RNA POLYMERASE FROM MICROCOCCUS LUTEUS: COMPARATIVE EFFECT OF RIBOSYL AND DEOXYRIBOSYL OLIGOMERS ON THE HOMOPOLYMER - DIRECTED REACTION

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SUMMARY: The deoxyribosyl oligomer, $dA(pdA)_4$, was found to stimulate poly U-directed AMP incorporation by M. luteus RNA polymerase. The oligomer concentration required for saturation is about half the template concentration, suggesting that formation of a stoichiometric complex with poly U is involved in the mechanism of stimulation. Comparison of stimulation by ribosyl and deoxy- oligomers at 25° shows the ribosyl oligomer is far more effective. At 25°, only the ribosyl oligomer forms a complex with poly U. At 10°, where both oligomers form complexes with poly U, stimulation by both oligomers is comparable.

Ribosyl oligomers complementary to a homo- polynucleotide template stimulate the polymerization reaction catalyzed by both M. luteus (1,2) and E. coli (3,4) RNA polymerase. These oligomers can act as initiators of the product strand and presumably bypass the rate-limiting initiation step (2,3). An integral part of the mechanism of oligomer stimulation may be the ability to form a stoichiometric complex between the oligomer and the template (1,3,4). Thus, the ribosyl oligomers, $rA(prA)_{3-6}$, exert a maximum effect at concentrations and temperatures optimizing complex formation with the template (1,4).

Stoichiometric complex formation could aid product release as well as product initiation (5), however. Our previous work has indicated that under certain circumstances, release of the product may be rate-limiting (5-7). Therefore the effects of an oligomer which is unlikely to initiate a product strand have been examined. In this experiment, deoxyribosyl adenylic oligomer was compared with ribosyl adenylic oligomers for effects

on poly U-directed AMP incorporation. Our results indicate that at temperatures below the T_m of each oligomer:template complex, stimulation by ribosyl and deoxyribosyl oligomers is comparable.

METHODS AND MATERIALS

Poly U (6S) and the ribosyl oligomers $rA(prA)_4$ and $rA(prA)_5$ were purchased from Miles Laboratories. Molar extinction coefficients per nucleotide of 9.2×10^3 and 11.0×10^3 at 260 mμ were used to adjust solutions of poly U and the oligomers, respectively (5,8). Melting curves of polymer·oligomer complexes were performed with a Beckman DB Spectrophotometer equipped with a T_m Analyzer.

The deoxyribosyl oligonucleotide, $(dpA)_5$, was prepared by the polymerization of protected deoxyadenosine-5'-phosphate, similar to the general method of Narang *et al* (9). To remove the 5'-terminal phosphate, bacterial alkaline phosphatase (Worthington, BAPF) was incubated with $(pdA)_5$ as previously described (10). The reaction mixture was then chromatographed on paper (n-propanol:NH_4OH:water = 55:10:35) for 36 hours and the band corresponding to $dA(pdA)_4$ eluted with 0.01M Tris buffer, pH 7.5, and used for the enzyme studies reported here. Degradation of $dA(pdA)_4$ by spleen diesterase and snake venom diesterase (10) yielded pdA/dA ratios of 4 ($\pm$0.2). Solutions of the deoxyribosyl oligomer were adjusted using a molar extinction coefficient of 11.0×10^3 at 260 mμ.

The standard 1.0 ml reaction mixture for RNA polymerase activity (5) contained 100 μmoles of Tris buffer (pH 7.5), 2.5 μmoles of $MnCl_2$, 100 nmoles of poly U, and 400 nmoles of ^{3}H-ATP (Schwarz Bio Research; specific activity, 8.3 Ci/mole). Dephosphorylated ribosyl and deoxyribosyl oligomers were added as indicated. After a 20 minute preliminary incubation of these ingredients at either 10° or 25°, as indicated, 10 - 40 μg of highly purified *M. luteus* RNA polymerase (5) was added to start the reaction. Throughout the reaction, 0.1 ml aliquots

were removed, streaked onto 2 x 3 cm pads of Whatman No. 3 paper, washed batchwise, and counted in a Beckman Model 200B liquid scintillation counter as previously described (7).

