Molecular modelling of the domain structure of factor I of human complement by X-ray and neutron solution scattering

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Factor I is a typical multidomain protein of the complement system. It regulates complement activation by proteolytic degradation of C3b or C4b in the presence of factor H, complement receptor type 1, membrane cofactor protein or C4b-binding protein as cofactor. It is constructed from five presumed independently folded domains, namely a factor I module, a CD5-like domain, two low-density-lipoprotein receptor type A domains and a serine-proteinase domain. X-ray and neutron solution scattering was used to study the arrangement of these domains in factor I. Factor I was determined to be monomeric in solution, with an $A_{280}^{1\%1\text{cm}}$ of 12.3–14.1. Its radius of gyration (R_G) was 3.96 nm by X-rays in a high positive solute–solvent contrast, and 3.84 nm by neutrons at infinite solute–solvent contrast. The cross-sectional radius of gyration (R_{xs}) was likewise found to be 1.64 nm by X-rays and 1.55 nm by neutrons. The R_G

data were not noticeably dependent on the solute–solvent contrast, whereas the R_{xs} data showed a small dependence. The maximum dimension of factor I was determined to be 12.8 nm from the R_G and R_{xs} data, and 14–15 nm from the X-ray and neutron distance distribution functions. This length is too short to account for a linear arrangement of the domains in factor I. Small sphere models were developed for factor I in which the largest domain was modelled from the crystal structure for β -trypsin. The attachment of either an elliptical cylinder or a two-armed V-shaped structure to this domain to represent the remaining four small domains gave good scattering curve-fits for factor I, and were compatible with experimental sedimentation coefficients. The non-extended domain models for factor I imply that the steric accessibility of each domain will be reduced, and this may be important for its functional activity.

INTRODUCTION

Factor I is one of the six serine proteinases (SPs) involved in the activation and control of the complement system of immune defence. Its function is to regulate the formation of the C3 convertases by proteolytic degradation of the activated complement components C4b and C3b (Hourcade et al., 1989), and for this reason was earlier known as ‘C3b/C4b inactivator’ (Crossley, 1981). For cleavage of C3b (or C3u) and C4b (or C4u), a cofactor protein must first form a complex with C3b or C4b. Factor H acts as a cofactor for the cleavage by factor I of C3b, whereas C4b-binding protein (C4BP) is a requisite cofactor for C4b degradation, again enhancing cleavage by factor I of the α' chain (Fujita et al., 1978; Gigli et al., 1979). The cell-surface protein complement receptor type 1 (CR1) is another cofactor for both C3b and C4b degradation that involves factor I (Fearon, 1979; Medof et al., 1982); another is the membrane cofactor protein (Hourcade et al., 1989). C4b is cleaved twice by factor I to form C4c and C4d, whereas C3b is cleaved twice in the α' chain to form C3f and iC3b. Further breakdown of iC3b to C3dg plus C3c may be mediated by factor I, with CR1 (only) as cofactor, but it is possible that another protease may mediate this degradation step (Becherer et al., 1989). Factor I is unusual in that no proenzymic form exists and that it is not normally inhibited by any known protein proteinase inhibitor, nor by the commonly used SP inhibitors such as di-isopropyl fluorophosphate, benzamidine and phenylmethanesulphonyl fluoride (Crossley and Porter, 1980; Ekdahl et al., 1990).

The complete cDNA-derived amino acid sequence of factor I has been established (Catterall et al., 1987; Goldberger et al., 1987). Factor I consists of a heavy chain (polypeptide M_r 35400)

and a light chain (polypeptide M_r 27600), each of which has three putative N-linked glycosylation sites. The light chain (243 amino acids) consists entirely of an SP domain. Starting from the N-terminus, the heavy chain (318 amino acids) contains one region of sequence similarity known as the factor I module (FIM), another one corresponding to a CD5-like domain approx. 100 residues long, and two more to the class-A low-density-lipoprotein receptor (LDLr) domain (Haefliger et al., 1989; DiScipio and Hugli, 1989; Freeman et al., 1990; Schneider, 1989; Reid and Day, 1989; Sim et al., 1993). Another report suggesting a 60-residue similarity to the short consensus/complement repeat is not statistically valid (Goldberger et al., 1987; Haefliger et al., 1989; Sim et al., 1993). Another suggestion that residues 12–59 of factor I exhibited similarity with the epidermal-growth-factor domain has now been superseded by the recognition of the FIM as a distinct structural motif (Catterall et al., 1987; Reid and Day, 1989). Of particular interest is that the FIM and LDLr domains occur also in several of the late complement components that form the lytic membrane-attack complex.

Knowledge of the arrangement of the five domains recognized to date in the factor I sequence is essential for any proposed molecular mechanism for the function of factor I in the complement system. Surprisingly little structural information is available on factor I, apart from sedimentation coefficient and electron-microscopic data (Cooper, 1975; DiScipio, 1992). No information is available on the structure of the FIM, CD5 or LDLr domains. The use of both X-ray and neutron solution scattering is a powerful means for determining overall structures under conditions close to physiological, and is a valuable complement to other techniques such as electron microscopy and

Abbreviations used: FIM, factor I module; LDLr, low-density-lipoprotein receptor; SP, serine proteinase; C4BP, C4b-binding protein.

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hydrodynamic studies (Perkins, 1988a,b). Use of the known crystal structures for the common SPs is a useful constraint on the interpretation of the scattering data [see the accompanying paper (Perkins et al., 1993)]. Here we determine the molecular dimensions of factor I in conditions close to physiological, and assess possible arrangements for the five domains within this structure. The structure of factor I can be compared with similar models for those of C4 and C4BP in order to assess the nature of the possible interactions between these proteins.

