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Electron microscopy of 26 S complex containing 20 S proteasome

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A high molecular weight protease complex (26 S complex) involved in the intracellular protein degradation of ubiquitinated proteins was purified from rat liver and studied by electron microscopy. The most prevalent molecular species with best preserved symmetrical morphology had two large rectangular terminal structures attached to a thinner central one having four protein layers. We concluded that they were the closest representation of the 26 S complex so far reported. The central structure was identified as 20 S proteasome and the terminal one as recognition units for ubiquitinated proteins.

Proteasome; 26 S protease complex; Protein degradation system (intracellular); Ubiquitin recognition complex

1. INTRODUCTION

Certain types of intracellular protein degrading systems have protease activities which can be regulated by ubiquitin and ATP [1,2]. It has recently been shown that one of the enzyme complexes of this type, called 26 S complex, is associated with a multiprotease complex known as proteasome [3–5]. Association between 20 S proteasome and the rest of the complex is labile under the purification conditions so far employed and the structure of the intact 26 S complex has remained elusive. We have recently been successful in obtaining the complex from rat liver as a stable entity in the presence of glycerol and ATP [5] (with modifications to be published by Tanaka) and the present report deals with the electron micrograph of the complex in the closest form to the intact one.

2. MATERIALS AND METHODS

The 26 S complex of rat liver was purified by density gradient centrifugation of 10–40% glycerol in Tris-HCl buffer containing 2 mM ATP according to the method of Orino et al. [5] (on a human leukemia cell system) with modifications. Details of the modifications will be published by Tanaka together with biochemical properties of the complex including its noted stability in the presence and absence of ATP. A key modification to be mentioned here was the use of Q-Sepharose chromatography (Pharmacia, Uppsala, Sweden) in the final stage of purification. The purified complex with the activity to degrade ubiquitinated lysozyme in the presence of ATP as determined according to [5] was applied to a TSK G4000SW column (Tosoh, Tokyo) and eluted with dust-free phosphate buffer (50 mM, pH 6.9) prepared in ultrapure water (Milli-Q Labo, Millipore, Japan Ltd., Tokyo) containing 30% glycerol without ATP. This procedure was necessary not for further purification of the complex but for ensuring a clean background in electron microscopy. About 80% of the applied material was recovered in a single peak, excluding its dilute front and hind edges which accounted for the rest of the material. Thus the active material obtained in the last step of purification was almost fully recovered in this procedure. Immediately after sample recovery, i.e. within 10 min of sample application to the column, the solution was diluted to a final protein concentration of about 50 μg/ml with the elution buffer containing 2 mM ATP and examined by electron microscopy.

Negative staining of samples was carried out on a carbon-coated Formvar film which was glow-discharged prior to sample application. The sample solution was deposited on the film and excess solution was removed by blotting. Three percent uranyl acetate solution was applied over the sample and blotted within 30 s. The sample was dried in air and observed by a Hitachi H7000 electron microscope.

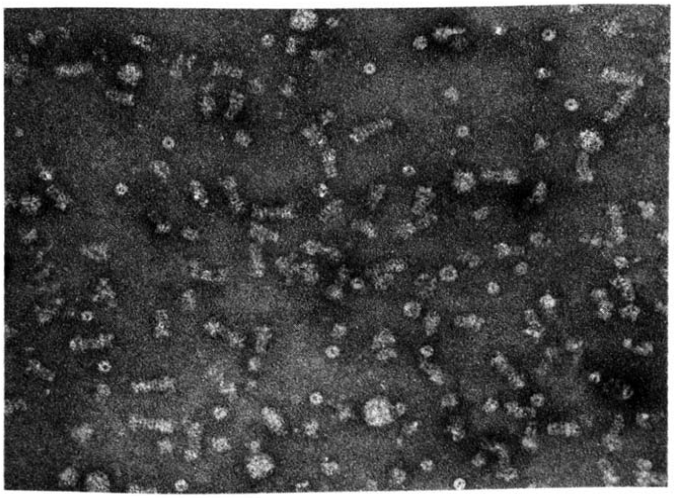

Fig. 1. Electron micrograph of the purified 26 S complex. Bar = 100 nm.

