Conformation zoning of large molecules using the analytical ultracentrifuge

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A substantial proportion of large molecules made naturally or by artificial means exist as linear chains. In biology this includes DNA, mRNA, many important classes of sugar polymers (polysaccharides) and denatured proteins. In physical science this includes polyethylene, polyvinylchloride and many important polymers used in plastics and also the many new ones being explored for use in drug delivery. Crucial to how many of these large molecules function is their conformation *in solution* (either aqueous or organic), a realm unfortunately outside the grasp of high-resolution techniques such as X-ray crystallography. We have now however devised a quick and accessible method for identifying the conformation type or "Zone" of a molecule: Zone A (extra rigid rod type); Zone B (rigid rod type); Zone C (semi-flexible type), Zone D (completely random coil) and Zone E (compact or highly branched particle). To perform this "Conformation Zoning" requires a few milligrams of material and access to one of the new types of high-speed Centrifuge which are now proliferating in academic and industrial establishments. © 1997 Elsevier Science B.V.

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1. Introduction: conformation zones

How large molecules behave in solution is the subject of increasing interest amongst biological and physical scientists, and there have been several exciting developments in methodology devised to study this behaviour. One of the more interesting developments has been the revival of analytical ultracentrifugation as a molecular probe [1,2], for which there have been outstanding recent advances in instrumentation, optimising the ease and precision of data capture. Whereas most of the attention appears to have been directed towards folded structures such as proteins, the latter represents only a minority of the vast array of large molecules ("macromolecules") a substantial proportion of which are based on a linear chain (or parallel chains) template which can be linked together by non-covalent interactions. These large linear molecules in solution, along with their folded (e.g. proteins) or branched (e.g. starch amylopectin) counterparts can adopt a variety of overall conformations depending on their chemical structure and the medium in which they are dispersed (Fig. 1A).

For example a DNA can exist as a rigid rod shape or "Zone A" structure or a tightly condensed structure (Zone E) depending on the environment in which it is dispersed. As a further example, sugar polymers (polysaccharides) can have a range of conformations from extremely rigid rod structures (Zone A) through to perfectly random flexible coils (Zone D). How do we assign these conformation types? Although a small minority of macromolecules – of which globular proteins are an example – can be characterised precisely by high resolution crystallographic and magnetic resonance techniques, the majority are not accessible, particularly those which cannot be crystallized or are highly polydispersed: in biology this includes DNA and mRNA, polysaccharides – until recently the Cinderella molecules of biology – and a vast array of glycoconjugates. For these types of molecule, dilute solution ultracentrifugation-based characterisation methods are invaluable. We propose now a completely novel but nonetheless simple way to assign a molecular conformation type or "zone"

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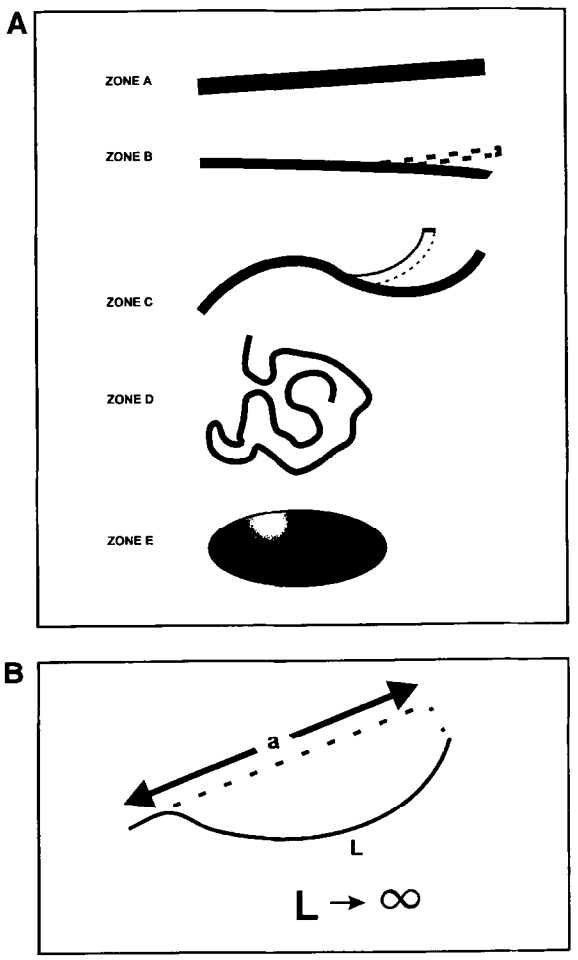

