RELATION BETWEEN SOLUBLE DNA-DEPENDENT RNA POLYMERASE AND "AGGREGATE" RNA POLYMERASE

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In animal nuclei, two RNA polymerase activities have been described : the insoluble "aggregate" enzyme which contains firmly bound deoxynucleohistone acting as endogenous primer (Weiss, 1960) and a soluble DNA-dependent enzyme (Ramuz et al., 1965) with the same characteristics as purified bacterial RNA polymerase. Most of the properties of these soluble and "aggregate" RNA polymerases are similar, but while the "aggregate" enzyme activity is stimulated by the addition of ammonium sulphate or KCl (Goldberg, 1961) to the incubation medium, the activity of the animal or bacterial soluble enzyme is highly inhibited by these salts at the same ionic strength (Ramuz et al., 1965).

We now present results indicating that the "soluble" and the "aggregate" animal enzymes could be two forms of the same enzyme, respectively free or bound to deoxynucleohistone. Since we have not so far greatly purified the animal soluble RNA polymerase (Ramuz et al., 1965), the experiments reported here were performed with E.coli RNA polymerase as source of soluble enzyme ; but we have obtained similar results using the soluble enzyme partially purified from liver nuclei.

Material and Methods.

Purified E.coli RNA polymerase was fraction 4 enzyme (Cham-

berlin and Berg, 1962) stored in a liquid nitrogen bath. With calf thymus DNA as primer, the rate of the RNA synthesis was linear during the first 10 min. F1+F2b histones were prepared according to Johns and Butler (1962). Treatment of incubation mixtures and measurement of acid-insoluble radioactivity were carried out as already related (Busch et al., 1962).

Results and Discussion.

The following conclusions can be drawn from results presented in the Tables :

1) When ammonium sulphate (final concentration, 9 percent saturated) was added to the incubation medium at the same time as the soluble enzyme, the enzymatic activity dropped markedly (Table I). The same degree of inhibition was observed by pre-incubating DNA and enzyme alone (2 min. at 37°) before the addition of nucleoside triphosphates and ammonium sulphate. Once the RNA synthesis had started, however, the addition of the same concentration of the salt resulted in a very much less inhibition (Table I). Therefore we conclude that ammonium sulphate prevents the formation of the enzyme-DNA complex or dissociates it. These results confirm the existence of two possible complexes between the enzyme and DNA, an enzyme-DNA complex and an enzyme-DNA-cRNA[+] complex (Fox et al., 1965 - Bremer and Konrad, 1964) and show that, after the synthesis of RNA has begun, the enzyme is more tightly bound to DNA.

2) When ammonium sulphate was added to the incubation medium after the reaction had been blocked with F1+F2b histones, the RNA polymerase activity resumed and was greatly stimulated (Table II). The measured activity was the same whatever the quantity of histones previously added (Table II), but was proportional to the

[+]cRNA : complementary RNA (Fox et al., 1965).

Table I. - Effect of ammonium sulphate on E.coli RNA polymerase activity.

Reaction mixture	Time of addition of A.S.+ to the reaction mixture	mμmoles incorporated
Complete	-	4.30
Complete + A.S.	0 min	0.14
Complete + A.S.	1 min	2.70
Complete + A.S.	2 min	3.30

As indicated in the table, ammonium sulphate (0.025 ml 100 percent saturated) was added at various times after the addition of 10 μg of purified enzyme to the reaction mixture at 37°. Incubation time was 10 min at 37°.
Complete reaction mixture (0.25 ml) contains : 40 mM Tris HCl pH 7.8, 1 mM $MnCl_2$, 4 mM $MgCl_2$, 12 mM 2-mercaptoethanol, 0.5 mM each of CTP, ATP and GTP, 0.5 mM α-P^{32}-UTP and 30 μg calf thymus DNA.
+A.S. : ammonium sulphate.

amount of enzyme initially bound (unpublished results). Thus, the activity measured in the presence of ammonium sulphate is only related to the amount of RNA polymerase previously bound to DNA in an enzyme-DNA-cRNA complex.

3) The stimulation by ammonium sulphate was related to the ionic strength (Table III). The optimal concentration of salt was nearly the same as for the stimulation of an "aggregate" enzyme (Goldberg, 1961).

4) The base composition of the RNA synthesized after the addition of histones was shifted to a higher GC content (Table IV § II) (in these conditions RNA polymerase activity dropped by 80 percent). Similar results were obtained with bacterial (Hurwitz et al., 1963) and with "aggregate" (Liau et al., 1965) enzymes. When the RNA synthesis was resumed by addition of ammonium sulphate, a return to a more DNA-like base composition (Table IV § III) was observed. It is noteworthy that results obtained by Widnell (1965) show that the RNA synthesized with an

Table II. - Effect of ammonium sulphate on activity of E.coli RNA polymerase bound to DNA or to reconstituted deoxynucleohistone.

