Structural comparisons of the native and reactive-centre-cleaved forms of α_1 -antitrypsin by neutron- and X-ray-scattering in solution

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α_1 -Antitrypsin is the best-characterized member of the serpin (serine-proteinase inhibitor) superfamily. Its solution structure was studied by high-flux neutron-scattering and synchrotron X-ray-scattering. Neutron data show that its absorption coefficient $A_{280,1\text{cm}}^{1\%}$ is 5.4. The neutron radius of gyration R_G at infinite contrast for native α_1 -antitrypsin is 2.61 nm, characteristic of a moderately elongated structure, and its cross-sectional R_G is 1.34 nm. The internal inhomogeneity of scattering densities within α_1 -antitrypsin is high at 29×10^{-5} . The X-ray R_G is 2.91 nm, in good agreement with the neutron R_G of 2.82 nm in $^1\text{H}_2\text{O}$. This R_G is unchanged in reactive-centre-cleaved α_1 -antitrypsin. These parameters are also unchanged at pH 8 in sodium/potassium phosphate buffers up to 0.6 M. The neutron and X-ray curves for native α_1 -antitrypsin were compared with Debye simulations based on the crystal structure of reactive-centre-cleaved (papain) α_1 -antitrypsin. After allowance for residues not visible in the crystallographic electron-density map, and rejoining the proteolysed site between Met-358 and Ser-359 by means of a relatively minor conformational re-arrangement, good agreement to a structural resolution of 4 nm is obtained with the neutron data in two contrasts and with the X-ray data. The structures of the native and cleaved forms of α_1 -antitrypsin are thus similar within the resolution of solution scattering. This places an upper limit on the magnitude of the presumed conformational changes that occur in α_1 -antitrypsin on reactive-centre cleavage, as indicated in earlier spectroscopic investigations of the Met-358–Ser-359 peptide-bond cleavage. Methods for scattering-curve simulations from crystal structures are critically assessed. The R_G data lead to dimensions of $7.8 \text{ nm} \times 4.9 \text{ nm} \times 2.2 \text{ nm}$ for native α_1 -antitrypsin. The high internal inhomogeneity and the asymmetric shorter semi-axes of 4.9 nm and 2.2 nm suggest that the three oligosaccharide chains of α_1 -antitrypsin are essentially freely extended into solvent in physiological conditions. This conclusion is also supported by the Debye simulations, and by modelling based on hydrodynamic parameters.

INTRODUCTION

The human serine-proteinase inhibitors of plasma show a remarkable degree of sequence homology and have been grouped together in a superfamily termed serpins (serine-proteinase inhibitor). The most abundant member of this superfamily in plasma is α_1 -antitrypsin (α_1 -AT) (M_r 51 500), sometimes termed α_1 -proteinase inhibitor (Carrell *et al.*, 1982). Other members include antithrombin III (Carrell *et al.*, 1981), ovalbumin (Kurachi *et al.*, 1981), α_1 -antichymotrypsin (Chandra *et al.*, 1983; Bao *et al.*, 1987), β -plasminogen activator (Ny *et al.*, 1986), α_2 -antiplasmin (Lijnen *et al.*, 1987) and the C1 inhibitor component of complement (Bock *et al.*, 1986). α_1 -AT exhibits its highest affinity towards neutrophil elastase (Travis & Salvesen, 1983). In a healthy individual, α_1 -AT is present at a plasma concentration of 1.3 mg/ml (Jeppsson *et al.*, 1978), but this substantially increases under inflammatory conditions, and is sometimes much decreased in disease. It acts by presenting a reactive site as an ideal substrate to the proteolytic enzyme, and a stable stoichiometric 1:1 complex is formed with the enzyme to inhibit it. This reactive site is the Met-358–Ser-359 peptide bond, which is situated 37 residues from the C-terminus of α_1 -AT (Carrell *et al.*, 1980; Johnson & Travis, 1978). Under certain conditions *in vivo*, a proteolytically modified form of α_1 -AT, in which the reactive site is cleaved, is produced by abortive interaction with many proteinases. This 'split' α_1 -AT is no

longer able to inhibit proteinases. Inhibition by proteolytic cleavage may be important for the regulation of the inhibitor molecule (Carrell *et al.*, 1982; Carrell & Owen, 1985).

Split α_1 -AT is a more stable form of the protein and has a known crystal structure (Löbermann *et al.*, 1982, 1984). Its secondary structure contains three β -sheets and eight α -helices. Since the newly formed C- and N-termini at Met-358 and Ser-359 lie at opposite ends of the molecule and are separated by 7 nm, a significant conformational change must have occurred on cleavage. Using c.d. and fluorescence, Bruch *et al.* (1988) confirmed that the secondary structures of the native and split forms of four serpins (included α_1 -AT) were significantly different. However, no experimental data are available on whether the conformational transition is a localized or a more global re-arrangement of the structure.

Neutron-scattering and synchrotron X-ray-scattering in solution are techniques that are able to provide structural information under physiological conditions to nominal resolutions of 2–4 nm (Perkins, 1988*a,b*). In principle, therefore, any large conformational change between the native and split forms of α_1 -AT should be detectable by both techniques. In addition, they enable comparisons of the physiological structure with those observed in higher phosphate concentrations, including 2.5 M, that from which split α_1 -AT was crystallized. The scattering curves determined experimentally under physiological conditions can also be compared quantitatively with scattering curves

Abbreviation used: α_1 -AT, α_1 -antitrypsin.

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calculated from the crystal co-ordinates of split α_1 -AT. This permits both a careful comparison of the two structures and a critical assessment of the methods used for these calculations. In particular, the comparison allows the average conformation of the three oligosaccharide chains in α_1 -AT relative to the protein surface to be deduced. This is not possible by using other physical measurements of solution structures.

