SIZE AND SHAPE OF HUMAN Cl-INHIBITOR

Erich ODERMATT, Hans BERGER* and Yoh SANO

Biozentrum der Universität Basel, Abteilung für biophysikalische Chemie, Klingelbergstr. 70, 4056 Basel, Switzerland

Received 29 June 1981

1. Introduction

Cl-inhibitor, a plasma protein with a very high amount of carbohydrates (34% by wt, 17% of which is sialic acid) is composed of a single polypeptide chain of M_r 105 000 [1–4]. It inhibits a variety of enzymes of the complement system as well as others (e.g., plasmin, kallikrein, Hageman factor). The exact nature of the reaction of this inhibitor with the enzymes is unclear. With activated $\overline{C1s}$, its most likely in vivo substrate, a 1:1 complex is formed which remains stable even in SDS and urea.

It has been shown that Cl-inhibitor is a lysyl-type inactivator [5]. Evidence has been provided that Cl-inhibitor contains terminal sialic acid residues and penultimate galactose residues [6]. In vivo infused asialo-Cl-inhibitor is rapidly cleared from the serum of rabbits while asialo-agalactose-Cl-inhibitor remains uncleared for about the same time as the native molecule, indicating that exposure of galactosyl residues might render the molecule recognizable by receptors. Interestingly, removal of the carbohydrate moiety does not alter the inhibitory activity of the molecule [6].

Concerning the structure, only hydrodynamic data are available so far. The extremely small sedimentation coefficient of 3.7 S [3] for a molecule of this size indicates a very elongated structure. Our electron micrographs provide for the first time more detailed information on the structure of this interesting molecule.

Abbreviations: SDS–PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; M_r, relative molecular mass

Nomenclature: The complement terminology used conforms to the recommendations of the World Health Organisation Committee on Complement Nomenclature (Bull. WHO (1968) 39, 935–938)

* To whom correspondence should be addressed

2. Materials and methods

Fresh human plasma was obtained from the Blutspendezentrum, Basel. Cl-inhibitor was prepared according to [7]. Further purification was achieved on a hydroxylapatite column. The protein was homogeneous as judged by SDS–PAGE and by analytical ultracentrifugation (Beckman model E, equipped with a photoelectric scanner), with a sedimentation coefficient of 3.7 S in agreement with [3]. The activity of the inhibitor was determined by the formation of a complex with activated $\overline{C1s}$ (SDS–PAGE) and by its ability to inhibit the esterase activity of $\overline{C1s}$ using *p*-tosyl-L-arginine methyl ester as a substrate.

Sample preparation for the electron microscope was done essentially as in [8]. The rotatory shadowing technique as well as the negative staining method was employed.

DEAE-Sephacel and con A–Sepharose were purchased from Pharmacia, hydroxylapatite from Bio-Rad Labs; α-methyl-D-mannoside and *p*-tosyl-L-arginine methyl ester were products of Sigma.

3. Results

Fig.1(a–e) presents typical electron micrographs of the Cl-inhibitor obtained by the rotatory shadowing technique. It is a very elongated structure. Clearly a head and a tail can be distinguished. The length distribution of 100 molecules is shown in fig.2. About 43% of the molecules fall into the range between 33 and 36 nm. The diameters of the head and the tail cannot be accurately determined from these pictures [8]. Negatively stained samples (fig.1f) do not exhibit the high contrast of rotatory shadowed specimen; however, their diameters are more precisely represented. We estimate them to be ~4 nm for the head

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.50

and 2 nm for the tail.

Fig.3 compares a simplified picture of the molecule as derived from electron microscopy with a hydrodynamically equivalent rotational cylinder. The

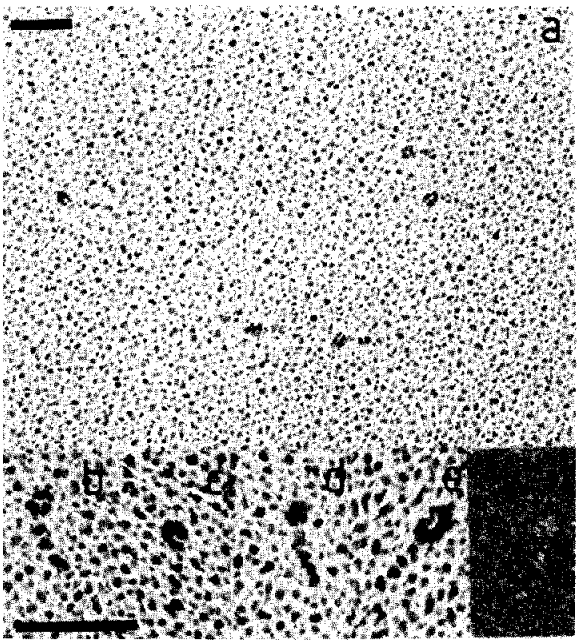

Fig.1. Electron micrographs of human Cl-inhibitor obtained by the rotatory shadowing technique (a–e). Fig.1f shows a typical negatively stained molecule. The bar corresponds to 50 nm.

