Vol. 110, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 27, 1983 Pages 443-448

DNase Inhibition by the Adenovirus DNA-Binding Protein Exhibits Specificity for the Enzyme but not for the Secondary Structure of the DNA

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Received December 7, 1982

The adenovirus-specific DNA-binding protein (DBP) has been shown to inhibit the hydrolysis of single-stranded DNA by a DNase isolated from KB cells, (Nass, K., and Frenkel, G.D. (1980). J. Virol. 35, 314-319). The specificity of the inhibition has now been investigated. The DBP inhibits the hydrolysis of single-stranded DNA by several different DNases (DNase II, KB DNase, S1 nuclease) under a variety of reaction conditions, but it has no effect on DNase I-catalyzed hydrolysis of single-stranded DNA. The DBP also inhibits the rate of hydrolysis of double-stranded DNA by KB DNase and DNase II, but has no effect on DNase I-catalyzed hydrolysis of this substrate. The DBP also inhibits the dephosphorylation of 5'-phosphoryl-terminated DNA by bacterial alkaline phosphatase but stimulates the phosphorylation of 5'-hydroxyl-terminated DNA by polynucleotide kinase.

Many proteins with specific affinity for DNA have been isolated from both procaryotic and eucaryotic cells (1,2). Among these "DNA-binding proteins" (DPBs) there are a number for which no specific enzymatic activity has been demonstrated, although they are known to be involved in DNA replication and/or genetic recombination (1,2). In some cases the binding of the protein to DNA may itself be the essential "activity" of the protein, serving such purposes as causing a conformational change in the DNA (1,2) or protecting it from degradation by DNase (3-6).

A eucaryotic DBP which has been the subject of extensive investigation is the adenovirus-coded protein (7). Studies on a mutant virus have shown that this protein is essential for viral DNA replication (7-10), but its precise role in this process has remained unclear. We have previously reported (11) that the DBP is able to inhibit the enzymatic hydrolysis of DNA by a DNase from KB cells (12). This "activity" can serve as an assay for the DBP, as well as a probe for various features of its binding to DNA. The inhibition

0006-291X/83/020443-06$01.50/0

may also be an indication of a function which the DBP may play in DNA replication--protection of the replicating viral DNA molecules (13). In this paper we report our finding that the inhibition of DNase by the DBP exhibits specificity relative to the enzyme but not to the secondary structure of the DNA substrate.

MATERIALS AND METHODS

Materials. The adenovirus DBP was isolated from Ad5-infected KB cells as previously described (11). DNases I and II were obtained from Worthington, S1 nuclease from Calbiochem. KB DNase, purified as described previously (11), is probably identical to the enzyme described by Wang et al. (12). Bacterial alkaline phosphatase and polynucleotide kinase were obtained from Bethesda Research Laboratories. [γ-^{32}P]ATP was purchased from New England Nuclear. Adenovirus [^{3}H]DNA was purchased from Bethesda Research Laboratories; DNA denaturation was carried out with alkali.

DNase Reactions. Hydrolysis of DNA was measured by the formation of acid-soluble nucleotide, as described previously (13). Incubations were for 30 min at 37°C, unless otherwise indicated. For DNase II the reaction mixture (0.3 ml) contained 67 mM sodium acetate buffer (pH 4.9), adenovirus [^{3}H]DNA (3.7 x 10^{4} cpm/nmole) and enzyme diluted in 90 mM sodium acetate buffer (pH 4.9) containing 10 mM EDTA and 0.5 mg bovine serum albumin per ml. For S1 nuclease the reaction mixture (0.3 ml) contained 67 mM sodium acetate buffer (pH 4.9), 50 mM NaCl, 2 mM $ZnSO_4$, 5% glycerol, denatured adenovirus [^{3}H]DNA, and enzyme diluted in 30 mM sodium acetate buffer (pH 4.9) containing 5% glycerol and 0.5 mg bovine serum albumin per ml. For DNase I the reaction mixture (0.3 ml) contained 67 mM Tris-HCl buffer (pH 8.1), 6.7 mM $MgCl_2$, adenovirus [^{3}H]DNA and enzyme diluted in 10 mM Tris-HCl buffer (pH 8.1), containing 20 mM NaCl. For KB DNase the reaction mixture was as described previously (11).

Polynucleotide Kinase Reaction. The reaction measures the transfer of the γ-phosphate group of [γ-^{32}P]ATP to the 5'-hydroxyl terminus of DNA (14). The reaction conditions and assay procedures were essentially as described by Richardson (14), except that 10% trichloroacetic acid was used in place of 0.7 N perchloric acid, and the 5'-hydroxyl-terminated DNA was prepared as described previously (15).

Alkaline Phosphatase Reaction. The reaction measures the release of radioactivity from DNA which has been labelled with ^{32}P at its 5' termini by treating 5'-hydroxyl-terminated DNA (see above) with polynucleotide kinase and [γ-^{32}P]ATP as described previously (15).

RESULTS AND DISCUSSION

In earlier work (11) we demonstrated that the DBP inhibits the rate of hydrolysis of single-stranded DNA by KB DNase. We have now found that the DBP is able to inhibit the hydrolysis of denatured DNA by some other DNases, such as DNase II and nuclease S1 but to different degrees (Table 1). Not all DNases are inhibited by the DBP however; the hydrolysis of single-stranded DNA by DNase I was unaffected by the DBP (Table 1).

