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Crystallization of a large single crystal of a B-DNA decamer for a neutron diffraction experiment by the phase-diagram technique

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Crystallization of a large single crystal of a B-DNA decamer, d(CCATTAATGG), for a neutron-diffraction experiment has been accomplished by an analysis of its solubility phase diagram and a large single crystal was successfully crystallized at around the minimum solubility point of the oligonucleotide: 30%(*v*/*v*) MPD, 100 m*M* $MgCl_2$ pD 6.6 using 0.4 ml D_2O solutions of the DNA (sample concentration 1.5 m*M*). It is confirmed that the resulting crystal (dimensions: 1.7 × 1.3 × 0.6 mm) diffracts sufficiently well for neutron data collection.

1. Introduction

Water has long been recognized as an important determinant of nucleic acid structure; the stability (Record *et al.*, 1981), polymorphism (Leslie *et al.*, 1980) and flexibility (Levitt, 1982; Yanagida *et al.*, 1982) of the DNA double helix are all hydration-dependent. Moreover, specific hydration patterns play a role in nucleotide-sequence recognition by proteins and drugs (Otwinowski *et al.*, 1988). The most prominent feature of the hydration of B-DNA duplexes is a chain of water molecules penetrating deeply into the minor groove. This spine of hydration was first observed in X-ray crystallographic analysis of the B-DNA duplex of the self-complementary dodecamer (Shui *et al.*, 1998; Drew & Dickerson, 1981). In these studies, the observed hydration structure was obtained from the network of only the O atoms of the water molecules, as it is difficult to identify the H atoms in an X-ray crystallographic analysis. In order to more fully understand the role of water molecules in nucleotide-sequence recognition by proteins, the location of the H atoms in this network structure of water molecules is important.

Recently, the high-resolution neutron diffraction method has been established to locate H-atom positions in proteins unambiguously (Niimura *et al.*, 1997; Niimura, 1999). Neutron fibre-diffraction studies of DNA have been reported and the hydration structure has been described (Shotton *et al.*, 1997), but the resolution of those studies was limited to about 3 Å and the information on base-pairing specificity is either lacking or very limited. High-resolution neutron diffraction from a single-crystal experiment is indispensable in order to determine the precise network structure of hydration in the minor and major grooves. In order to carry out such a high-resolution neutron experiment, a large single crystal of volume in excess of 1 mm^3 is necessary.

2. Materials and methods

The decamer d(CCATTAATGG) was selected as a sample because the vapour-diffusion crystallization conditions for standing drops of this oligonucleotide have already been reported (Goodsell *et al.*, 1994). In order to obtain a large single crystal suitable for a high-resolution neutron-diffraction experiment, it was necessary to first obtain a phase diagram of this decamer d(CCATTAATGG). So far, there have only been reports of the phase diagrams of some $d(pGpT)_n{\cdot}(pApC)_n$, n = 2, 3, 4 oligonucleotides (Malinina *et al.*, 1987) and the Z-DNA hexanucleotide d(CGCGCG) (Malinina *et al.*, 1991; Minasov *et al.*, 1992; Ho *et al.*, 1991); there have not been any reports of phase diagrams for B-DNA, nor of the existence of large single crystals suitable for neutron diffraction experiments.

The DNA decamer was synthesized with a DNA synthesizer. The purity of the DNA was analyzed with HPLC and impurities arising from other macromolecules or species were less than 3.9%. Salt impurities were removed by dialysis and the final electrical conductivity of the outer solution of the dialysis was 2.1 μS cm^{-1}, corresponding to a concentration of 0.08 m*M* NaCl, which was negligibly small compared with the precipitant concentration used in the crystallization experiments.

Firstly, a preliminary phase diagram was produced by the vapour-diffusion method using the method reported by Goodsell *et al.* (1994). The final precise phase diagram was

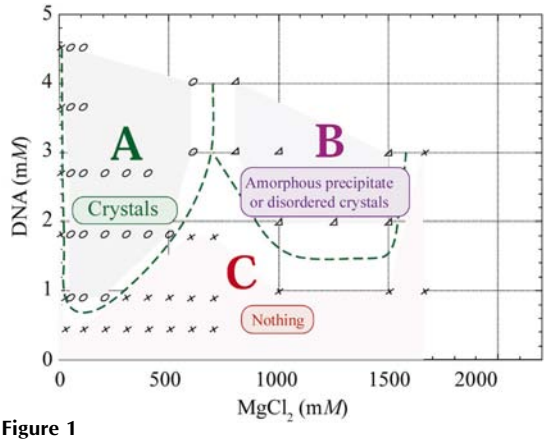

Figure 1
The experimentally determined phase diagram of the solubility of the DNA decamer d(CCATTAATGG) with $MgCl_2$ concentration in H_2O. The broken lines show the boundary between regions: circles, triangles and crosses in the phase diagram correspond to the presence of crystals, amorphous precipitate and clear solutions (*i.e.* no crystals), respectively. Solutions were kept in an incubator at 279 K for 20 d with an MPD concentration of 30%(*v*/*v*) and pH of 7.0 (buffer solution of 0.1 *M* sodium cacodylate). (The melting of a crystal has not been attempted. The true solubility curve might be shifted downwards slightly.)