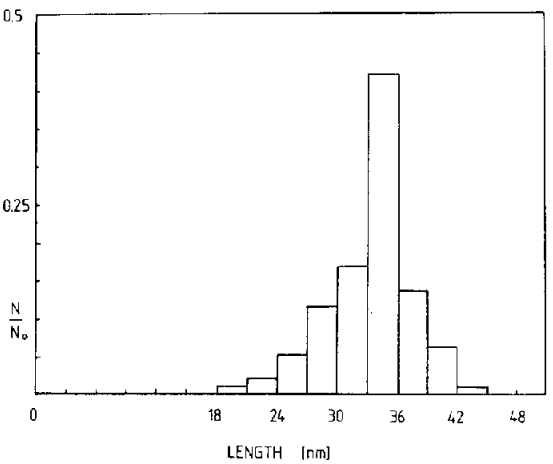

Fig.2. Length distribution of rotatory shadowed human Cl-inhibitor; 100 molecules were measured. The length was determined between the end of the tail and the center of the head domain. N/N_0 denotes the fraction of molecules which fall in a range of 3 nm.

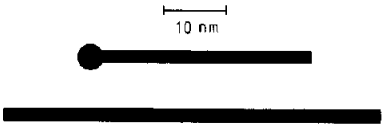

Fig.3. Upper part: Model of Cl-inhibitor constructed with the length derived from rotatory shadowing micrographs. The diameter of the head and of the tail were taken from negatively stained molecules. Lower part: Hydrodynamically equivalent model of the Cl-inhibitor (M_r 105 000, s = 3.7 S, $\bar{v}$ = 0.67 cm³/g) assuming a rod-like shape with a diameter of 2 nm. The bar corresponds to 10 nm.

hydrodynamic model was calculated according to [9] and applying the Svedberg equation under the assumption that the diameter of the cylinder is 2 nm.

4. Discussion

Electron micrographs of human Cl-inhibitor reveal a highly elongated molecule consisting of a globular and a rod-like domain. Rotatory shadowing allows determination of the length of the molecule while the diameter of the two domains can be estimated from negatively stained pictures. A simplified model presented in fig.3 consists of a globular structure having a diameter of 4 nm and a rod-like domain of length 33 nm and diameter 2 nm. Assuming a diameter of 2 nm a hydrodynamically equivalent cylinder was calculated according to [9] and the Svedberg equation using M_r 105 000, a degree of hydration of 0.5, a diameter of 2 nm and a partial specific volume of 0.67 cm³/g [3]. It confirms qualitatively the result of the electron microscopy that Cl-inhibitor is a very elongated structure. The quantitative difference in length can be attributed to the influence of the head as well as to protrusions of the tail not visible in the electron microscope. Side chains of the amino acid residues, especially lysine, as well as the carbohydrate chains can easily double the diameter observed by negative staining. A non-circular cross-section of the tail would also increase the frictional coefficient and result in a shorter model.

It is likely that it consists of more than one strand because we could resolve the tail by electron microscopy. Myosin, a coiled coil structure, has a mass/length ratio of ~1400 [10]. Applying this ratio to the inhibitor, this gives 1400 × 33 nm ≃ 46 000. The remaining mass can be distributed in a globular head of diameter 4.8–5.5 nm depending on the degree of

hydration. This indicates that a structure similar to that of myosin might be present in the tail.

The pictures clearly show two domains. Since evolutionary principles would be eliminated domains that are not involved in the inhibition of $\bar{C}1s$, there might be more than one function for the molecule. This and the important question, which domain bears the inhibitory site, remain open.

Acknowledgements

We are very grateful to A. Lustig for the analytical ultracentrifugation. T. Schulthess provided valuable assistance in the preparation of the inhibitor. We also thank J. Engel for helpful discussions. This work was in part supported by grant 3.556.79 from the Swiss National Science Foundation.

References

[1] Pensky, J., Levy, L. R. and Lepow, I. H. (1961) J. Biol. Chem. 236, 1674–1679.
[2] Pensky, J. and Schwick, H. G. (1969) Science 163, 698–699.
[3] Haupt, H. Heimburger, N., Kranz, T. and Schwick, H. G. (1970) Eur. J. Biochem. 17, 254–261.
[4] Harpel, P. C. and Cooper, N. R. (1975) J. Clin. Invest. 55, 593–604.
[5] Minta, J. O. and Aziz, E. (1981) J. Immunol. 126, 250–255.
[6] Minta, J. O. (1981) J. Immunol 126, 245–249.
[7] Reboul, A., Arlaud, G. J., Sim, R. B. and Colomb, M. G. ((1977) FEBS Lett. 79, 45–50.
[8] Engel, J., Odermatt, E., Engel, A., Madri, J. A., Furthmayr, H., Rohde, H. and Timpl, R. (1981) J. Mol. Biol. in press.
[9] Broersma, S. (1960) J. Chem. Phys. 32, 1626–1631.
[10] Lowey, S., Slayter, H. S., Weeds, A. G. and Baker, H. (1969) J. Mol. Biol. 42, 1–29.