Table 1. Effects of DBP on the hydrolysis of DNA by various nucleases[a]

	Single-stranded DNA			Double-stranded DNA		
	Hydrolysis (pmol)		Inhibition (%)	Hydrolysis (pmol)		Inhibition (%)
Enzyme	-DBP	+DBP		-DBP	+DBP	
KB DNase	66	2	97	41	1	98
DNase II	89	41	54	80	23	71
S1 nuclease	64	30	53	ND	ND	ND
DNase I	71	74	--	81	86	--

[a] Reactions were carried out with 1.1 nmol/ml DNA, in the absence or presence of 0.6 µg of DBP. ND: Not Done.

Although the DBP was originally reported to bind only to single-stranded DNA (16), a subsequent report described its binding to the termini of double-stranded DNA as well (17). We therefore investigated the ability of the DBP to inhibit the hydrolysis of double-stranded DNA. Figure 1 shows that the DBP is able to significantly decrease the rate of hydrolysis of double-stranded DNA by DNase II. The specificity of the DBP with this substrate appears to be similar to its specificity with single-stranded DNA. Thus, hydrolysis of double-stranded DNA by KB DNase and DNase II is inhibited by the DBP, but hydrolysis by DNase I is not (Table 1).

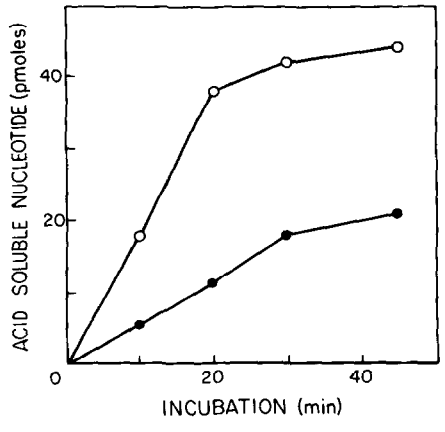

Fig. 1. Time course of hydrolysis of double-stranded DNA by DNase II. The reaction was carried out as described in Materials and Methods for the times indicated. DNA concentration was 1.7 nmol/ml. (O) No DBP; (O) 0.3 µg DBP.

Since DNase II cleaves DNA endonucleolytically (18), it is a priori difficult to see how binding of the DBP to the ends of double-stranded DNA molecules could affect cleavage by the DNase. However, the inhibition assay measures the decrease in the rate of formation of acid-soluble nucleotide, i.e. oligonucleotides of length 10 approximately. Cleavage of only a single phosphodiester bond is required to produce an acid soluble oligonucleotide from the DNA termini, whereas two bond cleavages are required for internal oligonucleotides. Thus, the product measured early in the reaction is likely to result primarily from cleavages occurring near the ends of the molecules, even for an endonuclease. Thus, the effect of the DBP could be to inhibit these terminal cleavages. The greater the percentage of the DNA molecule which is proximal to an end, the more the terminal hydrolysis should predominate in the reaction.

Binding of the DBP to the ends of double-stranded DNA might be expected to affect the activity of enzymes other than nucleases. We examined the effect of the DBP on the activity of bacterial alkaline phosphatase and polynucleotide kinase at DNA termini. The reactions measured were the removal of the phosphate from a 5'-phosphoryl-terminated DNA by the phosphatase (19), and the transfer of the γ-phosphate of ATP to a 5'-hydroxyl-terminated DNA by the kinase (14). The results (Table 2) showed that both reactions were in fact affected by the DBP, but that the phosphatase reaction was inhibited whereas the kinase reaction was stimulated.

DNase inhibition by the DBP does not appear to show specificity in terms of the secondary structure of the substrate DNA. The hydrolysis of single-

Table 2. Effect of DBP on alkaline phosphatase and polynucleotide kinase.

Enzyme	Product Formed (fmol) -DBP	+DBP[a]
alkaline phosphatase	1.3	0.7
polynucleotide kinase	4.2	15.1

[a] 1.2 µg

stranded and double-stranded DNA by a given DNase is equally inhibited by the DBP (Tables 1). There does however appear to be specificity in terms of the DNase. Thus, there is a quantitative difference in the degree of inhibition of KB DNase, S1 nuclease and DNase II. More significantly, DNase I is completely unaffected by the DBP, in its hydrolysis of both single-stranded and double-stranded DNA. This enzymatic specificity is also reflected in the differential effect of the DBP on two other enzymatic activities on DNA: inhibition of hydrolysis of 5'-phosphoryl groups by phosphatase and stimulation of phosphorylation of 5'-hydroxyl groups by kinase. This patterns of specificity raises the possibility of interaction of the DBP with the enzymes (3). An alternative possibility remains equally feasible however: that interaction of the DBP with the DNA produces an effect on the latter which affects various enzymes differently in their interaction with the substrate, and in fact affects some not at all. These differences could be due to structural or enzymatic differences between the enzymes. Further study of these different effects of the DBP on various enzymes acting on DNA may yield some insight into the details of the molecular interaction between the DBP and DNA.

Acknowledgements - This work was supported in part by grant CA28084 from the National Institutes of Health and NP-198 from the American Cancer Society.

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