Short Communication

Preparation of Oriented Ca- and Mg-DNA by Means of the Wet Spinning Method

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The wet spinning method for DNA was developed to allow the preparation of samples of oriented DNA and DNA-ligand complexes for a variety of physicochemical procedures.[1–4] The work was carried out at the MRC Group for Bacteriological Bioengineering,[5,6] where physicochemical studies of oriented DNA were also initiated,[4,6] partly in cooperation with specialists at other laboratories. These and subsequent studies in the fields of NMR spectroscopy,[7,8] ESR spectroscopy,[9] polarized optical spectroscopy,[10] Brillouin,[11] Raman[12] and neutron[13] scattering, electrical conductivity,[6,14] mechanochemistry[15] and X-ray diffraction[16,17] have given information about such fundamental properties of DNA as its hydration, conformation, helix-coil transition, electrical conductivity and its interaction with radiation, alkali-metal ions and drugs (the references given serve only as examples taken from a large number of papers).

As suggested earlier[4] it should be of interest to prepare and study samples of oriented DNA with counterions other than the alkali metals, such as alkaline-earth and transition-metal ions.[18] However, attempts to wet-spin Ca- and Mg-DNA (alkaline-earth-metal counterions), obtained from calf-thymus NaDNA by dialysis, have not been successful. The material had a tendency to gel when the DNA solution was extruded through the spinneret and came into contact with the ethyl alcohol of the spinning bath. This is probably a result of the greater tendency of DNA with divalent counterions to aggregate.[19] The purpose of the present communication is to highlight a modification of the wet spinning method, based on the exchange of the Na counterions of spun NaDNA against Ca or Mg ions, which can be used successfully in the preparation of oriented Ca- and Mg-DNA.

Experimental

The approach used here was first to spin the DNA (calf-thymus DNA from Pharmacia) as NaDNA, as described in Ref. 2 or 16, and thereafter to exchange the Na ions of the spun deposit against the divalent counterions by bathing the cylinder with the spun NaDNA for three days in 75 % (v/v) ethyl alcohol containing $CaCl_2$ or $MgCl_2$ (0.3–0.5 M). This procedure was inspired by an earlier work on the wet spinning method[16] where the Na ions of wet-spun NaDNA were exchanged for Li ions by bathing the cylinder with spun NaDNA deposit in a solution of ethyl alcohol containing 1 M LiCl. This bathing procedure is expected to give an even more effective exchange in the present case of divalent counterions. To obtain the Ca- and Mg-DNA with an excess of salt ($CaCl_2$ and $MgCl_2$, respectively), a final bath of 75% ethyl alcohol with 0.25–0.3 M salt was used. In the drying procedure,[2,16] finally, the spun deposits merged into films of oriented Ca- and Mg-DNA.

X-Ray diffraction patterns were taken at 75 % relative humidity from folded concertina-like packs of the DNA films (total thickness 0.3 mm) mounted in holders described earlier.[16] Nickel-filtered Cu K_α radiation and a collimator of 500 μm were used. The exposure time was about 3 h.

Results and discussion

The modification of the wet spinning method described above gave films of oriented Ca- and Mg-DNA which, due to the presence of excess salt, were both in the crystalline B form, as may be seen from Figs. 1 and 2 (cf. the crystalline B form patterns of wet-spun oriented LiDNA in Refs. 2, 16 and 17). The patterns show both good orientation and crystallinity. The occurrence of the B form is in agreement with earlier X-ray diffraction work on fibers of CaDNA[20]

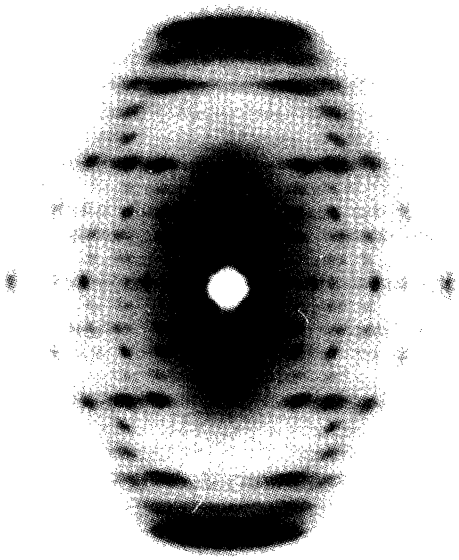

Fig. 1. X-Ray diffraction pattern from wet-spun, oriented CaDNA at 75 % relative humidity.

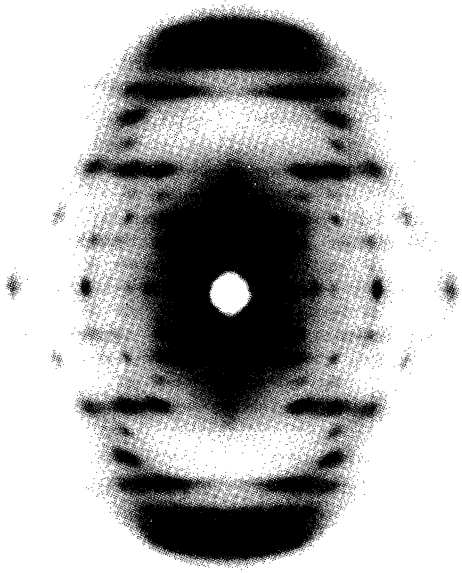

Fig. 2. X-Ray diffraction pattern from wet-spun, oriented MgDNA at 75 % relative humidity.

and MgDNA[21] prepared by pulling the fibers from a gel. It should be pointed out that without exchange of the counterions, the NaDNA would have occurred in the A form.[2,16,17]

Attempts were also made to use bathing solutions of ethyl alcohol with chlorides of Ba and Sr (alkaline-earth metals), and Cd, Cu and Mn (transition metals), but of these only Cd-, Sr- and Mn-DNA gave useful X-ray diffraction patterns, which, in spite of their low quality, could be interpreted as showing the B form (Ba could not be used for these bathing experiments owing to the low solubility of $BaCl_2$ in solutions of ethyl alcohol). Since it could be suspected that part of the failure with these salts would be due to a low pH, leading to a partial denaturation of the DNA, attempts were made to titrate with NaOH or ammonia. However, (colored) precipitates were mostly formed and during this process the pH returned to its original value. Although the titration was repeated several times with the same solution it was not possible to adjust the pH favorably. Thus the improvement brought about by this titration procedure was poor, except perhaps in the case of SrDNA since $SrCl_2$ did not form any precipitate. However, the quality of the X-ray diffraction patterns obtained was much lower than for those displayed in Figs. 1 and 2.

It has been shown that high quality films of oriented Ca- and Mg-DNA can be prepared with a modification of the wet spinning method involving a bathing procedure. As described in Refs. 3 and 10, oriented DNA samples of practically any dimensions can be prepared (films, parallel-epipedic samples, ribbons) which can be studied with a variety of physicochemical methods, as indicated in the introduction. We are thus engaged in studies of wet-spun oriented Ca- and Mg-DNA involving NMR spectroscopy, neutron scattering, polarized optical spectroscopy and mechanochemistry to elucidate the effects of these divalent alkaline-earth-metal counterions on various properties of DNA. Polyelectrolyte theory[22] will be applied to correlate the results with those obtained for DNA with the monovalent alkali-metal counterions.

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