Large scale preparation of positively supercoiled DNA using the archaeal histone HMf

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ABSTRACT

A technique to prepare relatively large quantities (≥100 µg) of highly positively supercoiled DNA is reported. This uses a recombinant archaeal histone (rHMfB) to introduce toroidal supercoils, and an inexpensive chicken blood extract to relax unrestrained superhelical tension. Preparation of positively supercoiled pUC19 DNA molecules, >50% of which have linking number changes ranging from +8 to +17, is demonstrated. Advantages include the high degree of positive supercoiling that can be achieved, control over the extent of supercoiling, easy production of relatively large quantities of supercoiled DNA, and low cost.

There is growing interest in the biological roles of superhelical tension, given the evidence for its presence in both prokaryotic and eukaryotic cells (1–3) and its apparent involvement in diverse cellular processes (4–10). Superhelical tension also alters the structure and dynamics of DNA in ways that may affect the selection of targets by genotoxic chemicals such as DNA intercalators (2,11–13). The full range of superhelical tension has not been experimentally accessible as methods to prepare positively supercoiled DNA are limited; therefore, to help solve this problem, we have developed a straightforward and inexpensive method that generates preparations of circular DNAs with high levels of positive supercoiling.

The method exploits the observation of Musgrave et al. (14) that the archaeal histones from Methanothermus fervidus (designated HfM) form nucleosome-like structures in which the DNA molecule is wrapped around the protein core in a right-handed superhelix. HfM binding to a circular DNA molecule, followed by relaxation of unrestrained supercoils and removal of all proteins, results in a DNA molecule that is positively supercoiled. In contrast, exposing circular DNAs that contain eukaryal nucleosomes (15) to this procedure results in negatively supercoiled DNA molecules. Critical reagents are a clean preparation of circular DNA, purified recombinant HfMB (rHMfB; 16) and a topoisomerase-containing extract obtained from chicken erythrocytes. The technique works equally well with commercially prepared topoisomerase I and the empirically determined quantities of wheat germ topoisomerase I from Promega (Madison, WI) are noted throughout the following procedure as an example. Furthermore, the results shown here to illustrate the procedure were obtained using plasmid pUC19 DNA, isolated from lysates of Escherichia coli DH5α, using Qiagen Gigaprep kits (Qiagen, Santa Clarita, CA). Another commercial plasmid isolation kit (Wizard Megaprep, Promega; Madison, WI) was evaluated but the plasmid preparations obtained apparently contained materials that interfered with the topoisomerase I activity (data not shown).

Preparation of rHMfB. rHMfB preparations were purified from E.coli IM105 cells that contained pKS323, an expression plasmid that carries the hmfB gene, exactly as described by Starich et al. (17). [The pKS323 plasmid can be obtained from Dr Kathleen Sandman (ksandman@postbox.acs.ohio-state.edu).]

Preparation of chicken blood extract (CBE) that relaxes superhelical tension. As a less expensive alternative to purchasing purified topoisomerase I, the procedure of Camerini-Otero and Felsenfeld (18) was used to prepare a topoisomerase-containing extract from chicken erythrocytes, here referred to as chicken blood extract (CBE). These preparations contained no detectable endonuclease or protease activities, and retained activity for at least 6 months when stored at –80°C.

Production of positively supercoiled pUC19 DNA. Negatively supercoiled pUC19 molecules isolated from E.coli were relaxed by incubation at 25 µg/ml with CBE in 200 mM NaCl, 20 mM Tris–HCl (pH 8), 0.25 mM EDTA–Na2 and 5% glycerol for 1 h at 37°C. The CBE:DNA ratio must be determined empirically for each batch of CBE; the present studies were performed with 0.5 µl CBE/µg pUC19 or 4 U wheat germ topoisomerase I/µg pUC19. The relaxed molecules were purified by exposure to proteinase K (100 µg/ml) for 2 h at 37°C in the presence of 2% SDS, phenol/chloroform extraction, ethanol precipitation and passage through a Sephadex G-50 spin column. The purified plasmid (25 µg/ml) was then complexed with 1.2–1.4 mass equivalents of rHMfB by incubation in 10 mM Tris–HCl (pH 8), 2 mM K3PO4, 1 mM EDTA–Na2 and 50 mM NaCl for 15 min at 37°C. To remove unrestrained negative supercoils, the reaction conditions were adjusted by the addition of 1/3 vol of 186 mM Tris–HCl (pH 8), 3 mM K3PO4, 8 mM EDTA–Na2 and 72 mM NaCl, followed by addition of 1/10 vol (adjusted) of CBE and incubation for 45 min at 37°C. Again, the CBE:DNA ratio must be determined empirically; the present studies were performed
with 6 µl CBE/µg pUC19 or 12 U wheat germ topoisomerase I/µg pUC19. As a control, a population of negatively supercoiled pUC19 molecules was subjected to the same treatments except that CBE dilution and storage buffers were added instead of CBE. Plasmid DNAs were quantified using the Hoechst dye 33258 (Sigma, St Louis, MO) fluorescence assay (19).

**Results with pUC19.** An example of the positive supercoiling achieved with pUC19 is shown in Figure 1. Maximal supercoiling was obtained with a rHMIB:DNA mass ratio of 1:1.4 and, although the highest resolvable topoisomer had a change in linking number (ΔLk) of +11 (Fig. 1B), >30% of the DNA molecules had electrophoretic mobilities consistent with even higher ΔLk, with the maximum ΔLk estimated to be +17.

This technique is not limited by the size of the DNA substrate, it allows the extent of positive supercoiling to be controlled, and it generates substantial quantities of positively supercoiled product. We routinely prepare >100 µg of positively supercoiled pUC19 as a single batch, and multiple batches can be prepared concurrently. Positive supercoiling has been introduced into plasmids and cosmids with a wide range of different sizes, including large plasmids such as the 94.5 kb F-factor from E. coli (data not shown). The extent of positive supercoiling is determined by the ratio of rHMIB to DNA (14) and, by optimizing this ratio, substantial quantities of a plasmid DNA with a specific superhelical configuration can be obtained by band excision and extraction from a one-dimensional agarose gel.

The use of rHMIB offers several advantages for the preparation of positively supercoiled DNA. Intercalators such as ethidium bromide (13) do not generate the high levels of positive supercoiling achieved with rHMIB and, although high levels of superhelical tension can be obtained by using reverse gyrase (20,21), the purification of this enzyme is complicated and lengthy in comparison with the simple rHMIB purification procedure (17). Approximately 50 mg of rHMIB can be purified from a 10 l culture of E. coli and this is sufficient protein to prepare ~30 mg of positively supercoiled pUC19. Using the CBE rather than purchasing purified topoisomerase I results in a very substantial reduction in cost: ~120 ml of CBE can be obtained from 250 ml of chicken blood ($100–200) in <1 day that, when used in this procedure, is functionally equivalent to ~240,000 U of commercial topoisomerase I. It should be noted, however, that we have obtained identical results using commercially available topoisomerase I from TopoGEN Inc. (human; Columbus, OH), Promega (wheat germ; Madison, WI) and Gibco-BRL (calf thymus; Grand Island, NY); other sources are likely to give similar results. As mentioned earlier, there is potential for inhibition of topoisomerase I by DNA contaminants introduced during DNA isolation.

Circular DNAs, with the high and defined levels of positive supercoiling generated by this procedure, should now be very valuable in evaluating the role of this superhelical tension in DNA interactions with small chemical ligands, and also with both sequence specific and non-specific DNA-binding proteins.

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