Quantity of histone added 1 min 30 sec after the beginning of the incubation	Addition at 3 min after the beginning of the incubation	mμmoles incorporated
0.0	0.125 μmole UTP^{32}	3.00
7.5 μg	"	1.45
30.0 μg	"	0.69
0.0	0.125 μmole UTP^{32} + 0.025 ml A.S. 100 percent saturated	1.77
7.5 μg	"	1.92
30.0 μg	"	1.93

The reaction mixture (0.25 ml) contains : 40 mM Tris HCl pH 7.8, 1 mM $MnCl_2$, 4 mM $MgCl_2$, 12 mM 2-mercaptoethanol, 0.5 mM each of CTP, ATP and GTP, 0.25 mM UTP, 30 μg calf thymus DNA.
In order to obtain E.coli RNA polymerase bound to deoxynucleohistone, F1+F2b histones were added 1 min 30 sec after the addition of 10 μg of purified enzyme to the incubation mixture at 37°. The enzymatic activity of the bound enzyme was then measured by addition of α-UTP^{32} 3 min after the beginning of the incubation. Where indicated 0.025 ml of saturated ammonium sulphate (A.S.) was added with UTP^{32}. Incubation lasted 12 min more at 37°.

Table III. - Effect of various concentrations of ammonium sulphate on activity of E.coli RNA polymerase bound to deoxynucleohistone.

Ammonium sulphate, final concentration in percent	mμmoles incorporated
0	0.80
1	0.93
3	1.87
5	2.75
7	2.80
9	2.46
12	1.98

Reaction mixture : see legend of table II.
In order to obtain E.coli RNA polymerase bound to deoxynucleohistone, 30 μg F1+F2b histones were added 1 min 30 sec after the addition of 10 μg of purified enzyme to the reaction mixture at 37°. 1 min 30 sec later, 0.125 μmole α-P^{32}-UTP was added with the appropriate quantity of ammonium sulphate and the incubations were stopped 15 min after the addition of enzyme.

Table IV. - Base composition of the RNA synthesized using E.coli RNA polymerase bound to DNA or deoxynucleohistone.

	primer	addition of A.S.	base composition in per cent A	U	G	C
I	DNA	none	29.7	29.6	21.4	19.3
II	deoxynucleohistone	none	26.4	26.2	26.0	21.4
III	deoxynucleohistone	+	29.0	28.5	23.3	19.2

The reaction mixture (0.25 ml) contains : 40 mM Tris HCl pH 7.8, 1 mM $MnCl_2$, 4 mM $MgCl_2$, 12 mM 2-mercaptoethanol, 0.5 mM each of CTP, ATP, GTP and UTP, 30 µg calf thymus DNA.
At time indicated below α-P^{32}-labelled nucleoside triphosphates were added. Incubations were conducted as follows :

I. The reaction was initiated at 37° by addition to the incubation mixture of 10 µg of purified RNA polymerase. 3 min later the labelled nucleoside triphosphate was added. The incubation lasted 12 min more at 37°.(Labelled nucleotide,0.5mM)
II. As I, but 1 min 30 sec after the initiation of the reaction 30 µg of F1+F2b histones were added.
III. As II, but 3 min after the initiation of the incubation 0.025 ml of saturated ammonium sulphate (A.S.) was added at the same time than the labelled nucleoside triphosphate.

"aggregate" enzyme at high ionic strength is also more DNA-like than the GC rich RNA synthesized at low ionic strength.

We have obtained essentially the same results as those reported here by using 10 fold purified liver soluble RNA polymerase and whole histones. At the same ionic strength, KCl had the same effect as ammonium sulphate. Therefore it seems that the differences in the effects of ammonium sulphate (or KCl) on soluble and "aggregate" RNA polymerases might be related to the state of the enzyme which is either free or bound to endogenous DNA in an enzyme-DNA-cRNA complex. Our results strongly suggest that the stimulating effect of ammonium sulphate on "aggregate" enzyme activity is the consequence of two phenomena having opposite effects : a decrease in the forces binding histones to DNA and an inhibition of the RNA polymerase which is already complexed with DNA and the newly synthesized cRNA, but cannot function before

histones are removed. In this respect it is interesting to note that the RNA synthesis catalyzed by E.coli polymerase bound to rat liver chromatin is more than 3 fold stimulated by incubating at high ionic strength (Doly et al., 1965).

Since we have observed that the activity measured at high ionic strength is proportional to the amount of enzyme present in the form of an enzyme-DNA-cRNA complex, it can be assumed that the activity of the nuclear "aggregate" enzyme measured in the presence of ammonium sulphate reflects only the amount of bound enzyme, while at low ionic strength the measured activity is also related to the amount of histones.

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