

TECHNICAL COMMENTS

Polypropylene Tube Surfaces May Induce Denaturation and Multimerization of DNA

Claire Gaillard and François Strauss (1) found that DNA fragments containing the sequence $(CA)_{30}$ spontaneously associated in a series of products of reduced mobility, and that these products bond to the nuclear nonhistone high mobility group (HMG) proteins 1 and 2. We have obtained evidence strongly suggesting that this association is mediated by partial denaturation of the DNA resulting from interaction with certain polypropylene tube surfaces. This phenomenon has implications for the possible role of surfaces (including cellular surfaces) in affecting the conformation of DNA and should be taken into consideration by investigators conducting experiments that are sensitive to low levels of denaturation.

We incubated a ^{32}P -labeled 124-bp fragment of DNA containing the sequence $(CA)_{30}-(GT)_{30}$ (fCA30) (2) in ordinary polypropylene (pp) microfuge tubes (3) and produced a ladder of apparently multimeric complexes as observed by Gaillard and Strauss; however, no ladder was seen when the incubation was carried out in borosilicate glass tubes (3) or in pp tubes that have been commercially coated to have low-binding properties ("lube" tubes; Marsh Biomedical Products, Rochester, New York). Moreover, the association was inhibited when an excess amount of a single-stranded oligonucleotide (lacking any significant homology to the DNA fragment) was present in the solution before adding the fragment.

To determine whether the association actually required the presence of a pp surface, as opposed to its being a solution phenomenon that was simply inhibited by contaminants released from the glass and lube tube surfaces, we preincubated fCA30 in a glass tube for several hours, then transferred the

DNA to a pp tube and continued the incubation for another several hours. If the glass emitted some diffusible inhibitor of the reaction, there should have been no association after transferring to the pp tube. However, association did in fact occur after transfer, indicating that the pp surface was required. Because single-stranded DNA binds more tightly to many surfaces than does double-stranded DNA, it is likely that the inhibitory effect of the oligonucleotide results from its binding to putative sites on the pp surface that mediate the association. Similar experiments involving transfer from lube tubes into pp tubes did not result in association, indicating that a diffusible inhibitor was present in the lube tubes. It is likely that, on transferring the solution, this inhibitor also prevents association by binding to active sites on the pp surface. Thus, it is important to avoid the use of lube tubes for manipulating or storing DNA in order to observe this phenomenon. To minimize background levels of denaturation and complexation while using pp tubes, it is best to store DNA samples in the frozen state and avoid small volumes that increase the ratio of pp surface to sample volume.

Parallel experiments were performed using a 62-bp control fragment (fC) lacking any long repeats and another, approximately 120-bp fragment (fGA37) (4) containing an internal $(GA)_{37}$ repeat instead of a CA repeat. Multimerization was observed with fGA37, but not with fC.

When various surfaces and inhibitors were used, the same conditions that led to multimerization of fCA30 and fGA37 also led to the appearance, in the case of fC and fGA37, of a faster migrating fraction that comigrated with the denatured fragment. (In the case of fCA30, the denatured fragment

was poorly resolved from the duplex under our conditions.) When fCA30 was deliberately subjected to alkaline denaturation followed by renaturation (performed in lube tubes, or in the presence of an excess of oligonucleotide to disallow the pp-dependent association, or in both) the resulting pattern of products was similar to that seen for spontaneous association in pp tubes.

The simplest explanation of these results is that the formation of slower migrating products is mediated by partial or complete denaturation followed by misaligned annealing at the repetitive sequence. Further intermolecular pairing of single-stranded ends could lead to multimeric branched structures, which would be expected to bind HMG1 and HMG2, as observed by Gaillard and Strauss, because those proteins are known to bind to branched or distorted DNAs (5).

Because we observed little or no denaturation or multimerization using tubes from one supplier (3), it may be that minor constituents of the plastic that vary with manufacturing procedures contribute to the surface activity. A fuller account and discussion of these results will be presented elsewhere (6).

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REFERENCES AND NOTES

1. C. Gaillard and F. Strauss, *Science* **264**, 433 (1994)
2. Fragment fCA30 was cut from a plasmid kindly provided by F. Strauss.

3. Ordinary polypropylene tubes that demonstrated surface activity were 0.5 and 1.5 ml (non-"lube") microfuge tubes obtained from Marsh Biomedical Products (Rochester, NY) and from E&K Scientific Products (Campbell, CA). Similar tubes from United Scientific Products (San Leandro, CA) showed little or no denaturation or multimerization. Borosilicate glass tubes (10 mm × 75 mm) were obtained from Baxter (Deerfield, IL). In a typical reaction, ³²P-labeled DNA fragments (~10 nM) in 9- μ l volumes containing 0.85 to 1.24 M NaCl, 6.5 mM tris-HCl (pH 7.45), and 0.65 mM EDTA were incubated at 37°C for 16 to 22 hours, then analyzed on polyacrylamide gels.
4. Fragment fC was the 62-bp Hind III/Xba I fragment from pBSISK⁺ (Stratagene), having the sequence AGAGGGAATTCTCCTT inserted at the Sma I site. The fragment fGA37 was the Eae I/Hind III fragment from pGA37, a derivative of pUC9, bearing a (GA)₃₇ insert in the EcoRI site, approximately 120 bp in length. This plasmid, kindly provided by R. D. Wells [D. A. Collier and R. D. Wells, *J. Biol. Chem.* **265**, 10652 (1988)], was derived from a plasmid originally constructed by T. Evans.
5. P. M. Pil and S. J. Lippard, *Science* **256**, 234 (1992); M. E. Bianchi, M. Beltrame, G. Paonessa, *ibid.* **243**, 1056 (1989); M. E. Bianchi *et al.*, *EMBO J.* **11**, 1055 (1992); E. N. Hughes, B. N. Engelsberg, P. C. Billings, *J. Biol. Chem.* **267**, 13520 (1992).
6. B. P. Belotserkovskii and B. H. Johnston, in *Biological Structure and Dynamics*, R. H. Sarma and M. H. Sarma, Eds. (Adenine, Schenectedy, NY), in press; _____, in preparation.

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Response: Belotserkovskii and Johnston suggest that the phenomenon which we described in our report is catalyzed by the surface of polypropylene tubes. This is an interesting observation, which we have also seen, independently, since the publication of our report, and with which we agree entirely. We plan to describe our results on this point (1).

Belotserkovskii and Johnston also suggest that the interaction of DNA with polypropylene induces a denaturation, and that this denaturation is responsible for the formation of multistranded complexes. Their results seem to be in agreement with this suggestion, but do not prove it unambiguously. It seems likely that the interaction with polypropylene induces a change of conformation of DNA (1), but is this a denaturation in the classical sense? We have performed denaturation-reassociation experiments similar to theirs, but we did not obtain the same result: Under conditions where interaction of DNA with polypropylene was inhibited, the formation of multistranded complexes was hardly detectable in our hands.

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