Fig. 1. (A) The five conformation zones for large molecules. (B) Length parameters for a linear macromolecule. L = contour length, a = Persistence length (defined as the projection length along the initial direction of a chain of length L and in the limit of $L \rightarrow$ infinity).

based on a single experiment in the analytical ultracentrifuge, with the appropriate optical system (such as the XL-A ultracentrifuge with absorption optics [5] and the XL-I ultracentrifuge with both absorption and interferometric optical recording systems [16]).

2. Some basic macromolecular characteristics

Let us first consider some of the basic characteristics of linear polymer chains in solution. Two of the most important parameters representing a polymer chain are its molecular weight (M), and its contour length, L. The mass per unit length (M_L) is defined as simply M/L and is known or can be measured from e.g. electron microscopy [3] X-ray fibre diffraction studies [4]. M itself can be measured by a variety of techniques such as sedimentation equilibrium [2,5] or light scattering analysis [6]. A further significant parameter (Fig. 1b) is the persistence length [7], a, which is a measure of how far a polymer chain "persists" along the same direction as one of its ends starts out in, before Brownian motion etc. drags it away. Its value takes into account complicated behaviour such as excluded volume effects in flexible chain "Zone D" polymers or draining effects in rigid chain "Zone A and B" polymers or both (semi-rigid or semi-flexible "Zone C" chains) [8]. There are other equivalent parameters (such as the Kuhn length l), but to keep things simple we just stick with M, L, $M_L = M/L$ and a as our fundamental parameters.

3. Sedimentation parameters

Let us now consider the large amount of data that has been published on the sedimentation properties of macromolecules of *known* class or zone (A–E). (In general the persistence lengths vary from $\sim$200 nm for Zone A macromolecules progressively down to $\sim$ 1 nm for Zone E): The key experimental parameters are the sedimentation coefficient s_0 (seconds) and the concentration dependence parameter of the sedimentation coefficient, k_s (cm^3/g). s_0 is simply the sedimentation rate per unit centrifugal field extrapolated to $c \rightarrow 0$, and comes, along with k_s from fitting the relation $1/s = (1/s_0)(1+k_s c)$ to the sedimentation coefficient s measured at a variety of molecular concentrations, c (g/cm^3).

The sedimentation coefficient has to be adjusted so as to allow for buoyancy effects of the particular solvent used during the sedimentation experiment, to give the intrinsic sedimentation coefficient $[s]$ defined by $[s] = \{s_0\eta_0/(1-v\rho_0)\}$, where η_0 is the viscosity of the solvent, ρ_0 is the density of the solvent and v a parameter known as the "partial specific volume" (essential the reciprocal density of the polymer) which is well known for a wide range of polymers [8]and can be precisely measured without undue difficulty ($\sim$0.73 cm^3/g for proteins, $\sim$0.5–0.6 cm^3/g for nucleic acids $\sim$0.50–0.60 cm^3/g for polysaccharides and $\sim$0.65–0.70 cm^3/g for polystyrene).