MATERIALS AND METHODS

Protein purification of factor I

Factor I was prepared from outdated human plasma (Regional Blood Transfusion Service, Oxford, U.K.), by the method of Hsiung et al. (1982) as modified by Sim et al. (1993). This procedure consists of extraction of factor I from plasma by passage over a column of MRC-OX21 [mouse anti-(human factor I) monoclonal antibody] covalently bound to Sepharose. Factor I was eluted from the antibody column with a pH 11.5 wash or by the use of 3 M MgCl₂ at pH 6.8 and was subsequently purified to homogeneity by gel filtration on Sephacryl S-200. As described for C3 and its fragments (Perkins and Sim, 1986), factor I was initially concentrated to about 2 mg/ml by ion-exchange on DEAE-Sephacel, using a step-elution procedure, and was then gel-filtered on a Sephacryl S300/Ultrogel AcA34 column equilibrated in 12.4 mM sodium phosphate/200 mM NaCl/0.5 mM EDTA, pH 7.0. The gel-filtration step removed traces of aggregates and equilibrated the protein in the buffer used for scattering experiments. A final concentration step was done using B15 Minicon concentrators (Amicon) (Perkins and Sim, 1986). The protein concentration (*c*) for solution scattering was calculated from the sample absorption at 280 nm using sequence data and the modified Wetlaufer procedure (Wetlaufer, 1962; Perkins, 1986).

Scattering data for factor I were collected using buffers containing 0.2 M NaCl, 12.5 mM sodium phosphate, with 0.1 mM, 0.5 mM or 1.0 mM EDTA, pH 7.0. For neutron experiments, samples were dialysed at 6 °C into their buffers containing 0, 80 or 100 % ²H₂O for at least 36 h with four changes of buffer. For X-ray work, dialysis into 0 % ²H₂O buffers was carried out likewise. The final dialysate was used for the buffer background run.

Neutron and X-ray solution scattering experiments

Neutron scattering data were obtained in three independent data collection sessions on Instrument D17 at the Institut-Laue-Langevin, Grenoble, France. The use of a sample-detector distance (*SD*) of 3.46 m and 1.40 m, a wavelength (*λ*) of 1.106 nm and detector-main beam angles of 0° or 20° resulted in a *Q* range of 0.07–3.21 nm⁻¹ (*Q* = 4π sin *θ* / *λ*; 2*θ* = scattering angle). Other experimental neutron data collection details are given in the accompanying paper (Perkins et al., 1993). Individual sample counting times typically ranged between 13 and 20 min in 100 % ²H₂O buffers to between 50 and 200 min in 0 % ²H₂O buffers.

After two preliminary sessions, final X-ray scattering data were obtained in two further sessions using the low-angle solution scattering camera at Station 8.2 (Townes-Andrews et al., 1989) at the Synchrotron Radiation Source, Daresbury, Warrington, U.K. Experiments were performed with beam currents in the range 128–253 mA and a ring energy of 2.00 GeV. Samples were measured for 10 min for protein concentrations that ranged between 1.0 and 4.1 mg/ml. The use of a 500-channel quadrant

detector with *SD* values of 3.54 m and 4.16 m resulted in a usable *Q* range between 0.06 and 2.22 nm⁻¹, with considerably improved signal-to-noise ratios over twice the *Q* range compared with the use of a linear detector in the preliminary sessions with factor I. Other details of experimental X-ray data collection are given in the accompanying paper (Perkins et al., 1993).

Analysis of scattering data

Scattering theory in application to factor I is briefly summarized. At small *Q* values, analysis of the scattering curves by the Guinier equation gives the radius of gyration (*R_G*) (which is a measure of macromolecular elongation) and the forward scattered intensity at zero *Q* [*I*(0)] (which is a measure of composition) (Guinier and Fournet, 1955; Glatter and Kratky, 1982):

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

This expression is valid for *I*(*Q*) values in a *Q* range corresponding to *Q* · *R_G* values less than 1.5. Absolute or relative molecular masses (*M_r*) are obtained from the *I*(0)/*c* values, where *c* is the sample concentration (Kratky, 1963; Jacrot and Zaccari, 1981). If one of the molecular dimensions is longer than the other two (by as little as 2:1:1), the radius of gyration of the cross-section *R_{xs}* and the cross-sectional intensity at zero angle [*I*(*Q*) · *Q*]_{*Q*→0} can be obtained from the *Q* range beyond that used in the *R_G* analyses (Pilz, 1982):

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

Hjelm (1985) has considered the analysis of macromolecules with a non-uniform cross-section along their major axis. This shows that *R_{xs}* and [*I*(*Q*) · *Q*]_{*Q*→0} parameters can still be derived, although these now become the weight average of the individual *R_{xs}*² values of the rod and the mean [*I*(*Q*) · *Q*]_{*Q*→0} value. This complication can be analysed by the use of the sphere modelling below to consider explicit arrangements of domains. The neutron and X-ray Guinier *R_G* and *R_{xs}* analyses were performed jointly using a common interactive analysis and plotting program SCTPL3.

In neutron-contrast-variation studies, the protein matchpoint (at which factor I is invisible to neutrons) was obtained from plots of √{[*I*(0)]/c*T_st*} and √{[*I*(*Q*) · *Q*]_{*Q*→0}/c*T_st*} against volume % ²H₂O (*T_s*, sample transmission; *t*, path length). The matchpoint was predicted from the amino acid and carbohydrate composition on the basis that the protein volume was unhydrated and there was 10 % of non-exchange of the main-chain NH protons (Perkins, 1986).