MATERIALS AND METHODS

Purification of α_1 -AT

α_1 -AT purification was based on the thiol-disulphide interchange procedure described by Laurell *et al.* (1975). Briefly, in a typical preparation, 500 ml of fresh frozen plasma was thawed and made 25 mM in 2-mercaptoethanol. An equal volume of saturated $(\text{NH}_4)_2\text{SO}_4$ was then added with stirring, and the resulting precipitate was removed by centrifugation and discarded. A further addition of $(\text{NH}_4)_2\text{SO}_4$ was made (160 g/l; final saturation 72%), and the precipitate was collected by centrifugation. This second precipitate was redissolved in 0.1 M-Tris/HCl buffer, pH 8.1, containing 5 mM-EDTA and 5 mM- NaN_3 , then dialysed exhaustively against the same buffer to remove any residual 2-mercaptoethanol. The dialysed material was loaded on to a GSH-Sepharose column [170 ml bed volume, previously activated by charging with 5,5'-dithiobis-(2-nitrobenzoate), and washed and equilibrated with 0.1 M-Tris/HCl buffer, pH 8.1, containing 5 mM-EDTA and 5 mM- NaN_3], and the column was washed with the same buffer, then buffer containing 1.0 M-NaCl. (The 1.0 M-NaCl wash was necessary to remove a proteinase that otherwise co-purified with α_1 -AT and inactivated the purified inhibitor by specific cleavage within the reactive-centre loop.) α_1 -AT was eluted from the column with 0.1 M-Tris/HCl buffer, pH 8.1, containing 0.5 M-NaCl, 5 mM-EDTA, 5 mM- NaN_3 , 1 mg of 5,5'-dithiobis-(2-nitrobenzoate)/ml and 0.2 mg of dithiothreitol/ml and dialysed, initially against 0.1 M-Tris/HCl buffer, pH 8.1, containing 50 mM-NaCl, 5 mM-benzamidine and 10 mM-2-mercaptoethanol [to effect the total removal of the protein-bound derivative of 5,5'-dithiobis-(2-nitrobenzoate), 3-carboxy-4-nitrobenzenethiol], then against the same buffer in the absence of 2-mercaptoethanol. The α_1 -AT pool was loaded on to a DEAE-Sepharose column (230 ml bed volume) equilibrated in the above final dialysis buffer, and, after a washing with 1 column volume of equilibration buffer, α_1 -AT was eluted with a gradient (8 column volumes) to equilibration buffer containing 0.4 M-NaCl. α_1 -AT-containing fractions were again pooled, concentrated to 20–25 ml and applied to a Sephadex G-150 (superfine grade) column (2 litres bed volume) equilibrated with 20 mM-sodium/potassium phosphate buffer, pH 7.0, containing 0.5 M-NaCl and 10 mM- NaN_3 . The peak fractions only of α_1 -AT were pooled, treated with 5 mM-2-mercaptoethanol then 10 mM-iodoacetamide to block the single thiol group of α_1 -AT and hence the potential for dimerization, dialysed to remove excess reactants and concentrated to approx. 20 mg/ml. Throughout the procedure α_1 -AT was detected by single-dimension crossed immunoelectrophoresis (Laurell, 1966) and its purity was assessed by SDS/PAGE (on 10% gels; buffer system of Laemmli, 1970). The final product gave a single band only.

α_1 -AT was either held at 4 °C (for imminent use) or stored frozen at –70 °C until required. Samples that had been frozen were prepared for scattering studies by repeating the gel filtration on Sephadex G-150 to remove any aggregates that had formed. Only the peak fraction(s) were retained. These were pooled and reconcentrated to approx. 20 mg/ml. Immediately before scattering analysis all samples were filtered through a 0.2 μm -

pore filter (Gelman) into sterile glass or plastic vials, then held at 4 °C until dialysis against the appropriate scattering buffer.

Reactive-centre-cleaved α_1 -AT was generated by papain cleavage (Johnson & Travis, 1977). Although this is reported to react abortively at the reactive-centre Met-358–Ser-359 peptide bond only, SDS/PAGE and h.p.l.c. analysis of the digestion indicated that at least two proteolytic events occurred during generation of the stable cleaved product. Before use, papain (Worthington Corp.) was activated by incubation in 20 mM-sodium/potassium phosphate buffer, pH 7.0, containing 5 mM-cysteine and 10 mM-EDTA. Under the reaction conditions used, papain/ α_1 -AT ratio 1:1000 (w/w) at 37 °C in 20 mM-sodium/potassium phosphate buffer, pH 7.0, containing 10 mM-EDTA, 5 mM-cysteine and 5 mM-NaCl, an initial fast cleavage was essentially complete after 10 min. However, at this time a second, slower, cleavage was also evident. As the first cleavage could not be effected in the total absence of the second, digestion was continued for 6 h, at which time both cleavages were complete. Papain digestion was terminated by the addition of iodoacetamide (5 mM). *N*-Terminal sequence analysis (performed by Dr. L. Packman at the Protein Sequencing Facility, Department of Biochemistry, University of Cambridge) shows that, in addition to cleavage between Met-358 and Ser-359 at the reactive site, cleavages at Gly-5–Asp-6 and Asp-12–Thr-13 also occur. Sequence analysis of papain-digested α_1 -AT before and after dialysis against scattering buffers demonstrated that, as expected, the *C*-terminal peptide (residues 359–394) was retained after dialysis, but the small *N*-terminal peptides Glu-Asp-Pro-Gln-Gly and Asp-Ala-Ala-Gln-Lys-Thr-Asp were lost. The sequence analysis also showed that native α_1 -AT remains intact, as expected.

Collection of neutron data for α_1 -AT was initially performed in 12 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 0.2 M-NaCl. X-ray data were measured in a range of concentrations from 50 mM-phosphate up to the crystallization buffer 2.5 M-sodium/potassium phosphate buffer, pH 8.0 (Löbermann *et al.*, 1982). $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ was dissolved to 3.0 M in $^1\text{H}_2\text{O}$ or $^2\text{H}_2\text{O}$, back-titrated with stirring at room temperature to pH 8.0 by additions of known amounts of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, and diluted to its final desired phosphate concentration. The α_1 -AT samples were dialysed against the appropriate buffers with stirring at 4 °C and with four changes over at least 36 h. Optical absorbances were recorded at 280 nm immediately afterwards, and the scattering data were obtained within the following 24–48 h. SDS/PAGE analysis of samples before and after scattering analysis showed that they had the expected band patterns, and that no radiation-induced damage or non-specific proteolysis had occurred.

Neutron- and X-ray-scattering experiments

Neutron data were obtained on Instrument D17 at the Institut Laue Langevin in Grenoble, France. Guinier data were based on a sample-to-detector distance of 3.44 m and a wavelength λ of 1.095 nm (Q range of 0.016–0.615 nm^{–1}, where $Q = 4\pi \sin \theta / \lambda$ and 2θ is the scattering angle). Data at large Q were obtained by using a sample-to-detector distance of 1.41 m and λ of 1.095 nm (Q range of 0.045–1.451 nm^{–1}). Samples were measured in rectangular quartz Hellma cells of path thickness 1 mm for 0% $^2\text{H}_2\text{O}$ buffers (200 μl) and 2 mm for 70%, 80% and 100% $^2\text{H}_2\text{O}$ (400 μl) buffers at 20 °C. Counting times were 3–16 min in $^2\text{H}_2\text{O}$ buffers and 1–6 h in $^1\text{H}_2\text{O}$ buffers. Data reduction in Grenoble utilized standard software and procedures (RNILS, SPOLLY, RGUI and RPLT; Ghosh, 1981; Perkins *et al.*, 1990), and the final analyses in London employed the interactive Guinier and large-angle plotting program SCTPL2 (Perkins *et al.*, 1990).