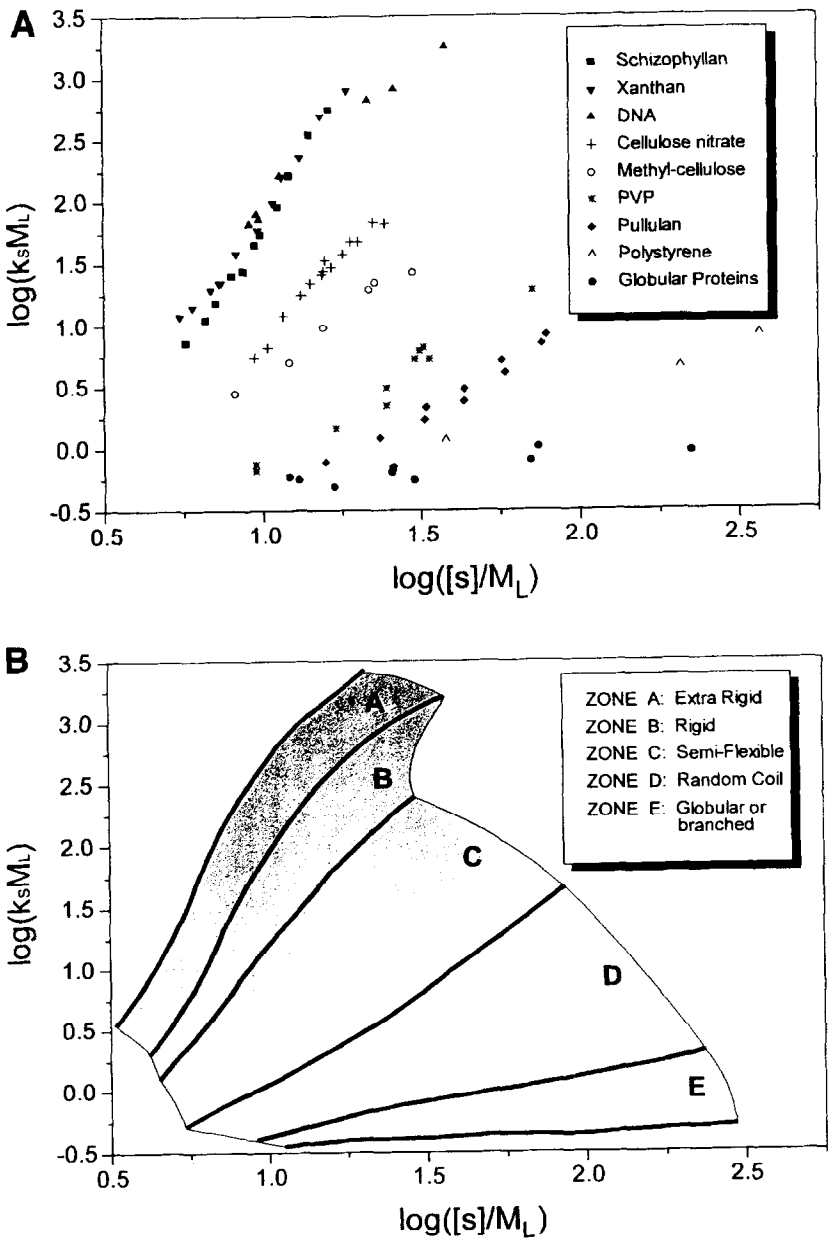

Fig. 2. Sedimentation conformation zoning. (a) Dependence of $\log(k_s M_L)$ versus $\log([s]/M_L)$for 82 macromolecules of known conformation type [9–15]: 10 Schizophyllans (M_L = 215 Daltons. A^{-1}), 12 Xanthans (M_L = 194 Daltons. A^{-1}) and 7 DNA's (M_L = 195 Daltons.A^{-1}) (Zone A); 14 cellulose nitrates (M_L = 55 Daltons.A^{-1}) (Zone B); 6 methyl celluloses (M_L = 36 Daltons. A^{-1}) (Zone C); 10 polyvinylpyrrolidones (M_L = 44 Daltons.A^{-1}), 10 pullulans (M_L = 33.8 Daltons.A^{-1}) and 3 polystyrenes (M_L = 41 Daltons. A^{-1}), (Zone D); 10 Globular proteins (Zone E). All molecules in dilute solution conditions. (b) Corresponding "sedimentation conformation zoning" plot.

