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Electron microscopic demonstration of a common structural motif in human complement factor C3 and rat α_1-inhibitor 3 (murinoglobulin)

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Electron microscopy of two homologous giant proteins revealed that complement factor C3 and α_1-inhibitor 3 have a common structural motif of a semicircularly bent string 18–20 nm long with two or three bumps indicating globular domains. C3 had a structure similar to the letter C with a small but distinct hole in the center. α_1-Inhibitor 3 was a more complete ring sometimes ajar at one corner. When the latter was treated with a proteinase, it became slightly flattened and adopted a squarish C-shape.

Complement factor C3; α_1-Inhibitor 3; Electron micrograph; Homologous protein structure

1. INTRODUCTION

Complement factor C3 and α_1-inhibitor 3 are distantly related proteins both belonging to the α_2-macroglobulin superfamily of proteins [1,2]. C3 is a well-known major member of the complement cascade and made of two polypeptide chains with molecular masses of 110 000 and 70 000, which are the consequences of post-translational processing of a single polypeptide with a molecular mass of 180 000 [3], whereas rat α_1-Inhibitor 3 is made of a single chain of molecular mass 200 000 [4]. The function of C3 is to release an anaphylatoxin upon interaction with C3 convertase and expose a thiolester bond which becomes temporarily activated and forms a covalent bond with cell surface hydroxyl groups to induce the complement cascade which eventually brings upon lysis of the cell [3]. α_1-Inhibitor 3, on the contrary, is known as a proteinase inhibitor with a similar activity to human α_2-macroglobulin although its molecular mass is only one-fourth of the latter [4]. The thiolester bond of α_1-inhibitor 3 does not have a well-defined function, except that it can form a covalent bond with the inhibited proteinase molecule. Both proteins have not been studied by X-ray crystallography and elucidation of their gross structural features by electron microscopy is needed for the understanding of the structure-function relationship of the two distantly related proteins with diversified functions.

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2. MATERIALS AND METHODS

2.1. *Proteins*

Human complement C3 and rat α_1-inhibitor 3 were prepared according to the method of Muto et al. [5] and Saito et al. [4], respectively, and the purity was checked by polyacrylamide gel electrophoresis in the presense and absence of sodium dodecyl sulfate [6]. The proteinase inhibitory activity of α_1-inhibitor 3 was confirmed using trypsin and chymotrypsin as proteinases and azocasein as substrate.

2.2. *Electron microscopy*

Electron micrographs were taken on an Hitachi HU 11B electron microscope (Hitachi, Japan). Purified protein samples were passed through a TSK G4000SW gel column (Tosoh) mounted on an HPLC system (BIP-I pump and UVIDEC 100-V monitor, Jasco, Japan) with 50 mM sodium phosphate (pH 7.0) as elution buffer. The final concentration of protein samples was adjusted to be between 30 and 100 μg/ml. A drop of protein solution was placed on a glow-discharged carbon-coated Formvar film formed on a copper grid and the excess solution was blotted. A staining solution of 3% uranyl acetate was then applied to the membrane and blotted. The resulting stained sample was dried in air and used as a negatively stained specimen for electron microscopy. Photographs were taken at the nominal magnification of ×50 000 and enlarged in printing when necessary.

3. RESULTS

3.1 *Electron micrograph of complement factor C3*

Fig. 1a and b show low and high magnification photographs of complement C3 after negative staining with uranyl acetate. The low magnification photograph in fig.1a confirms that the sample was homogeneous in size with a clear background. When enlarged in printing in fig.1b, C3 showed distinct structural characteristics resembling the letter C made of a thick

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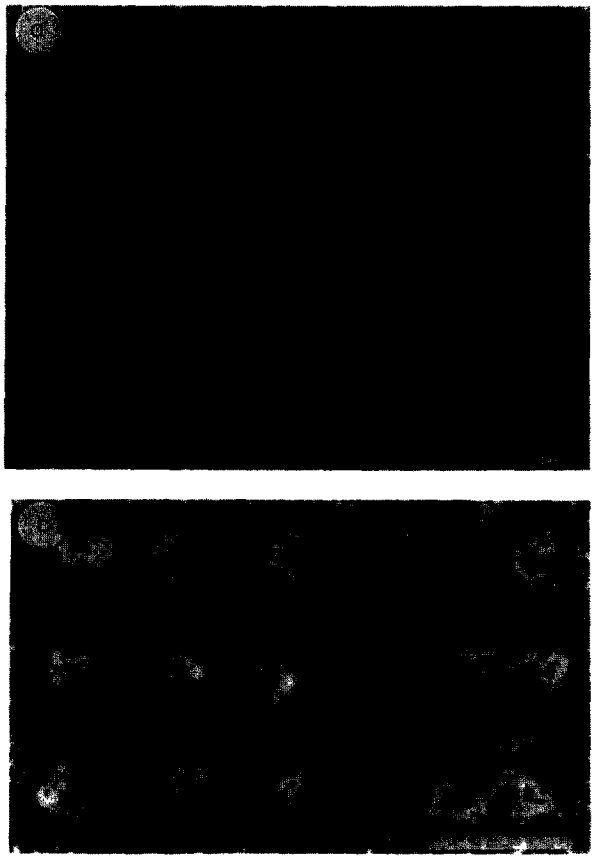