X-ray data were obtained on the Synchrotron Radiation

Source at Daresbury, Warrington, U.K., operating at 2.00 GeV. The low-angle solution scattering cameras at Station 2.1 (beam currents of 61 mA to 52 mA) and Station 8.2 (261 mA to 75 mA) were used (Townsend-Andrews *et al.*, 1989) in a total of four sessions. At Station 2.1, the detector-to-sample distance was 2.52 m, with a wavelength of 0.155 nm (Q range of 0.189–3.206 nm⁻¹). At Station 8.2 the detector-to-sample distances of 2.605 m, 2.608 m and 3.83 m and the wavelength of 0.159 nm resulted in Q ranges of 0.051–1.534 nm⁻¹ and 0.039–1.012 nm⁻¹ respectively. Fresh wet slightly stretched rat tail collagen (diffraction spacing of 67 nm) was used to calibrate the detector Q axis. Samples were contained in round Perspex [poly(methyl methacrylate)] cells with a volume of 20 μ l, a path thickness of 1 mm and mica window thicknesses close to 10 μ m, held in position by Teflon inserts, and measured nominally at 20 °C. Samples were measured for 10–20 min in dilution series, in alternation with buffer in the same sample cell and with equal data collection times to reduce the occurrence of background subtraction errors. Data were measured in ten separate time slices as a check for radiation damage, after which the ten curves were averaged. Data reduction was performed by using OTOKO at Daresbury to subtract the buffer background and correct for the detector response measured with a uniform radioactive source, and final data analyses utilized SCTPL2 conjointly with the neutron data.

Analyses of Guinier data

At small Q , the Guinier equation gives the radius of gyration R_G and the forward scattering at zero scattering angle $I(0)$ (Perkins, 1988a,b):

$$\ln I(Q) = \ln I(0) - R_G^2 \cdot Q^2/3$$

In a given solute-solvent contrast, R_G measures the degree of elongation of a glycoprotein. If this structure is sufficiently elongated, the R_G of the cross-sectional structure R_{XS} and the cross-sectional intensity at zero angle $[I(Q) \cdot Q]_{Q \rightarrow 0}$ are obtained from:

$$\ln[I(Q) \cdot Q] = \ln[I(Q) \cdot Q]_{Q \rightarrow 0} - R_{XS}^2 \cdot Q^2/2$$

The matchpoint is obtained from a graph of $\sqrt{[I(0)/cT_s t]}$ against % ²H₂O (T_s is the sample transmission; t is the sample thickness), and from the cross-sectional analyses by using $\sqrt{[I(Q) \cdot Q]_{Q \rightarrow 0}/cT_s t}$. Experimental matchpoints can be compared with the amino acid sequence on the basis of the unhydrated shape of α_1 -AT, the use of crystallographic volumes (non-hydrated) and the 10% non-exchange of the main-chain amide protons (Perkins, 1986). The dependence of the R_G on the internal scattering densities within α_1 -AT is given by the Stuhmann equation (Ibel & Stuhmann, 1975):

$$R_G^2 = R_c^2 + \alpha/\Delta\rho - \beta/\Delta\rho^2$$

where $\Delta\rho$ is the difference in scattering density ρ between the solute and solvent. In Fig. 3(b), R_c is the R_G at infinite contrast and corresponds to the intercept at zero $\Delta\rho^{-1}$, while the slope α reflects the radial distribution of scattering-density inhomogeneities. The subscripts G and XS correspond to the overall and cross-sectional Guinier analyses. If the outermost scattering densities are higher than those nearer the centre, α will be positive. β corresponds to the distance between the centres of two distinct components of very different scattering densities and is negligible for glycoproteins. All linear-regression analyses of the R_G , $I(0)/c$, matchpoint and Stuhmann graphs used the OXSTAT and MINITAB statistics programs. The Guinier analyses lead to the triaxial dimensions of α_1 -AT. If this resembles an elliptical cylinder, its length $L = \sqrt{[12(R_G^2 - R_{XS}^2)]}$. From the intensity parameters, $L = \pi \cdot I(0)/[I(Q) \cdot Q]_{Q \rightarrow 0}$. The two shorter semi-axes

A and B are obtained from the total dry (neutrons) or hydrated (X-rays) volume $V = \pi ABL$ and $R_{XS}^2 = (A^2 + B^2)/4$. For X-ray data, the volume of the hydration shell is calculated assuming 0.3 g of water/g of glycoprotein and 0.0245 nm³ per water molecule (Perkins, 1986).

Debye sphere modelling

Small spheres were used to model the scattering curves with the use of the Debye equation (Perkins & Weiss, 1983; Perkins, 1985). The crystal co-ordinates of α_1 -AT were subdivided into cubes of side 0.608 nm such that their total volume is the dry volume of the crystallographically visible residues. The curve simulations were based on the overlapping spheres of volume 0.608³ nm³ set at the centre of each cube. The sphere diameter is 0.754 nm, which is much less than the highest nominal resolution $2\pi/Q_{\max}$ of the curve, i.e. 4.3 nm for $Q_{\max} = 1.45$ nm⁻¹ (Fig. 5). The contrast-dependence was modelled by subdividing the 291 spheres into an outer hydrophilic surface region of 163 spheres and an inner core of 128 spheres on the basis of the volumes of these two components. From compositions, the neutron-scattering densities of the two regions should be assigned as 49.4% ²H₂O and 31.6% ²H₂O respectively (Perkins, 1986). It was found more effective to use instead 60% ²H₂O and 30% ²H₂O respectively. This model was converted into the hydrated X-ray model by expanding the co-ordinates by 1.10, which was derived from the cube root of the ratio of the hydrated and dry volumes. The cube size is now 0.668 nm. For X-rays, the outermost spheres correspond to the hydration shell as well as the carbohydrate and hydrophilic amino acid residues. Electron densities were taken as 334 e/nm³ for solvent, 435 e/nm³ for the outermost 216 spheres of the model, and 389 e/nm³ for the innermost 75 spheres.

The R_{G-C} value is calculated from:

$$R_G^2 = \sum_{k=1}^n (R_k^2 + r_k^2)$$

for n spheres, each with an R_G denoted R_k and located at a distance r_k from the centre of mass of the model. The Stuhmann α value is calculated from:

$$\alpha_G = n^{-1} \sum_{k=1}^n (\rho_k - \rho_m) r_k^2$$

where ρ_k is the scattering density of the sphere and ρ_m is the mean scattering density. The full Debye curve is calculated from Rolbin *et al.* (1973):

$$\frac{I(Q)}{I(0)} = g(Q) \left(n_1 \cdot \rho_1^2 + n_2 \cdot \rho_2^2 + 2\rho_1^2 \sum_{j=1}^m A_j^{11} \frac{\sin Q \cdot r_j}{Q \cdot r_j} + 2\rho_2^2 \sum_{j=1}^m A_j^{22} \frac{\sin Q \cdot r_j}{Q \cdot r_j} + 2\rho_1 \cdot \rho_2 \sum_{j=1}^m A_j^{12} \frac{\sin Q \cdot r_j}{Q \cdot r_j} \right) (n_1 \cdot \rho_1 + n_2 \cdot \rho_2)^{-2}$$