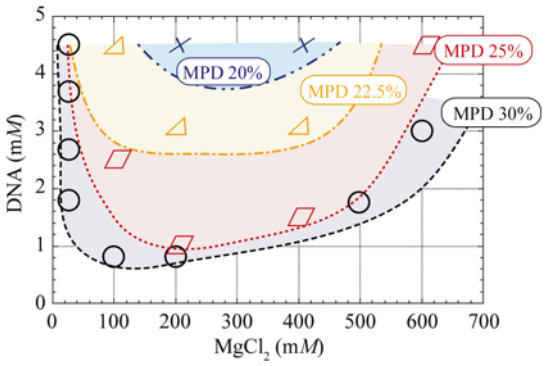

Figure 2
The phase diagram of DNA solubility *versus* $MgCl_2$ concentration at various MPD concentrations at a fixed temperature of 279 K and pH 7.0 in H_2O. Circles, squares, triangles and crosses mean that crystals have appeared at MPD concentrations of 30, 25, 22.5 and 20%(*v*/*v*), respectively. The broken lines show the estimated solubility curves at different MPD concentrations.

then prepared using a microbatch system, in which the drops were kept at 279 K for 20 d (Fig. 1).

3. Results and discussion

In the diagrams there are three regions, A (circles), B (triangles) and C (crosses), which correspond to the existence of crystals, amorphous precipitate and a clear solution, respectively. Fig. 1 shows the variation of DNA and $MgCl_2$ concentration with the pH kept constant at 7.0 (using 0.1 *M* sodium cacodylate buffer) and the concentration of MPD (2-methyl-2,4-pentandiol) held at 30%(*v*/*v*). The most distinctive feature of the phase diagram is that there is a minimum in the DNA-solubility curve; that is, the DNA solubility decreases abruptly to less than 1 m*M* at about a $MgCl_2$ concentration of 100 m*M* and then gradually increases as the $MgCl_2$ precipitant concentration is increased. In Fig. 2, in which the MPD concentration was varied, it was found that the DNA solubility increased with a decrease of MPD concentration.

We have attempted DNA crystallization at various points in region A of Fig. 1 and found that in general crystal quality becomes worse and crystal size becomes smaller as the solution conditions are moved further from the minimum point. In addition, the crystal quality also decreases as the MPD concentration decreases. However, we have succeeded in growing a large single crystal at conditions near the minimum solubility point. A photograph of a single crystal (with dimensions 1.7 × 1.3 × 0.6 mm) grown from 0.4 ml D_2O[1] solutions of DNA (1.5 m*M*), $MgCl_2$ (100 m*M*), MPD [30%(*v*/*v*)] and pD 6.6 is shown in Fig. 3. This is the largest single crystal of an oligomer B-DNA reported thus far (Wing *et al.*, 1980). The reproducibility of growing such large crystals at these solution conditions is quite satisfactory.

A preliminary neutron diffraction pattern from the crystal has been obtained with the BIX-3 (Niimura, 1999; Tanaka *et al.*, 2002) diffractometer at the JRR-3M reactor. One typical raw data frame is shown in Fig. 4. The program *DENZO* (Otwinowski & Minor, 1997) was used for data processing and analysis. This crystal is trigonal, with unit-cell parameters $a = b = 32.9$, $c = 97.4$ Å and a resolution limit of 2.6 Å. It is confirmed that this crystal is suitable for neutron diffraction analysis and the collection of a complete data set of Bragg reflections is presently scheduled.

[1] In a neutron-diffraction experiment, in order to avoid the high background arising from the incoherent neutron scattering of H atoms, crystallization in D_2O solution instead of H_2O is generally conducted. As the phase diagrams shown in Figs. 1 and 2 are the results from H_2O solutions, we had to check whether these results are applicable for D_2O solutions; we found that the difference is negligibly small.

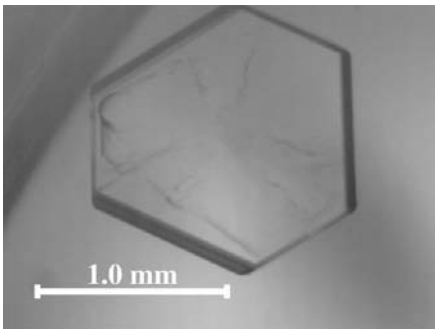

Figure 3
A photograph of the largest single crystal grown under the following conditions: 0.4 ml D_2O solutions of DNA (1.5 m*M*), $MgCl_2$ (100 m*M*), MPD [30%(*v*/*v*)] at pD 6.6. The dimensions of this crystal are about 1.7 × 1.3 × 0.6 mm.

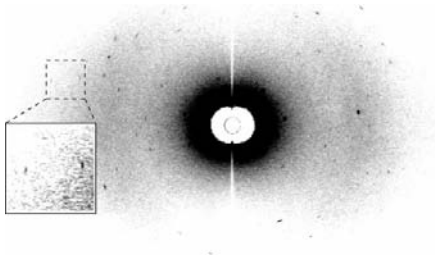

Figure 4
A preliminary neutron diffraction pattern from the single crystal recorded using the neutron diffractometer BIX-3. The wavelength of the incident neutron beam was 2.33 Å. The exposure time was 10 h. After this measurement, the DNA crystal was not damaged by the thermal neutron irradiation. The insert shows a magnification of resolution 2.6 Å.

Finally, we note that such a large single crystal of DNA might also be useful in other fields of molecular spectroscopy and the recent 'nanometre technology' DNA manipulation.

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