4. The conformation zone plot

Now consider a plot of $k_s M_L$ versus $[s]/M_L$. In Fig. 2a we have accumulated a large amount of data [9–15] (more than 80 data points) for molecules of known conformation class (including our own recent data for methyl cellulose [11] and for pullulan [13]) and from this *empirically* constructed the conformation zones of Fig. 2b. Although for convenience lines – drawn simply as guides – have been used to separate each zone, it should be stressed that the boundaries between each must be regarded as a continuum (one zone merges into the next etc.)

It is quite clear from Fig. 2 that there is a definite empirical relationship between $k_s M_L$ and $[s]/M_L$ specific for each conformation class or Zone. Measurement of $[s]$ with k_s along with knowledge of M_L

(from chemical structure, electron microscopy or x-ray scattering) is therefore quite sufficient to pinpoint the conformation type of a macromolecule. This procedure represents a considerable advantage over earlier methods which either could not distinguish between flexible coils and compact spheres, or required fractionation of a macromolecule into different molecular weight species: the sedimentation conformation zoning plot distinguishes between the 5 zonal types without the need for molecular fractionation and multiple measurements. Further, since modern ultracentrifuges permit the measurement of several solution concentrations in the same run (multiple hole rotors and appropriate data multiplexing), s_0 and k_s can be measured in a single experiment [16].

It can be seen also from Fig. 2 that the plots appear to converge to a single point: this is not surprising since in the limit L (polymer) $\rightarrow L$(monomer unit) the distinction between the various conformation types must vanish.

5. Theoretical basis

An accidental discovery? This empirical finding – although at first sight apparently fortuitous – on close scrutiny is no accident and has a clear theoretical basis:

The problem of predicting the form of the relationship between $\log(k_s/M_L)$ and $\log([s]/M_L)$ can be simplified down, for a log–log relationship, to considering simply the exponent of the first bracketed term with respect to its argument (the second bracketed term). This will be the slope of the plot. We consider two extreme cases: infinitely long rigid rods and perfect spheres:

For infinitely long rods, treated as prolate ellipsoids, (of semi-axes $a > b$) it has been known for over 40 years [17] that the frictional ratio (the ratio of the friction coefficient of the molecule to that of a sphere of the same mass and volume) is, for a given width, directly proportional to the axial ratio (a/b), and that hence the sedimentation coefficient can be treated as a linear function of mass/unit length. From this, it immediately follows that the size-related term s/M_L is linear in $(a/b)^{-1}$, and the problem is to determine the exponent of k_s/M_L with respect to $(a/b)^{-1}$. This falls out simply. From the fact that k_s varies for highly elongated particles simply with the cube of the frictional ratio, and that the frictional ratio itself, as noted by Peacocke and Schachman [17] a linear function of (a/b), and since M_L is itself not a function of (a/b), it follows at once that the exponent will have the value $3-(-1)=4$. In other words, the log–log plot will have a slope of 4.

By contrast, for perfect spheres the terms k_s and M_L are both invariant in (s/M_L) and hence the predicted slope of the log–log plot must be zero. Basically the difference between the two cases (long rod vs. sphere) arises predominantly from the fact that the regression coefficient k_s is a shape (extension) related function for the former case but not for the latter. Since it is difficult to conceive of a shape which would generate friction more rapidly with respect to extension than an infinitely long rod, we may presume that all other shapes would generate log–log plots with a slope intermediate between zero and 4.

Inspection of the empirical data of Fig. 2 shows a clear confirmation of all these theoretical predictions.

6. Concluding remarks

The molecular conformation itself – as represented by its Zone – will be influenced by (i) the equilibrium rigidity of the chain (ii) the (cross-sectional) diameter of the chain; (iii) the thermodynamics of polymer–solvent interaction, with the most important being the equilibrium rigidity of the chain.

