A PROBABLE MECHANISM FOR APURINIC ACID INHIBITION OF DNA SYNTHESIS

N. Burr FURLONG, F. B. THOMAS and Carol G. MINTZ

Department of Biochemistry, The University of Texas System Cancer Center, M. D. Anderson Hospital & Tumor Institute, Houston, Texas 77025, USA

and

The University of Texas, Graduate School of Biomedical Sciences at Houston, Houston, Texas 77025, USA

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1. Introduction

Heike's [1] observation that apurinic acid (APA) inhibits DNA synthesis both in vivo and in vitro was investigated and confirmed by Müller et al. [2] who found that the inhibition of DNA polymerase from L5178Y (mouse lymphoma) cells was non-competitive with respect to DNA template concentration. This group also reported that the most effective fraction of APA on a molar basis had an average molecular weight of 19 500 while on a weight-to-volume basis a fraction of M.W. 2880 exerted the strongest inhibition. Further, an APA with reduced aldehyde groups was much less effective and the nucleotide, nucleoside and nucleobase decomposition products had no inhibitory effect.

The observations suggested to us that APA might inhibit DNA polymerase by interfering with the template–primer relationships which are thought to be necessary for enzyme activity [3, 4]. In confirming a similar study by Smellie [5], we have earlier shown [6] that the reaction catalyzed by a partially purified mammalian DNA polymerase requires both template and primer for maximal incorporation of precusor into DNA and that the reaction is stimulated only by primers (oligodeoxynucleotides) with a free 3' hydroxyl group; inhibition results if this group is blocked. Fragmented APA would be expected to possess many pyrimidine runs complementary to purine runs on a DNA template and, thus, might quite successfully anneal as a primer. However, almost all of the terminal 3' positions are occupied by phosphate or phosphate–carbohydrate remnants and are not available for addition of substrate.

If APA inhibition of DNA polymerase is the result of a 'blocked primer' mechanism, treatment of the APA with a phosphatase or diesterase or both should reduce or reverse the ability to inhibit. These expectations are, in fact, confirmed by the experiments to be described.

2. Methods

Apurinic acid was prepared, by the method of Tamm et al. [7], from 105 mg of calf thymus DNA (Sigma) and approximately 40 mg of APA were obtained in the final lyophilized product. Four solutions of 4 mg/ml APA were made up in 0.05 M Tris–HCl (pH 8.61 at 4°C0, 0.025 M $MgCl_2$ and 0.5 mg/ml bovine serum albumin (to facilitate the coprecipitation of nucleases). Bacterial alkaline phosphatase (35 μg/ml) and snake venom diesterase (50 μg/ml) were added to appropriate solutions. All solutions were incubated at 37°C for 15 min before being immersed in water at 95°C for 5 min. Precipitated proteins were removed by centrifugation.

Except for the substitutions of primers and templates detailed herein, the assays and preparation of

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DNA polymerase have been described previously [8]. Native calf thymus DNA was heated in the presence of the APA test solutions in assay buffer to 85°C and slowly cooled to room temperature to increase the likelihood of annealing the primer and template. The DNA and APA solutions were mixed in ratios of 25 mg to 10 mg and 25 mg to 50 mg, respectively, and the incorporation of $dCTP^{32}$ into DNA was assayed at 15 and 60 min.

3. Results and discussion

The extents to which the various APA solutions altered a standard DNA polymerase reaction are given in table 1. Each APA solution was also tested in an assay without template and no incorporation could be detected in any of these cases. Thus, APA or APA derivatives could not serve as both template and primer. The enzyme used in these assays was partially purified from the cytoplasmic fraction of Walker 256 carcinosarcoma tissue (rat). This enzyme is most active with a DNA template which has been partially degraded enzymatically, but significant incorporation also occurs with renatured DNA.

Our finding of approximately 30% inhibition when twice as much APA as DNA was present agrees well with the observations of Müller et al. [2]. Our results indicate that snake venom diesterase alone is ineffective in reversing the inhibition of APA. This enzyme attacks a variety of phosphodiesters, but its activity on polynucleotides is diminished by blocking groups on the 3′ position. Bacterial alkaline phosphatase is active on a wide variety of phosphate esters and treatment of APA with this enzyme quite obviously abolishes APA inhibition of DNA polymerase. The combination of enzymes appears to have a synergistic effect on reversing the inhibition of APA, for not only is the inhibition of the APA preparation reduced, but, at higher APA to DNA ratios, the reaction is actually enhanced particularly at the early time point. The stimulation produced by 3′-OH oligodeoxynucleotides in our earlier study was also found to be especially prominent in the initial phase of the synthetic reaction. Müller et al. [2], comment on an alkaline nuclease which they feel may be responsible for the 'recovery' of cell proliferation after APA administration. Our observations raise the possibility that a non-specific alkaline phosphate might also play a role in reversing inhibition in vivo.

Table 1
The effect of APA solutions on DNA polymerase

APA solution	Amount (mg)	Percent Inhibition 15′	60′
APA untreated	10	10.5	14.8
	50	18.6	18.6
SVD treated APA	10	15.0	22.4
	50	14.4	17.4
Phosphatase treated APA	10	3.7	0.1†
	50	−5.0*	−0.3*†
Combined treatment APA	10	6.1	9.4
	50	−14.6*	0.3†

The average enzymatic activity of duplicate assays with no addition of APA solutions was 255 ± 5 pmoles/hr/mg protein.

* Negative inhibition signifies stimulation.
† Non-significant variation.

The finding by the Mainz group that APA was non-competitive with respect to reversal by DNA is also compatible with our evidence that APA can not substitute as template. Thus, APA would only compete with endogenous primer probably present as a minor contaminant of the enzyme preparation and the ratio of primer to template would not be altered by adding more DNA solution.

On the basis of these considerations, then, we conclude that a likely mechanism for the APA interference with DNA synthesis is by competition with natural primer molecules with consequent blockage of chain elongation. Of course, this in no way diminishes the highly significant observation by the Mainz group that such molecules are taken up into living cells to produce cytostasis, for, if our mechanism is correct, it should be relatively easy to design an APA or polypyrimidine derivative with a group on the terminal 3′-position that is very resistant to enzymatic cleavage and thus produce a cytostatic agent of low toxicity, high specificity and long duration.

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