INHIBITION OF CELL FREE PROTEIN SYNTHESIS BY HOMOPOLYNUCLEOTIDES

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Nirenberg and Matthaei (1961), Martin et al. (1962), and Lengyel et al. (1961), have reported that a cell free system from E. coli polymerises amino acids under the direction of artificial polynucleotides and naturally occurring RNA's. Nathans et al. (1961), have described a similar cell free system, which under the direction of RNA isolated from an E. coli bacterio-phage, incorporates amino acids into the peptides of the coat protein of this phage.

The experiments described here show that artificial polynucleotides act as specific inhibitors of the protein synthesis, induced by this phage RNA. Nirenberg and Matthaei (1961), already reported that polyadenylic acid inhibits the polyuridylic acid induced synthesis of polyphenyalanine.

All four polynucleotides tested inhibited the protein synthesis (Fig. 1) induced by the phage RNA.

The inhibition was most marked with Poly I and decreased with Poly A, Poly C, and Poly U, in that order. Since the sedimentation constants of all the polymers used are in the same range (see legend to Fig. 1) structure and composition, rather than size, seem to be responsible for their inhibitory action.

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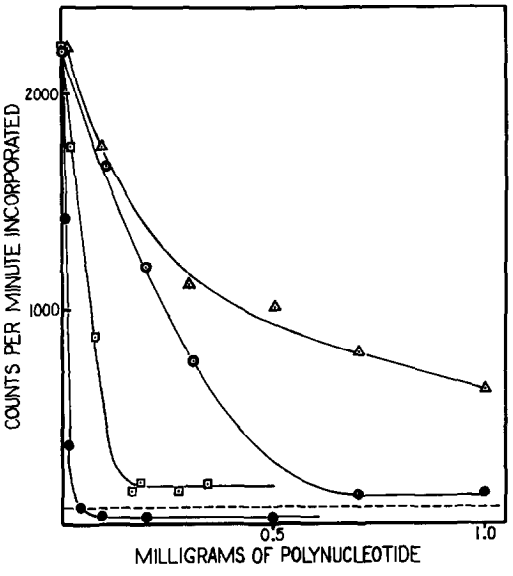

Figure 1: Effect of varying amounts of homopolynucleotides on the stimulation of C^{14}-L-threonine incorporation induced by 0.100 mg. MS_2 phage RNA.
C^{14}-L-threonine incorporation: (△) in the presence of increasing amounts of Poly U (sedimentation constant S20.W = 5.85). (⊙) in the presence of increasing amounts of Poly C (sedimentation constant S20.W = 4.25). (⊡) in the presence of increasing amounts of Poly A (sedimentation constant S20.W = 5.20). (●) in the presence of increasing amounts of Poly I (sedimentation constant S20.W = 6.54). ---in the absence of added MS_2 phage RNA and polynucleotides. The reaction mixture contained in a total volume of 0.5 ml; 50μ moles tris-HCl, pH = 7.6; 5μ moles $MgAC_2$; 25μ moles KCl; 3μ moles mercaptoethanol; 0.5μ moles ATP; 3.5μ moles phosphoenolpyruvate - K - salt, 10μg pyruvate kinase; 0.2μ moles GTP; 0.05μ moles of each 19 L-amino acids, threonine omitted; 0.005μ moles C^{14}-L-threonine (200.000 counts per minute). 0.5 mg E. coli sRNA; 1 mg preincubated E.coli S30 protein (Nirenberg et al. 1961), 0.100 mg MS_2 phage RNA and polynucleotides as indicated.
Samples were incubated at 37° for 60 minutes precipitated with 5% TCA and washed as described by Siekevitz (1952).

The stronger inhibition by Poly I, as compared to the other polymers, could be explained by the ease with which this polymer forms base-pairs with itself and other polynucleotides (Rich 1958, Davies and Rich, 1958). The results illustrated in Table (1) and Figure (2) confirm this hypothesis. When a 1% solution of Poly I is aged, aggregation occurs as shown by a drastic increase in viscosity and sedimentation constant, at the same time Poly I is no longer inhibitory. Subsequent heating results in deaggregation and a reappearance of the original inhibition. A second aging results in a somewhat smaller loss of inhibition (see Table 1).

TABLE 1

Effect of aggregation of Poly I on C^{14}-L-Threonine incorporation, induced by phage RNA.

