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Purification of histone core octamers and 2.15 Å X-ray analysis of crystals in KCl/phosphate

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Intact histone octamers, produced by a new method quickly and in bulk, were crystallized in KCl/phosphate, and the X-ray data were analysed to 2.15 Å, confirming a $P6_5$ space group. This environment preserves the high-resolution structure of the octamers and will be useful for studying them with other functionally important molecules. The octamers form into left-handed superhelices hexagonally spaced by 158.65 Å, having a pitch of 102.57 Å with six octamers per turn. A dipotassium tetraiodo mercurate derivative had good phasing power and should prove valuable in refining the structure after molecular-replacement analysis with lower resolution coordinates; the heavy atom was isomorphously placed at a unique site between the two H3-cysteine residues in the octamer.

1. Introduction

The histone octamer, $(H3.H4)_2{\cdot}2(H2A.H2B)$, forms the core around which *ca* two turns of DNA wind for packaging into nucleosomes in eukaryotic cells. The nucleosome is involved in the regulation of transcription, where ~28 lysines in the basic domains of the histones become acetylated exclusively in active genes (Allfrey *et al.*, 1964; Hebbes *et al.*, 1988). Indeed, transcription repression in silenced genes is characterized by methylated DNA and the associated repressor (MeCP9) recruits a histone deacetylase complex (Eden *et al.*, 1998; Jones *et al.*, 1998; Nan *et al.*, 1998).

Co-activators with histone acetyltransferase activity can target acetylation to specific core histones in the promoter regions of an active gene (Utley *et al.*, 1998; Kuo & Allis, 1998). Indeed, the multiple-protein assembly SW1/SNF required for the function of several heterologous gene activators binds directly to nucleosomes with ATP hydrolysis, to produce an altered nucleosome structure enhancing transcription-factor binding (Côté *et al.*, 1998; Steger & Workman, 1996). Nucleosomes associated with promoter DNA from a eukaryotic gene can dynamically bind with the transcription factor recognizing the DNA sequence, allowing histone–DNA contacts to be broken. This has been demonstrated by an enhanced DNAase1 sensitivity (Boyes *et al.*, 1998). RNA polymerase can move through the histone octamer in transcription. This seems to involve a transfer of the octamer from a position in front of the polymerase to a position behind it, whilst maintaining its attachment to the DNA (Studitsky *et al.*, 1994; Felsenfeld *et al.*, 1996), apparently without splitting the histone octamers (Protacio & Widom, 1996; O'Neill *et al.*, 1993).

The high-resolution structure of the histone octamer and its association with DNA, RNA polymerase, high-mobility group proteins, deacetylases, histone acetyl transferases *etc.* is

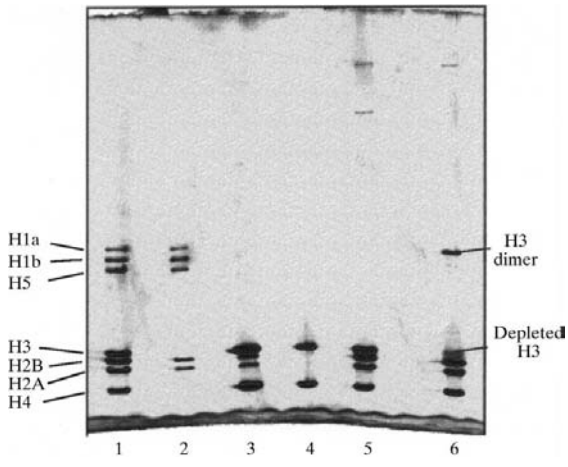
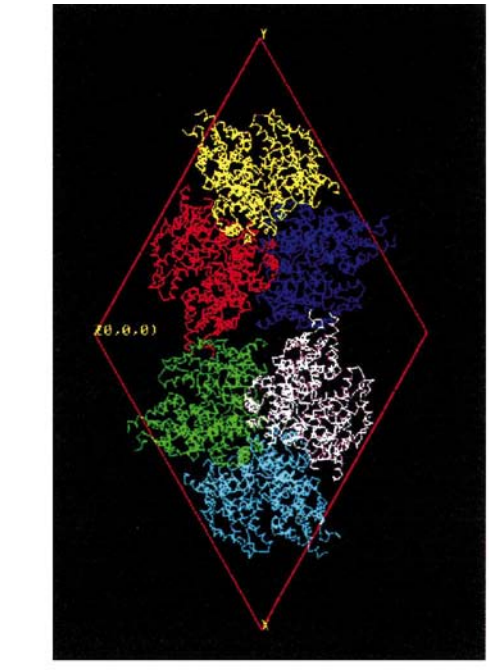