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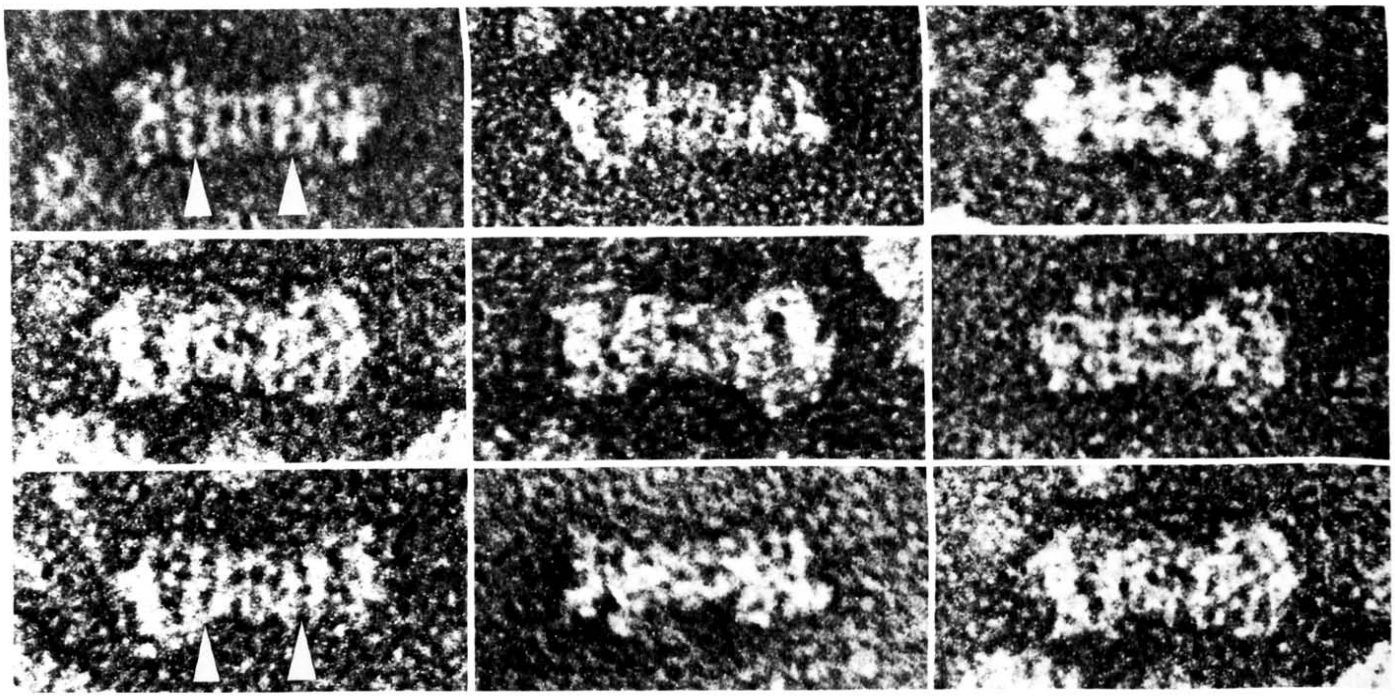

Fig. 2. The dumbell structures with two terminal domains which we consider the closest forms to the intact 26 S complex. Bar = 50 nm.

3. RESULTS AND DISCUSSION

Fig. 1 gives an example of the electron micrographs of the molecular species contained in the peak fraction of TSK G4000SW column elution. We are able to identify at least six kinds of well-definable images in this and similar fields and they are given in Figs. 2–4.

Fig. 2 is a collection of dumbbell-shaped complexes with an average overall length of 45–50 nm (n = 50). Since they were the most prevalent and largest species