where the model is constructed from n_1 and n_2 spheres of different scattering densities ρ_1 and ρ_2 ; $g(Q) = 3(\sin Q \cdot R - Q \cdot R \cdot \cos Q \cdot R)^2/Q^6 \cdot R^6$ (the squared-form factor of the spheres of radius R); A_j^{11} , A_j^{22} and A_j^{12} are the number of distances r_j for that increment of j between the spheres 1 and 1, 2 and 2 and 1 and 2 in that order; m is the number of different distances r_j . Before comparison with the experimental neutron-scattering curves, the calculated curve incorporated corrections for the wavelength spread of 10% on Instrument D17 and the beam divergence in radians (Cusack, 1981; Perkins & Weiss, 1983), based on the empirical parameters of 16% and 0.016 radians (Cusack, 1981). Since the X-ray cameras utilize point collimation, the calculated and experimental $I(Q)$ curves are directly comparable without corrections. To facilitate quantitative com-

parisons, a residual R was employed, which is defined by analogy with protein crystallography (Blundell & Johnson, 1976):

$$R = \Sigma |I(Q)_{\text{expt.}} - I(Q)_{\text{calc.}}| / \Sigma |I(Q)_{\text{expt.}}|$$

Hydrodynamic modelling of α_1 -AT

Frictional coefficients f are calculated from:

$$f = \frac{M_r(1 - \bar{v} \cdot \rho_{20,w})}{N_a \cdot s_{20,w}^0}$$

where $\bar{v}$ is the partial specific volume, $\rho_{20,w}$ is the density of water at 20 °C, N_a is Avogadro's constant, and $s_{20,w}^0$ is the sedimentation coefficient. Frictional ratios f/f_0 are calculated by using $f_0 = 6\pi\eta r$, in which the macromolecule is assumed to be a hydrated sphere of radius r . The value of $\bar{v}$ is derived from the consensus volumes data set of Perkins (1986), which is based on the sum of the electrostricted water shell volume and the dry volume. Frictional coefficients were calculated by using non-overlapping spheres by the modified Oseen tensor procedure of Bloomfield (Garcia de la Torre & Bloomfield, 1977a,b; Perkins, 1989). The crystal structure was subdivided into 31 dry cubes of side 1.216 nm such that the overall dimensions are close to those of the co-ordinates. Since relatively few spheres are used, allowance for the void spaces between the non-overlapping spheres is necessary. The effective triaxial lengths of the model

should be reduced by $(1 - \pi/4)d$ (d is the sphere diameter) before comparison with the crystallographic dimensions. To allow for this, a hypothetical hydration of 0.39 g of water/g of glycoprotein in place of 0.30 g of water/g is used to calculate the total hydrated volume of the 31 spheres (Perkins, 1989), i.e. a hydration factor of 1.12. The 31 'dry' co-ordinates were thus rescaled by a factor of 1.39 to give a final sphere diameter of 1.69 nm (Fig. 6). If the protein surface is uniformly covered by a single layer of water molecules of thickness 0.36 nm (which corresponds to a hydration of 0.3 g of water/g of protein; Perkins, 1986, 1989), good agreement with the dimensions of the crystal co-ordinates can be obtained.

RESULTS AND DISCUSSION

Neutron-scattering of native α_1 -AT in physiological buffers

Fig. 1(a) shows that, under physiological conditions, neutron-scattering in 100% $^2\text{H}_2\text{O}$ gave good linear Guinier plots for native α_1 -AT in an acceptable $Q \cdot R_G$ range of 0.3–1.1. Dilution series for α_1 -AT in the range $c = 2.4$ to 13.7 mg/ml (Fig. 2) showed that the R_G increased by 20%; sedimentation experiments also gave a similar result (Kress & Laskowski, 1973). The extrapolation to zero c gave an R_G of 2.40 ± 0.03 nm for 12 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 0.2 M-NaCl and 2.37 ± 0.03 nm for 50 mM-sodium/potassium phosphate buffer, pH 8.0. No dependence

Fig. 1. Guinier R_G and R_{XS} plots of neutron and X-ray data for native and split α_1 -AT

The buffer in both cases is 50 mM-sodium/potassium phosphate buffer, pH 8.0. (a) Neutron Guinier plots of the dilution series for native α_1 -AT in 100% $^2\text{H}_2\text{O}$ at concentrations of 12.3 mg/ml ($\blacktriangle$), 9.8 mg/ml ($\circ$), 7.0 mg/ml ($\bullet$) and 4.8 mg/ml ($\triangle$). The Q range employed for fitting is 0.17 – 0.57 nm $^{-1}$ between the $Q \cdot R_G$ range as arrowed. The slopes increase slightly with increasing dilution; the relative vertical displacements reflect the dilutions. (b) Neutron cross-sectional plot of native α_1 -AT at 8.0 mg/ml. The Q range used for fitting is 0.61 – 1.42 nm $^{-1}$, as indicated by the arrowed $Q \cdot R_{XS}$ range. (c) X-ray Guinier plots of native ($\circ$ and $\bullet$) and split ($\triangle$ and $\blacktriangle$) α_1 -AT at concentrations of 16.8 mg/ml ($\circ$), 12.6 mg/ml ($\bullet$), 18.9 mg/ml ($\triangle$) and 14.2 mg/ml ($\blacktriangle$). The Q range used for fitting the data is 0.15 – 0.57 nm $^{-1}$ between the $Q \cdot R_G$ range as shown. The curves were displaced arbitrarily for clarity. (d) X-ray cross-sectional plot of the samples in (c) by X-ray-scattering. The $Q \cdot R_{XS}$ values are based on a Q range of 0.60 – 1.20 nm $^{-1}$. Note that the vertical scale is halved in (d) relative to (a), (b) and (c).

Fig. 2. Concentration-dependence of the R_G of native α_1 -AT

The R_G values were measured by neutron-scattering in 100% $^2\text{H}_2\text{O}$ in 50 mM-sodium/potassium phosphate buffer, pH 8.0 (O), in 0.50 M-sodium/potassium phosphate buffer, pH 8.0 (●), and in 12 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 0.2 M-NaCl (▲). The R_G at zero α_1 -AT concentration was obtained from linear regression with the use of MINITAB.

was observed for the $I(0)/c$ parameter (results not shown). At larger scattering angles, good cross-sectional Guinier plots were also obtained. Fig. 1(b) shows that the mean R_{XS} of 1.22 nm was obtained in a satisfactory $Q \cdot R_{XS}$ range of 0.8–1.8.

