interpretation, segments from two such rods run antiparallel to each other, and are related by the crystallographic twofold axis to the same segments of the other two chains. These four portions of polypeptide chain in the center of the molecule are in close contact with each other and are presumably composed of the nonpolar regions of the molecule (Fig. 1).

In detergents and nonpolar solvents, melittin is a monomer (e.g., reference 3). In going from tetramer to monomer, it seems likely that nonpolar side chains are exposed to the solvent. Thus the amphipathic properties of melittin may be governed in part by its tetrameric structure of 222 symmetry, and in part by the tetramer-monomer equilibrium.

The shape of the melittin molecule as displayed in the 6-Å electron density map of form II is consistent qualitatively with the unit cell dimensions of the form I crystals.

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NUCLEOSOME AND DNA-PROTEIN CONDENSED STRUCTURES IN SOLUTION FROM FLOW BIREFRINGENCE AND INTRINSIC VISCOSITY

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Highly sensitive streaming birefringence measurements combined with intrinsic viscosity are used to characterize the shape anisometry and optical anisotropy of nucleosomes over a range of salt concentration >30 mM KCl and of structures obtained by the condensation of high molecular weight DNA with polylysine.

These measurements appear useful for several reasons. (*a*) Both streaming birefringence and intrinsic viscosity are hydrodynamic properties based upon the rotational diffusion of macromolecular particles and hence are inherently more sensitive to details of particle anisometry than are hydrodynamic properties based upon translational diffusion. (*b*) An established body of both hydrodynamic and continuum dielectric optical theory is available with which to interpret streaming birefringence results. The theory has been tested experimentally in various ways, and appears to be adequate, at least for highly anisotropic systems such as DNA (Oriel and Schellman, 1966; Harrington, 1970). Extinction angles (i.e., mean orientation angles of particles in a velocity gradient) are entirely hydrodynamic properties, and hence can be interpreted through the rotational coefficient to characterize particle anisometry and to estimate absolute dimensions. The ratio of Maxwell coefficient to intrinsic viscosity is proportional to the absolute particle anisotropy. (*c*) The high optical anisotropy of DNA relative to that of associated protein permits certain details of tertiary structure and

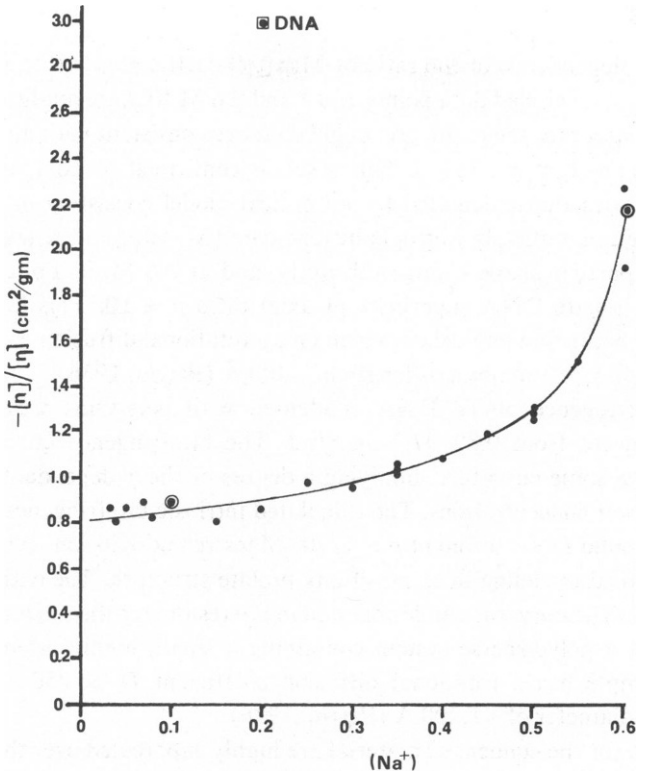

Figure 1 The salt dependence of nucleosome conformation between 0.03 and 0.6 M KCl. Dramatic changes are observed near the upper end of this range. For comparison, results for pure DNA of ~150 bp are also shown (Harrington, 1970). Data points at 0.1 and 0.6 M are analyzed in detail.

shape anisometry to be estimated from the observed optical anisotropy compared to optical models involving the DNA alone. (*d*) The method is essentially independent of solvent.

EXPERIMENTAL METHODS

Basic instruments include a special, highly sensitive photoelectric streaming birefringence apparatus and a cartesian-diver, rotational viscometer. Both instruments were constructed in our laboratory. The birefringence apparatus is based upon a design in which the total flow cell annulus is scanned photoelectrically (Zimm, 1958). This design has an inherently advantageous ratio of signal-to-optical noise, which is further improved by using additional signal averaging and computerized data smoothing procedures.

