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Supramolecular structure of the recombinant murine small heat shock protein hsp25

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The size and shape of the recombinant murine small heat shock protein, hsp25, have been analyzed by hydrodynamic and electron microscopic methods. According to these studies recombinant hsp25 exists in large complexes with a sphere-like shape and diameters of 15–18 nm. The molecular mass of these complexes amounts to about 730 kDa indicating that they are composed of about 32 monomers.

Heat shock protein; Supramolecular structure; Analytical ultracentrifugation; Electron microscopy

1. INTRODUCTION

In the stationary phase of the Ehrlich ascites carcinoma (EAC) a protein (p25) with a molecular mass of about 25 000 accumulates which has been isolated, cloned and sequenced [1–4]. These studies reveal a high sequence homology of p25 with the small heat shock protein hsp27 and to a lesser degree with the α-crystallins [4]. Since protein p25 is inducible under stress conditions [5], it is now designated as hsp25. Like small heat shock proteins of other species [6–9] hsp25 appears in a non-phosphorylated and at least two phosphorylated isoforms [1,2]. A characteristic feature of the small heat shock proteins of various organisms [9–13] as well as the α-crystallins [14–16] is their occurrence in rather large aggregates. To analyze whether recombinant hsp25 also forms higher order structures, hydrodynamic and electron microscopic experiments have been carried out. As will be shown recombinant hsp25 is organized in high molecular mass complexes of 15–18 nm diameter consisting of about 32 subunits.

2. EXPERIMENTAL PROCEDURES

2.1. *Preparation of recombinant hsp25*

Recombinant mouse hsp25 was expressed in *E. coli* BL 21 (DE3) as described previously [4]. After lysis of *E. coli* cells, hsp25 was precipitated with ammonium sulfate at 35% final saturation and further purified by ion-exchange chromatography on DEAE-Sepharose 6B-CL. The protein was eluted from the column by a gradient of 50–200 mM NaCl. A purity of more than 95% was achieved as shown by two-dimensional polyacrylamide gel electrophoresis. Hsp25 was dialyzed against buffer containing 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 0.1% NaN_3 and 0.5 mM DTE (buffer 1).

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2.2. *Ultracentrifuge experiments*

Sedimentation velocity and diffusion measurements were carried out in a Beckman Model E ultracentrifuge with ultraviolet-absorption optics, monochromator and photoelectric scanner by means of a capillary-type double sector synthetic boundary cell. Diffusion coefficients (D) were measured at a rotor speed of 6000 rpm from the time-dependent broadening of the boundary registered at 280 nm and corrected to the viscosity of water and 20°C. Additionally, in the same experiments sedimentation coefficients (s) were calculated from the moving boundary at rotor speeds of 20 000 rpm and the data obtained were again corrected for density and viscosity of water at 20°C. The partial specific volume ($\bar{\nu}$) was calculated from the amino acid composition [4] and the apparent specific volume increments of amino acids according to Cohn and Edsall [17]. The molecular mass (M) of the hsp25 was determined from s, D and $\bar{\nu}$ using the Svedberg formula. Information about the shape was obtained by the frictional ratio (f/f_0) calculated from the equation (1) with the solvent density ϱ.

$$f/f_0 = 10^{-8}\left[\frac{1-\varrho\bar{\nu}}{D^2\cdot s\cdot\bar{\nu}}\right]^{1/3} \quad (1)$$

The diameter (d) of spherical proteins was calculated by formula (2).

$$d = 2\left[\frac{0.75\cdot M\cdot\bar{\nu}}{\pi\cdot N_A}\right]^{1/3} \quad (2)$$

with N_A being the Avogadro number.

2.3. *Electron microscopy*

Recombinant protein hsp25 was diluted to a concentration of 100 μg/ml with buffer 1 and negatively stained with 1% uranyl acetate. Negative staining was carried out by a double-carbon film technique. Carbon evaporated onto freshly cleaved mica was used as sample support [18] and covered with a second carbon film floating off from mica [19]. Samples were investigated in a Philips EM 400T at 80 kV and a magnification of 60 000.

3. RESULTS

3.1. *Ultracentrifuge experiments*

In sedimentation experiments a sharp symmetrical

boundary was observed indicating homogeneity of the hsp25 sample. By evaluation of the moving boundary, sedimentation coefficients of 20–22 S were determined (Fig. 1). These high values are evidently due to the existence of high molecular mass complexes of hsp25. Dilution of the samples with buffer 1 or buffer 1 containing 500 mM KCl resulted in a slight increase of sedimentation coefficients. This behaviour indicates a high stability of the hsp25 complexes. From linear regression analysis, the sedimentation behaviour of hsp25 can be described as

$$s_{20,w} = (21.23 - 0.60\ c)\ S,\ (c = mg/ml).$$

Diffusion coefficients calculated from the overlaying experiments amount to $(2.64 \pm 0.08) \cdot 10^{-7}$ cm^2/s. They are nearly independent of the protein concentration (Fig. 1).