This concentration-dependence is unusual for plasma glycoproteins. From their analysis of the crystal structure, Löbermann *et al.* (1984) have proposed that α_1 -AT probably has a large dipole moment, since the acidic and basic charged groups are positioned asymmetrically on the structure. To test whether this was the source of the concentration-dependence, a further dilution series of α_1 -AT was studied in 0.50 M-sodium/potassium phosphate buffer, pH 8.0. As a control, the values of $I(0)/cT_s$ and $[I(Q) \cdot Q]_{Q \rightarrow 0}/cT_s$ were calculated and found to be independent of phosphate concentration, as expected. Fig. 2 shows a diminished dependence of the R_G on phosphate concentration, and the extrapolated R_G of 2.39 ± 0.03 nm is within error of those for 12 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 0.2 M-NaCl and 50 mM-sodium/potassium phosphate buffer, pH 8.0. The concentration effect on R_G is thus attributed to the interactions of the dipole moments of different molecules.

Full contrast variation of native α_1 -AT was carried out in 0%, 70%, 80% and 100% $^2\text{H}_2\text{O}$. To verify that the neutron data correspond to monomeric α_1 -AT, its relative molecular mass M_r was determined from the $I(0)/c$ value in 0% $^2\text{H}_2\text{O}$ (Jacrot & Zaccari, 1981), where c was calculated from the absorption coefficient $A_{280,1\text{cm}}^{1\%}$ of 5.0–5.4 (Bundy & Mehl, 1959; Kress & Laskowski, 1973; Bruch *et al.*, 1988). By using an M_r of 51 500 (calculated from sequence data), $I(0)/c$ gave an A_{280} determination of 5.4 ± 0.2 , in good agreement with Bruch *et al.* (1988), and showing also that α_1 -AT is monomeric in solution. It may be noted that the molecular composition of α_1 -AT leads to too low a predicted A_{280} of 3.86; it is, however, known that deviations from the calculation can occur when the A_{280} is less than 10 (Wetlaufer, 1962; Edelhoch, 1967; Perkins, 1986). The neutron matchpoint was determined for comparison with that predicted from the sequence. In Fig. 3(a) the matchpoint was determined as $40.3 \pm 1.1\%$ $^2\text{H}_2\text{O}$, which agrees well with a prediction of 40.3% $^2\text{H}_2\text{O}$ (Table 1). The limited cross-sectional data of $\sqrt{[I(Q) \cdot Q]_{Q \rightarrow 0}/cT_s t}$ (four points in two contrasts; results not shown) gave a comparable matchpoint of $42.1 \pm 1.2\%$ $^2\text{H}_2\text{O}$. It is concluded that neutron-scattering 'visualizes' α_1 -AT as a non-hydrated monomeric macromolecule.

The contrast-variation R_G data were analysed in the Stuhrmann

Fig. 3. Neutron contrast-variation analysis of native α_1 -AT, with 2.7 to 12.2 mg/ml concentrations in 12 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 0.2 M-NaCl

(a) The matchpoint determination was based on extrapolations of the $I(0)/c$ values to zero c (16 points in four contrasts), and calculating $\sqrt{[I(0)/cT_s t]}$ from these (c is the α_1 -AT concentration; T_s is the sample transmission; t is the path length). The error in the matchpoint is thus higher than usual. The matchpoint from regression analysis is $40.3 \pm 1.1\%$ $^2\text{H}_2\text{O}$. (b) The Stuhrmann plot of the R_G^2 against the reciprocal solute-solvent contrast difference $\Delta\rho^{-1}$ is also based on the R_G data extrapolated to zero c (Fig. 2). The regressed line gives an R_{G-C} of 2.61 ± 0.02 nm and the slope gives a Stuhrmann α of $29 \times 10^{-5} \pm 5 \times 10^{-5}$.

plot of Fig. 3(b). At infinite contrast R_{G-C} was determined to be 2.61 ± 0.02 nm. The elongation ratio R_{G-C}/R_0 is thus 1.35 (where R_0 is the R_G of the sphere of the same dry volume as α_1 -AT), which is close to the average of 1.28 ± 0.10 found for many globular proteins (Perkins, 1988b). The corresponding R_{XS} analysis gave an R_{XS-C} of 1.34 ± 0.01 nm. The slopes α were strongly positive, where α_G is $29 \times 10^{-5} \pm 5 \times 10^{-5}$ and α_{XS} is $13 \times 10^{-5} \pm 1 \times 10^{-5}$. It is concluded that the surface regions in α_1 -AT have a higher scattering density than those near the core. Since the hydrophilic amino acid and carbohydrate residues have a mean matchpoint of 49.4% $^2\text{H}_2\text{O}$, while hydrophobic amino acid residues have one of 31.6% $^2\text{H}_2\text{O}$, hydrophilic residues are seen to be predominant in the outer regions of the α_1 -AT structure, as expected.

Some notable differences are seen on comparison of α_1 -AT with α_1 -acid glycoprotein (M_r 38 000; 44% carbohydrate; Perkins *et al.*, 1985). α_1 -Acid glycoprotein has a compact conformation in low-salt buffers with an R_G/R_0 ratio of 1.32 and an α_G of $7 \times 10^{-5} \pm 2 \times 10^{-5}$, whereas its expanded form in high-salt buffers (where the oligosaccharide chains are extended into solution) has an R_G/R_0 ratio of 1.67 and an α_G of $43 \times 10^{-5} \pm 4 \times 10^{-5}$. The α_G of $29 \times 10^{-5} \pm 5 \times 10^{-5}$ for α_1 -AT is considerably larger than that for the compact form of α_1 -acid

glycoprotein, even when allowance is made for the difference in R_{G-C} in the precision of measurement of α or in the ratio of hydrophilic to hydrophobic residues (54:46 for α_1 -AT and 68:32 for α_1 -acid glycoprotein). Since α is proportional to r_k^2 (see the Materials and methods section), these data suggest that the oligosaccharide chains on α_1 -AT are extended into solution under physiological conditions.

The Guinier analyses at infinite contrast lead to estimates of the triaxial dimensions of α_1 -AT (see under 'Analyses of Guinier data' in the Materials and methods section). The ratio of intensities give a length L of 7.6 ± 0.5 nm (four values), in agreement with an L of 7.8 ± 0.1 nm from the R_G data. α_1 -AT is thus determined to be $7.8 \text{ nm} \times 4.9 \pm 0.1 \text{ nm} \times 2.2 \pm 0.1 \text{ nm}$ in size. The reported crystallographic dimensions of $6.7 \text{ nm} \times 3.2 \text{ nm} \times 3.2 \text{ nm}$ (Löbermann *et al.*, 1984) should be increased to $7.2 \text{ nm} \times 3.4 \text{ nm} \times 3.4 \text{ nm}$ to correspond to the correct total volume of α_1 -AT. Even though the shorter axes of the protein component are more similar in the crystal structure, molecular graphics showed that the three oligosaccharide sites of α_1 -AT are located in such a way that one short axis will be significantly longer if the oligosaccharide chains are extended into solution. The Guinier data thus support the suggestion of extended oligosaccharide conformations.

