Volume 271, number 1,2, 89–92 FEBS 08899 October 1990

Hydrodynamic data show that C$\bar{1}$ inhibitor of complement forms compact complexes with C$\bar{1}$r and C$\bar{1}$s

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Received 4 July 1990

The C$\bar{1}$ inhibitor of the complement cascade forms stoichiometric complexes with C$\bar{1}$r and C$\bar{1}$s and controls the activation of first component C1 of complement. Literature sedimentation coefficients $s^{\circ}_{20,w}$ for the complexes formed between C$\bar{1}$ inhibitor, C$\bar{1}$r and C$\bar{1}$s were analysed using frictional ratios and the hydrodynamic sphere approach. A head-and-tail two-domain model for C$\bar{1}$ inhibitor was combined with cylindrical hydrodynamic models for the six-domain structures of C$\bar{1}$r and C$\bar{1}$s. The hydrodynamic data show that the heavily glycosylated N-terminal domain of C$\bar{1}$ inhibitor is positioned close to the two complement 'short consensus repeat' domains found in the centre of C$\bar{1}$r and C$\bar{1}$s.

C$\bar{1}$ inhibitor; C1r; C1s; Complement; Sedimentation coefficient; Hydrodynamic simulation

1. INTRODUCTION

C1, the first component of the classical pathway of the complement cascade, is a complex formed between two large distinct multidomain subunits, C1q and tetrameric $C1r_2C1s_2$. C1q provides the recognition element of C1 for binding to immune complexes, while the enzymatic activity of C1 resides in two proteins C1r and C1s that bind to C1q. The control of C1 activation to C$\bar{1}$ is an essential aspect of the complement cascade [1]. This is achieved by C$\bar{1}$ inhibitor, a member of the SERine Proteinase INhibitor (SERPIN) superfamily [2].

C$\bar{1}$ inhibitor is a two-domain protein which binds with the six-domain proteins C$\bar{1}$r and C$\bar{1}$s, the activated forms of C1r and C1s, and removes these from C$\bar{1}$ [2]. The serpin domain of C$\bar{1}$ inhibitor reacts with the serine proteinase domain found in each of C$\bar{1}$r and C$\bar{1}$s. Little is known of the structure of their complexes. The solution structures of the domains within C$\bar{1}$ inhibitor, C1r and C1s have been recently studied by neutron scattering and hydrodynamic analyses [3,4]. Improved determinations of their molecular weights from sequence data are now available [5–7]. Extensive sedimentation coefficient $s^{\circ}_{20,w}$ data on these proteins and the complexes formed between them have been published by Colomb and coworkers, however, these data have not been interpreted by structural analyses [8,9]. It is now possible using the method of hydrodynamic sphere modelling with these data to analyse structures for the interaction between C$\bar{1}$ inhibitor and each of C$\bar{1}$r and C$\bar{1}$s [10,11]. This will identify the arrangement of the domains in C$\bar{1}$ inhibitor relative to those within C$\bar{1}$r and C$\bar{1}$s in the complexes.

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2. RESULTS AND DISCUSSION

2.1. *Hydrodynamic analyses of C$\bar{1}$ inhibitor, C$\bar{1}$r and C$\bar{1}$s*

Neutron scattering analyses show that C$\bar{1}$ inhibitor is 18 nm in length, and can be constructed from a serpin domain of size 7 × 3 × 3 nm, and an N-terminal domain of length 15 nm [4]. Most reports of the sedimentation coefficient $s^{\circ}_{20,w}$ of C$\bar{1}$ inhibitor fall in a range of 3.67–3.8 S (Table I). A distinct two-domain head-and-tail hydrodynamic sphere model of length 15 nm to 18 nm gives a good account of these $s^{\circ}_{20,w}$ data [4]. Simple hydrodynamic rods were shown to lead to overestimated lengths for C$\bar{1}$ inhibitor [4], and were not used further in the modelling of the complexes below.

C1r and C1s are each constructed from six domains denoted I to VI (Fig. 1) [6,7]. Calculations of their overall lengths from neutron scattering and electron microscopy suggest values of 25, 16 or 21–26 nm [3,12,13]. The use of both the hydrodynamic sphere and rod methods to analyse $s^{\circ}_{20,w}$ values for C1r and C1s leads to lengths between 17 and 20 nm in satisfactory agreement with these other studies [3]. C1r II is a proteolysed form of C1r that contains only the serine proteinase domain VI and two complement 'short consensus repeat' (SCR) domains IV and V. This has a hydrodynamic length of 9–11 nm [3].