Since glycoproteins are inhomogeneous in their scattering densities, the *R_G* and *R_{xs}* data depend on the solvent scattering density. This dependence is described by the Stuhrmann equation (Ibel and Stuhrmann, 1976):

$$R_G^2 = R_{G-c}^2 + \alpha_G / \Delta\rho - \beta_G / \Delta\rho^2$$

where *R_{G-c}* is the *R_G* at infinite contrast, Δ*ρ* is the difference in scattering density (ρ) between the solute and solvent, α_{*G*} is the radial inhomogeneity of scattering density fluctuations within the protein, and β_{*G*} corresponds to the distance between the centres of two components of very different scattering densities. α_{*G*} is positive if the outermost scattering densities are higher than those nearer the centre. β_{*G*} is not usually measurable for glycoproteins. The analogous expression is valid for the neutron *R_{xs}* data, with the corresponding Stuhrmann parameters denoted by *R_{xs-c}*, α_{*xs*} and β_{*xs*}. The X-ray data correspond only to high positive Δ*ρ* values and cannot be analysed in this way.

Triaxial molecular dimensions are obtained from the Guinier and cross-sectional analyses. If factor I is assumed to be an

elliptical cylinder in shape, its length (L) is given by $\sqrt{[12(R_G^2 - R_{XS}^2)]}$ (Glatter and Kratky, 1982). Alternatively, L can be calculated from the intensity ratios, where L is derived from $\pi \cdot I(0)/[I(Q) \cdot Q]_{Q \rightarrow 0}$, and this is not dependent on the assumed shape (Perkins et al., 1986). The two semi-axes, A and B , of the elliptical cylinder are calculated by combining the dry (neutrons) or hydrated (X-rays) volume (V) ($V = \pi ABL$) with the R_{XS} , where $R_{XS}^2 = (A^2 + B^2)/4$ for an elliptical cylinder. The hydrated volume is obtained on the basis of a hydration of 0.3 g of water/g of glycoprotein and 0.0245 nm³ per water molecule (Perkins, 1986) [see the accompanying paper (Perkins et al., 1993)].

Indirect transformation of the scattering data in reciprocal space, $I(Q)$, into those in real space, $P(r)$, was carried out using the ITP81 program of Glatter (1982). This offers an alternative calculation of the R_G that is based on the full scattering curve, and gives the maximum dimension of the macromolecule L [for details, see the accompanying paper (Perkins et al., 1993)]. For factor I, the neutron $I(Q)$ contained 93 data points extending out to 3.01 nm⁻¹ and was fitted with eight splines with the maximum assumed dimension (D_{max}) set as 18.0 nm, whereas the X-ray $I(Q)$ contained 133 data points extending out to 2.23 nm⁻¹ and was fitted with eight splines with D_{max} set as 20.0 nm.

Modelling of the overall structure of factor I

Calculations of the full scattering curves for factor I using small Debye spheres followed standard procedures (Glatter and Kratky, 1982) in application to neutron and synchrotron X-ray scattering data (Perkins and Weiss, 1983; Perkins and Sim, 1986; Perkins et al., 1993). To constrain the simulations, the unhydrated volume of factor I was calculated from the composition using sequence data and the assumption that all six carbohydrate sites on factor I were occupied by either high mannose or tetra-antennary complex-type oligosaccharides respectively (total volumes of 92.1 nm³ or 104.1 nm³) (Chothia, 1975; Perkins, 1986). The neutron and X-ray curve-modelling procedure used was that described in the accompanying paper (Perkins et al., 1993). The final Debye models were generated with each sphere volume set as that of the cube of side 0.54 nm (dry model) or 0.589 nm (hydrated model). Note that a good curve-fit does not lead to the determination of a unique structure as the result of the spherical averaging which is implicit in solution scattering.

Modelling of the sedimentation coefficient ($s_{20,w}^0$) was based on the hydrated volume as in the X-ray curve-fitting. The frictional coefficient (f) was calculated from:

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

where $\rho_{20,w}$ is the density of water at 20 °C, N is Avogadro's constant, and $\bar{v}$ is the partial specific volume. The calculation of f from sphere models was performed using the program GENDIA (Garcia de la Torre and Bloomfield, 1977a,b) and the hydrated X-ray scattering model (but with non-overlapping spheres of diameter 0.589 nm), as described in the accompanying paper (Perkins et al., 1993).

RESULTS AND DISCUSSION

Neutron scattering studies of factor I

Neutron scattering was used to investigate the overall structure of factor I in ionic conditions close to physiological using 0, 80 and 100% ²H₂O buffers. A concentration range of 1.3–4.5 mg/ml gave satisfactory counting statistics (Figure 1a), and no con-

centration effects could be detected in this range. Linear Guinier R_G plots were reproducibly observed in an acceptable $Q \cdot R_G$ range between 0.3 to 1.3. Likewise linear cross-sectional Guinier R_{XS} plots could be obtained in an acceptable $Q \cdot R_{XS}$ range of 0.7 to 1.2 (Figure 1c).

Analysis of the Guinier $I(0)$ data for factor I is directly related to its composition. The total of 565 amino acid residues results in an M_r of 63300 for the protein component. For factor I, higher M_r values of 88000–93000 have been measured from hydrodynamic data or SDS/PAGE (Cooper, 1975; Pangburn et al., 1977; Fearon, 1977; Crossley and Porter, 1980), and another, of M_r 75000, was measured using SDS/PAGE (Hsiung et al., 1982). The difference in M_r is attributed to carbohydrate; however, no accurate carbohydrate content is available. Pangburn et al. (1977) report that factor I is at least 10.7% carbohydrate by weight. If six high-mannose oligosaccharide chains are present (each of assumed structure Man₆GlcNAc₂), these result in a final M_r of 74500, which is 15.0% carbohydrate (w/w). If six tetra-antennary complex-type oligosaccharide chains are present, this gives an M_r of 85300, which is 25.7% carbohydrate (w/w) and is close to the majority of biochemical determinations of the M_r . It is, however, known that high glycosylation levels in a glycoprotein lead to aberrantly high- M_r values when visualized by SDS/PAGE (Gordon, 1975). The predicted M_r values of 74500–85300 thus define an error range within which the scattering analyses were performed.

