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Conformation of plasmid DNA and of DNA–histone chromatin-like complexes by laser light scattering

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Classical fluctuation and scattering theory has enabled the study of equilibrium properties such as molecular mass, size and intermolecular interactions of synthetic and biological macromolecules. Photon correlation and light beating spectroscopy, using laser radiation, allows the measurement of dynamic properties such as diffusion coefficients and, perhaps, the characterization of internal motion. The recently discovered plasmid DNA molecules are excellent objects of study, being monodisperse, in a convenient molecular mass range, and obtainable in supercoiled, relaxed circular and open linear conformations. Structural transitions can be studied in a variety of solvents and folding properties of DNA investigated. Chromatin can be reconstituted by interaction of DNA under well chosen experimental conditions with a selected group of histones, basic proteins of the nucleoprotein complex in the chromosome. The plasmid DNA–histone interaction leads to well-defined monodisperse chromatin-like complexes; their formation, composition and solution properties have been studied by laser light scattering and related physico-chemical methods.

Introduction

The study of the intensity of light scattered from dilute solutions of macromolecules has been a rewarding field of investigation for over three decades (the classical papers in this field have been collected by McIntyre & Gornick 1964). It received great impetus as a result of the World War II activities of Peter Debye in connection with the synthetic rubber programme and consequently Hermann Mark was able to gather a brilliant group of young scientists comprising, at one time or another, Bruno Zimm, Paul Doty, Walter Stockmayer and many others, at the fledgling Polymer Institute of the Brooklyn Polytechnic. Both theory and experiment progressed rapidly, based on ideas that had been available in the literature since the turn of the century; for instance, Einstein's theory of density fluctuations, leading to the thermodynamic analysis of scattering from pure liquids, later extended by Debye to the analysis of equilibrium fluctuations of solute concentration; Debye's own analysis in 1915 of the structure factor, or rotationally averaged angular dependence of scattering of X-rays in solutions; the work of Cabannes and Krishnan on the nature of the polarization of the incident and scattered light, and finally the empirical experimental studies of Putzeys and Brosteaux on the scattering from solutions of proteins and other biopolymers. Moderate confusion, already envisaged by Debye, arose from the fact that, for mixed solvents, the treatment of fluctuations had to be extended over more than the two classical components, solute and solvent (Stockmayer 1950); in the case of charged macromolecular species in solutions of simple salts, usually encountered in the study of the biological systems, it was possible, following the definitions of George Scatchard, to treat fluctuations of conveniently defined electroneutral components (Casassa & Eisenberg 1964).

Basically, from the angular and concentration dependence of the total intensity of the

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'elastically' scattered light, it is possible to derive the number of particles per unit volume (from which molecular masses may be defined by suitable definitions of mass concentration units), the model-independent radius of gyration R_g of the particles (as well as other, model-dependent, size and shape parameters, under favourable circumstances) and the interparticle interaction parameter, usually expressed as the second virial coefficient A_2. In the case of a distribution of sizes of otherwise chemically or optically identical macromolecules, suitably weighted average quantities may be derived.

In the following we will discuss early results on scattering from DNA solutions, leading to modern development based on the introduction of the laser into light scattering technology. Next we shall describe recent results on conformational states of the Col E_1–plasmid DNA and its complexes with histones. The high histone content of these complexes led us to study the binding of additional histones to nucleosome core particles. Finally, we mention the use of sunlight to study macromolecular dynamics by correlation of scattering intensity fluctuations. References quoted in this article are not exhaustive. They are amply sufficient, though, for a correct evaluation by the interested reader of the historical topics under discussion.