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00145793/90/$3.50

Table I

Comparisons of experimental and calculated sedimentation coefficients

Macromolecule	M_r ($\times 10^{-3}$)	Hydrated volume (nm^3)	$s^o_{20,w}$ (S)		Frictional ratio, f/f_o	Length (nm)
			Experimental	Calculated		
Monomers						
C1r	86	143	5.1	5.0	1.27	17
C1r II	50	84	4.1	3.8	1.09	9
C1s	79	132	4.5	4.5	1.36	19
C1 inhibitor	71	117	3.67–3.8	3.8, 3.5	1.54	18, 15
Dimers						
C1r·C1 inhibitor	157	260	6.5	6.5, 6.3	1.49	21, 18
C1r II·C1 inhibitor	121	201	5.8	5.5, 5.3	1.40	21, 17
C1s·C1 inhibitor	150	249	6.1	6.1, 5.9	1.54	21, 20
Tetramers						
$C1s_2$·C1 $inhibitor_2$	300	499	7.7	7.9, N.C.	1.94	39, 39
C1r·C1s·C1 $inhibitor_2$	307	509	8.5	8.3, N.C.	1.79	37, 37

For C1 inhibitor, the sources of $s^o_{20,w}$ data are summarised in [4]; those for C1r, C1r II and C1s are summarised in [3]. Those for the C1 inhibitor complexes are given in [8,9]. The hydrated volume corresponds to the sum of the dry glycoprotein volume and the hydration shell volume assuming a hydration of 0.3 g H_2O/g glycoprotein and an electrostricted water molecule volume of 0.0245 nm^3, and this leads to partial specific volumes v [16,17], e.g., 0.721 ml/g for C1 inhibitor. Frictional ratios f/f_o are calculated from $f = M_r(1 - v\varrho)/N_a \cdot s^o_{20,w}$ for the macromolecule (f, frictional coefficient; N_a, Avogadro's constant; M_r, molecular weight) and from $f_o = 6\pi a\eta$, where f_o is the frictional coefficient of the sphere (radius a) with the same hydrated volume as the macromolecule (ϱ, buffer density; η, buffer viscosity). When hydrodynamic spheres are used, this hydration is increased to 0.39 g in order to compensate for the void spaces between the non-overlapping spheres [11]. For C1 inhibitor, the protein spheres are 1.69 nm diameter and the carbohydrate spheres are 0.95 nm diameter [4]. The C1r, C1r II and C1s models are those of [3], with sphere diameters of 4.186 nm, 4.417 nm and 3.786 nm in that order. Calculations were performed using the program GENDIA [18]. The results for the C1 inhibitor calculations correspond firstly to the compact carbohydrate structure, then the extended one, as presented in [4]. N.C., not computable for reason of computer memory limitations.

2.2. *Complexes formed between C1 inhibitor and C1r and C1s*

Literature $s^o_{20,w}$ data from sucrose density gradient ultracentrifugation [14] are summarised in Table I for the complexes of C1 inhibitor with each of C1r, C1r II and C1s [8,9]. The most recent molecular weight determinations from sequences are given in Table I. Note that the revised value for C1 inhibitor is now 71000 as a result of DNA and carbohydrate sequencing and supersedes previous estimates close to 100000 [2,4]. The partial specific volumes of C1r, C1s and C1 inhibitor lie in a restricted range of 0.721–0.725 ml/g [3,4] as required [14]. The frictional ratios f/f_o (Table I) for C1r, C1s and C1 inhibitor are similar in a range of 1.3–1.5, while that for C1r II is less at 1.1, as expected from the hydrodynamic lengths summarised above (Table I). Calculations of f/f_o for the three dimeric complexes in Table I show that these fall in a similar range of 1.4–1.5, and therefore that the dimers are as elongated as uncomplexed C1 inhibitor. The f/f_o values for the tetrameric complexes are larger at 1.8–1.9, and are more elongated than the monomers and dimers.

Hydrodynamic sphere models were used to account for the $s^o_{20,w}$ data (Table I). It is known that the serine proteinase domain VI in C1r, C1r II and C1s is positioned at one end of the six-domain structure of hydrodynamic length 17–20 nm [13,15]. The modelling of the complexes is accordingly constrained so that domain VI is close to the serpin domain of C1 inhibitor. Two calculations of the $s^o_{20,w}$ and length are reported for C1 inhibitor and its complexes in Table I. These correspond to controls in which the 26% carbohydrate content of C1 inhibitor is either indistinguishable from the protein surface or positioned in an extended configuration as in Fig. 1(a) [4].