Figure 1 Guinier analyses of factor I by neutron and X-ray scattering

Closed circles correspond to the $I(Q)$ data employed in determining the R_G and R_{XS} values, and the straight lines correspond to the best fit through these points. Statistical error bars were shown only when these were large enough to be visible, as in the 0% ²H₂O neutron data. (a) Neutron R_G Guinier plots for factor I in 100% and 0% ²H₂O buffers. The Q range used for the R_G values was 0.08–0.34 nm⁻¹. Sample concentrations (c) were 3.5 and 2.4 mg/ml for the 100% ²H₂O data and 4.7 mg/ml for the 0% ²H₂O data. In 100% ²H₂O, the mean R_G was 3.86 ± 0.12 nm (six values) for c between 1.3 and 4.5 mg/ml. In 0% ²H₂O, the mean R_G was 3.84 ± 0.11 nm (four values) for c between 3.1 and 4.7 mg/ml. (b) X-ray R_G Guinier plots for factor I in ¹H₂O buffers (after exposure times of 10 min). The Q range used for the R_G values was 0.16–0.34 nm⁻¹. Concentrations were 4.1, 3.1 and 2.1 mg/ml. The mean R_G was 3.96 ± 0.12 nm (six values) for c between 1.5 and 4.1 mg/ml. (c) Neutron R_{XS} Guinier plots for factor I in 100% and 0% ²H₂O buffers. The Q range used for the R_{XS} values was 0.45–0.81 nm⁻¹. Concentrations were 2.4 mg/ml and 1.3 mg/ml for the 100% ²H₂O data and 4.7 mg/ml for the 0% ²H₂O data. In 100% ²H₂O, the mean R_{XS} was 1.48 ± 0.05 nm (seven values) for c between 0.8 and 3.4 mg/ml. In 0% ²H₂O, the mean R_{XS} was 1.66 ± 0.03 nm (three values) for c between 3.1 and 4.7 mg/ml. (d) X-ray R_{XS} Guinier plots for factor I, using the same curves used in (b) above. The Q range used for the R_{XS} values was 0.40–0.72 nm⁻¹. The mean R_{XS} was 1.64 ± 0.04 nm (ten values) for c between 1.5 and 4.1 mg/ml.

The neutron M_r of factor I was calculated from the Guinier $I(0)/c$ values measured in 0% $^2\text{H}_2\text{O}$, and this required the concentrations c . The M_r values of 74500 and 85300 led to calculated $A_{280}^{1\%, 1\text{cm}}$ values of 14.1 and 12.3 respectively. This range of A_{280} values is more accurate than the previously assumed values of 10.0 (Crossley and Porter, 1980; Nagasawa et al., 1980) or 5.0 (Goldberger et al., 1984). The mean of four M_r determinations from $I(0)/c$ was calculated to be either 72000 (starting from a sequence M_r of 74500) or 63000 (from a sequence M_r of 85300). Since the individual M_r determinations exhibited variability (which is caused by the high incoherent scattering background of $^1\text{H}_2\text{O}$ and low sample concentrations), and the statistical error in the M_r determinations is ± 27000 , no choice between the M_r values was made. Both values are deemed to be consistent with the sequence M_r values, and show that factor I is monomeric in solution.

The analysis of $I(0)$ values in different contrasts leads to the matchpoint determination of factor I, at which its mean scattering density is the same as the buffer. The matchpoint graph of $\sqrt{[I(0)]/cT_s t}$ versus the volume percentage of $^2\text{H}_2\text{O}$ (not shown) was based on the following mean values: 0% $^2\text{H}_2\text{O}$, 0.304 ± 0.056 (four values); 80% $^2\text{H}_2\text{O}$, -0.306 ± 0.022 (three); 100% $^2\text{H}_2\text{O}$, -0.462 ± 0.041 (six). The corresponding matchpoint graph using the $\sqrt{[I(Q) \cdot Q]_{Q \rightarrow 0}}/cT_s t$ data was based on the following values: 0% $^2\text{H}_2\text{O}$, 0.051 ± 0.002 (three values); 80% $^2\text{H}_2\text{O}$, -0.046 ± 0.001 (two); 100% $^2\text{H}_2\text{O}$, -0.069 ± 0.005 (seven). These intensity data have been referenced to the incoherent scattering of water as an absolute standard. MINITAB linear regressions gave matchpoints of $40.1 \pm 1.7\%$ $^2\text{H}_2\text{O}$ for the $I(0)$ data and $42.4 \pm 1.3\%$ $^2\text{H}_2\text{O}$ for the $[I(Q) \cdot Q]_{Q \rightarrow 0}$ data. The calculation from the two compositions gave matchpoints of 42.5% $^2\text{H}_2\text{O}$ and 43.1% $^2\text{H}_2\text{O}$ for M_r values of 74500 and 85300 respectively. The satisfactory agreement of the observed and calculated matchpoint determinations show that factor I remains monodisperse in the three $^2\text{H}_2\text{O}$ buffers employed in the present study.