Using the partial specific volume ($\bar{v}$) of 0.732$_6$ cm^3/g calculated as described in section 2, the molar mass M_{sD} of hsp25 was determined to be 730 000 ± 25 000 g/mol. This value exceeds the molar mass calculated from the amino acid composition (M_1 = 22 950) 31.8 times on the average, indicating that the hsp25 complexes consist of about 32 monomers.

The tendency of hsp25 complexes not to dissociate on continuous dilution with buffers of moderate or higher concentrations of electrolytes (Fig. 1), points to hydrophobic contacts between the subunits. Therefore, the stability against non-ionic detergents has been analyzed by the addition of different amounts of Lubrol PX or octylglucoside. As can be seen from Fig. 2, as much as 330 mol of Lubrol PX or 1300 mol of octylglucoside per mol monomeric hsp25 are necessary to induce dissociation of the hsp25 complexes.

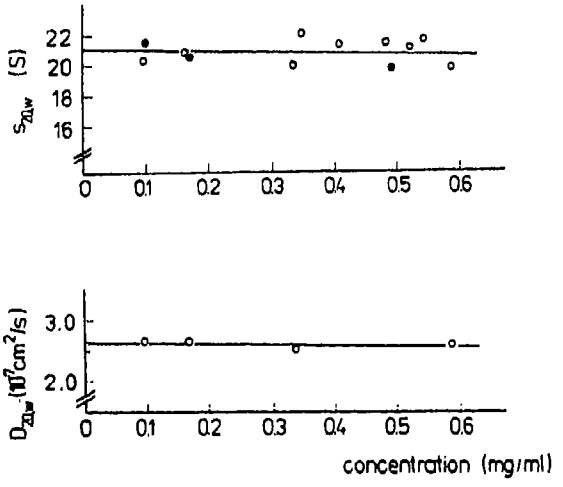

Fig. 1. Sedimentation and diffusion coefficients of hsp25 at different concentrations in 50 mM Tris-HCl, pH 7.4, with 10 mM $MgCl_2$ (○) or additionally 500 mM KCl (●) measured at 20 ± 1°C.

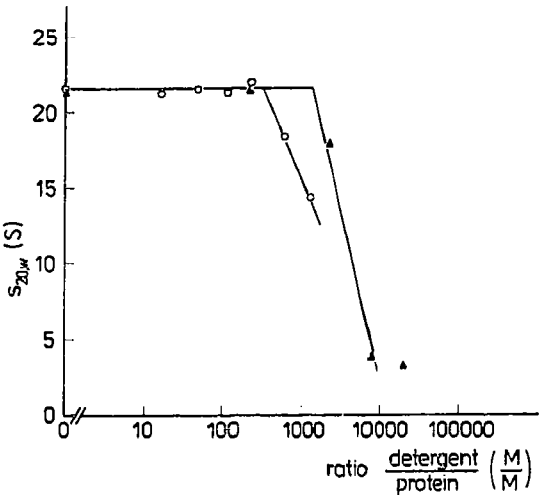

Fig. 2. Sedimentation coefficients of hsp25 in 50 mM Tris-HCl, pH 7.4 with 10 mM $MgCl_2$ and different detergent/protein ratios. (○) Lubrol PX, (▲) octylglucoside. Protein concentration 0.3–0.5 mg/ml.

3.2. *Electron microscopy*

Recombinant hsp25 is visualized after negative staining in the electron microscope in the form of nearly globular particles with diameters ranging from 15 to 18 nm (Fig. 3). The profiles of the particles vary from circular to elliptical ones. More rarely, triangular and polygonal profiles were observed. The different profiles probably reflect different orientations of the particles on the supporting film, although other reasons such as distortions during preparation cannot be excluded. The substructure of the particles is not well resolved and does not allow their subunit composition to be recognized.

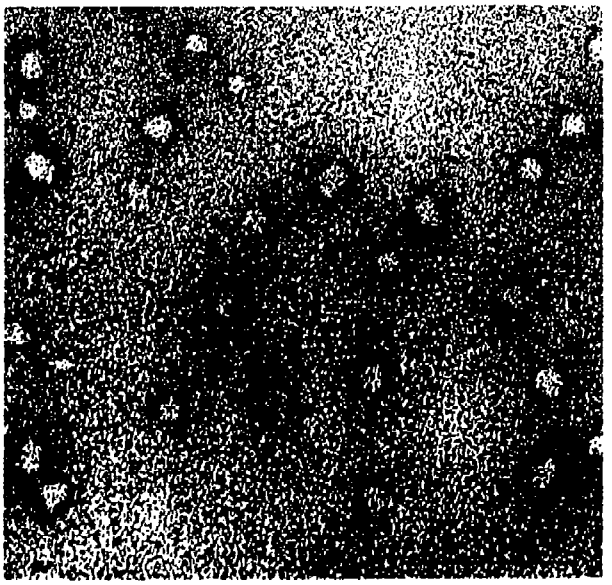

Fig. 3. Electron micrograph of negatively stained hsp25. Magnification 200 000×.