SCATTERING FROM DNA SOLUTIONS: EARLY RESULTS

The developments indicated above were adequate for the analysis of the properties in solution of a large number of synthetic and biological (globular proteins, for instance) macromolecular systems. Evident failure was encountered in the analysis of scattering from nucleic acids, in particular DNA solutions, owing to circumstances which became evident only in recent years (Eisenberg (1971) has reviewed problems encountered in the study of scattering of light and X-rays from nucleic acid solutions). A favourite object of study was preparations of DNA obtained from the calf thymus gland in the form of linear polydisperse fragments, hopefully exhaustively free from traces of proteins with which they strongly interact in their natural form. These were reduced in mass from the large native value of 10^{11} (the approximate size of the intact chromosomal DNA chain) to the relatively small value of about 20×10^6, as the result of extreme sensitivity to even the mildest unavoidable shear in the purification procedure. The intact structure, meant to preserve and reproduce the complete genetic apparatus of the respective animal, is exquisitely folded with histone and non-histone proteins in complexes, about which more will be said below. It might appear to the uninitiated that the study of these DNA chains, drastically reduced from their native size, might be quite useless, and yet a great deal of important structural and functional information could be obtained as long as the base-stacked double helical structure of DNA was conserved; as a matter of fact this celebrated DNA model was obtained from the analysis of X-ray diffraction of such fragmented DNA chains (Watson & Crick 1953).

In classical light scattering, the angular dependence of scattering, in the limit of vanishing concentration c (mg/ml), and the concentration dependence, in the limit of vanishing scattering angle θ, are analysed by the two equations

$$\frac{Kc}{\Delta R(\theta)} = \frac{1}{M} P^{-1}(\theta); \quad c \to 0, \tag{1}$$

$$\frac{Kc}{\Delta R(0)} = \frac{1}{M} + 2A_2 c; \quad \theta \to 0, \tag{2}$$

where K is a constant, $R(\theta)$, the Rayleigh ratio (corrected for solvent scattering), is a measure of the light scattered at angle θ, M is the molecular mass, $P(\theta)$ reflects the reduced scattering intensity due to interference from different parts of the same molecule ($P(0) = 1$), and A_2 is the second virial coefficient (Zimm 1948).

For small values of the dimensionless parameter $X \equiv q^2 R_g^2$ ($q = (4\pi n/\lambda) \sin \frac{1}{2}\theta$ is the scattering vector, n and λ are the refractive index and the wavelength (*in vacuo*) respectively), $P^{-1}(\theta)$ or, more precisely, $P^{-1}(X)$ can be expanded in powers of X, for small X

$$P^{-1}(X) = 1 + \tfrac{1}{3}X + \dots . \tag{3}$$

The complete expression has been calculated for various models (Tanford 1961).

The double stranded linear DNA structure freely coils in solution, under the influence of brownian motion, with continuous curvature of the chain. Its configuration is best described by the persistence length a of the wormlike Kratky & Porod (1949) coil. This is a statistical concept representing the sum of the average projections of all chain elements, in an indefinitely long chain, on the direction of the first element. According to Landau & Lifshitz (1958) $a = EI/kT$, where E is the Young modulus and $I = \frac{1}{4}\pi r^4$, the moment of inertia of the cross section of a uniform flexible rod with radius r. For chains containing a large enough number of persistence lengths, Gaussian coil behaviour becomes an adequate representation; the Kuhn (1934) equivalent chain is composed of linear statistical elements (of length equal to twice the value of a) connected by freely rotating links.

For the calf thymus DNA chains considered above ($M = 20 \times 10^6$) we calculate (on the basis of an average molecular mass of 660 per base pair and a 0.34 nm contribution, per base pair, to the contour length in the high humidity B conformation of the DNA) a contour length L equal to 10^4 nm; with $a = 50$ nm (Voordouw *et al.* 1978), we may use the Gaussian limit $R_g^2 = \frac{1}{3}aL$ to calculate $R_g = 400$ nm. Classical light scattering studies with conventional light sources were usually not carried below a value of $\theta = 30°$, from which we calculate a lower limit of $X = 12$ with $\lambda \approx 500$ nm. From Debye's equation for the scattering of Gaussian coils we calculate $P^{-1}(X) = 6.6$. Clearly, extrapolation to $\theta = 0$ ($P(0) = 1$) was out of the question, and simple minded application of the limiting law, equation (3), yielded $M \approx 6 \times 10^6$, together with improbable and widely scattering values for R_g and the persistence length a. When this situation was eventually appreciated, it was possible, with a great deal of effort, to reduce θ to $10°$, for which we calculate $X \approx 1.4$ and $P^{-1}(\theta) \approx 1.5$, a situation only marginally more satisfactory.