The Stuhrmann analysis of the dependence of the R_G values on the solute-solvent contrast (Figure 2) resulted in a radius of gyration at infinite contrast R_{G-C} of 3.84 ± 0.03 nm and a slope α_G of $2(\pm 8) \times 10^{-5}$, which is close to zero. Calculation of the elongation ratio or anisotropy R_G/R_0 (where R_0 is the R_G of the sphere with the same dry volume as factor I) gave a value of 1.77 (± 0.01). Since the R_G/R_0 values for typical globular proteins are close to 1.28 (Perkins, 1988b), it is seen that factor I possesses an extended solution structure. This structural anisotropy is similar to that found with the complement components C3, C4, C5 and C9 which are moderately elongated in shape (Perkins et al., 1990; Smith et al., 1992).

The Stuhrmann analysis of the R_{XS} data (Figure 2) gave an R_{XS-C} value of 1.56 ± 0.01 nm and a positive slope α_{XS} of $11(\pm 2) \times 10^{-5}$. The combination of the mean R_G and R_{XS} data in 0, 80 and 100% $^2\text{H}_2\text{O}$ gave lengths (L) of 12.2 ± 0.1 nm, 11.9 ± 0.1 nm and 12.3 ± 0.6 nm in that order. The alternative calculation based on the ratio of intensities gave L values in good agreement, of 13.1 ± 2.5 nm, 13.3 ± 2.5 nm and 14.1 ± 1.4 nm respectively. The mean L value is thus 12.8 ± 0.8 nm. Use of the dry volume of 92.1 nm^3 (M_r 74500) resulted in mean axial dimensions $L \times 2A \times 2B$ of $12.8 \text{ nm} \times 5.7 \text{ nm} \times 1.6 \text{ nm}$ for factor I, where the S.D.s of 2A and 2B are ± 0.6 nm and ± 0.2 nm respectively. Use of the dry volume of 104.1 nm^3 (M_r of 85300) gave similar dimensions within error of $L \times 2A \times 2B$ of $12.8 \text{ nm} \times 5.7 \text{ nm} \times 1.9 \text{ nm}$. To a first approximation, factor I is seen to have an elongated cross-sectional shape.

Two full neutron scattering curves $I(Q)$ in reciprocal space extending to $Q = 3 \text{ nm}^{-1}$ were transformed into distance dis-

Figure 2 Neutron-contrast-variation analyses of the structure of factor I

The Stuhrmann plot of R_G^2 and R_{XS}^2 against the reciprocal solute-solvent contrast difference ($\Delta\rho^{-1}$) is shown. The mean R_G^2 (●) and R_{XS}^2 (○) values are given at each contrast. Statistical error bars were shown only when these were large enough to be visible. MINITAB regressions gave an R_{G-C} of 3.84 ± 0.03 nm at zero $\Delta\rho^{-1}$ and a slope (α_G) of $2(\pm 8) \times 10^{-5}$ (13 points), and an R_{XS-C} of 1.56 ± 0.01 nm at zero $\Delta\rho^{-1}$ and a slope (α_{XS}) of $11(\pm 2) \times 10^{-5}$ (12 points).

Figure 3 Distance distribution functions $[P(r)]$ calculations (Glatter, 1982) based on the neutron and X-ray $I(Q)$ data for factor I

(a) The neutron $P(r)$ curve corresponds to the solid line with statistical error bars, using a factor I sample at 3.5 mg/ml measured in a negative solute-solvent contrast in a 100% $^2\text{H}_2\text{O}$ buffer. The neutron maximum length of factor I lies between 13.5 and 18 nm, based on a calculation with eight splines and $D_{\max}$ set at 18 nm. (b) The X-ray $P(r)$ curve is depicted by the continuous line; this corresponds to factor I at 4.1 mg/ml in a positive solute-solvent contrast. The X-ray maximum length of factor I is estimated as 15 ± 4 nm. The broken line corresponds to the neutron $P(r)$ curve from (a).

tribution functions $P(r)$ in real space (Figure 3a). The best $P(r)$ analysis showed that the $I(0)$ value from $P(r)$ was within 1% of the Guinier value, whereas the R_G was 10% larger. The statistical errors of Figure 3(a) and the larger-than-expected R_G value suggest that the maximum dimension of factor I is at least 13.5 nm and that 18 nm is the upper limit. The $P(r)$ curve is thus consistent with the L value of 12.8 ± 0.8 nm from the Guinier analyses. The most frequently occurring distance within factor I is deduced from the maximum in $P(r)$ to be at $r = 3.2$ nm.

The neutron contrast variation α_G and α_{XS} values can be compared with those for other glycoproteins after these have

been normalized on the basis of their proportionality to R_G^2 and R_{XS}^2 . Using a panel of eight proteins of known α_G values (lysozyme, myoglobin, β -trypsin, trypsinogen, α_1 -acid glycoprotein, α_1 -antitrypsin, C1 inhibitor and C9), α_G for factor I is predicted to be $47(\pm 22) \times 10^{-5}$. This is higher than the observed α_G value close to zero. Using a panel of seven proteins of known α_{XS} values (α_1 -antitrypsin, C4, C4u, C4b, C5, C9 and factor H), α_{XS} for factor I is predicted to be $12(\pm 4) \times 10^{-5}$. This agrees well with the observed α_{XS} value of $11(\pm 2) \times 10^{-5}$. In terms of its average cross-sectional properties, the distribution of hydrophilic and hydrophobic residues in factor I is similar to that expected for elongated glycoproteins. The low α_G of factor I is thus the result of a relatively even distribution of hydrophilic and hydrophobic residues along the longest axis of the macromolecule, as encountered for C1q of complement (Perkins et al., 1984) and also C3, C4 and C5 of complement.