Good agreement with the experimental $s^o_{20,w}$ data for the five complexes was obtained only for the most compact models that were tested, e.g., that for C1s when aligned in the $+x$ direction from the contact with the serpin domain in Fig. 1. These models position the N-terminal domain of C1 inhibitor close to the two SCR domains IV and V at the centre of C1r and C1s [3]. Table I shows that the C1 inhibitor complexes with C1r, C1r II and C1s have hydrodynamic lengths between 18 and 21 nm, which are similar to those of 15–19 nm for free C1 inhibitor, C1r and C1s. The calculated and experimental $s^o_{20,w}$ values for the dimers

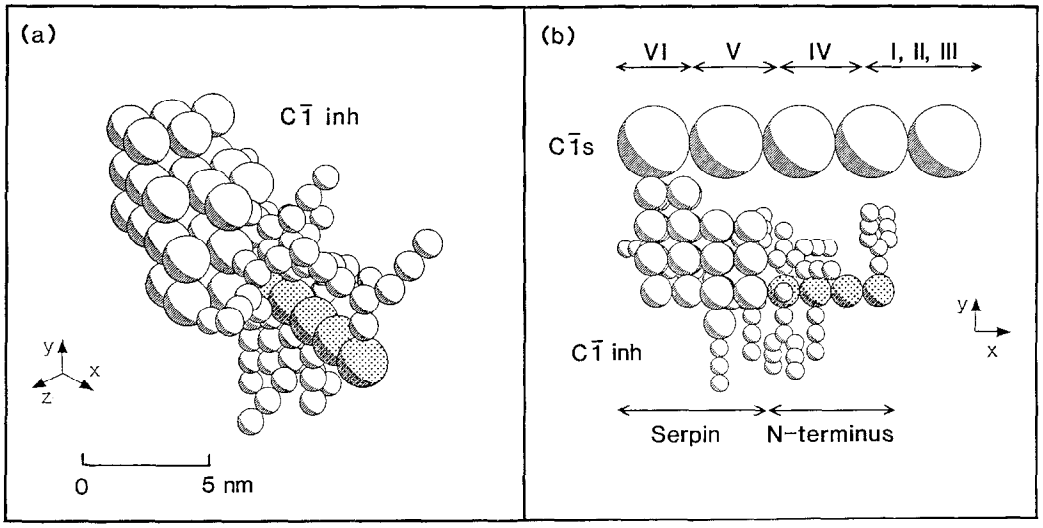

Fig. 1. Schematic model for the interaction of C1 inhibitor with C1s in a dimeric complex. (a) Perspective view of the hydrodynamic sphere model successfully used to account for the experimental $s^{o}_{20,w}$ of C1 inhiibtor [4]. The serpin domain is at the rear; the N-terminal domain (5 spheres as stippled) with extended oligosaccharide chains is in the foreground. (b) A side-view of the C1 inhibitor complex with C1s indicates the family of models successfully used for the dimer in Table I. In C1s, I, II and III correspond to the 'interaction' domains, IV and V to the two complement 'short consensus repeat' domains, and VI to the serine proteinase domain. The 5 spheres used to represent C1s result from the modelling and do not relate to the domain structure of C1r or C1s. The hydrodynamic data report on the overall length of the macromolecule, so the contact shown between the two components of the complex can only be schematic.

agree to within 0.0–0.3 S, which is acceptable given that the accuracy of each of the calculations and the experiments is generally within ± 0.2 S [11].

In contrast, tests of more extended dimer models lead to large differences with the experimental data. Models in which C1r and C1s extend along the other possible ($-x$, $+y$, $+z$ and $-z$) directions from the contact site (Fig. 1) give rise to calculated $s^{o}_{20,w}$ values that are 0.4 S to 0.7 S less than the experimental values for the compact carbohydrate C1 inhibitor model and between 0.6 S to 0.9 S for the extended carbohydrate C1 inhibitor model. The magnitude of these differences argues against the existence of these structures for the dimeric complexes.

In the two tetrameric complexes of C1 inhibitor with C1r and C1s, the $+x$ dimers were positioned in an end-to-end linear arrangement. The sphere(s) corresponding to domains I, II and III in each of C1r and C1s are in tangential contact with each other. Biochemically, these contacts correspond to those found in the $C1s_2$ dimer and $C1r_2C1s_2$ tetramer. This doubled the length of the tetramer relative to that of the monomer (Table I). Good agreements between the calculated and experimental $s^{o}_{20,w}$ data were obtained with these models (Table I). This is further support for the compact nature of the interaction between C1 inhibitor and each of C1r and C1s.

3. CONCLUSIONS

Hydrodynamic analyses of literature $s^{o}_{20,w}$ data on C1 inhibitor and its complexes can now be interpreted using recent sequence and structural data. The f/f_o ratios and the sphere modelling both support the result that the complexes contain compact arrangements of C1r, C1s and C1 inhibitor, and this result can be refined using other techniques.

A hypothesis can be proposed for the role of the N-terminal domain of C1 inhibitor. Models such as those of Fig. 1(b) show that the steric accessibility of the two SCR domains VI and V at the centre of C1r and C1s will be reduced by the N-terminal domain of C1 inhibitor after the serpin domain of C1 inhibitor has interacted with the serine proteinase domain VI of C1r and C1s. It can be postulated that this reduced accessibility will influence the binding affinity of C1r and C1s to C1q and likewise for that of C1s with C4 of complement. In other words, the inhibitors of multi-domain serine proteinases may themselves require a multi-domain structure in order to perform their physiological role.

Acknowledgement: Financial support from the Wellcome Trust is gratefully acknowledged.

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