SCATTERING FROM DNA SOLUTION: MODERN DEVELOPMENTS

The rather unsatisfactory situation described above has been radically improved in recent years as a result of (*a*) the availability of smaller, monodisperse DNA samples, intact in their original biological form (Helinski & Clewell 1971) and (*b*) the introduction of a new scattering technology, based on the use of high intensity, monochromatic, coherent laser beams (Cummins & Pike 1974). The use of easily focused fine laser beams allowed considerable reduction of sample size (a prime consideration when working with scarce biological materials) and reduction, without undue effort, of the lower limit of θ to $6°$ (Jolly & Eisenberg 1976). As will be seen below, reliable total intensity scattering data can now be obtained on well defined experimental systems.

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In addition, completely novel experiments could now be designed, based not on the time averaged fluctuations in solute concentration, but rather on the time evolution of fluctuations deriving from translational, rotational and internal modes of motion of the particles under investigation. The dominating quantity derivable by the now well established methods of photon correlation spectroscopy is the translational diffusion coefficient D_t and, for small, rigid, isotropic objects, this is the only quantity that can be gleaned from the experiments. In the case of large, flexible, anisotropic macromolecules, such as the DNA particles of interest to us here, additional parameters characteristic of molecular motions are convoluted with D_t and can, in principle, provide interesting dynamic information of a higher order on molecular motion of the DNA molecules. We have shown that an 'apparent' translational diffusion coefficient derived from DNA solutions strongly depends on the scattering vector q, and the true value of D_t is obtained only by extrapolation to low values of q (Jolly & Eisenberg 1976). This is truly a new dimension in the use of light scattering as applied to systems of biological interest and, in conjunction with related experimental methods, is capable of providing new information of significant value (for a comprehensive recent review see Schurr 1977).
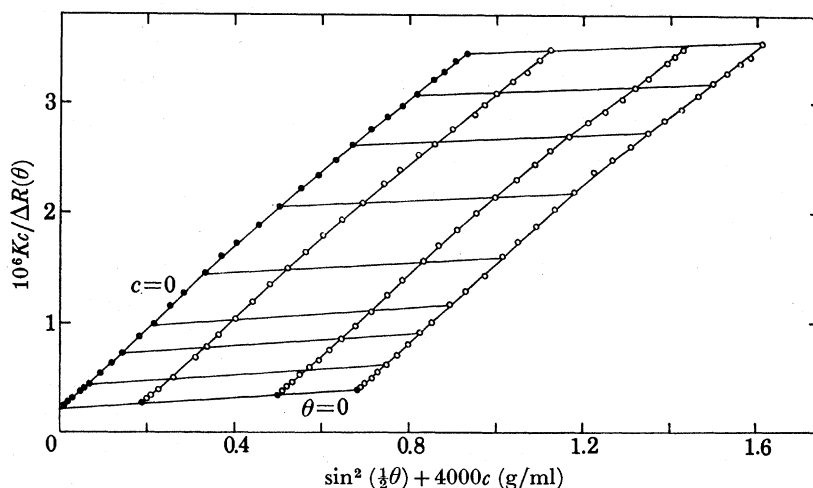

FIGURE 1. Total intensity light scattering Zimm plot for Col E_1–plasmid DNA, form III, over wide angular range. Experimental conditions: 23 °C, 0.2 M NaCl, 0.002 M $NaPO_4$ pH 7.0, 0.002 M EDTA.

THE COL E_1–PLASMID DNA MOLECULE

The Col E_1–plasmid DNA is an extrachromosal DNA of moderate molecular mass. It has a well defined function in the life cycle of the *Escherichia coli* bacterium. A large number of copies, up to 3000, of the plasmid are produced per bacterium in the presence of the anti-biotic chloramphenicol. Replication of chromosomal DNA ceases under these conditions (Clewell 1972). Thus, from bacterial cultures harbouring the plasmid DNA, it is possible to isolate large amounts (10–20 mg) of pure superhelical (form I) DNA, free of the much higher molecular mass chromosomal DNA. Mild digestion of the covalently closed superhelical form I with pancreatic nuclease releases the topological constraints and yields the circular relaxed DNA form II. Col E_1 DNA presents one restriction site to the EcoR1 restriction enzyme, and therefore action of this enzyme on the form I DNA yields a completely homogeneous population of linear form III coils of plasmid DNA. These three DNA forms have been studied by light

