STIMULATION OF AMINO ACID INCORPORATION BY RNA OBTAINED FROM DIFFERENT RIBOSOMAL FRACTIONS OF E.COLI

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Received July 15, 1965

In previous papers Lindigkeit et al. (1964, 1965) described the separation of the ribosomal fraction of bacteria on a DEAE-cellulose column into several fractions differing from each other among other properties, in their content of rapidly labelled RNA which was composed of a mixture of DNA-like RNA and ribosomal precursor RNA. The problem we were interested in was to which extent the in vitro incorporation of amino acids was stimulated by RNA preparations obtained from these different fractions and in which way the stimulation activities were influenced by the content of DNA-like RNA.

Experimental

The ribosomal fraction used in our experiments was obtained from bacteria that had been cultivated in the presence of 500 µg of chloramphenicol (ClA)/ml with the aim of accumulating ribosomal precursors. In order to obtain the RNA in a possibly undamaged state after elution from the column cooled columns of about -2° were used and the samples were precipitated with ethanol of -15° immediately after elution. The different fractions were designated by Roman figures viz. I,II,III,IIIa and IV. As previously shown, Fraction I consisted of ribosomes containing 37% protein whereas

the Fractions II and IV contained less protein. The RNA preparations of the different fractions are characterized by their elution patterns after separation on the MAK-column, by their nucleotide composition and by their ability to form double strands with DNA. Due to the lower protein content and the higher specific activities of these fractions observed after short-term incorporation of ^{32}P and in inhibition experiments with ClA they had come to be regarded as RNA precursors. Further separation of the Fractions II and III on a Sephadex column and degradation experiments with RNase showed, however, that these fractions were composed of both ribosomal precursors and ribosomes (unpublished). The preparation of RNA from ribonucleoprotein precipitates was carried out by using phenol and dodecyl sulphate with the addition of bentonite. Stimulation experiments were performed in an in vitro system according to Nirenberg and Matthaei (1961). To characterize the system used in our experiments Table 1 lists the stimulation values for ribosomal RNAs prepared from normal bacteria and from bacteria that had been cultivated for 1 hour in the presence of 500 µg ClA per ml.

Table 1

Stimulation of ^{14}C-lysine (1 µc per ml of reaction mixture) incorporation by RNA obtained from normal cells (n-RNA) or cells that had been incubated for 1 hour in the presence of 500 µg ClA per ml (ClA-RNA).

Additions	Counts/min.
-	800
n-RNA (0.5 mg)	1640
ClA-RNA (0.5 mg)	2155

Conditions of incubation were the same as employed by Nirenberg and Matthaei (1961). Incubation period 50 minutes at 37° C. The radioactivity was measured with a 2 π counter.

The RNA preparations from the different fractions of the DEAE-cellulose column (subsequently referred to as RNA I, RNA II, RNA III and RNA IV) had first been tested for their stimulation activity on ^{14}C-phenylalanine incorporation. RNA I which was practically identical with pure ribosomal RNA had hardly any effect on the in vitro incorporation of amino acids, as can be seen from Figure 1.

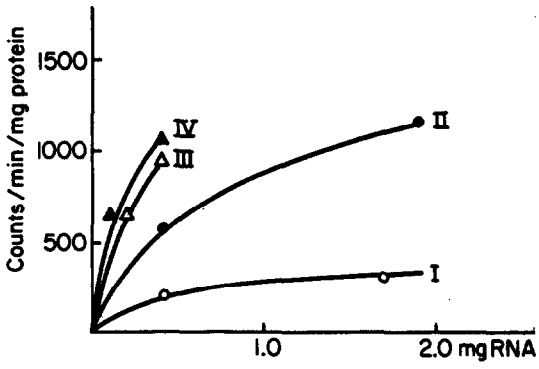

Figure 1
Stimulation of ^{14}C-phenylalanine incorporation (1 µc/ml) by RNA obtained from the different fractions of the DEAE-cellulose column. Conditions of incubation as described in the legend of Table 1.

This agrees with experimental results of Nirenberg and Matthaei (1961) and Willson and Gros (1964) who as well did not find any stimulation activity of ribosomal RNA. Among the other RNA preparations derived from the remaining fractions, RNA IV was found to have the highest stimulation activity followed by RNA III and RNA II in the order of their respective stimulation activities. Similarly graded stimulation activities of the different RNA fractions were found to occur in experiments studying the ^{14}C-lysine incorporation, as can be seen from Table 2.

In order to check a possible loss of stimulation activity after column separation all stimulation activities of the different RNA fractions were added up and compared with that of RNA obtained from the ribosomal fraction without further separation on the DEAE-cellulose column. No appreciable loss of stimulation activity was found to occur after column separation. Moreover, it

was shown that about 50% of the stimulation activity were accounted for by RNA IV.

RNA-Fraction	RNA mg	Stimulation counts/min.
I + II	0.6	150
	1.0	230
III	0.6	515
IV	0.6	600
	1.0	775
C1A-RNA	0.6	335
	1.0	595

Table 2
Stimulation of ^{14}C-lysine incorporation by RNA obtained from the different fractions of the DEAE-cellulose column. Conditions of incubation as described in Table 1.