X-ray scattering of native and split α_1 -AT in high-phosphate buffers

Synchrotron X-ray-scattering was employed to compare (i) the physiological structure with that in high concentrations of phosphate, (ii) the native and split structures of α_1 -AT, and (iii) the hydrated structure with the dry one seen by neutron-scattering. Figs. 1(c) and 1(d) show that excellent R_G and R_{XS} Guinier plots were obtained for both native and split α_1 -AT in 50 mM-sodium/potassium phosphate buffer, pH 8.0. The concentration-dependence of the R_G values is visible also in Fig. 1(c). Occasionally a slight time-dependent aggregation of α_1 -AT was detected by an increase in $I(Q)$ at a very low Q . Its occurrence was facilitated by increased beam currents just after beam refills, increased phosphate concentration, the prior presence of trace aggregates or the use of native α_1 -AT. Accordingly the ten time-slices of each run were examined to check that the Guinier parameters were unaffected.

Data collection in sodium/potassium phosphate buffers up to 1.2 M were affected by worsening sample transmissions with increased phosphate concentrations, and satisfactory signal-to-noise ratios could be obtained only up to 0.6 M-sodium/potassium phosphate buffer, pH 8.0 (Fig. 4). Data analyses showed that, unlike the corresponding neutron data in 100% $^2\text{H}_2\text{O}$, the $I(0)/c$ values increase (results not shown). This appears to reflect the different solute-solvent scattering density and radiation in use. This dependence decreased with increase in the phosphate concentration, and the $I(0)/cT_s$ and $[I(Q) \cdot Q]/Q_{\rightarrow 0}/cT_s$ data after extrapolation to zero c were constant within error in the different phosphate buffers (results not shown), thus confirming the good quality of the Guinier data. The R_G values exhibited a statistical spread comparable in magnitude with the observable R_G -dependence on α_1 -AT concentration, which is attributed to fluctuations in the background subtraction. Accordingly the mean R_G from each experiment is reported in Fig. 4. No concentration-dependence is seen for the R_{XS} data, and mean values are again given in Fig. 4.

As the phosphate concentration is increased to 0.6 M, no change is seen in the R_G or R_{XS} values of native α_1 -AT, showing that the structure is stable under these conditions. Three different experiments in 50 mM-sodium/potassium phosphate buffer, pH 8.0, gave R_G values of 2.80 ± 0.09 nm, 2.93 ± 0.06 nm and 3.00 ± 0.04 nm. These are similar to but slightly larger than the

Fig. 4. Dependence of the Guinier parameters on the phosphate concentration

The upper plot shows the R_G -dependence upon sodium/potassium phosphate buffer concentration for the X-ray data (Δ , ∇ and $\circ$) for native α_1 -AT. X-ray data for split α_1 -AT ($\blacktriangle$) and 67% split 33% native α_1 -AT ($\bullet$) are also shown. Error bars are shown only when significant in the X-ray data and increase at higher phosphate concentrations. The lower plot shows the corresponding R_{XS} -dependence on phosphate concentrations by X-rays, the same symbols being used.

neutron R_G of 2.82 ± 0.03 nm in water, since both experiments correspond to positive solute-solvent contrasts, but the X-ray data now reflect a hydration shell surrounding α_1 -AT. Likewise, two experiments gave 'hydrated' R_{XS} values of 1.73 ± 0.19 nm (19 values) and 1.82 ± 0.13 nm (seven values) that are slightly larger than the neutron 'dry' R_{XS} value of 1.53 ± 0.10 nm measured in $^1\text{H}_2\text{O}$ in 12 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 0.2 M-NaCl. The corresponding data for split α_1 -AT are also shown in Fig. 4, and no difference in R_G or R_{XS} from the native form is observed. This suggests that the conformational transition between the native and split forms of α_1 -AT involves only a localized re-arrangement of the protein structure, and that the loss of the N -terminal peptides (see the Materials and methods section) has a negligible effect on the structure.

Molecular modelling of α_1 -AT

The scattering data were compared directly with the crystal structure of split α_1 -AT (Löbermann *et al.*, 1984) by converting the latter into a Debye sphere model for scattering-curve simulations (see the Materials and methods section). Only 374 of the 394 amino acid residues are present in the structure, since Gly-1-Asp-19 and Lys-394 are not visible in the electron-density map. This protein structure was subdivided into the equivalent unhydrated volume of 246 cubes of side 0.608 nm, which facilitates the representation of the oligosaccharide chains as a single cube for each residue (Perkins *et al.*, 1985). Both tetragonal and hexagonal split α_1 -AT co-ordinates were taken from the Protein Data Bank (Bernstein *et al.*, 1977). After addition of hydrogen atom co-ordinates (Perkins, 1982), and with the use of a unit weighting of atoms, the R_G of these structures was calculated as 2.12 nm and 2.13 nm respectively by using $R_G^2 = \sum r_i^2/n$, where r_i is the distance of each atom from the centre of gravity. This agrees with the R_G of 2.15 nm calculated from the sphere model. Since the two co-ordinate sets gave very similar results, only the tetragonal model was used further.

Carbohydrate and the N -terminal residues were added to the model. The three N -linked oligosaccharides at Asn-46, Asn-83 and Asn-247 were assumed to be biantennary with 11 residues each (Vaughan *et al.*, 1982). Although only partly visible, the

Fig. 5. Comparisons of the curve simulations with the α_1 -AT models

(a) Neutron data in $^2\text{H}_2\text{O}$ in 50 mM-sodium/potassium phosphate buffer, pH 8.0 (high negative solute-solvent contrast difference), for native α_1 -AT are compared with the smeared simulated curves. The vertical arrows indicate the Q ranges of 0.17–0.57 nm^{-1} and 0.61–1.42 nm^{-1} used for the R_G and R_{XS} determinations respectively (Figs. 1a and 1b). Curve 1: the unhydrated native α_1 -AT structure with extended oligosaccharide and the use of two densities; R of 0.006 (see the Materials and methods section). Curve 2: non-hydrated native α_1 -AT structure with compact oligosaccharide and two densities; R of 0.010. Curve 3: non-hydrated native α_1 -AT with extended oligosaccharide and use of a single density; R of 0.020. Curve 4: native α_1 -AT with two densities and a hydration of 0.3 g of water/g of α_1 -AT; R of 0.024. (b) Neutron data in $^1\text{H}_2\text{O}$ in 12 mM-sodium phosphate buffer, pH 7.0, containing 1 mM-EDTA and 0.2 M-NaCl (high positive solute-solvent contrast difference) for native α_1 -AT are compared with the four simulated curves numbered as in (a) but recalculated for this contrast. The R values for curves 1, 2, 3 and 4 are 0.017, 0.019, 0.017 and 0.029 in that order. (c) X-ray data in 50 mM-sodium/potassium phosphate buffer, pH 8.0 (high positive solute-solvent contrast difference), for native α_1 -AT are compared with models based on a hydration of 0.3 g of water/g of α_1 -AT in curves 1 and 2. The vertical arrows indicate the Q ranges of 0.33–0.57 nm^{-1} and 0.60–1.20 nm^{-1} used for the R_G and R_{XS} determinations respectively (Figs. 1c and 1d). Curve 1: native α_1 -AT, hydrated to 0.3 g of water/g of α_1 -AT, with extended oligosaccharide and two densities; R of 0.011. Curve 2: native α_1 -AT, hydrated to 0.3 g of water/g of α_1 -AT, with compact oligosaccharide and two densities; R of 0.028. Curve 3: non-hydrated native α_1 -AT with extended oligosaccharide and two densities; R of 0.035. Curve 4: native α_1 -AT, hydrated to 0.67 g of water/g of α_1 -AT, with extended oligosaccharide and two densities; R of 0.028.