Synchrotron X-ray scattering studies of factor I

As a corroboration of the neutron data, synchrotron X-ray scattering (corresponding to high positive solute-solvent contrasts) was used with factor I in the concentration range 1.5–4.1 mg/ml. Analyses of the ten time frames during selected sample runs showed that beam irradiation did not lead to time-dependent changes in the scattering curves of factor I. Linear Guinier R_G and R_{XS} plots within satisfactory $Q \cdot R_G$ and $Q \cdot R_{XS}$ ranges were obtained (Figures 1b and 1d). The M_r of factor I was determined to be 89 000–78 000 (± 7000) (six determinations for c between 2.1 to 4.1 mg/ml) by reference to four other proteins of known M_r and composition measured in the same beamtime session. This calculation is within error of the neutron M_r and the two possible sequence M_r values, and shows that the data correspond to monomeric, monodisperse factor I. The mean R_G was determined to be 3.96 ± 0.12 nm (eight determinations), and the mean R_{XS} was 1.64 ± 0.04 nm (ten). These values are within error of the neutron R_G and R_{XS} data measured in 0% $^2\text{H}_2\text{O}$ buffers of 3.84 ± 0.11 nm and 1.66 ± 0.03 nm.

Combination of the overall and cross-sectional X-ray Guinier analyses lead to a length (L) of 12.7 ± 0.6 nm from the R_G and R_{XS} data, and one of 13.9 ± 0.8 nm from the ratio of intensities, which are both in good agreement with the neutron value 12.8 ± 0.8 nm. The molecular dimensions $L \times 2A \times 2B$ of factor I were determined as $12.8 \text{ nm} \times 6.3 \text{ nm} \times 1.8 \text{ nm}$ and $12.8 \text{ nm} \times 6.2 \text{ nm} \times 2.1 \text{ nm}$ for M_r values of 74 500 and 85 300 respectively, of hydrated volume 122.6 nm^3 and 138.9 nm^3 . Since the S.D.s of $2A$ and $2B$ were ± 0.2 nm and ± 0.1 nm respectively, these two results are within error of each other, and also agree with those of factor I by neutrons.

The X-ray $P(r)$ curve gave a maximum dimension of factor I of 15 ± 1 nm, which is consistent with other determinations. The maximum in the X-ray $P(r)$ curve is at $r = 3.6$ nm, which is slightly larger than, but within error (± 0.2 nm) of, the neutron $P(r)$ maximum at 3.2 nm. This similarity reflects the low positive value of α_G observed in Figure 2, and confirms the overall low inhomogeneity of scattering density within factor I. Larger differences between the X-ray and neutron $P(r)$ in Figure 3(b) were seen only at large r , where the precision of the $P(r)$ calculation is reduced.

Scattering curve simulations for factor I

The full neutron and X-ray scattering curves for factor I were modelled using small spheres in order to assess possible arrangements of the five domains within factor I. Calculations were

performed twice to assess the effect of the M_r values of 74 500 and 85 300.

Neutron curve-fitting was initially based on a simple elliptical cylinder to assess the overall dimensions of factor I (Figure 4a). Sphere models were constructed to follow the R_{G-C} of 3.83 ± 0.11 nm and the approximate dimensions of $12.8 \text{ nm} \times 5.7 \text{ nm} \times (1.6\text{--}1.9 \text{ nm})$ ($\pm 0.8\text{--}0.2$ nm) derived from the Guinier analyses. For an M_r of 74 500 (volume of 92.1 nm^3), the testing of 29 models showed that good fits to the 100% and 0% $^2\text{H}_2\text{O}$ data could be obtained with a 268-sphere model (cube side 0.70 nm; volume 91.9 nm^3) in an array of $19 \times 8 \times 2$ with dimensions of $13.3 \text{ nm} \times 5.6 \text{ nm} \times 1.4 \text{ nm}$. This model had an R_G of 3.85 nm. The goodness-of-fit (R) coefficient (Smith et al., 1990) was acceptable at 0.020 for the 100% $^2\text{H}_2\text{O}$ curve and 0.021 for the 0% $^2\text{H}_2\text{O}$ curve. A single-density model gave good curve-fits in both contrasts, in accordance with the low Stuhmann α_G value. The relatively low M_r of factor I and the resulting poorer counting statistics for the 0% $^2\text{H}_2\text{O}$ curve did not permit an assessment of whether two-density models would give improved curve-fits. For the M_r of 85 300 (volume of 104.1 nm^3), the above elliptical cylinder model was adapted by the addition of an extra segment of spheres in an array of $19 \times 2 \times 1$ at the centre of the model to give a total of 304 spheres in place of 268 spheres. The dimensions of this model were very similar at $13.5 \text{ nm} \times 6.3 \text{ nm} \times 1.4 \text{ nm}$. This model had an R_G of 3.96 nm. It gave good curve-fits to both the 100% and 0% $^2\text{H}_2\text{O}$ data with goodness-of-fit parameters (R) of 0.016 and 0.022 respectively.

Further sphere models for factor I were developed to take account of its five-domain structure. Its heavy chain was divided into four segments: i.e. Lys¹–Thr⁸⁹ for the FIM domain and a short N-terminal segment (plus two oligosaccharide sites), Ala⁹⁰–Thr¹⁹⁸ for the CD5 domain (plus one oligosaccharide), Gln¹⁹⁹–Cys²³⁷ for the first LDLr domain and Cys²³⁸–Arg³²¹ for the second LDLr domain and a C-terminal segment. The light chain Ile³²²–Val⁵⁶⁵ (plus three oligosaccharides) was assigned as the SP domain. The volumes of these five segments were 16.8, 17.5, 5.3, 11.7 and 41.1 nm^3 respectively. If each segment was spherical in shape (the most compact shape possible), their diameters are 3.2, 3.2, 2.2, 2.8 and 4.3 nm respectively. If these five spheres were arranged in a linear structure, the sum of the diameters corresponds to a minimum overall length of 15.7 nm (or 16.2 nm for a total volume of 104.1 nm^3). Since it is more likely that the five domains are non-spherical, this minimum length of 15.7–16.2 nm would be increased if all five domains were aligned within factor I with their longest axes co-linear with the major axis of factor I. Since factor I has a length of 13 nm, this shows that the five domains in factor I cannot be arranged as a linear structure.