RESULTS AND DISCUSSION

Our studies comparing ribosyl and deoxyribosyl oligomers have utilized $rA(prA)_5$ and $dA(pdA)_4$, the oligomers most readily available to us. Although these two oligomers are not identical in length, our results indicate little difference in the effect of $rA(prA)_4$ and $rA(prA)_5$. The T_m of each oligomer: poly U complex in a 1:1 mixture has been determined under simulated assay conditions (Fig. 1). The T_m of the complex[1] between $dA(pdA)_4$ and $r(U)_n$ is 22°, considerably below the 37° T_m of the complex between $rA(prA)_5$ and $r(U)_n$. If oligomer stimulation depends upon complex formation between the template and the oligomer, any effect of the deoxyribosyl oligomer on poly U-directed poly A synthesis should be maximum around 10° where most of the oligomer:template complex would be intact. By the same reasoning, $rA(prA)_5$ should be effective both at 10° and at higher temperatures.

At 25° (Fig. 2A), both $rA(prA)_4$ and $rA(prA)_5$ stimulate linear, poly U-directed AMP incorporation about 30-fold, in agreement with our previous results (1). The deoxyribosyl oligomer also stimulates linear AMP incorporation although the extent of stimulation is only about 3-fold, considerably less than that seen with the ribosyl oligomers. Since at 25° the $rA(prA)_5$·poly U complex is about 95% intact but the $dA(pdA)_4$·poly U complex is only about 30% intact (Fig. 1), the two oligomers were compared in the RNA polymerase assay at 10° where both oligomer:poly U complexes should be intact. Under these conditions, the deoxyribosyl oligomer can stimulate the reaction almost as effectively as the ribosyl oligomer (Fig. 2B). These

1. Although the stoichiometry of these complexes has not been determined under these conditions, it is most likely to be 1A:2U (8, 18, 19).

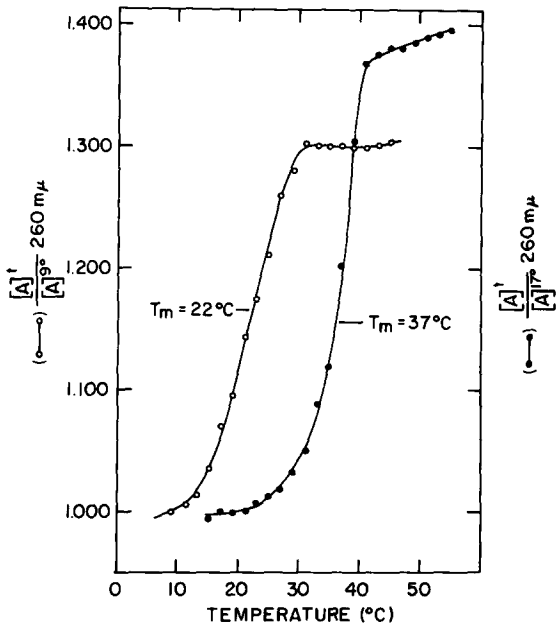

Fig. 1 Melting temperatures of oligo A·poly U complexes. Each melting mixture contained 50 nmoles/ml of poly U and 50 nmoles/ml of either $dA(pdA)_4$ (o——o) or $rA(prA)_5$ (●——●) in 0.1 M Tris buffer pH 7.5 and 1.5 x 10^{-3}M $MnCl_2$. Absorption at 260 mμ is given relative to the absorption at 9° or at 17° for the complexes with $dA(pdA)_4$ or $rA(prA)_5$, respectively. Since the stoichiometry of these complexes is likely to be 1A:2U, the mixtures contain excess amounts of oligo A.

studies support the previous suggestion (1,4) that complex formation between oligomer and template is an important aspect of the mechanism of oligomer stimulation. It should be noted that at 10°, the extent of stimulation by both oligomers (8 - 10 fold) is smaller than that exerted by the ribosyl oligomer at 25° (30 fold) but greater than that exerted by the deoxyribosyl oligomer at 25° (3 fold).

The importance of complex formation is also suggested by examining the oligomer concentration required for optimal stimulation (Fig. 3). As shown, stimulation by $dA(pdA)_4$ is maximum between 50 and 100 nmoles, corresponding to a 1:2 or a 1:1 complex between oligomer and template, as previously reported for $rA(prA)_4$ and $rA(prA)_6$ (1). Further, the profile of

