column. Factor or low molecular weight RNA in fig. 1a was prepared by ultrafiltration of the redissolved reticulocyte pH 5 precipitate. The relative yield of the active fraction was enhanced by

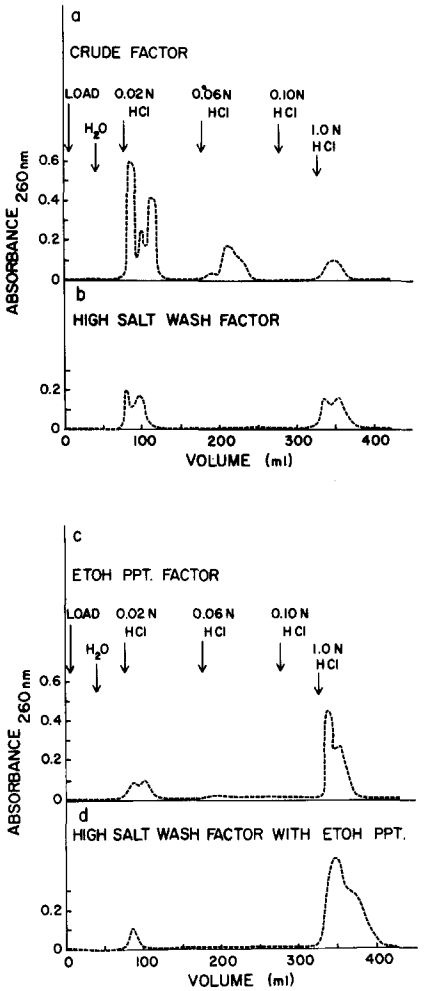

Figure 1. Analysis of different 'tcRNA' preparations on Dowex-1 columns. Samples were eluted from the columns by step-wise increments in HCl concentration as indicated in the figures. Biological activity was exclusively associated with the fraction which eluted last from the column. The elution profile of the following samples are depicted; fig. 1a, the ultrafiltrate of a pH 5 precipitate from reticulocyte lysate; fig. 1b, the ultrafiltrate of a 0.5 M KCl wash of reticulocyte ribosomes; fig. 1c, the ethanol precipitate of an ultrafiltrate of a pH 5 precipitate; fig. 1d, the ethanol precipitate of an ultrafiltrate of a 0.5 M KCl wash.

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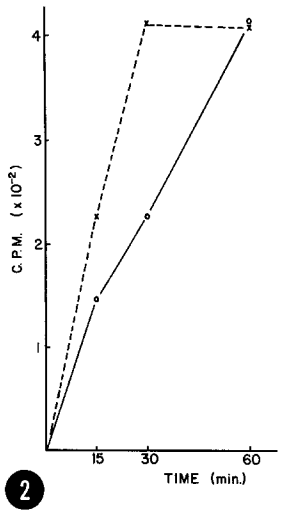
ultrafiltration of the ribosomal 0.5 M KCl wash (fig. 1b). Precipitation with two volumes of absolute ethanol significantly increased the proportion of active RNA in either preparation (fig. 1c and 1d). Although no identification was made of the early eluting peaks, their point of elution is compatible with their tentative identification as adenine compounds, e.g.



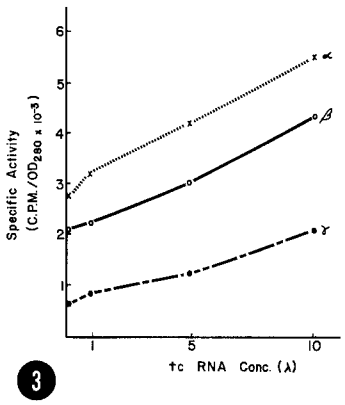


Figure 2. The rate of protein synthesis by a lysate cell-free system prepared from cord blood reticulocytes. The solid line represent total TCA precipitable activity in control incubations. The dashed line represents total TCA precipitable activity in samples containing added cord blood 'tcRNA' from the start of the reaction. Lysates were reacted at 37° C.

Figure 3. Effect of 'tcRNA' prepared from cord blood reticulocytes upon the synthesis of specific globin chains by a lysate prepared from cord blood reticulocytes. The RNA preparation had an absorbance of 0.307/ml at 260 nm. Lysates were reacted for 30 min. at 37° C.

ATP at 0.6 N HCl. Although the last peak is apparently still not homogeneous, no distinct activities have as yet been found associated with different parts of the last peak.

The results in figure 2 indicate that the time of assay may be important in observing an effect of added RNA. After 15 min. and 30 min. of incubation protein synthesis was stimulated 54% and 83% respectively. After 60 min. of incubation, however, there is no difference between control and incubations with added RNA. This is due to the sudden cessation of synthetic activity in the reaction with RNA. No samples in this series of experiments exceeded the total level of protein synthesis shown in figure 2. Presumably, this is due to the accumulation of an inhibitor in the lysate whose appearance is dependent on the level of protein synthesis which has occurred (7).

RNA extracted from cord blood reticulocytes stimulated the synthesis of all three globin chains, γ, β, and α synthesized by the lysate prepared from cord blood reticulocytes (fig. 3). The rate of synthesis of all three globin chains was stimulated almost equally. This is in contrast to the effect of RNA extracted from reticulocytes of patients with β-thalassemia (1), which stimulated β-globin synthesis preferentially.

DISCUSSION

The existence of a low molecular weight RNA which plays a role in the translational control of protein synthesis seems well established. Evaluation of the biological significance of the new RNA, however, has been difficult due to apparently conflicting reports. Kennedy et al. reported that the tcRNA blocks the translation of heterologous mRNAs but had no effect on translation of homologous mRNAs (4). The present report confirms the stimulatory effect of tcRNA upon the translation of homologous mRNAs reported by Fuhr and Natta (1) and Bogdanovsky et al. (2). In addition, the kinetic study indicates that the time of assay may be important in observing a stimulatory effect in lysates incubated without added hemin. In general, lysate systems prepared from human reticulocytes synthesize globin linearly for longer durations than rabbit systems in the absence of added hemin due presumably to a higher endogenous level of heme. Parenthetically, work in progress in this laboratory indicates that the stimulatory effects of hemin and tcRNA are additive. Although the new RNA may function to provide specificity to translation (1,2,3), it would appear from the present experiments to have no ability to prevent the formation of the inhibitor (8) in reticulocyte lysate systems. The difference in activity observed between the RNA prepared from β-thalassemia reticulocytes and cord blood reticulocytes can not be resolved at present. It is not known whether the previously reported specific effect upon β-globin synthesis is due to an apparent accumulation of β-specific tcRNA which is related to the

pathogenesis of β-thalassemia or represents tcRNA that is merely more readily extracted due to the reported deficit of β-globin messenger (9) in reticulocytes of patients with β-thalassemia.

In summary, additional evidence is presented for the existence of a low molecular weight RNA which functions in the translational control of protein synthesis. This RNA may be instrumental in effecting specificity at the level of initiation, but it does not appear capable of replacing heme in the lysate system, and thereby block the formation of an inhibitor of protein synthesis.

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