Table 1. Compositional and scattering data for α_1 -AT

Amino acid residues:	
Total	394
Volume (nm^3)*	57.4
Carbohydrate residues:	
Total	36
Volume (nm^3)*	7.9
Total M_r from sequence	51 500
Percentage carbohydrate by mass	14.1
Overall $\bar{v}$ (ml/g)†	0.732
Overall $\Sigma b/M_r$	
In $^1\text{H}_2\text{O}$ (fm)	0.2248
In $^2\text{H}_2\text{O}$ (fm)	0.3813
Predicted $A_{280,1\text{cm}}^{1\%}$ ‡	3.86
Literature A_{280}	5.0–5.4
Neutron A_{280} determination	5.4 ± 0.2
Predicted neutron matchpoint (% $^2\text{H}_2\text{O}$)§	40.3
Experimental matchpoint (% $^2\text{H}_2\text{O}$)	
From $I(0)$	40.3 ± 1.1
From $[I(Q) \cdot Q]_{Q \rightarrow 0}$	42.1 ± 1.2
Stuhrmann analysis	
R_{G-C} (nm)	2.61 ± 0.02
α_G	$29 \times 10^{-5} \pm 5 \times 10^{-5}$
R_{XS-C} (nm)	1.34 ± 0.01
α_{XS}	$13 \times 10^{-5} \pm 1 \times 10^{-5}$

* The dry volume is taken from Chothia (1975) (amino acids) and Perkins (1986) (carbohydrates) and used in matchpoint and modelling calculations. The carbohydrate composition is calculated assuming two biantennary and one triantennary oligosaccharides.

† Partial specific volumes $\bar{v}$ were calculated from the consensus volume set of Perkins (1986) and are close to those determined by the Cohn & Edsall (1943) compilation. These values were used in sedimentation simulations.

‡ Calculated from the content of tyrosine, cysteine and tryptophan residues by the Wetlaufer (1962) procedure and rescaled by using a factor of 1.03 (Perkins, 1986). Literature determinations of A_{280} are reported in Bundy & Mehl (1959), Kress & Laskowski (1973) and Bruch *et al.* (1988).

§ Calculated by using the assumption of the non-exchange of 10% of the main-chain peptide hydrogen atoms (Perkins, 1986).

crystal structure shows that all three are seen in an extended configuration (Fig. 6a). Each oligosaccharide was thus modelled by 11 cubes in an extended arrangement (Fig. 6b). It is possible that the oligosaccharide chains may be folded against the protein surface. Such a model (Fig. 6b) has an R_G that is 0.22 nm smaller than that of the extended structure. The 19 N -terminal residues were represented by a further 12 cubes close to His-20. Robson and Chou-Fasman secondary-structure predictions (Garnier *et al.*, 1978; Chou & Fasman, 1978) suggest that six to 11 of these residues constitute an α -helix of length 1–2 nm. The 19 residues were taken to be fully exposed to solvent since they contain up to ten charged groups and since this N -terminal section is readily cleaved by proteolysis (see the Materials and methods section). The N -terminus in native α_1 -AT was thus modelled as a $6 \times 2 \times 1$ array of spheres of length 3.6 nm extending away from the protein surface, which increases the R_G by 0.04 nm.

A model for native α_1 -AT requires also the rejoining of Met-358 and Ser-359 in split α_1 -AT (Fig. 6a). Löbermann *et al.* (1984) proposed that residues 359–394 are fixed in the two structures, and residues 343–358 have been relocated. This is supported by the susceptibility of residues 351–358 to proteolytic attack (Kress *et al.*, 1979), and also by the ability of the C -terminal residues 359–394 to bind tightly to split α_1 -AT by non-covalent forces (Löbermann *et al.*, 1982). Inspection of the α_1 -AT crystal structure suggested that the minimum conformational change required to form native α_1 -AT involves residues 348–358. Since these correspond to seven cubes, those closest to residues 348–358

Fig. 6. Comparative views of the crystal structure and models for α_1 -AT

(a) The α -carbon co-ordinates of tetragonal split α_1 -AT are shown (Löbermann *et al.*, 1984). The α -carbon atoms of Asn-46 and Asn-83 are on the $+z$ face of the structure and are indicated by larger spheres, and their associated carbohydrate residues where visible in the electron-density map are numbered as shown. That for Asn-247 is on the $-z$ face. Residues Met-358 and Ser-359 are arrowed. (b) The final extended Debye sphere model for native α_1 -AT is shown in the same orientation as in (a), as employed for curves 1 and 2 of Figs. 5(a), 5(b) and 5(c) (see the text). The three oligosaccharide groups are shown in an extended conformation (curve 1) and are assumed to possess biantennary structures. For comparison, the compact conformation (curve 2) is shown also. (c) The hydrodynamic model for native α_1 -AT is depicted also in the same orientation. The protein core is represented by 32 spheres of diameter 1.69 nm. In the extended model, each of the three carbohydrate chains is represented by eight spheres of diameter 0.95 nm. The compact model is shown as 36 spheres of diameter 1.69 nm. It should be noted that (i) the mean distance between the α_1 -AT molecules in a 20 mg/ml solution of α_1 -AT is 16 nm; (ii) the maximum length of the extended model shown in (b) is 11 nm; (iii) the oligosaccharide chains extend by 3.5 to 3.9 nm from the protein surface; (iv) the upper and lower structures in (b) and (c) as shown are displaced by 13 nm.

were relocated to rejoin Met-358 and Ser-359 on the protein surface. The R_G of the native α_1 -AT model is increased by 0.03 nm, which is not detectable experimentally.