scattering and other (mainly sedimentation and viscosity) methods. While form III is completely homogeneous by all criteria used, forms I and II contain a small percentage contamination of dimeric and possibly trimeric components, and additionally samples of the superhelical form I contain an unavoidable small number of molecules in the circularly relaxed form II (Voordouw *et al.* 1978).

The results of the classical total intensity light scattering studies on Col E_1–plasmid DNA are exemplified by the conventional Zimm double extrapolation plot of form III (figure 1) taken over a wide range of scattering angles. The $P^{-1}(\theta)$ functions, in the small angle region only, for all three forms, extrapolated to vanishing concentration, are given in figure 2. For the analysis of the linear form III (curves *a–c*), a few words of background explanation are necessary.
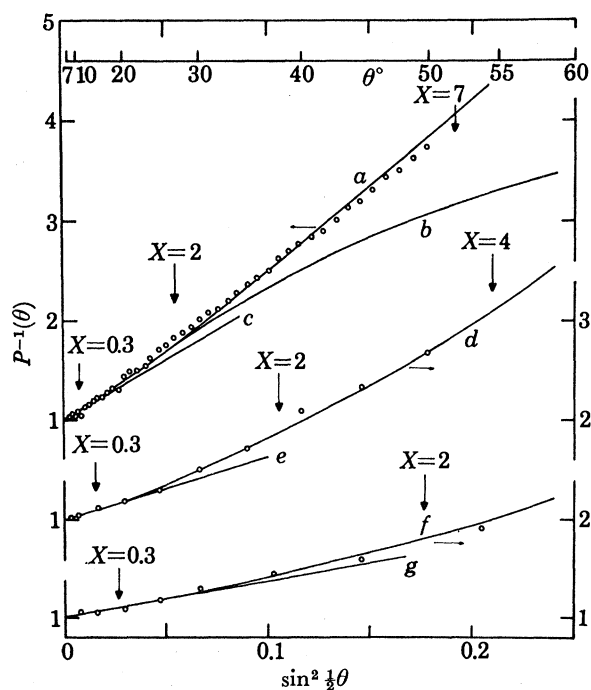

FIGURE 2. Angular dependence at low angles of scattering of Col E_1–plasmid DNA, form I, supercoiled (lower set of data); form II, circular rings (middle set of data); form III (top set of data) at low angles of scattering. Theoretical curves (*a*) Gaussian coils $R_g = 186$ nm; (*b*) thin rods, same R_g as (*a*); (*c*) Zimm limiting slope, same R_g as (*a*); (*d*) Gaussian ring, $R_g = 134.2$ nm; (*e*) Zimm limiting slope, same R_g as (*d*); (*f*) Supercoiled Y with equal arms, $R_g = 103.5$ nm; (*g*) Zimm limiting slope, same R_g as (*f*). Experimental conditions as in figure 1. Data extrapolated to vanishing DNA concentration.

Classical expressions of $P(X)$ for random Gaussian coils and for rigid rods are available in closed form. For values of $X < 2 (P^{-1}(X) \approx 1.75)$ it is not possible to distinguish between Gaussian coils and rigid rods having the same value of R_g. The linear Zimm limiting slope (equation 3) is valid for values of $X < 0.3$ $(P^{-1}(X) = 1.1)$ only, a limit rarely achieved in practice with sufficient accuracy. Scattering curves for the Kratky–Porod persistent wormlike coil are available in approximate form; for values of $X < 7$ $(P^{-1}(X) \approx 4)$ it is not possible to distinguish between the Gaussian and the persistent chain (Sharp & Bloomfield 1968; Yamakawa & Fuji 1974).