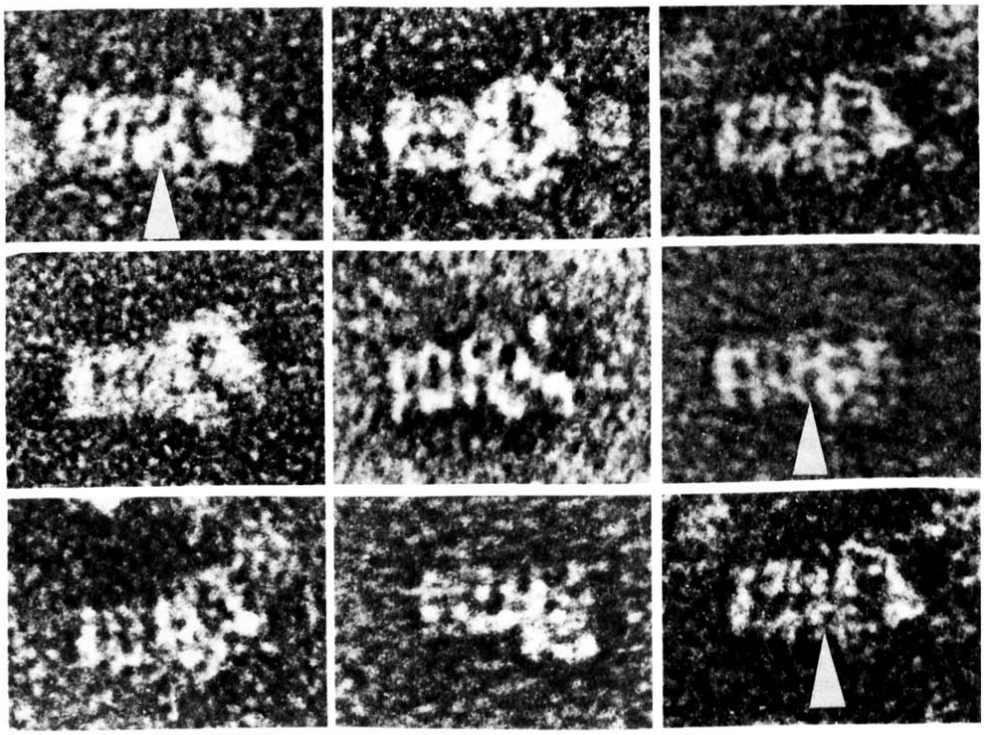

Fig. 3. Smaller 26 S complexes with only one terminal domain. Bar = 50 nm.