Nucleosome core particles were prepared using a modification of Lutter's "soluble chromatin" procedure (Lutter, 1970) as developed by G. Bunick (Oak Ridge, Tennessee). Prior to study the nucleosomes were stored in the cold without freezing. The nucleosomal DNA was not sized in our laboratory but has been estimated to be 146 ± <4 bp.[1]

Condensed DNA systems were made by reacting 1/2 molecules of T4 phage DNA with polylysine (MW 30,000–70,000) at a phosphate/lysine ratio of ~2 under low salt conditions and <0.25 μgm/ml DNA. Samples were concentrated by ultrafiltration (1000 Å Millipore filters) and fractionated on 5–25% sucrose density gradients. A sharply sedimenting peak at S = 300 was collected and used for studies reported.

[1]G. Bunick, private communciation.

RESULTS

The ionic strength dependence of the ratio of Maxwell coefficient, [n], to intrinsic viscosity, $[\eta]$ is shown in Fig. 1. Encircled data points at 0.1 and 0.6 M KCl are analyzed independently. At the lower ionic strength, the extinction angle data are consistent with an oblate particle of axial ratio p 1/2 (Finch et al., 1977). This result is confirmed by comparing the observed optical anisotropy with that calculated for an optical model consisting of 1.75 superhelical turns of DNA about an optically isotropic histone core (Maestre and Kilkson, 1965). Above ~0.3 M KCl, the particle shape changes abruptly, and at 0.6 M, its optical anisotropy has become that of a prolate DNA superhelix of axial ratio $p \simeq 10$. This latter result is also consistent with the extinction angle data which give a rotational diffusion coefficient $D_r = 2 \times 10^{-4} s^{-1}$, corresponding to a major axis length of ~400 Å (Perrin, 1936).

The flow birefringence of T4 DNA condensed with polylysine was obtained over a concentration range of from 0.89–97.2 μgm/ml. The birefringence curves at the highest concentrations show some curvature, implying a degree of shear-dependent aggregation, but are linear at the lower concentrations. The calculated intrinsic birefringence is commensurate with a scramble-wound DNA toroid of $p \simeq 1/20$ (Maestre and Kilkson, 1965), but cannot be rationalized by optical modeling in terms of any prolate structure. The extinction angle data are shown in Fig. 2. The curvature and inflection in the results for the unfractionated material is characteristic of a polydisperse system containing a small, highly orientable component. The S = 300 sample has a rotational diffusion coefficient $D_r \simeq 250\ s^{-1}$, leading to an estimated particle diameter of ~1,200 Å (Perrin, 1936).

The CD spectra for the condensed material are highly suppressed over that for the original DNA. These spectra exhibit considerable ψ-like character (Lerman, 1971), and appear similar to those of nucleosomes in low salt solutions (Fasman et al., 1978).

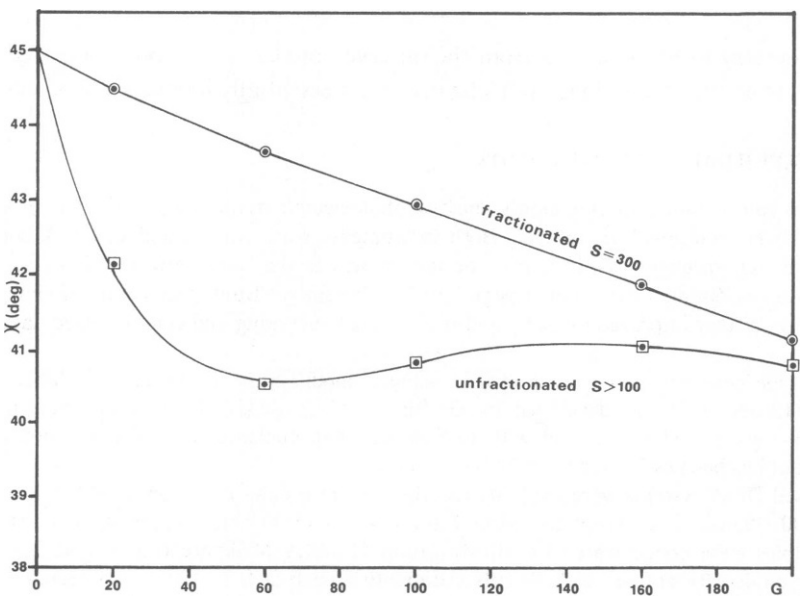

Figure 2 Extinction angles, χ, extrapolated to zero concentration are shown as a function of velocity gradient, G, for: ⊛. fractionated S = 300 peak; and ⊛, unfractionated sample containing a broad band of S ≥ 100. The curvature observed in the latter is characteristic of polydisperse samples.

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