We have, in figure 2, fitted the experimental data on form III DNA to the Gaussian coil expression, curve *a*, with $R_g = 186$ nm. All data correspond to $X < 7$, which justifies the

procedure used. Curve *b* corresponds to the rigid rod, and curve *c* is the limiting linear behaviour. It will be appreciated that a quite reasonable 'limiting' straight line (considering the experimental inaccuracy) through all points up to $\theta = 50°$ would have yielded an incorrect value for R_g.

We next consider the circular form II. The theoretical curve *d* has been calculated according to the theory of Casassa (1965) for a Gaussian ring with $R_g = 134.2$ nm. This is the best fit to the experimental points. The theory predicts $R_g^{coil}/R_g^{ring} = \sqrt{2}$, and this is well observed by our experiments $186/134.2 = 1.39$. Curve *e* is the Zimm limiting slope. Curve *f* is for form I and has been calculated by Jolly & Campbell (1972) for a Y-shaped molecule with $R_g = 103.5$ nm; curve *g* is the limiting slope.
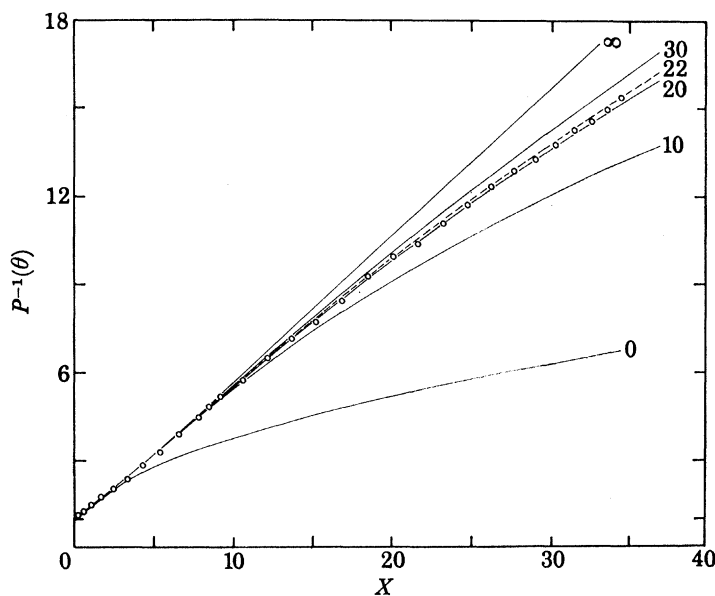

FIGURE 3. Angular dependence of scattering of Col E_1–plasmid DNA, form III, at high scattering angles. Experimental points from figure 1, at vanishing DNA concentrations, normalized to unity at $X = 0$ (from the data of low angles) and scaled to $X \equiv q^2 R_g^2$ ($R_g = 186$ nm). Theoretical curves for $L' \equiv L/2a = \infty$ (Gaussian coils), $L' = 30, 22, 20, 10$ (stiff coils) and $L' = 0$ (rigid rods).

The three conformational forms are well described. In figure 3, we present $P^{-1}(\theta)$ for form III over an extended angular range, up to $\theta = 150°$. The theoretical curves have been calculated for various values of the parameter $L' = L/2a$, the number of statistical elements per macromolecular chains; $L' = \infty$ corresponds to the random coil, $L' = 0$ to the rigid rod; intermediate values of L' correspond to calculations for stiff coils (Sharp & Bloomfield 1968). The experimental points have been scaled with the use of $R_g = 186$ nm (from the low angle data) and they follow closely the curve expected with $L' = 22$. Molecular quantities derived from these and other experiments are summarized in table 1.

We now turn to figure 4, in which 'apparent' translational diffusion constants D are derived from the decay curves with time in an autocorrelation experiment of fluctuations of the scattered light. For small isotropic particles, the decay time τ is exponential and $D = 1/\tau q^2$. For DNA the decay curve is a simple exponential at small values of q only; a two-exponential or single forced exponential fit at higher values of q yields increasing 'apparent' values of D

with increasing q. That the limiting values at low q indeed yield the correct translational diffusion coefficient has been tested by obtaining a value $M_{s,D}$ (the molecular mass derived from the Svedberg equation)

