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New crystals of the histone core octamer diffract to higher resolution, 2.65 Å

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Abstract

A new crystal form of the histone octamer, crystallized in 1.6 *M* KCl, 1.6 *M* phosphate, diffracts to appreciably better than 2.6 Å resolution. The crystals have space group $P6_1$ or $P6_5$ and lattice parameters $a = b = 158.29$, $c = 103.27$ Å, $\alpha = \beta = 90$, $\gamma = 120°$, with one molecule per asymmetric unit. The new crystals promise to yield more detail of the histone basic domains and a higher resolution structure for the histone octamer.

1. Introduction

In eukaryotic chromosomes at metaphase and in chromatin at other phases of the cell cycle the DNA is organized into looped domains (for review see Mirkovitch, Gasser & Laemmli, 1987). Each domain contains between 10^4 and 10^5 base pairs (bp) of DNA packaged with histone proteins into nucleosomes. The strings of nucleosomes connected by DNA are coiled at least into one higher order structure and higher orders still in metaphase chromosomes.

Nucleosomes are composed of a histone octamer core, 2(H2A.H2B).$(H3.H4)_2$ around which 146 bp of DNA wind in *ca* 1.8 turns to form a nucleosome core particle. A further approximately 22 bp of DNA, associated with histone H1 (H5 in avian erythrocytes) complete approximately two turns (168 bp) of DNA around the histone octamer and there is a further 'linker' length of DNA between 0 and 70 bp in multicellular organisms which is species and cell-type specific (for review see van Holde, 1988).

The core histones are known to have globular regions with a typical fold (Arents, Burlingame, Love, Wang & Moudrianakis, 1991) and N- and C-terminal domains which are rich in lysine and arginine (for review see Crane-Robinson & Bohm, 1985). The basic domains are directly implicated in the binding to DNA phosphate groups. Their behaviour can be regulated by chemical modifications, especially acetylation of specific lysine sites which occurs when genes are active (Thorne, Kmiceikt, Mitchelson, Sautiere & Crane-Robinson, 1990). Detailed structural knowledge of the basic domains is a prerequisite to understanding the functions of the core histones in inactive and active genes.

Previous crystallographic studies of the nucleosome core particle (Richmond, Finch, Rushton, Rhodes & Klug, 1984; Uberbacher & Bunick, 1985; Richmond, Searles & Simpson, 1988; Struck, Klug & Richmond, 1992) and the histone octamer (Arents, Burlingame, Love, Wang & Moudrianakis, 1991) have not been able to provide this information; the former owing to lack of resolution and the latter because small-angle scattering results (Wood *et al.*, 1991) indicate that the highly basic regions are disordered in the published octamer crystal form and only about 70% of the protein is observed in the electron-density map to 3.1 Å resolution (Wang, Rose, Arents & Moudrianakis, 1994).

2. Method and results

We have successfully developed efficient methods for quickly preparing large quantities of pure histone octamers in phosphate to mimic the histone environment when interactions with DNA phosphates are present. It is believed that the basic domains will be less disordered in this environment which is closer to the physiological conditions.

We have crystallized the octamers in 1.6 *M* KCl, 1.6 *M* phosphate (equimolar monobasic and dibasic) (Lambert, Carter, Shariff & Baldwin, in preparation). The crystals are shown in Fig. 1. They are needle shaped with perfectly hexagonal cross-section. Typically they grow within three weeks to about $0.8 \times 0.25 \times 0.25$ mm upwards from a precipitate and on breaking them off at the base they float, but are nearly buoyant-density matched. Their density, by sedimentation studies of crystals on progressively lowering the phosphate content of the mother liquor is close to 1.25 g ml^{-1}.

A typical X-ray diffraction pattern on station 7.2 ($\lambda = 1.488$ Å) at the Daresbury X-ray Synchrotron-Radiation Source is shown in Fig. 2. Data were collected using the MAR image plate; the storage ring current ranged between 160 and 140 mA at an energy of 2 GeV. The crystals were flash frozen to 100 K under optimum conditions. They diffract to well beyond 2.6 Å resolution but we have obtained a 96.7% complete data set to 2.65 Å resolution, with an R_{merge} of 9.7% ($R_{merge} = \sum|I-\langle I\rangle|/\sum I$).

