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NOTES

Dextran Sulfate as a Contaminant of DNA Extracted from Concentrated Viruses and as an Inhibitor of DNA Polymerases

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Dextran sulfate is commonly used with polyethylene glycol to concentrate viruses before extraction of their DNA. However, dextran sulfate then easily contaminated such DNA and acted as a potent inhibitor of DNA polymerases from *Bacillus subtilis* (III), phage PBS2, and phage T4. Dextran sulfate only weakly inhibited *Micrococcus luteus* and *Escherichia coli* DNA polymerase I preparations.

A general method for the concentration of a wide variety of viruses from lysates involves the addition of polyethylene glycol and dextran sulfate and the collection after low-speed centrifugation of the virus particles at the interface of the polyethylene glycol-dextran sulfate two-phase system (1, 2). The viruses can then be suspended in buffer (2) or in 1% dextran sulfate (1), followed by addition of KCl, which precipitates most of the dextran sulfate and leaves the viruses in suspension. The overall increase in concentration is typically 100-fold in 80 to 100% yield (1, 2). Bacteriophages can then be purified further by CsCl step gradient centrifugation (2, 9). We have used these procedures effectively (13) to concentrate viable *Bacillus subtilis* phage PBS2, for use in infections and for extraction of its DNA (which uniquely contains uracil instead of thymine).

Dextran sulfate (sodium salt; Sigma Chemical Co., St. Louis, Mo.) is a glucose polymer with $\alpha_{1\rightarrow6}$ linkages and $\alpha_{1\rightarrow3}$ branch points (a *Lactobacillaceae* dextran with an average molecular weight of 500,000), which has been modified by chlorosulfonic acid to introduce about 2.2 sulfate groups per glucose residue (11). We now report that this anionic polymer is a contaminant of extracted PBS2 DNA prepared from phage concentrated as described above. Furthermore, this dextran sulfate is a potent inhibitor not only of the PBS2-induced DNA polymerase (13) but also of other DNA polymerases as well.

During purification of the PBS2 phage-induced DNA polymerase (R. A. Hitzeman and A. R. Price, Fed. Proc. **36**:847, 1977), we observed that PBS2 DNA (extracted from phage concentrated with dextran sulfate, as described above) could not be used as a template by the purified polymerase. However, when the PBS2 DNA was purified by equilibrium CsCl density gradient centrifugation, an inhibitor of polymerase activity was pelleted (apparent density greater than 1.8 g/ml), and the purified DNA then served as a good template.

The inhibitor which contaminated the above PBS2 DNA preparations was characterized by various physical, chemical, and enzymatic treatments. The quantitatively similar inhibition of polymerase activity per glucose residue, using the phenol-sulfuric acid colorimetric method (7), finally demonstrated that the inhibitor was dextran sulfate. About 0.04% of the original dextran sulfate (its original concentration [1, 13] was 1% in the viral suspension) was carried over into the DNA preparations, giving up to 15% dextran sulfate by weight in these preparations. Such contaminated DNA did not serve as a template for the purified PBS2 DNA polymerase; however, crude extracts of phage-infected cells were able to use these DNA preparations (13), because the extracts contained an enzyme which destroyed the inhibiting activity of dextran sulfate (Hitzeman, unpublished data). Furthermore, dextran sulfate contaminated the PBS2 DNA preparations made not only from phage purified as described above, but also from lysates of cells infected by these original dextran sulfate-contaminated phage. Only when plaque-purified phage were concentrated by polyethylene glycol without dextran sulfate (16) was the PBS2 DNA found to be free of inhibitor and to be a good template for all DNA polymerases tested.

Figure 1 shows the sensitivity to inhibition by dextran sulfate of purified PBS2- and T4 phage-induced DNA polymerases, of *Escherichia coli* and *Micrococcus luteus* DNA polymerase I preparations, and of purified *B. subtilis* DNA polymerase III. The DNA polymerase I preparations were relatively insensitive to dextran sulfate; it was necessary to add 18 μg of dextran sulfate per ml to get 40% inhibition (with 55 or 240 mM NaCl present). In contrast, the PBS2-induced DNA polymerase, the T4-induced DNA polymerase, and the *B. subtilis* DNA polymerase III were inhibited 50% by dextran sulfate at 0.28, 0.22, and 0.11 μg/ml, respectively, in 240 mM NaCl. When 55 mM NaCl was present, 50% inhibition by dextran sulfate was observed at 0.02, 0.004, and 0.05 μg/ml for the PBS2, T4, and *B. subtilis* enzymes, respectively. The greater sensitivity in low- versus high-salt concentrations suggests that dextran sulfate inhibition involves an ionic interaction with the polymerases.