The curve-fitting to the neutron and X-ray data for native α_1 -AT in three contrasts is summarized by curves 1 and 2 in Fig. 5. The model with extended carbohydrate chains gives the three good curve 1 fits for native α_1 -AT. The residual R is 0.006, 0.017 and 0.011 in that order for (a), (b) and (c) in Fig. 5. The calculations based on the compact carbohydrate model give worsened curve fits, as shown by visual inspection of the curves 2 in Fig. 5 and the R of 0.010, 0.019 and 0.028. This is clearest in Fig. 5(c), since this contrast corresponds to the strongest contribution of carbohydrate to the scattering curve. In positive contrasts, X-rays result in better signal-to-noise ratios at large Q than neutrons. The R_{G-C} of the extended carbohydrate model (curve 1) is 2.58 nm, which agrees well with 2.61 nm from the experimental determination (Table 1). The compact carbohydrate model (curve 2) gives worsened agreement, with an R_{G-C} of 2.36 nm. The α of the extended model is 29×10^{-5} , which is in better agreement with the α of $29 \times 10^{-5} \pm 5 \times 10^{-6}$ by experiment (Table 1) than that of the compact model with an α of 20×10^{-5} . The density-weighted X-ray R_G of the extended model is 2.97 nm, in good agreement with the experimental X-ray values of 2.80 to 3.00 nm (Fig. 4). It is concluded that the solution structure of native α_1 -AT is similar to that seen in the crystal structure if the carbohydrate and N -terminal moieties are in extended conformations.

Two sets of control calculations indicate the importance of incorporating the internal scattering density and hydration into these models.

(a) Given the large α values, it is necessary to incorporate the internal inhomogeneity of scattering densities in the neutron simulations (see the Materials and methods section). If this is

neglected, it leads to simulated curves at large Q that underestimate the neutron $I(Q)$ data in negative contrasts in $^2\text{H}_2\text{O}$ (curve 3 of Fig. 5a; R of 0.020) and overestimate those in positive contrasts in $^1\text{H}_2\text{O}$ (curve 3 of Fig. 5b; R of 0.017).

(b) Likewise the X-ray simulations require an adequate hydration shell of 0.3 g of water/g of α_1 -AT in addition to the internal inhomogeneity of electron densities. Calculations for the dry model gave curves that generally overestimated the X-ray $I(Q)$ data (curve 3 of Fig. 5c; R of 0.035). Too great a hydration of 0.67 g of water/g of α_1 -AT leads to worsened agreements beyond $Q = 1 \text{ nm}^{-1}$ (curve 4 of Fig. 5c; R of 0.028). The poorer fits of curves 4 in Figs. 5(a) and 5(b) (R of 0.024 and 0.029 respectively) show that hydration is not appropriate for neutron curve modelling.

As an independent test of the α_1 -AT solution models, hydrodynamic models were derived from the crystal structure in order to calculate the frictional coefficient (see the Materials and methods section). The sedimentation coefficient $s_{20,w}^0$ of α_1 -AT has been reported as 3.3 S, 3.41 S and 3.45 S (Bundy & Mehl, 1959; Shamash & Rimón, 1966; Kress & Laskowski, 1973). The frictional coefficient is thus 6.7×10^{-8} – 7.0×10^{-8} g/cm per s. From this the frictional ratio f/f_0 is calculated as 1.29–1.35, to show that this has a moderately elongated structure, in agreement with the conclusion from the R_{G-C}/R_0 ratio of 1.35. The 31 hydrodynamic spheres for the protein core are of diameter 1.69 nm. One more was added to allow for the N -terminus. Each carbohydrate moiety was represented by eight spheres of diameter 0.95 nm in the extended model (Fig. 6c). In the compact model, these were replaced by four spheres of diameter 1.69 nm. The frictional coefficients are 7.2×10^{-8} and 6.4×10^{-8} g/cm per s respectively for the extended and compact models, which are greater than and less than the estimated experimental range. This suggests that the carbohydrate of α_1 -AT exists in a range of

extended conformations, and is consistent with the Debye modelling.

Conclusions

Large conformational and stability changes have been reported between the native and split forms of α_1 -AT (Löbermann *et al.*, 1984; Carrell & Owen, 1985; Bruch *et al.*, 1988). No evidence for a gross conformational change was found by solution scattering. This is explicable in terms of the observation by Löbermann *et al.* (1984) that, if β -strand 4A (residues 343–358) is removed from the centre of β -sheet A to re-form native α_1 -AT, the structural integrity may largely be conserved. In the modelling, this change corresponds to the relocation of just seven spheres out of the total of 291 in the split α_1 -AT model (Fig. 6b). This places an upper structural limit on the extent of the changes seen by spectroscopy (Bruch *et al.*, 1988).

How large is a conformational change before it is detectable by scattering? The neutron and X-ray R_g of deoxyhaemoglobin increased by 0.05–0.15 nm on the binding of O₂ (Conrad *et al.*, 1969; Schneider *et al.*, 1969); this corresponds to α -helix movements of 0.05–0.15 nm and subunit movements of 0.6 nm (Baldwin & Chothia, 1979). The X-ray R_g of aspartate transcarbamoylase increased by 0.25 nm after ligation to a substrate analogue (Moody *et al.*, 1979; Hervé *et al.*, 1985) and corresponds to 1.2 nm movements of the catalytic trimers and rotational reorientations of 10° to 15° between the regulatory and catalytic subunits (Krause *et al.*, 1987; Ke *et al.*, 1988). Sometimes no changes are seen by scattering, such as in the activation of chymotrypsinogen to α -chymotrypsin (Krigbaum & Godwin, 1968) or in the binding of pyridoxal 5'-phosphate to aspartate aminotransferase (Vergé *et al.*, 1983). The observation of change by solution scattering thus depends on the protein in question.

The conformational properties of oligosaccharides in glycoproteins are not readily accessed by physical techniques. The R_g parameter in solution scattering is of potential value, since this corresponds to the mean square distance of scattering vectors from the centre of scattering. Oligosaccharides usually lie on the protein surface, and will contribute maximally to the R_g . Even though the Stuhmann α is less accurately measured than the R_g , this may sometimes be of use. In the present study, the oligosaccharide chains of α_1 -AT were found to be largely extended into solution. These are mostly biantennary oligosaccharides, but may be replaced at Asn-83 and to a lesser extent at Asn-46 by triantennary oligosaccharides (Mega *et al.*, 1980; Vaughan & Carrell, 1981).

The present study has tested approaches for the modelling of scattering curves starting from a known crystal structure. It was found necessary in the simulations to allow for the different scattering densities of the hydrophilic and hydrophobic residues. This requirement can, however, be unnecessary if the protein has a low Stuhmann α for its R_g value. Neutron modelling is best performed with structures that are largely or fully non-hydrated; this is consistent with experience of neutron-scattering densities for glycoproteins (Perkins, 1986). In distinction, X-ray modelling requires hydrated structures. This is consistent with the calculation of partial specific volumes from the sum of the unhydrated volume and the electrostricted water shell surrounding the protein (Perkins, 1986), and also with the calculation of electron densities for X-ray-scattering studies by contrast variation (Perkins, 1988b).

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