Additions	Cpm
Complete system	3374
Complete system minus MS_2-RNA	150
Complete system + 25µg fresh Poly I	170
Complete system + 25µg aggregated Poly I	2927
Complete system + 25µg aggregated Poly I, heated 2 minutes at 80°C	134
Complete system + 25µg aggregated Poly I, heated 2 minutes at 80° and reaggregated at 0.05%	850

Reaction mixture was the same as in Fig. 1 including 0.100 mg MS_2-RNA. Aggregated Poly I refers to a 1% Poly I solution in water, kept for 3 days at 4°C and stored frozen for 3 days (S20W = 10 - 100). Freshly dissolved Poly I sediments as a single component with S20W = 6.54.

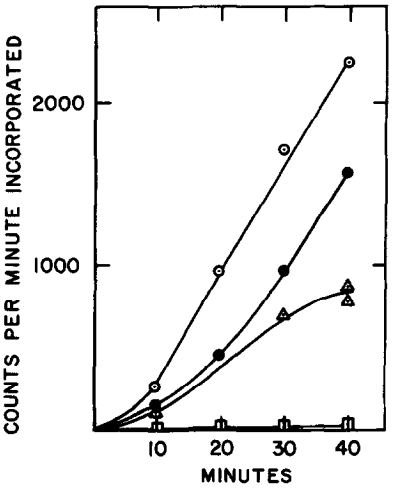

Figure 2: C^{14}-threonine incorporation in the presence of a preincubated mixture of MS_2 phage RNA and Poly I.
The following solutionsof water dialyzed, phage RNA were preincubated for 3 days at 4°C, frozen at -10°C for 3 days and tested for stimulation of C^{14}-threonine incorporation. (a) Phage RNA in water (2.5 mg/ml) (b) 5:1 mixture of phage RNA (2.5 mg/ml) and freshly dissolved Poly I in water. (c) Freshly dissolved Poly I, 2.0 mg/ml. (d) Freshly dissolved Poly I, 0.5 mg/ml. Reaction mixture was the same as described in Fig. 1 except that 0.200 mg phage RNA and 0.040 mg. Poly I were used. (⊙) phage RNA (soln. (a)). (⊡) preincubated mixture of phage RNA and freshly dissolved Poly I (soln. (b)). (●) phage RNA (soln. (a)) + Poly I alone, 2 mg/ml (soln. (c)). (△) phage RNA (soln. (a)) + Poly I alone, 0.5 mg/ml (soln. (d)).

The failure of aggregated Poly I to inhibit protein synthesis proves that the inhibition is not due to contamination of the polymer with RNA degrading enzymes. When freshly dissolved Poly I and phage RNA were incubated together, complete inhibition was observed, whereas preincubation of the two components alone, resulted in a concentration dependent loss of inhibition (see Fig. 2). The results of Table 1 and Fig. 2 can be explained if Poly I interacts with free phage RNA, thereby preventing the RNA from functioning as a template. Sedimentation studies proved that such an interaction exists even at concentrations of Poly I as low as 0.001% (W. Möller). The results shown in Fig. 3 suggest that the same mechanism applies in the case of Poly A since the inhibition varies at the ratio of Poly A to phage RNA, being complete at a value close to 1:1. It remains to be shown whether the described phenomena are of significance in the regulation of protein synthesis in the cell.

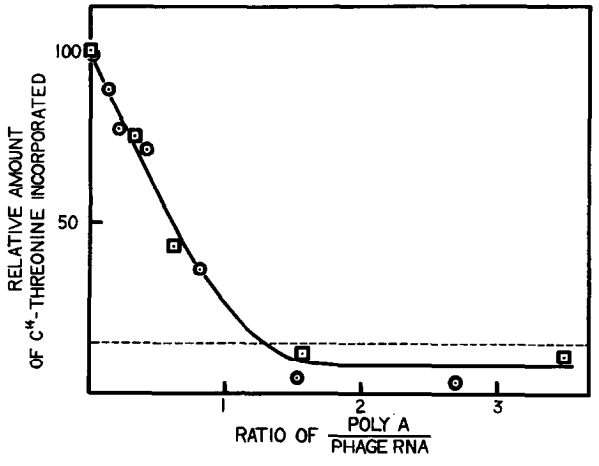

Figure 3: C^{14}-threonine incorporation as a function of the weight ratios of Poly A to MS_2 phage RNA.
The reaction mixture and the assay conditions were the same as in Fig. 1, MS_2 RNA and Poly A as indicated. (⊙) in the presence of 0.250 mg MS_2 RNA. (⊡) in the presence of 0.100 mg MS_2 RNA. --in the absence of MS_2 RNA and Poly A.

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