The SP domain was modelled starting from the β -trypsin sphere model in the accompanying paper (Perkins et al., 1993) and the sequence alignment in the accompanying paper (Perkins and Smith, 1993). The β -trypsin model of 188 spheres was converted into the SP domain of factor I by adding 73 spheres. Of these, 39 spheres correspond to the three oligosaccharide chains at Asn⁴⁴⁶, Asn⁴⁷⁶ and Asn⁵²⁸. In the β -trypsin crystal structure, these correspond to Gly¹³³, Ser¹⁶⁷ and Ser²⁰². All were located on the same face of the structure, away from the catalytic triad (Figure 4). All three were taken to be either the high-mannose or the tetra-antennary complex types to correspond to the M_r values of 74 500 and 85 300. The remaining 34 spheres correspond to amino acid residues which were added to allow for three large sequence insertions between β -strands D and F, H and I, M and N, and one more at the C-terminus in β -trypsin, as well as several minor residue insertions and deletions. In summary, the volumes of the FIM, CD5, LDLr-1, LDLr-2 and SP domains correspond to 106, 111, 34, 74 and 261 spheres, in that

Figure 4 Comparisons of the curve simulations for factor I with neutron scattering data

The factor I curve (continuous line) calculated from the model shown on the right is compared with the experimental curves (●) measured at 3.5 mg/ml in a 100% $^2\text{H}_2\text{O}$ buffer and at 4.7 mg/ml in a 0% $^2\text{H}_2\text{O}$ buffer on Instrument D17. Error bars due to data-counting statistics are shown when these are large enough to be seen. (a) Scattering-curve simulations are shown for a simple ellipsoidal model of factor I, based on 268 spheres of diameter 0.70 nm. (b) Simulations were based on a family of models in which an SP domain derived from the small-sphere model for β -trypsin is attached to an ellipsoid to represent the putative FIM, CD5, and two LDLr domains for a total M_r of 85300. A total of 652 spheres (diameter 0.54 nm) was used. The putative location of three carbohydrate chains on the SP domain is marked by 'dotted spheres' (○), as also in (c) below. (c) Simulations were now based on another family of models in which the SP domain is now attached to a V-shaped structure which represents the four remaining domains. A total of 668 spheres (diameter 0.54 nm) was used for a total M_r of 85300.

order for a total volume of 92.1 nm³, and 131, 124, 34, 74 and 299 for a total volume of 104.1 nm³.

In one class of neutron models, factor I was considered as the association of the SP domain with an elliptical cylinder for the remaining FIM, CD5, LDLr-1 and LDLr-2 domains. For the M_r of 74500 (volume 92.1 nm³), the elliptical cylinder was composed of 323 spheres. Testing showed that the most satisfactory curve fit resulted from an array of $14 \times 8 \times 3$ spheres (7.6 nm $\times$ 4.3 nm $\times$ 1.6 nm in size), totalling 585 spheres in all (Figure 4b). This resulted in an R_g value of 3.72 nm. The R coefficient of the curve-fits was acceptable at 0.022 for the 100% $^2\text{H}_2\text{O}$ data and 0.021 for the 0% $^2\text{H}_2\text{O}$ data. For the M_r of 85300 (volume 104.1 nm³), an array of $13 \times 9 \times 3$ spheres (7.0 nm $\times$ 4.9 nm $\times$ 1.6 nm) gave the best fit with a total of 652 spheres. This gave an R_g value of 3.77 nm. The R coefficients of the curve-fits were good at 0.015 for the 100% $^2\text{H}_2\text{O}$ data and 0.022 for the 0% $^2\text{H}_2\text{O}$ data.

In a second class of neutron models, factor I was considered as the association of the SP domain with a V-shape for the other domains. The first arm was constructed from 108 spheres (the two LDLr domains) and the second arm contained 217 or 255 spheres (the FIM and CD5 domains). For the M_r -74500 model, trial-and-error calculations showed that the most satisfactory curve-fits were obtained with an inter-arm angle of 10°, and that the two arms were best set as arrays of $14 \times 4 \times 2$ and $18 \times 4 \times 3$

spheres. The final model contained 590 spheres (volume 92.9 nm³), and had an R_g value of 3.78 nm. The R coefficients of the curve-fits were good at 0.015 for the 100% $^2\text{H}_2\text{O}$ data and 0.021 for the 0% $^2\text{H}_2\text{O}$ data. For the M_r -85300 model, the best curve-fit was obtained with arrays of $14 \times 4 \times 2$ and $17 \times 3 \times 5$ spheres. This model contained 668 spheres (105.2 nm³), and led to an R_g value of 3.87 nm. The R coefficients of the curve-fits were also good at 0.011 for the 100% $^2\text{H}_2\text{O}$ data and 0.023 for the 0% $^2\text{H}_2\text{O}$ data.

For comparison with the X-ray scattering curves, the neutron models were hydrated assuming 0.3 g of water/g of glycoprotein. Good curve-fits in the Q ranges corresponding to the R_g Guinier fits were obtained out to a Q of 0.4 nm⁻¹ (results not shown). The curve-fits deteriorated in the R_{xs} region of the scattering curve, which was contrast-sensitive in the neutron experiments (Figure 2). This discrepancy was thus attributed to the high (15.0–25.7%) carbohydrate content of factor I, since carbohydrate residues are electron-rich and make a relatively large contribution to X-ray scattering curves.