$$M_{s,D} = RTs/D(1-\phi'\rho), \tag{4}$$

consistent with the mass average molecular mass M_w from total intensity light scattering (see table 1). In equation 4, s is the sedimentation coefficient and $1-\phi'\rho$ a buoyancy term appropriate for multicomponent systems (Eisenberg 1976).
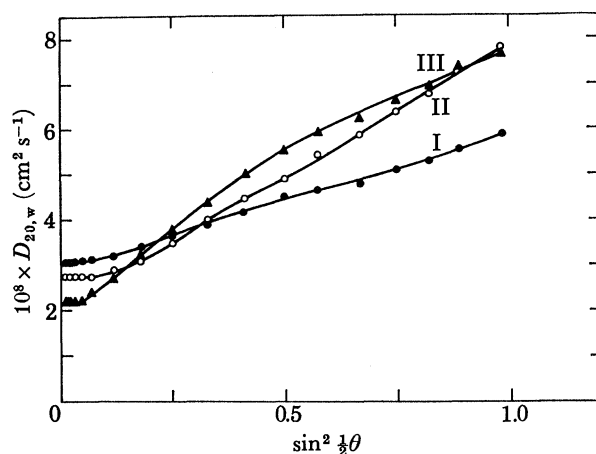

FIGURE 4. Apparent translational diffusion coefficients, $D_{20,w}$, obtained from a single exponential fit to the auto-correlation function as function of the scattering angle θ for the three forms of Col E_1–plasmid DNA. Experimental conditions as in figure 1. DNA concentrations: I, 0.275 mg/ml, II, 0.198 mg/ml, III, 0.258 mg/ml.

TABLE 1. COL E_1–PLASMID DNA, PHYSICAL PROPERTIES OF THREE CONFORMATIONAL FORMS IN SOLUTION AND OF THE SATURATED COMPLEX WITH CALF THYMUS HISTONES H2A, H2B, H3 AND H4

	$10^{-6} \times M_w$	$\dfrac{R_g}{nm}$	$s^0_{20,w}(S)$	$\dfrac{10^8 \times \langle D^0_{20,w} \rangle}{cm^2\,s^{-1}}$	$10^{-6} \times M_{s,D}$	$\dfrac{10^4 \times A_2}{mol\,ml^{-1}\,g^{-2}}$
Col E_1–I	4.59	103.5	24.5	2.89	4.69	0.70
Col E_1–II	4.15	134.2	18.8	2.45	4.37	4.8
Col E_1–III	4.30	186	16.3	1.98	4.39	$\tilde{5}.4$
complex	15.5	56.2	89	4.11	17.5	0.33

Measurements of the free, uncomplexed DNA in 0.2 M NaCl, 0.002 M NaPO$_4$ pH 7, 0.002 M EDTA. Measurements of the complex in 0.4 M NaCl, 0.1 M Tris pH 7.5, 0.01 M EDTA. Sedimentation and diffusion recalculated to standard conditions (water, 20 °C). Other experiments at 23 °C.

The behaviour (figure 4) at higher values of the scattering vector has not yet been properly analysed, yet it is without doubt indicative of dynamics, such as rotation or internal modes of motion, convoluted with the translational motion. Form I, which is the most compact and rigid, has the highest translational diffusion and lowest dependence of the apparent D on q; for form III the opposite is true, and form II is intermediate. We hope to be able to present a complete analysis of this interesting manifestation in a future communication. It has also previously been observed with less well defined systems (Jolly & Eisenberg 1976; Schurr, 1977) and has so far not been satisfactorily explained.

