THE EFFECT OF CHEMICAL MODIFICATION OF DNA ON ITS PRIMING ACTIVITY WITH RNA POLYMERASE[1]

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It is now well established that the process of mutagenesis involves a modification of DNA. Moreover, it has recently been proposed (Magee and Farber, 1962; Farber et al., 1962; Troll et al., 1963) that carcinogenic agents may act in a manner analogous to that of mutagenic agents. A need continues to exist, however, to find methods of great sensitivity which will detect DNA modification. The effect of a mutagenic agent and a carcinogenic agent on the Tm of DNA in vitro has recently been described (Troll et al., 1963). The present communication is concerned with the demonstration that the same agents, the phage mutagen hydroxylamine and the carcinogen 2-amino-1-naphthol, when allowed to react with DNA in vitro and removed from the DNA, modify the ability of such DNA preparations to act as primers in the RNA polymerase system. This test appears to be of greater sensitivity than the modification of the Tm.

Hydroxylamine and 2-amino-1-naphthol modified calf thymus DNA preparations after one day reaction were prepared as described

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(Troll et al., 1963). Heat denatured DNA was prepared by heating DNA solutions (100 μg/ml 0.01 M phosphate 0.001M citrate buffer pH 7.5) for 10 minutes and then cooling in an ice bath. The RNA polymerase (specific activity, 110) was prepared from Azotobacter vinelandii (Krakow and Ochoa, 1963). The modified test system consisted of 60.0 μg of calf thymus DNA primer, 0.05 ml of enzyme (0.0245 mg protein) in 0.007 M potassium phosphate, pH 6.8, containing bovine serum albumin (3.3 mg/ml), 0.5 μmoles each of ATP, GTP, UTP and CTP, 20.0 μmoles of Tris buffer pH 7.5, 5.0 μmoles of magnesium sulfate, 8.0 μmoles of mercaptoethylamine, 10.0 μmoles of putrescine, 5.0 μmoles of spermidine, and 0.125 μc of ATP-C^{14}. The total volume was 0.79 ml. For testing Poly A, Poly U or Poly C synthesis, only a single nucleotide was added e.g. UTP-C^{14} for Poly U. The reaction mixtures were incubated at 37°C for 30 minutes and the reaction was stopped by addition of 4 ml of 0.4 N perchloric acid, containing 0.02 M sodium pyrophosphate, and placed in an ice bath. The solutions were then filtered with suction through Millipore HA filters (24 mm diameter) to collect the acid precipitable polymers formed and washed once with the above perchloric acid mixture and once with 0.08 N perchloric acid containing 0.004 M pyrophosphate. The filter membranes were glued to planchettes, dried with a heating lamp, and the incorporated radioactivity determined in a gas flow container.

The results are summarized in Tables I and II. The most striking effect of the modified primer appears to be on the synthesis of RNA. The altered DNA's in both instances are poorer primers than heat denatured DNA. This is of particular interest in the case of the

TABLE I

Polymer Synthesis with Native Primer

Primer	Tm	RNA	Poly A	Poly U	Poly C
DNA	72	290.6	137.1	152.0	8.7
DNA+NH_2OH	72	22.4	70.7	110.6	20.8
DNA+ANOL	67	9.8	34.1	65.0	23.4

TABLE II

Polymer Synthesis with Heat Denatured Primer

Primer	RNA	Poly A	Poly U	Poly C
DNA	50.4	401.0	127.5	8.7
DNA+NH_2OH	18.2	230.6	117.1	24.2
DNA+ANOL	29.8	116.0	42.1	16.7

The values are given as mμM Nucleotide incorporated /30'/mg protein.

hydroxylamine modified material which, after one day reaction, has a Tm identical with that of native DNA, the first detectable change in Tm occurring after three days reaction. The effect of chemical modifications is clearly different from heat denaturation in the following aspects: 1) The inhibition of RNA synthesis is much greater with a primer modified chemically than with one modified by heat denaturation and 2) chemical modification causes a decrease in Poly A formation while heat denaturation causes an increase. The effects of a chemically modified primer on the synthesis of Poly A and Poly U are not as striking as those on RNA, but it is apparent

that the 2-amino-1-naphthol reacted DNA is a poorer primer than hydroxylamine reacted DNA in the synthesis of these two polymers.

The data obtained with heat denatured DNA is in agreement with observations reported in the literature using other RNA polymerase preparations. Thus it has been observed with E. coli RNA polymerase that thymus nucleic acid, heated to 100° for four minutes, results in a 50% decrease in the rate of RNA synthesis (Hurwitz and August, 1963). The increase in Poly A synthesis with a heated primer has been observed with this enzyme as well. Evidence has been presented that Poly A priming is caused by single-strands in the primer. It has been shown that phosphodiesterase, specific for single-stranded DNA, is capable of removing the priming effect for Poly A without loss of RNA priming, and that single-stranded DNA ϕX174 has high activity in priming for Poly A (Hurwitz and August, 1963). The mechanism of the chemical modification of DNA may be similar to the action of phosphodiesterase, namely that the chemical agents preferentially react with single-stranded polynucleotides.

The loss of single-strands, however, does not provide an explanation for the loss of RNA priming activity after chemical modification of DNA. Further studies are required to test whether this loss of priming activity is due to the alteration of one or more purine or pyrimidine bases or due to other modifications in the DNA molecule such as the formation of new covalent links observed with alkylating agents. (Geidushek, P., 1962).

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