3.3. *Model of the supramolecular structure of hsp25*

From the molecular mass of 730 kDa a composition of 32 monomers is proposed for the high molecular mass complexes of hsp25 (Fig. 4). When assuming a spherical shape for hsp25 monomers, a diameter of 3.76 nm is deduced from equation (2). A hexagonal packing of 32 of such monomers results in hsp25 complexes with diameters of 15.2 or 17.0 nm, respectively. These values are in good agreement with the diameters of hsp25 complexes determined by electron microscopy and the value of 1.35 determined for the frictional ratio.

The high stability of these complexes against electrolytes or detergents is interpreted to be based on the following properties of hsp25: the protein contains about 10% of the proline residues arranged especially in the N- and C-terminal regions of the polypeptide chain. These proline-rich regions of the molecules should be able to form only short β-strands interrupted by turns with hydrophobic and aromatic amino acids. They are, therefore, most likely involved in hydrophobic interactions between the monomers. A hexagonal arrangement of these monomers in the high molecular mass complexes would result in a particularly stable structure because each monomer is in contact with at least four neighbouring molecules.

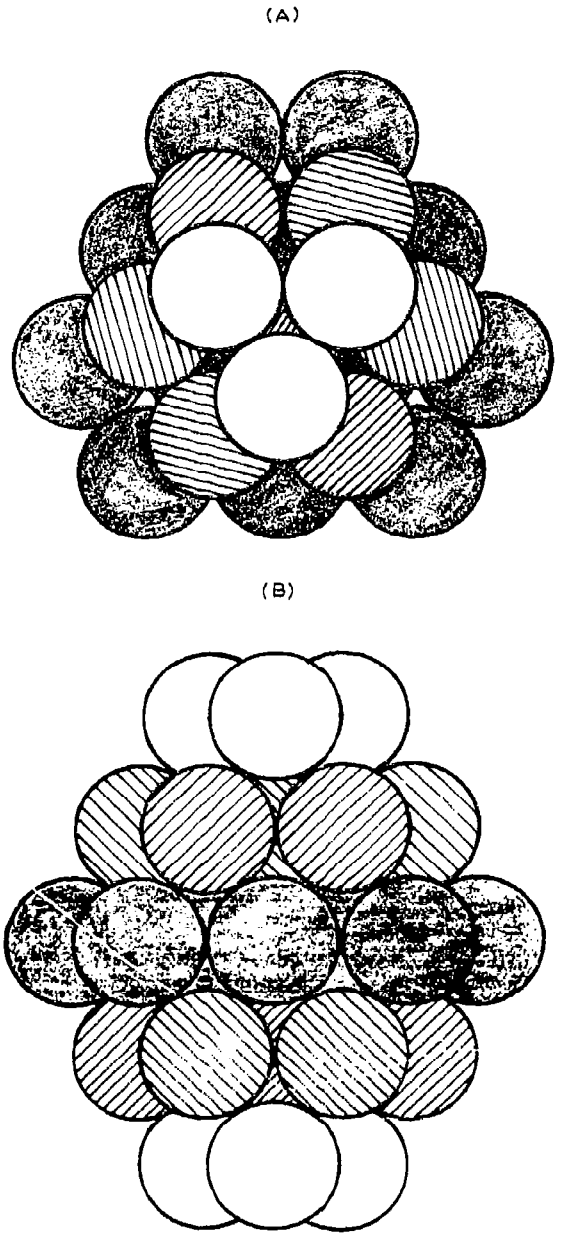

Fig. 4. Proposed model of hsp25 in different views. A hexagonal packing of the 32 sphere-like subunits is assumed.

4. DISCUSSION

Hydrodynamic and electron microscopic studies have shown that the recombinant murine small heat shock protein hsp25 is organized in large complexes with a molecular mass of about 730 kDa and diameters of 15 to 18 nm. From the molecular mass, shape and size of these complexes it is assumed that they are composed of 32 monomers arranged in hexagonal packing. Particles of similar size and morphology were also described for hsp28 of HeLa cells [9,10] as well as for α-crystallins [14-16] which, in contrast to the small heat shock proteins, consist of two different and shorter polypeptide chains [20,21]. Obviously the middle part of the protein molecules, which is characterized by a high degree of homology in the amino acid sequence with hsp25 is responsible for the formation of similar tertiary and quaternary structures. Models for the composition of the high molecular mass particles exist only for the α-crystallins [15,16] and are different from that described here for hsp25. Since the used recombinant hsp25 is unphosphorylated, the results demonstrate that the organization of hsp25 in large complexes is not dependent on phosphorylation. The analysis of phosphorylation and its influence on the supramolecular structure of hsp25 is presently under investigation [22].

Although the function of the small heat shock proteins is unknown so far, one can speculate that by the formation of the described high molecular mass particles under hyperthermic conditions constituents of the cell could be protected by complex formation with hsp25 or inclusion into the hsp25 assemblies.

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