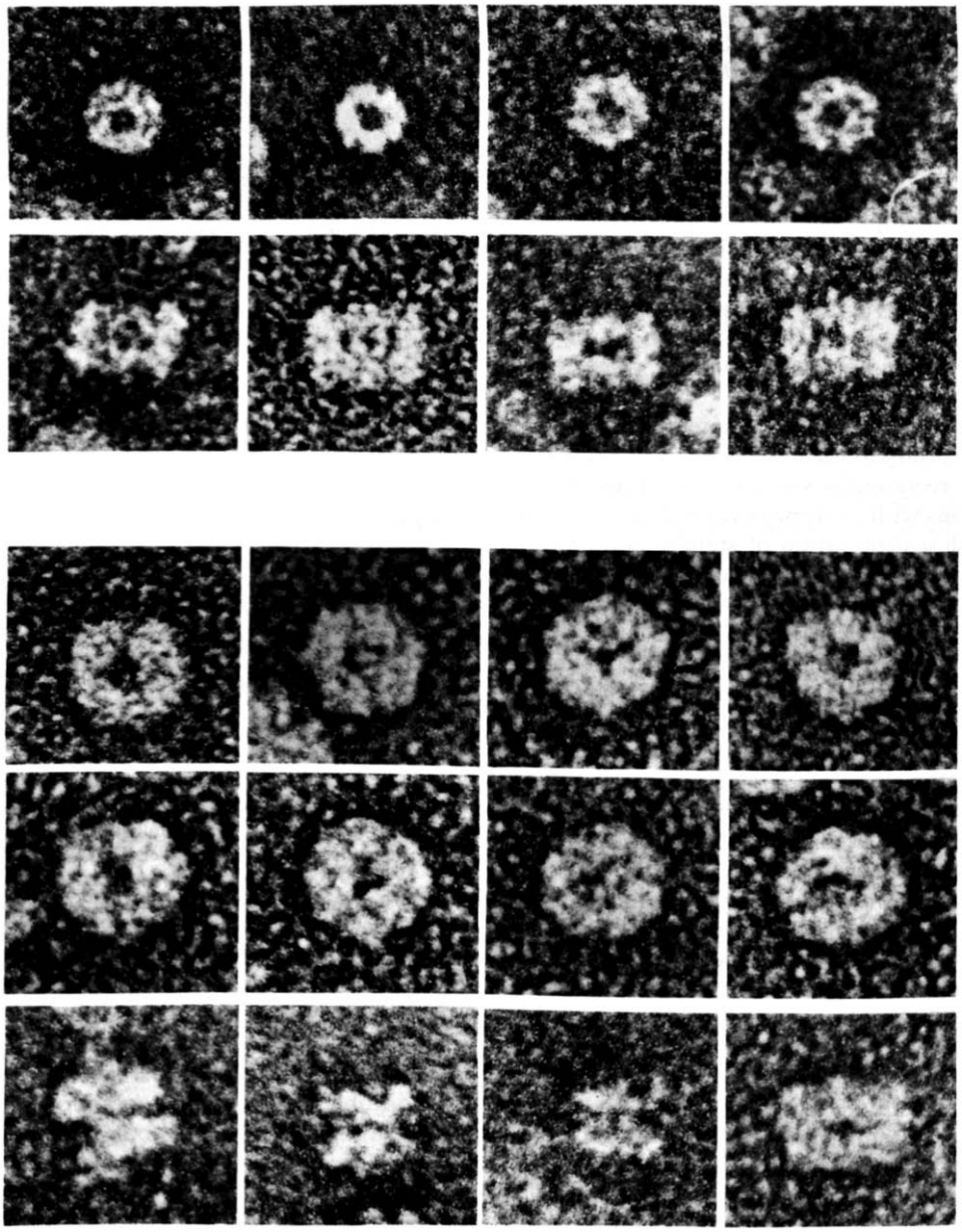

Fig. 4. Two kinds of ring structures and their presumed side views. (a) Smaller rings of 20 S proteasome and (b) its side views; (c) larger rings as candidates for the terminal domain and (d) their side views. Bars = 50 nm.

under the electron microscope and the structural symmetry is best preserved in them, we concluded that they were the closest representations of the 26 S complex. The dumbbell is made up of three parts: the central rectangle (ca. 13 × 17 nm) with three layers of stain penetration, plus two large irregular rectangles (ca. 20 × 13 nm) at both ends. We call the former the central (C) subset and the latter the terminal (T) subset. In some of the photographs, the proposed interfaces between C- and T-subsets are indicated by white arrows. Since the two T-subsets have very similar dimensions and appearances, we assume that they represent identical structures. The internal structure of the T-subset is characterized by a deep-penetration of the staining material along its longest axis.

It is tempting to view the 26 S complex as a cylindrical dumbbell with a long but slim central cylinder flanked by shorter but broader ones on the two ends. If so, the two terminal cylinders should have a larger diameter than the central one and the drying procedure for electron microscopy would have flattened the dumbbell at the two ends, distorting the exact geometry of the way the three domains are joined.

C-Subset looks very much like one of the characteris-

tic views of 20 S proteasome once suggested as a dimeric aggregate of disk-like proteasome [6]. More recent interpretations, however, tend to present it as the side view of cylindrical proteasome itself [7,8]. The stoichiometry of the 26 S complex can be expressed as TCCT or TCT depending on the identity of C-subset, and it is more likely now to be TCT.

Fig. 3 shows complexes with a proteasome-like domain attached to a single terminal domain. We consider these as defective 26 S complexes with one T-subset missing. This structure is surprisingly similar to the electron micrographs of Shelton et al. [9] of unidentified cytoplasmic particles which were later presented as 26 S complex [1].

Fig. 4a and b show rings with a diameter of 12–13 nm ($n = 30$) corresponding to the familiar view of proteasomes (a) and their rectangular side views (b). Figs. 4c and d show larger rings with an approximate diameter of 20 nm ($n = 30$) with a penetration of staining material in the center. These are probably the top (c) and the side views (d) of isolated T-subset of 26 S complexes. Identification of the above molecules as various parts of 26 S complexes is still tentative and the final conclusion must wait for a successful application of immuno-electron microscopy using subset-specific antibodies.

Fig. 5 is an illustration of 26 S complex based on our interpretation of the electron micrographs in Fig. 2. T-Subset is depicted as a triple-tiered ring with two thick protein layers flanking a thin one. C-Subset has four protein layers, the outer two being thicker than the central two layers, which is a characteristic of 20 S proteasome. An estimated molecular weight of the complex from its approximate size is well over 2×10^6. It will be most interesting to study the structure–function relationship of such an elaborate system for degrading ubiquitinated proteins in the cell.

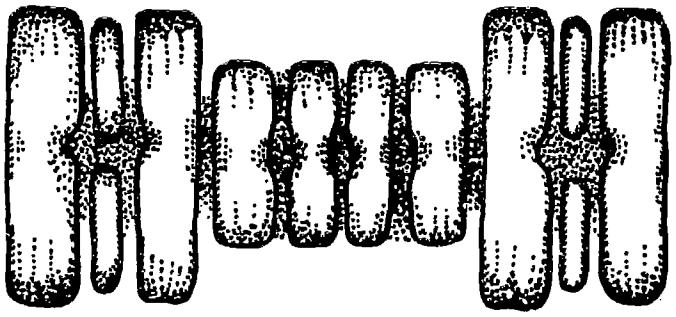

Fig. 5. Structural model of 26 S complex. Bar = 20 nm.

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