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Neutron-Scattering Studies of Chromatin Subunits in Solution under a Variety of Contrast Conditions

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Neutron-scattering studies of chromatin core particles in solutions containing various mixtures of D_2O/H_2O and small molecules (glycerol) show that the water closely associated (or bound) with the particles is largely in the outer DNA-rich regions. This confirms the fact that the particles contain a core composed of the hydrophobic regions of histone proteins.

Neutron-scattering studies of macromolecular particles in solution have usually used mixtures of D_2O and H_2O to provide contrast variation (Stuhrmann, 1974; Stuhrmann & Duee, 1975; Kneale, Baldwin & Bradbury, 1977). It has been usual to neglect the effect of water bound to the particles since the protons in this water will freely exchange with the solvent so that the bound water is always approximately contrast matched. However, if small molecules (*e.g.* glycerol) are used at various concentrations with the particles in D_2O, the bound D_2O is not contrast matched but contributes positively to the scattering-length density distribution within the particle. Bound H_2O contributes negatively in corresponding experiments using H_2O, so that studies of radius of gyration and zero-angle scattering of neutrons from particles in solution containing small molecules gives information about the distribution of bound water (Stuhrmann, Haas, Ibel, Koch & Crichton, 1976).

A study of chromatin core particles in glycerol and sucrose solutions containing various D_2O/H_2O mixtures has been undertaken and the zero-angle scattering and radius of gyration analysed. The core particles are produced by micrococcal nuclease digestion of chicken erythrocyte nuclei and have been studied in solution previously by D_2O/H_2O contrast variation using neutrons (Pardon, Worcester, Wooley, Tatchel, van Holde & Richards, 1975; Hjelm, Kneale, Suau, Baldwin, Bradbury & Ibel, 1977; Suau, Kneale, Braddock, Baldwin & Bradbury, 1977) and by low-resolution X-ray crystallography (Finch *et al.*, 1977). It has been quite well established that the core particle consists of a protein core, around which is wound one and a half to two turns of DNA. The particle maximum dimension is approximately 11 nm and it is believed that a large part of the histone protein is in the form of a core of radius ~3·2 nm. The particle has approximate axial ratios of 1:1:0·5.

In the combined contrast-variation study using small molecules as well as D_2O/H_2O it is convenient to consider only the 'wet' particle, which is defined as the particle plus associated water (which includes water bound to the particle plus water in regions near the particle which are inaccessible to small molecules). The scattering-length density within the wet particle includes a term $\Omega_E(\mathbf{r})$ [or in some references $\varrho_E(\mathbf{r})$], which arises because of D⇌H exchange in the wet particle, and in general this may be replaced by $x\Omega_E(\mathbf{r})$ when the combined small-molecule, D_2O/H_2O contrast-variation studies are made (Kneale *et al.*, 1977). x is unity for D_2O/H_2O contrast variation with no small molecules present and zero for experiments where the concentration of small molecules is varied at fixed D_2O/H_2O ratios. Experiments varying the D_2O/H_2O mixture at fixed small-molecule concentrations have values of x between 0 and 1, so that x can be used as a parameter to gain structural information. In fact, new scatter functions may be obtained to give information about the structure of water and exchangeable protons in the particle.

Fig. 1 shows graphs of the square root of the zero-angle scattering $\sqrt{I(0)}$ as a function of the solvent scattering density ϱ_{sol} for chromatin core particles. Similar graphs have been given by Stuhrmann *et al.* (1976) for ferritin. The slope of the graph gives V_C values of 186 nm^3 for pure H_2O/D_2O contrast variation and $V_F = 274$ nm^3 for small-molecule contrast variation at fixed D_2O/H_2O mixtures. V_F is the volume of the wet particle and V_C differs from V_F by $(\frac{1}{2}n_1 + n_2)V_W$, where V_W is the volume of a solvent water molecule, n_1 is the number of exchangeable protons in the dry particle and n_2 is the number of associated water molecules. $\frac{1}{2}n_1V_W$ is a volume which arises from D⇌H exchange in the particle, excluding the bound water, for the experiments where the D_2O/H_2O ratio of the solvent is changed. This volume (33 nm^3) has been determined in earlier studies (Hjelm *et al.*, 1977) so that the measurements of V_F and V_C give ~55 nm^3 for n_2V_W – the apparent volume of water associated with the chromatin core particle in solution.