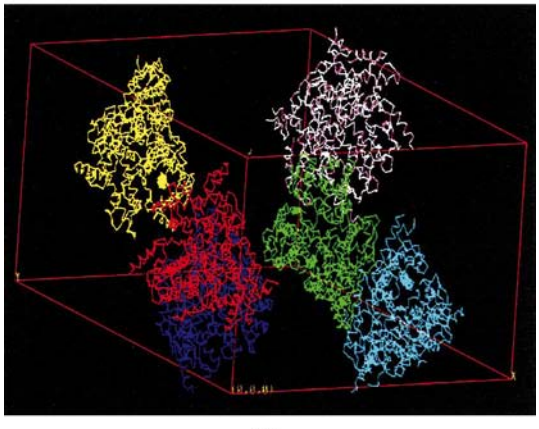
Figure 1
A 20% SDS–PAGE gel of the proteins present in the various stages of histone octamer purification. All samples were precipitated with trichloroacetic acid, washed with acetone and dissolved in reducing buffer containing 2-mercaptoethanol, with the exception of the sample in lane 6, which was not reduced in this way. Lane 1, the supernatant at the end of stage 1 (retained); lane 2, the supernatant at the end of stage 2 (discarded); lane 3, the pellet at the end of stage 2 (retained); lane 4, the pellet at the end of stage 3 (discarded); lane 5, the reduced protein from the supernatant at the end of stage 3; lane 6, the non-reduced protein from the supernatant at the end of stage 3 (crystallized).

therefore crucial for explication of multiple functions in relation to structure. The coordinates of the histone octamer itself to 3.1 Å resolution (Arents *et al.*, 1991; Arents & Moudrianakis, 1993; Moudrianakis & Arents, 1993; Wang *et al.*, 1994) and the coordinates of the histone octamer and its associated DNA in the nucleosome core particle to 2.8 Å resolution (Luger, Mader *et al.*, 1997; Luger & Richmond, 1998; Rhodes, 1997) are known from X-ray crystallography.

Higher resolution information on the histone octamer structure is needed, and therefore a KCl/phosphate medium has been developed to produce crystals which diffract to high resolution (Carter *et al.*, 1996), to provide a phosphate-rich environment for studying histone octamer interactions with other molecules and to allow fractionation of highly acetylated octamers.



(*a*)



(*b*)

Figure 2
(*a*) View down the *z* axis of the octamers in the unit cell. The top and bottom octamers in the figure are part of two superhelices of six octamers above and below the diagram, respectively. The left-hand two octamers are in a superhelix of six octamers to the left of the figure and the two octamers to the right are part of superhelices to the right. (*b*) The octamers in the unit cell, perspective view.

2. Histone octamer purification and crystallization

The histone octamer procedures of the present study involved only about 6 h of bench work. The procedure can be considered as three stages. In stage 1, 80 ml of 4 *M* NaCl were mixed with 40 ml saturated ammonium sulfate at 277 K and then swirled very quickly into 80 ml of a 2×10^9 chick erythrocyte nuclei per millilitre suspension, which formed a gel. After leaving on ice for 1 h, the gel became cloudy owing to precipitation of non-histone nucleoproteins and was centrifuged at 100000*g* for 18 h in a Beckman Ti45 rotor. The pellet was discarded and the supernatant, which gave an ultra-violet spectrophotometer trace which peaked at 277 nm wavelength with no absorbance due to DNA at 260 nm, contained only the core histones, H2A, H2B, H3 and H4, plus the linker histones H1a, H1b and H5, as shown in lane 1 of Fig. 1.

The supernatant was concentrated to 10 mg ml^{-1} using an Amicon pressurized-cell concentrator with a YM10 10 kDa molecular-weight-cutoff membrane in stage 2 of the procedure. An equal volume of saturated ammonium sulfate was added dropwise while stirring on ice and the suspension was centrifuged at 100000*g* for 1 h. The supernatant contained small quantities of histones H2A and H2B plus the linker histones (lane 2 of Fig. 1) and was discarded. The pellet contained only the core histones, with some excess of histones H3 and H4 (lane 3, Fig. 1). Lanes 2 and 3 showed that some dissociation of the octamer had occurred; similar effects have been well studied by others (*e.g.* Eikbush & Moudrianakis, 1978). The pellet from stage 2 of the procedure was dissolved in 100 ml of 10 m*M* Tris pH 7.4, 2 *M* KCl and the procedure in stage 2 was repeated for further purification of the histone core octamers.