Subsequently, we tried to find out whether the stimulation activity of the different RNA fractions was proportional to their content of DNA-like RNA. For this purpose ^{32}P-labelled RNA of the combined fractions I to IIIa and RNA of fraction IV which had been eluated from the DEAE-cellulose column at pH 7.4 and pH 10 respectively, were subjected to DNA-RNA double-strand formation according to a slightly modified method of Nygaard and Hall (1963). Parallel to this, the two corresponding non-radioactive fractions were tested for their stimulation of ^{14}C-lysine incorporation. Figure 2 shows the DNA-RNA double-strand formation of RNA preparations derived from the pH 7.4 and pH 10 fractions. The percentage of double-strand formation in relation to ^{32}P-labelled RNA found in RNA of fraction pH 7.4 was 1.4%, that of RNA of fraction pH 10 was 8%. Considering that in this method the amount of double-strand formation is only related to ^{32}P-labelled RNA, i.e. newly synthesized RNA, consideration of this factor would indicate that fraction pH 7.4 contains 0.21% and fraction pH 10 4.8% of DNA-like RNA.

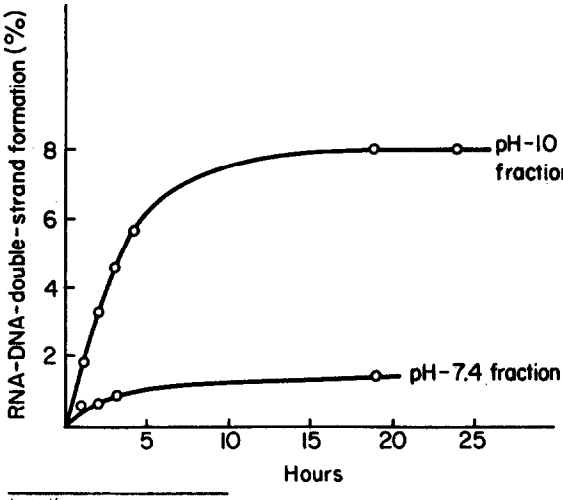

Figure 2
Double-strand formation of ^{32}P-RNA of the pH 7.4 and pH 10 fractions (as described in the paper) with DNA under experimental conditions as employed by Nygaard and Hall (1963). Percentage given relative to ^{32}P-labelled RNA.

Figure 3 shows the stimulation activities of these two RNA fractions on ^{14}C-lysine incorporation. If these stimulation activities are related to the amount of DNA-like RNA contained in these fractions it will be noted that the stimulating effect induced by RNA of fraction pH 7.4 is 5 to 6 times as high as that of RNA obtained from fraction IV whereas the stimulation activities of both fractions were found to be about the same if they were related to the total amount of RNA newly synthesized in the presence of ClA.

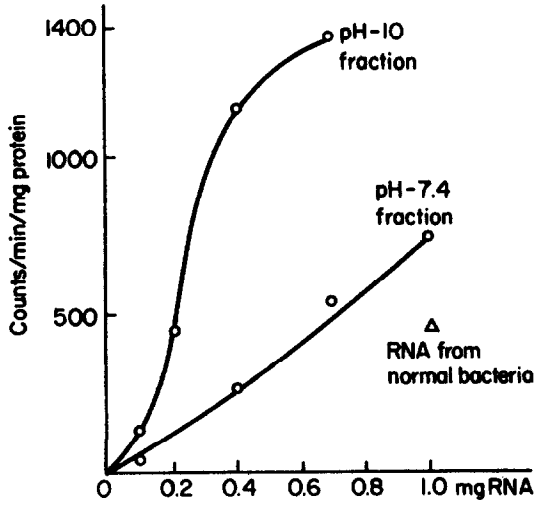

Figure 3
Stimulation of ^{14}C-lysine incorporation by RNAs of the fractions pH 7.4 and pH 10. Conditions of incubation as described in Table 1.

Discussion

Our experiments have shown, that the RNA containing the largest proportion of DNA-like RNA also possesses the highest stimulation activity though no strict proportionality could be established between these two fractions. If one, nevertheless, assumes that the entire stimulation activity is exclusively due to the presence of DNA-like RNA some auxiliary hypothesis will be required. According to results of Willson and Gros (1964) indicating that RNA exerts a stimulating effect only in a low-molecular state it might be assumed, for example, that this would be equally true for the DNA-like RNA of Fraction IV showing the pattern of pulse labelled RNA when separated on the MAK column. According to our own, hitherto unpublished experiments even the high-molecular RNA of Fraction IV yields complex formation with DNA. No auxiliary hypothesis will be necessary, however, if both DNA-like RNA and ribosomal precursor RNA are assumed to exert the same stimulating effect in vitro. The choice between these two alternative possibilities is complicated by the fact that, additionally, fraction pH 7.4 contains a small amount of DNA-like RNA possibly occurring in a form active for stimulation. Hitherto we have been unable to obtain ribosomal precursor RNA completely free from DNA-like RNA. Even under conditions of shift-up as described by Otaka, Osawa and Sibatani (1964) Fraction IV contained an amount of 8% of RNA capable of forming complexes with DNA.

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