55.5° of data were collected at 120 s exposure per 0.5° frame, 42 847 unique reflections were obtained from 466 281 measurements; the average multiplicity was 3.3. The data were processed using *DENZO* (Otwinowski, 1991). The crystals belong to space group $P6_1$ or $P6_5$ and have lattice parameters of $a = b = 158.29$, $c = 103.27$ Å, $\alpha = \beta = 90$, $= 120°$. The self-rotation function *POLARRFN* (Collaborative Computational Project, Number 4, 1994) and calculations of the volume of

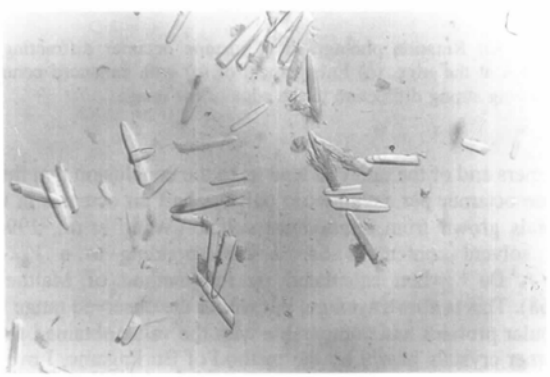
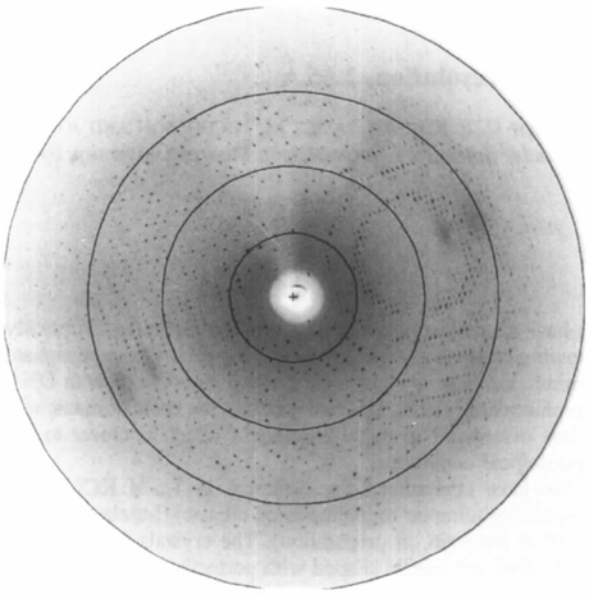
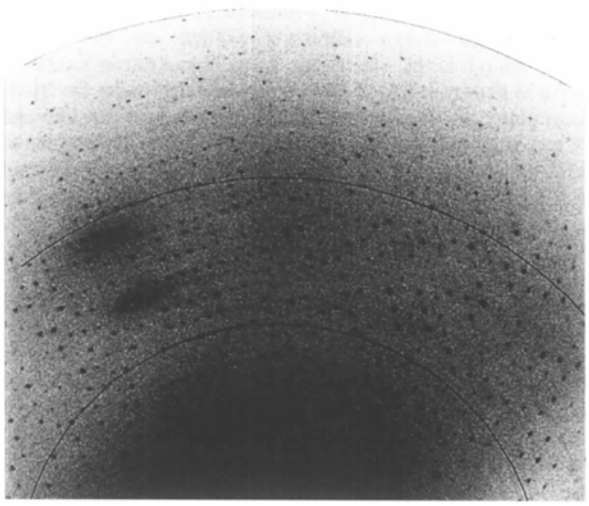


Fig. 1. Crystals of histone octamer grown in potassium phosphate. The crystals are hexagonal needles with dimensions of approximately $0.8 \times 0.25 \times 0.25$ mm.



(*a*)



(*b*)

Fig. 2. (*a*) Rotation photograph of histone octamer diffracting to 2.65 Å at the edge. (*b*) Enlargement of (*a*) with enhanced contrast showing strong diffraction to the edge of the image.

octamers and of the unit cell lead us to the conclusion that there is one octamer per asymmetric unit *cp*. half an octamer in the crystals grown from ammonium sulfate (Wang *et al.*, 1994). The solvent content is 64.4% corresponding to a V_M of 3.45 $Å^3\,Da^{-1}$ when calculated by the method of Matthews (1968). This is above average, but within the observed range for globular proteins and comparable with the value obtained from octamer crystals grown by the method of Burlingame, Love & Moudrianakis (1984), with $V_M = 3.89\ Å^3\,Da^{-1}$.

The octamer crystals, sealed in Pantak tubes with mother liquor at 293 K diffracted to 4 Å resolution. Slow cooling to 258 K increased the resolution to 2.8 Å. Cooling the crystals much further resulted in loss of diffraction. At room temperature and with this moderate cooling the crystals were susceptible to radiation damage; only approximately 5–6° of data could be collected without severe loss of resolution.

Crystal lifetimes and resolution were greatly enhanced when the crystals were flash frozen at 100 K, albeit with an increase in mosaicity, typically 0.27° at room temperature to 0.49° at 100 K. The crystals were briefly washed in mother liquor containing 20 to 30%(*v*/*v*) of glycerol and then flash frozen to 100 K by immersion in liquid N_2. However, we discovered that crystals allowed to soak in 1.6 *M* KCl, 1.6 *M* phosphate in 30% glycerol for at least half an hour, first crazed slightly but then annealed. When flash frozen, these crystals gave the highest resolution diffraction patterns and were stable in the X-ray beam for much longer than the time required to get a complete data set. Since the crystals on cooling from room temperature to 258 K or below show a much improved resolution it is likely that thermal motion (possibly in the basic domains) is at least one of the causes of the disorder in the crystal.

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