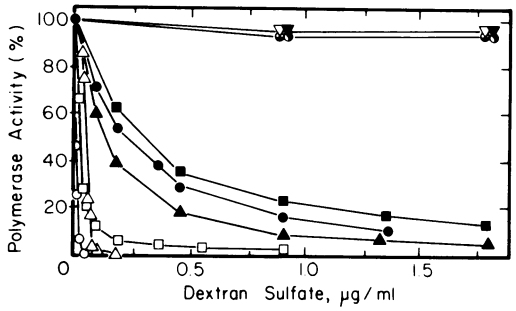

FIG. 1. *Inhibition by dextran sulfate of the activity of different DNA polymerases at two salt concentrations. About 0.3 μg of purified preparations of M. luteus DNA polymerase I (Miles Laboratories, Inc., Elkhart, Ind.; lot 31640), E. coli DNA polymerase I (Boehringer Mannheim Corp., New York; lot 15481), B. subtilis DNA polymerase III (Neal Brown), T4 phage-induced DNA polymerase (Nick Cozzarelli), or PBS2 phage-induced DNA polymerase (Hitzeman, manuscript in preparation) was added to each assay (total volume, 220 μl). Reaction mixtures contained 73 mM Tris-hydrochloride buffer (pH 8.5); 7 mM $MgCl_2$; 1 mM 2-mercaptoethanol; 0.1 mg of bovine serum albumin per ml; 2.3% glycerol; 37 μM each dGTP, dCTP, dTTP, and [8-^{3}H]dATP (8.2 μCi/ml); 225 μg of DNase-activated (to 6% acid solubility as described in reference 5) salmon sperm DNA per ml; and the indicated concentration of NaCl. Incubations were at 37°C, except for DNA polymerase III assays which were done at 30°C due to the lability of this enzyme at 37°C (4). Samples of 50 μl were removed at appropriate intervals to determine the initial rate of [^{3}H]dATP incorporation into acid-insoluble material, which was collected on paper disks for PBS2 polymerase assays (13) or on membrane filters for other polymerases (6). Rates per assay (100% activity) without added dextran sulfate were 190 and 340 pmol/min for the M. luteus enzyme in 55 (▽) and 240 (▼) mM NaCl, 660 and 230 pmol/min for the E. coli enzyme in 55 (◒) and 240 (◑) mM NaCl, 70 and 3.9 pmol/min for the B. subtilis enzyme in 55 (△) and 240 (▲) mM NaCl, 13 and 620 pmol/min for the PBS2 enzyme in 55 (□) and 240 (■) mM NaCl, and 22 and 36 pmol/min for the T4 enzyme in 55 (○) and 240 (●) mM NaCl.*

Figure 2 shows that dextran sulfate behaves kinetically as a competitive inhibitor with respect to denatured PBS2 DNA as a template-primer for the PBS2 phage-induced DNA polymerase. Under near-optimal conditions for this enzyme (7 mM $MgCl_2$ and 240 mM NaCl), the apparent K_m for denatured PBS2 DNA was 35 μg/ml. The K_i for dextran sulfate was 0.01 μg/ml, suggesting that binding of dextran sulfate may be 3,500-fold tighter than binding of DNA (on a weight basis). At the lower ionic strength (55 mM NaCl) used in Fig. 1, the PBS2 DNA polymerase was inhibited 50% by dextran sulfate at 0.02 μg/ml in the presence of 11,000 times that concentration of DNase-activated salmon sperm DNA as template. Thus, traces of dextran sulfate contaminating phage DNA can readily in-

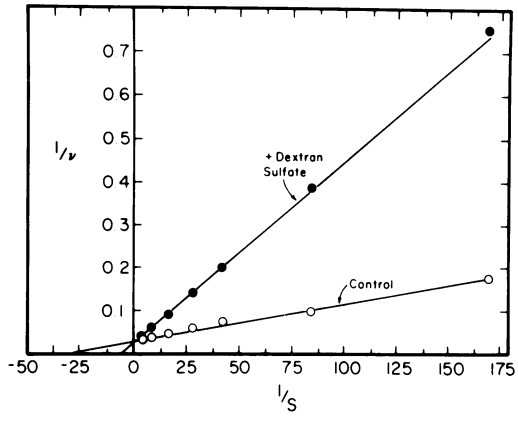

FIG. 2. *Double-reciprocal plot showing competitive inhibition by dextran sulfate of the PBS2 phage-induced DNA polymerase with respect to the concentration of denatured PBS2 DNA. Purified PBS2 DNA polymerase (10 μl of 11 μg/ml) was incubated as described in the legend to Fig. 1 with 240 mM NaCl and the indicated concentration of single-stranded PBS2 DNA (free of dextran sulfate contamination and with an average molecular weight of 6,000,000; S is given in units of milligrams per milliliter) in the absence (○) or presence (●) of dextran sulfate at 45 ng/ml. Samples of 50 μl were taken at intervals into tubes containing 5 μl of EDTA (0.25 M) and 10 μl of salmon sperm DNA (2 mg/ml) as carrier, followed by processing on paper disks (13) to determine the initial rate of ^{3}H incorporation (v, in units of nanomoles per minute per milliliter of enzyme) from [8-^{3}H]dATP (37 μM, 16.4 μCi/ml).*

activate it as a template-primer for DNA polymerase, explaining our results reported above for the early PBS2 DNA preparations.

Dextran sulfate has also been shown to be effective as an inhibitor of RNase (12), as a binding agent competitive against polyuridylic acid as mRNA for *E. coli* ribosomes (10), as an inhibitor of tRNA binding to ribosomes (14), as an inhibitor of poliovirus adsorption to kidney cells (3), and as a noncompetitive inhibitor (K_i = 0.05 μg/ml) versus DNA or ATP for T4 phage-induced polynucleotide kinase activity (15). In addition, our dextran sulfate-contaminated PBS2 DNA preparation inhibited *B. subtilis* RNA polymerase (J. Pero, personal communication). Therefore, it would seem advisable to avoid the dextran sulfate commonly used in concentrating viruses for nucleic acid extraction (1, 2) and instead to use polyethylene glycol alone (16). However, the potent inhibition seen here with dextran sulfate can be a useful tool in differentiating various DNA polymerases (Fig. 1) and their associated exonuclease activities (Hitzeman, manuscript in preparation). It might also be possible to couple dextran sulfate to a resin to provide a tight-binding affinity column for purification of some DNA polymerases.

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