Simulations of hydrodynamic data for factor I

Hydrodynamic sedimentation data serve as an independent control of the structure of factor I. The sedimentation coefficient

($s_{20,w}^0$) of factor I has been reported to be 4.5 S at 10.15 (Cooper, 1975). On the basis of the M_r values of 74 500 and 83 500, the calculated hydrated $\bar{v}$ of factor I for $s_{20,w}^0$ analyses is 0.714 ml/g and 0.708 ml/g respectively (Perkins, 1986). From hydrated volumes of 122.6 nm³ or 138.9 nm³, frictional ratios (f/f_0) of 1.36 or 1.52 were determined.

Hydrodynamic spheres were used to calculate the value of $s_{20,w}^0$ directly from the hydrated-sphere model used for X-ray modelling. The ellipsoid, SP/ellipsoid and SP/V-shape models (Figure 4) gave predicted $s_{20,w}^0$ values of 4.9 S, 4.6 S and 4.3 S in that order for the 92.1 nm³ model. The $s_{20,2}^0$ values were 5.5 S, 4.8 S and 4.7 S respectively for the 104.1 nm³ model. Since the precision of the experimental data is usually considered to be ± 0.3 S (Perkins, 1989), the second and third types of models are seen to be fully compatible with the experimental $s_{20,w}^0$ value of 4.5 S, irrespective of the presumed M_r for factor I, while the agreement for the ellipsoid model is at the limit of error. These agreements support the dimensions determined for factor I from the scattering analyses.

CONCLUSIONS

Factor I has a multidomain structure like many other complement proteins. The present study shows that its overall dimensions are not compatible with a fully extended arrangement of the five domains in solution. The overall length of factor I has been determined four times [from the Guinier data, $P(r)$ analyses, scattering and hydrodynamic modelling] to be close to 13–14 nm.

Figure 5 Comparison of the dimensions of factor I with the complement components C4 and C4BP

The scattering model of factor I (sphere diameter 0.54 nm) is shown in comparison with its cofactor complement component C4BP (sphere diameter 1.0 nm) and one of its substrates C4 (sphere diameter 0.8 nm) (Perkins et al., 1986, 1990). It should be noted that other scattering-equivalent models can offer equally satisfactory curve-fits to the data, and that the general form of these structures should not be over-rated.

It is gratifying that DiScipio (1992) independently reported a length of 13 nm by electron microscopy for factor I after we had completed the present study. A linear extended arrangement of factor I domains would be at least 15–16 nm in length, and would most probably be longer. Most of the other complement proteins studied so far possess extended domain structures (C1q, C1r, C1s, C1 inhibitor, C4BP, factor H and properdin) (Perkins et al., 1990). C9 is the only other complement protein studied by scattering to date to possess a non-extended domain structure (Smith et al., 1992).

The explicit consideration of possible domain arrangements in factor I was much assisted by the ability to model the largest one of the five in the light chain with spheres using its structural homology with β -trypsin. The two obvious possible arrangements for the four domains in the heavy chain are a zig-zag conformation with the long axes of the small domains arranged side-by-side (Figure 4b) or a V-shaped structure (Figure 4c). It is reasonable to expect that the FIM, CD5 and two LDLr domains are about 4 nm $\times$ 2 nm $\times$ 2 nm in size, by analogy to the short complement/consensus repeat in C4BP and the thrombospondin repeat in properdin (Perkins et al., 1986; Smith et al., 1991). A zig-zag conformation of four domains would be readily accommodated within the dimensions of 7–8 nm $\times$ 4–5 nm $\times$ 2 nm (Figure 4b). Alternatively a V-shaped structure would correspond to two LDLr domains of size about 8 nm $\times$ 2 nm $\times$ 2 nm located in the arm attached to the SP domain, and a pair of FIM and CD5 domains of size about 10 nm $\times$ 2 nm $\times$ 2 nm in the other arm (Figure 4c). It is not possible to discriminate between either of these possibilities by scattering, as they are equivalent in scattering. The electron-microscopic images obtained by DiScipio (1992) also do not distinguish between either of these possibilities. There, a bilobal structure with an overall length of 13.0 ± 1.4 nm and lobes of 4.9 ± 0.9 nm and 5.4 ± 0.8 nm in diameter was observed, in which one lobe may be V-shaped in appearance. We have attempted to model factor I with two solid lobes joined by a linker of various lengths and sizes, but the curve-fits were not promising. It can nonetheless be concluded that factor I does not contain an extended linear conformation of domains. Structural studies at higher resolution will be required to determine more precise details.

Irrespective of the packing arrangement of the domains in factor I, the present study implies that the domains are arranged in such a way that steric factors will be important for functional activity. It is possible that direct interaction of factor I with a cofactor protein or with the C3b/C4b cofactor complex causes a change in exposure of the reactive-site region. A comparative view of these low-resolution structures is shown in Figure 5. It is not possible to predict from the modelling if the active site of the SP domain would be sterically masked by the domains in the elliptical cylinder or V-shaped segments in Figures 4(b) or 4(c). Work by Ekdahl et al. (1990) suggests a conformational change in factor I on interaction with C3b, although this does not lead to cleavage of C3b in the absence of a cofactor protein. Whether these changes correspond to gross conformational rearrangements involving domain movements starting from the structures shown in Figures 4(b) and 4(c), or to localized ones involving a few residues, is not known. The accessibility of the catalytic site on the SP domain is clearly of great significance for complement activation. It is possible that one of the functional roles of the multidomain complement structures is that they not only offer high specificity in the protein–protein interactions that are involved in complement activation, but also a simple means of altering the reactivity of the individual components by steric displacements of individual domains or residues within each domain.

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