COMPLEXES OF COL E₁–PLASMID DNA AND HISTONES

We have next studied by both total intensity and quasi-elastic light scattering, as well as by other methods, purified monodisperse reconstitution complexes of Col E_1–plasmid DNA and saturating amounts of the four core histones H2A, H2B, H3 and H4 (Voordouw *et al.* 1977; Voordouw *et al.* 1978). It is feasible to reconstitute the lowest order 'bead' structure of chromatin, in the absence of magnesium ion and H1 histone, in which the four histones mentioned form a double tetramer, or octamer, around which DNA completely supercoils (Oudet *et al.* 1975). This basic repeating unit of chromatin, which can also be obtained by enzymatic digestion from chromatin as the so-called nucleosome core particle, is believed to be roughly cylindrical, with diameter = 11 nm and height 5.7 nm (Pardon *et al.* 1977; Finch *et al.* 1977). One of the questions which motivated our conformational studies of DNA in solution was how the tight DNA supercoiling in chromatin, or in other structures such as phage heads, for instance, is achieved by regular coiling and compacting an essentially bulkier structure. It should also
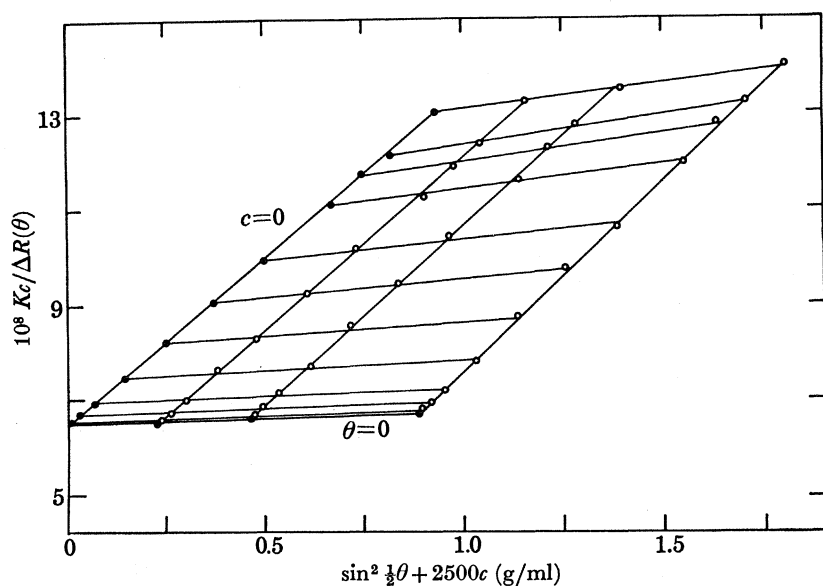

FIGURE 5. Zimm plot of the total intensity laser light scattering results for the saturated complex of linear Col E_1–III plasmid DNA with calf thymus histones H2A, H2B, H3 and H4, in 0.4 M NaCl, 0.1 M Tris pH 7.5, 0.01 M EDTA at 20.5 °C.
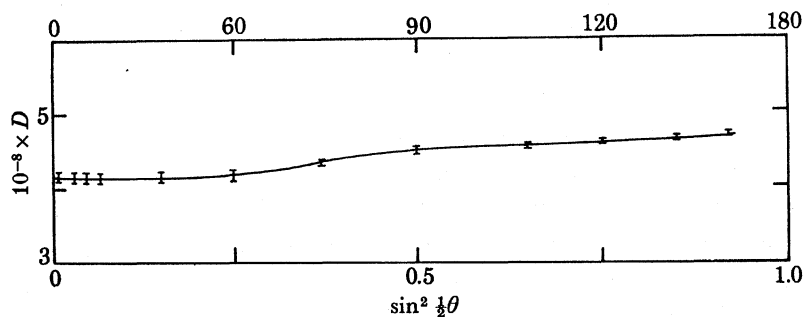

FIGURE 6. Apparent translational diffusion coefficient, $D_{20.5}$ obtained from a single exponential fit to the autocorrelation function of the quasi-elastic laser light scattering experiment as function of the scattering angle θ for the DNA–histone complex. Experimental conditions as in figure 5.

be mentioned here that the DNA–histone complex, at least at this organizational level, is rather unspecific, to the extent that the fact that bacterial plasmids do not at any instant of their life cycle contact histones, is not considered objectionable. No reconstitution is observed in the absence of histone H4.