In stage 3 of the procedure, the pellet was dissolved in 2 *M* KCl, 0.3 *M* monobasic phosphate, 0.3 *M* dibasic phosphate and the solution was concentrated to 20 mg ml^{-1} using the YM10 filter, as judged by the solution OD_{277}. The concentrate was dialysed against 2 *M* KCl, 0.475 *M* monobasic phosphate, 0.475 *M* dibasic phosphate overnight and the suspension was microcentrifuged for 30 min at 13000 rev min^{-1}. The small pellet contained the excess histones H3 and H4 observed at the end of stage 2 of the procedure (Fig. 1, lane 4), while the supernatant contained pure histone octamers with a typical yield of 100 mg (Fig. 1, lane 5). Lane 6 of Fig. 1 shows the histone octamers without reduction with 2-mercaptoethanol after TCA extraction: histone H3 is shown to run as an H3 dimer on the gel. A faint impurity band was observed (lanes 5 and 6) having a molecular weight of about 106 kDa. The band was likely to be very small quantity of a non-histone nucleoprotein co-purifying with the histone octamers under the prevailing conditions.

Octamers from the stage 3 supernatant were crystallized by dialysis in 50 µl cylindrical dialysis buttons into either 2 *M* KCl, 1.35 *M* phosphate or 1.6 *M* KCl, 1.6 *M* phosphate. In both these conditions crystals formed in 4–5 d and grew as hexagonal-tipped needles of length up to 2 mm and cross-section diameter up to 0.2 mm in about one and a half months. Crystals could be grown over a very narrow range of pH corresponding to 48–52% monobasic-, with 52–48% dibasic-phosphate molarity. Crystals could be grown in the solution range 2 *M* KCl, 1.35 *M* phosphate to 2 *M* KCl, 1.2 *M* phosphate.

3. X-ray data acquisition and data processing

Crystals were transferred on a loop in stages to a cryobuffer containing a final concentration of 20% glycerol and were then transferred to liquid nitrogen on a small loop made of a single strand of unwaxed dental floss and finally to the goniometer. X-ray diffraction data sets were taken at 1.488 Å wavelength on beamline 7.2 of the Daresbury Laboratory Synchrotron Radiation Source at a temperature of 100 K. Three data sets were collected from a single crystal of histone core octamers using rotation increments of 0.35°. Approximately 30° of

data could be collected before crystal deterioration, and so the goniometer was translated so that 30° data sets could be recorded from three parts of the crystal. The resolution achieved was 2.15 Å, but higher resolution is possible.

The X-ray data were processed using the *HKL* program suite (Otwinowski & Minor, 1997). Each individual data set yielded a merging R factor of typically 5–7% overall and approximately 30% in the last resolution shell from 2.25–2.15 Å. The three data sets were merged together and scaled giving unit-cell parameters $a = b = 158.648$ and $c = 102.573$ Å; $\alpha = \beta = 90$, $\gamma = 120°$. The resolution range was 20–2.15 Å, the mosaicity 0.25–0.45° and the merging R was 7.1% (33.1% in the highest resolution shell). 77448 reflections were used in the analysis corresponding to a completeness of 97.4% (97.1% in the highest resolution shell) and a multiplicity of 2.26 (2.16 in the highest resolution shell). The percentage of reflections with $I > 3\sigma(I)$ was 75.8% (56.6% in the highest resolution shell).

Molecular replacement (with *AMoRe*; Navaza, 1994) was attempted with the coordinates of one H3.H4.H2A.H2B protein group from 1hio (PDB code for the histone octamer to 3.1 Å; Arents *et al.*, 1991) or 1aoi (PDB code for the nucleosome core particle to 2.8 Å; Luger, Mader *et al.*, 1997). Of the two possible space groups $P6_1$ and $P6_5$ (Carter *et al.*, 1996), $P6_5$ was the correct solution with a correlation coefficient 23% higher than the best solution for $P6_1$. The correlation coefficient after the placement of the first group of four histones was already 10% higher than the first noise peak and the placement of the second group gave a correlation coefficient of 59 or 62% when using 1hio or 1aoi, respectively.

In the $P6_5$ space group, the octamers form left-handed superhelices hexagonally spaced by 158.65 Å and having a pitch of 102.57 Å, with six octamers per turn. Figs. 2(*a*) and 2(*b*) show the arrangement of octamers in the unit cell.