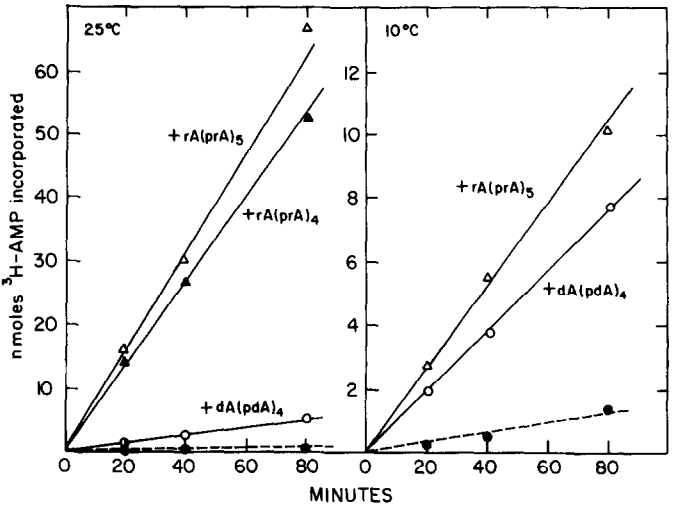

Fig. 2 Effect of ribosyl and deoxyribosyl adenine oligonucleotides on poly U-directed ^{3}H-AMP incorporation. The standard reaction mixture (see "Methods") was incubated either at 25° (A) or at 10° (B). Incorporation is shown in the absence of added oligonucleotides (●---●) and in the presence of 60 nmoles each of $rA(prA)_4$ (▲-▲), $rA(prA)_5$ (△—△), or $dA(pdA)_4$ (o——o).

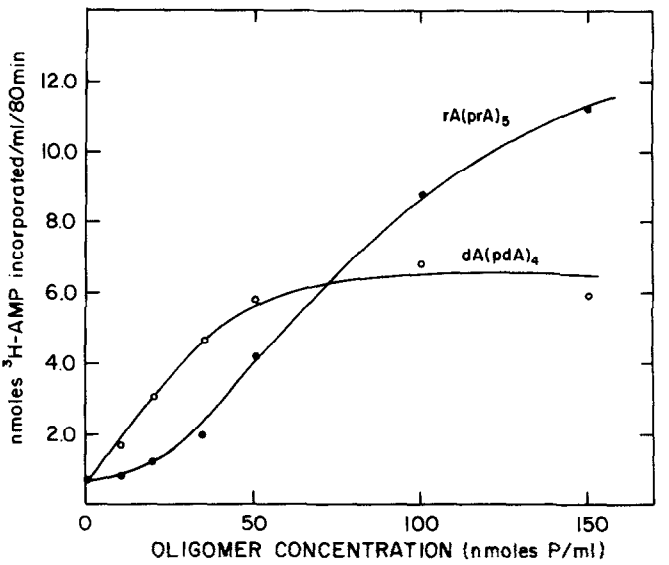

Fig. 3 Effect of concentration of adenine oligonucleotides on poly U (100 nmoles) -directed ^{3}H-AMP incorporation. To each standard reaction mixture (see "Methods") was added the indicated amount of either $dA(pdA)_4$ (o——o) or $rA(prA)_5$ (●——●). The temperature of the reaction was 10°C.

the $dA(pdA)_4$ concentration curve at 10° is quite similar to those of $rA(prA)_4$ and $rA(prA)_6$ conducted at 25° (1). The ribosyl oligomer, $rA(prA)_5$, however, shows an unexpected sigmoidal concentration dependence at 10°. As a result, the deoxyribosyl oligomer is more effective at low oligomer concentrations. The reason for the sigmoidal shape of the ribosyl oligomer concentration curve at 10° is unknown, but it is not due to decomposition or contamination as indicated by chromatographic analysis. These findings may reflect the fact that the degree of association for $dA(pdA)_4$·poly U at 10° is essentially the same (about 95%) as that for $rA(prA)_5$·poly U at 25°. At 10°, however, the degree of association or the stability of the $rA(prA)_5$·poly U complex is greater.

It may be concluded that deoxyribosyl oligomers as well as ribosyl oligomers can stimulate poly U-directed AMP incorporation. Further, the effectiveness of stimulation by either oligomer correlates with the extent of complex formation between oligomer and template, confirming the concept that the mechanism of oligomer stimulation depends on complex formation with the template (1,4). In the case of the ribosyl oligomers, however, there is evidence that they serve as initiators of the reaction product (3) bypassing the rate-limiting initiation step (2). In contrast, while deoxyribosyl oligomers effectively stimulate incorporation, there is no evidence that they initiate the reaction. That deoxyribosyl nucleotide triphosphates are not incorporated into the RNA product strand is, in fact, well established (13). Studies are currently under investigation in our laboratory to determine whether the deoxyribosyl oligomer stimulates by its action as an initiator or by some other mechanism. A possible alternative not involving initiation is that formation of an oligomer·template complex could enhance release of the product strand from the complex by preventing complex formation between the product and its single stranded template. Similar mechanisms, with product release as the rate-limiting step (i.e., translocation of the product from the active site

of the enzyme, thereby freeing that site for subsequent synthesis) can account for differences in kinetics between single and double stranded homopolymer templates (7).

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