Total intensity light scattering for the purified complexes is given in figure 5 and the diffusion constant D in figure 6. We note that the latter, in contrast to the three forms of the free DNA (figure 4) is almost independent of the angle of scattering. The comparison of the molecular parameters of the reconstitution complex of form III DNA with the original DNA (table 1) is instructive. We note, from molecular mass determinations by both classical light scattering (M_w) as well as from the Svedberg equation ($M_{s, D}$), that in the reconstitution complexes about 2.5–3 g of histones are bound per g of DNA. This is considerably in excess of the ratio of about 1.2–1 believed to characterize chromatin. We note that, although there is a big increase in molecular size, compaction of the structure leads to a considerable decrease in R_g. Repulsive interaction between particles, expressed as the second virial coefficient A_2, also decrease strongly upon complex formation. Higher order structures will also be studied following addition of magnesium ion and H1 histone.

BINDING OF ADDITIONAL HISTONES TO NUCLEOSOME CORE PARTICLES

The large unexpected excess of histones in the reconstituted structure, also documented by careful analysis of chemical composition, led us to perform an additional experimental study (Voordouw & Eisenberg 1978). We also had grounds to believe, from various indications in the literature, that a variable amount of histones to DNA in the chromatin structure might be related to a significant function (see, for example, Grellet *et al.* 1977). We recall here that native chromatin contains a considerable amount of non-histone proteins (in addition to histones), which together with H1 histone, are removed when the ionic strength is raised above 0.6 M NaCl (Van Holde & Isenberg 1975). Between 1.5 and 2 M NaCl, chromatin is completely dissociated into DNA and histones, and controlled lowering of the ionic strength to 0.4 M NaCl is the basis of the chromatin reconstitution procedure.

In the experiment referred to, nucleosome core particles from calf thymus and from chicken erythrocyte chromatin were prepared in normal fashion (Dr J. F. Pardon and Mrs M. Jacobs kindly provided well characterized chicken erythrocyte nucleosome core particles). It was then shown that at 0.5 M NaCl these core particles could bind additional histones (from an equimolar ratio of the four core histones) up to about a total ratio of about 2.5–3 g of histones per g of DNA, without significant change in the size and shape of the core particles. Monodisperse enriched particles were obtained with almost unchanged, or slightly decreased, diffusion coefficient derived from light scattering. Further work is in progress and for more detailed studies of these particles, which are rather small with respect to the wavelength of the light, small angle X-ray scattering studies are in progress. Neutron scattering contrast variation studies in mixed hydrogen–deuterium aqueous solvents can also provide valuable information on the location of nucleic acid and histones in the chromatin structure (Hjelm *et al.* 1977; Pardon *et al.* 1977).

MACROMOLECULAR DYNAMICS BY SUNLIGHT SCATTERING INTENSITY FLUCTUATION

We have mentioned above the difficulty in deconvoluting additional information from the dominating translational diffusion in studies of the decay of the autocorrelation at various angles. Such deconvoluted information has also been obtained in selected instances by the study of the depolarized scattered light. In a novel application, one of us (Z.K.) and S. Reich from this laboratory (S. Reich & Z. Kam, in preparation), suggest the use of sunlight, readily available in our country, for studying rotational and internal dynamics of single macromolecules directly, in an autocorrelation study. Scattering from Tobacco Mosaic Virus (TMV) particles is observed at right angles from sunlight collected by a Questar telescope, and the decay of fluctuations is analysed by the Malvern digital autocorrelator, also used in our other experiments. Owing to the essentially spatial and temporal incoherent nature of the source (experimental conditions are arranged such that, at most, one virus particle occupies one coherence volume at any one time), translational diffusion does not contribute to the correlation function, which becomes sensitive only to scattering fluctuations due to the rotational motion of the TMV particles. The autocorrelation function is built up within a few minutes of data collection and can be identified with rotational diffusion. Under these conditions very little correlation is obtained with standard isotropic polystyrene particles, yet when a laser is substituted as light source, the dominant translational diffusion autocorrelation is quickly recovered.

CONCLUSIONS

In this contribution we have aimed at showing, by a number of examples, the manifold applications of light scattering, both in the classical form of total intensity studies, as well as in the more modern applications of dynamical quasi-elastic scattering, to systems of biological interest. We have, in particular, shown that in conjunction with other experimental methods, valuable information may be obtained about chromatin, currently a much investigated nucleo-protein complex. We have also briefly sketched a possible use of an inexhaustible commodity, sunlight, in autocorrelation studies.

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