4. The dipotassium tetraiodo mercurate heavy-atom label

A heavy-atom derivative was prepared by dialysing or soaking crystals in 0.1 mM K_2HgI_4 for 2 d. Back-soaking took place during immersion of the crystals in the ascending concentrations of glycerol whilst preparing it for freezing. The heavy-atom position was determined using a difference Patterson transformation which clearly indicated a single peak for each Harker section (corresponding to $x = 0.186$, $y = 0.534$, $z = 0.0$); no extra peak could be found by difference Fourier analysis. The K_2HgI_4 molecule could be placed with respect to the molecular-replacement model and was found to be in a hydrophobic pocket in between the two H3 molecules (Fig. 3). As shown recently (Pannu *et al.*, 1998), these single isomorphous replacement phases could be incorporated during maximum-likelihood structure refinement to improve the map quality and remove some of the model bias introduced by molecular replacement.

Phase calculation and heavy-atom refinement with *MLPHARE* (Collaborative Computational Project, Number 4, 1994) using data from 20–4.5 Å resulted in an overall figure of merit of 0.32, and a phasing power (acentric) and an $R_{\rm Cullis}$ (centric) of 1.3 and 0.71, respectively. The Harker vectors, using results from the program *RSPS* (Collaborative Computational Project, Number 4, 1994) were $U_1 = 0.652$, $V_1 = 0.1863$, $W = 0.166$; $U_2 = 0.162$, $V_2 = 0.279$; $W_2 = 0.333$; $U_3 = 0.373$, $V_3 = 0.068$, $W_3 = 0.5$.

The heavy atom is completely isomorphous and is proving useful to further refine the electron-density map to 2.15 Å resolution (L. Chantalat, J. M. Nicholson, S. J. Lambert & J. P. Baldwin, in preparation).

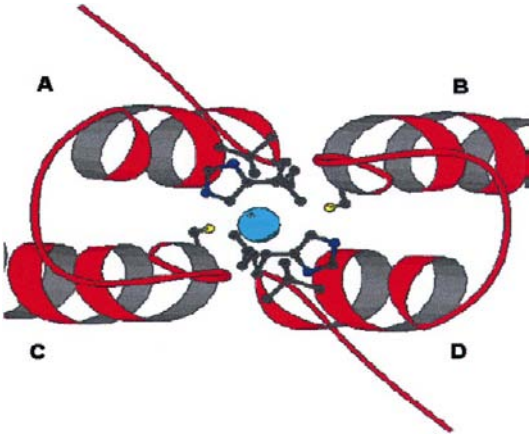


Figure 3
The four-helix bundle binding the two H3 molecules together, showing the tetraiodo mercurate molecule in a hydrophobic environment involving Cys110, His113, Leu126 and Ile130. Helices *A* and *B* are approximately parallel and they are approximately perpendicular to helices *C* and *D*, which themselves are parallel.

5. Discussion

A method for rapidly obtaining yields of 1 g (if scaled up) of histone octamers which allows the production of crystals diffracting to better than 2 Å resolution in KCl/phosphate has been developed. This is compared with other methods in which histones are expressed in bacteria and the octamers reassembled (Luger, Rechshteiner *et al.*, 1997; Moore *et al.*, 1997), DNA is stripped from nucleosome core particles or nucleosome multimers in high salt using a hydroxylapatite column (Norton *et al.*, 1989; Luger, Mader *et al.*, 1997; Harp *et al.*, 1996) or octamers are precipitated for crystallization in ammonium sulfate (Burlingame *et al.*, 1984; Godfrey *et al.*, 1990; Baxevanis *et al.*, 1991; Moehs *et al.*, 1992). The crystal structure results indicate that the intact histone octamers in KCl/phosphate are well ordered and therefore this is likely to be a useful medium for studying octamer complexes with other molecules, such as those discussed in §1.

Histone octamers produced in the present work contained H3 molecules which, under non-reducing conditions, run as dimers on SDS–PAGE gels. This illustrates the strength of the H3–H3′ interaction (*cf.* Fig. 3), but we confirm the results of Luger, Mader *et al.* (1997) that there is not a disulfide bridge between the cysteines of the H3 molecules in the intact histone octamer structure.

Molecular replacement, using known lower resolution coordinates of octamers (Arents *et al.*, 1991) and of octamers in nucleosome core particles (Luger, Mader *et al.*, 1997), with further refinement with an isomorphous dipotassium tetraiodo mercurate label, allows refinement of the electron-density map to 2.15 Å resolution. The resolution can be expected to be improved to well below 2 Å with brighter X-ray beams than are available on beamline 7.2 at the Daresbury Laboratory, CCD detectors and improvements already made in crystal preparation.

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