Fig.1. Electron micrographs of negatively stained complement factor C3. Scale bars represent 30 nm.

string. The outer diameter of the molecules is about 10–12 nm. The thickness of the string is not constant, less than 2 nm in thinner parts and close to 5 nm where it is thick. The string-like main body is thus divided into at least three semiglobular domains. The central cavity is about 2–3 nm in diameter and directly open to the outside. The shape of the molecule is similar to the results of Dahlback et al. [7] on the proteinase-treated C4, another homolog of α_2-macroglobulin, but the central cavity is more pronounced in our result.

3.2. *Electron micrograph of α_1-inhibitor 3*

Fig.2a shows a low magnification photograph of α_1-inhibitor 3 after the same treatment as above and confirms the homogeneity and cleanness of the sample. Fig.2b and c show, respectively, high magnification photographs of α_1-inhibitor 3 before and after the reaction with chymotrypsin. The native molecules in fig.2b look like closed rings but some of them are slightly ajar in one site. The diameter of the molecule is about 10 nm and that of the central hole is 2–3 nm. The ring structure often shows at least three globular domains as was the case for C3. The structure of α_1-inhibitor 3 is very similar to that of complement C3 except that the former looks like a more closed ring. After reaction with chymotrypsin, α_1-inhibitor 3 became more

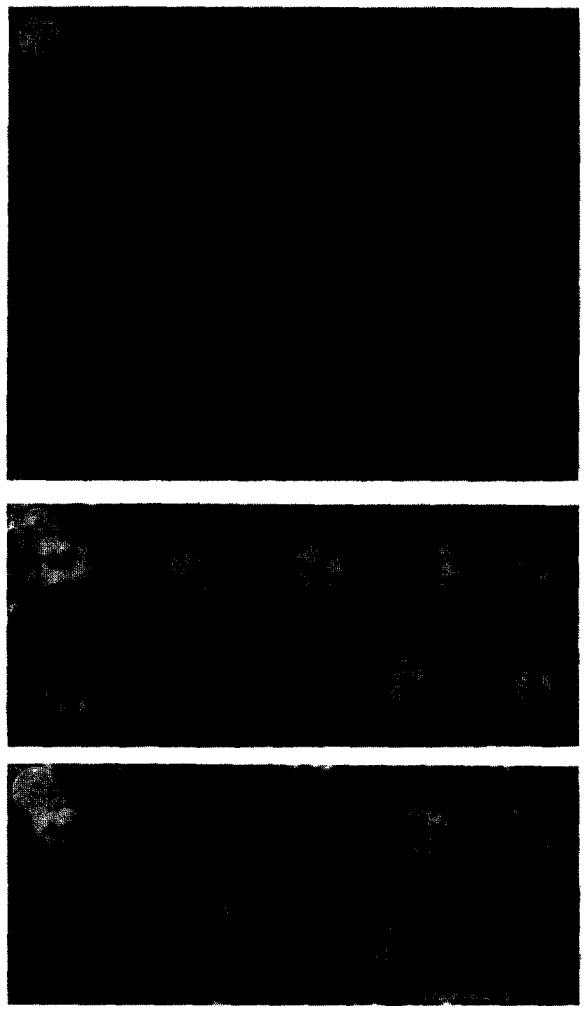

Fig.2. Electron micrographs of negatively stained α_1-inhibitor 3. Before interaction with chymotrypsin (a and b) and after (c). The same magnification factors apply for b and c. Scale bars represent 30 nm.

squarish and more open to the outside with a distinct channel connecting the inner cavity to the outer space. The presence of proteinase molecules within α_1-inhibitor 3 could not be confirmed by electron microscopy.

4. DISCUSSION

The electron micrographs of the two homologous proteins showed similar semicircular forms with a small hole in the center. In the case of complement C3, the string formed a thick C with a clear opening and a distinct hole in the center but in α_1-inhibitor 3, the polypeptide formed a closed ring but its proteinase treatment revealed a basic string unit similar to the one found in C3. Proteinase treatment of this protein cleaves only a limited number of peptide bonds in the bait region sequence and opening of the closed ring represents the major conformational change responsible for the binding of a proteinase molecule. Since, in

C3, the original giant polypeptide is already processed and split into two chains, although not exactly at the same position as the bait region of α_1-inhibitor 3, it is reasonable that the structure of proteinase-treated α_1-inhibitor 3 corresponded better to that of C3. In both cases, it was possible to detect two or three semiglobular domains and functional identification of them using immunoelectron microscopy will be essential to understand the structure-function relationship of these proteins.

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