Measurement of simple sedimentation – the ease and precision of measurement of which is getting greater and greater with the several major instrumental developments – should be sufficient to *unambiguously* specify the conformation class of a macromolecule. Furthermore, monitoring any *changes* in conformation type of a macromolecule in response to a change in the solvent environment it finds itself in (e.g. the condensation of DNA in the presence of polycations or the effect of changing salt ion concentration on the glycoproteins which dictate the protective properties of mucus in the alimentary, tracheobronchial or reproductive systems [18] or in response to genetic alteration (e.g. of plant cell wall polysaccharides [19]) will be considerably facilitated.

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References

[1] H.K. Schachman, Nature 941 (1989) 259–260.
[2] S.E. Harding, TrAC 13 (1994) 439–446.
[3] B.T. Stokke, A. Elgsaeter, Micron 25 (1994) 469–491.
[4] L.E. Alexander, X-Ray Diffraction Methods in Polymer Science, Krieger, Huntington, NY, 1979.
[5] G. Ralston, Introduction to Analytical Ultracentrifugation, Beckman Instruments, Palo Alto, CA, 1993.
[6] S.E. Harding, D.B. Sattelle, V.A. Bloomfield (Eds.), Laser Light Scattering in Biochemistry, Royal Society of Chemistry, Cambridge, 1992.
[7] H. Yamakawa, Modern Theory of Polymer Solutions, Harper and Row, New York, NY, 1971.
[8] E.H. Immergut, J. Brandrup, Polymer Handbook, 3rd ed., Wiley Interscience, New York, NY, 1989.
[9] T. Yanaki, T. Norisuye, H. Fujita, Macromolecules 13 (1980) 1462–1466.
[10] G. Pavlov, A. Kozlov, G. Martchenko, V. Tsvetkov, Vysokomol. Soedin. 24B (1982) 284–288.
[11] G. Pavlov, N. Michailova, E. Tarabukina, E. Korneeva, Prog. Coll. Polym. Sci. 99 (1995) 109–113.
[12] K. Kawahara, K. Ohta, H. Miyamoto, S. Nakamura, Carbohydrate Polym. 4 (1982) 335–356.
[13] G. Pavlov, E. Korneeva, N. Yevlampieva, Int. J. Biol. Macromol. 16 (1994) 318–323.
[14] J.M. Creeth, C.G. Knight, Biochim. Biophys. Acta 102 (1995) 549–558.
[15] C. Tanford, Physical Chemistry of Macromolecules, John Wiley, New York, NY, 1961.
[16] A. Furst, Eur. Biophys. J. 25 (1997) 307–310.
[17] A.R. Peacocke, H.K. Schachman, Biochim. Biophys. Acta 15 (1954) 198.
[18] P.Y. Tam, P. Verdugo, Nature 292 (1981) 340–342.
[19] C.J.S. Smith, C.F. Watson, J. Ray, C.R. Bird, P.C. Morris, W. Schuch, D. Grierson, Nature 334 (1988) 724–726.

Determination of sulphonated azo dyes in water and wastewater

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An overview of current analytical methodologies for the determination of sulphonated azo dyes in environmental samples is presented. Conventional analytical methods involving liquid chromatography and mass spectrometry with the thermospray (TSP) interface are reviewed, as well as the newly developed methods using atmospheric pressure ionisation (API) interfaces. The combination of capillary electrophoresis with mass spectrometry (CE–MS) as a new alternative for the analysis and confirmation of polar compounds (and particularly the sulphonated azo dyes) in environmental samples is also discussed. Finally, the extraction and preconcentration of sulphonated azo dyes from water samples involving various solid phase extraction cartridges are commented upon. © 1997 Elsevier Science B.V.

1. Introduction

Large quantities of dyes are produced and used in diverse applications including textiles, paint pigments, printing inks and food colouring. According to recent information, Western Europe is responsible for nearly 20% of the world dye production, which rose more than 10% annually to 2.2 billion lb. (10^9 kg) in 1994 [1]. The textile industry is the

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