The question of how this water is distributed is answered by the radius-of-gyration analysis which is shown in Fig. 2. The graphs of R_g^2 *versus* $1/\bar{\varrho}$ for contrast-variation studies in D_2O and H_2O with small molecules (Fig. 2*a*) show that the radius of gyration does not change much for different con-

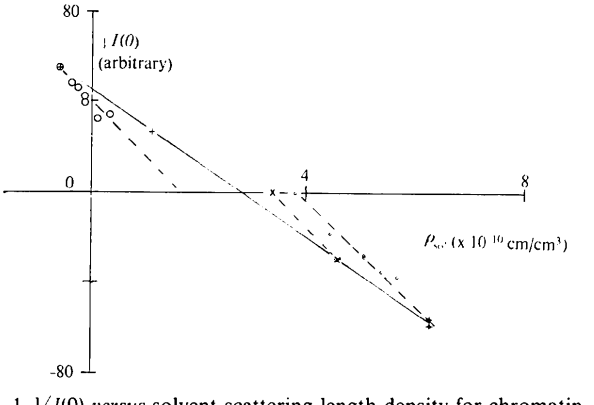

Fig. 1. $\sqrt{I(0)}$ *versus* solvent scattering-length density for chromatin core particles. ○ glycerol contrast variation in H_2O. ◦ glycerol contrast variation in D_2O. + contrast variation in D_2O/H_2O mixtures. × glycerol contrast variation in 75% D_2O.

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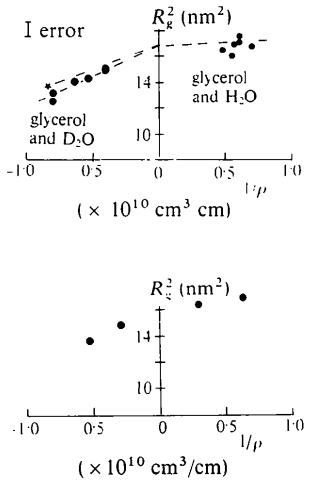

Fig. 2. The square of radius of gyration R_g^2 *versus* $1/\bar{\varrho}$ for chromatin core particles in H_2O/D_2O mixtures (*b*) and in glycerol/D_2O and glycerol/H_2O mixtures (*a*). $\bar{\varrho}$ is the contrast scattering-length density, $\varrho_{sol}-\varrho_{CM}$, where ϱ_{CM} corresponds to condition $I(0)=0$ in Fig. 1.

centrations of small molecules in H_2O, but R_g^2 decreases markedly for high glycerol concentrations in D_2O. This indicates that the first moment of the scattering density of the internal structure is very low for the experiments in H_2O and high and positive in D_2O; in fact it is more highly positive than the corresponding measurements with pure D_2O/H_2O contrast variation (Fig. 2*b*). This clearly indicates that the water associated with the particle is associated with the outer DNA regions so the two-level-step characteristic of the particle (protein and DNA) is reduced in H_2O and increased in D_2O (it should be remembered that bound water is part of the wet particle and contributes negatively to the particle scattering density in H_2O and positively in D_2O).

The radius-of-gyration analysis of Ibel & Stuhrmann (1975) can be used for a wet particle, with $\Omega_E(\mathbf{r})$ replaced by $x\Omega_E(\mathbf{r})$; the radius of gyration R_F for the wet particle at infinite contrast (4·04 nm) is obtained by extrapolating the graphs of Fig. 2(*a*) to zero $1/\bar{\varrho}$ while R_C (3·96) is the corresponding measurement for the pure D_2O/H_2O contrast variation studies of Fig. 2(*b*). From this we calculate the radius of gyration of the exchangeable protons associated with the particle to be ~4·2 nm again, indicating that they lie preferentially in the outer DNA rich regions of the particle.

We are at present completing some complementary studies of partial specific volumes of chromatin 1 particles before submitting a full report to *Nucleic Acids Research.*

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Neutron Scattering from Chromatin in Relation to Higher-Order Structure

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Chromatin fibres containing histone H1 as well as the DNA and histones H2*A*, H2*B*, H3 and H4 show the same off-meridional diffraction maxima at ~ 10 nm as reported by Carpenter, Baldwin, Bradbury & Ibel [*Nucleic Acids Res.* (1976), **3**, 1739–1746] for chromatin fibres depleted of histone H1. It is shown that the intensity of the maximum (as a function of solvent scattering-length density and deuteration of histones and DNA) can be quantitatively explained in terms of a globular protein component to the chromatin subunits in a matrix of hydrated DNA. The off-meridional diffraction maximum suggests that the chromatin is in the form of a flat helix and clearly the relative amounts of protein and hydrated DNA vary along the length of the chromatin fibre.