Volume 228, number 2, 263-267 FEB 05569 February 1988

Electron microscopy study of Q_β replicase

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Received 17 December 1987

Purified preparations of Q_β replicase have been studied by electron microscopy using a negative staining technique, and a three-dimensional model of the enzyme molecule has been constructed. The molecule of this four-subunit protein appears to be a compact structure having a size of 100 ± 10 Å; it is subdivided into two unequal bipartite subparticles. The conclusion has been made that all the constituent subunits, including the ribosomal protein S1, acquire a globular conformation when associated in the replicase complex.

Q_β replicase; Enzyme structure; Ribosomal protein S6; Electron microscopy

1. INTRODUCTION

Q_β replicase, an RNA-dependent RNA polymerase, is produced in *E. coli* cells infected with RNA-containing bacteriophage Q_β [1]. In addition to the phage genome-specified catalytic subunit (M_r 65317 [2]), the enzyme contains three host-derived proteins which normally take part in the translation process [3], namely the elongation factors Tu (M_r 43225 [4]) and Ts (M_r 30257 [5]) and the ribosomal protein S1 (M_r 61159 [6]). With the exception of EF-Tu whose low-resolution three-dimensional structure has been established [7], little is known on the spatial organization of the other subunits and their arrangement in the replicase molecule.

Some information is available on the structure of S1 protein (review [8]). Hydrodynamic and small-angle X-ray scattering studies of the isolated S1 protein have led to the conclusion that it is a highly elongated molecule with an axial ratio of 1:10, its length of 210–280 Å being comparable with the maximal ribosome dimension. Furthermore, it was suggested that the S1 protein retains its elongated shape upon binding to the ribosome, since coincidence between its radii of gyration in solution (60–80 Å) and within the glutaraldehyde-fixed 30 S subunits (60–65 Å) was observed [9].

Here we report the results of an electron microscopy study of the Q_β replicase molecule; the conclusion is made that all its subunits including S1 protein have a globular conformation.

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2. MATERIALS AND METHODS

Purified Q_β replicase (fig.1) with a specific activity of about 10000 U/mg was isolated by a modified Blumenthal's procedure [10] from *E. coli* Q13 cells infected by the Q_β amB86 mutant phage. Activity and concentration of the protein were determined as in [10,11], respectively.

Pure S1 protein was isolated from the ammonium sulfate fraction (45–55% saturation) of the 1 M NH_4Cl ribosomal wash [12] using poly(U)-cellulose chromatography [13]. Mouse anti-S1 antibodies were obtained as in [14] and purified using the affinity column prepared by coupling S1 protein to CNBr-Sepharose. The immunocomplexes were prepared by incubating a mixture of equimolar amounts of Q_β replicase and anti-S1-IgG for 5 min at 37°C.

The samples of Q_β replicase or the immunocomplexes were diluted with a buffer containing 10 mM Tris-HCl, pH 7.5 (20°C), 200 mM ammonium acetate, 5 mM $MgCl_2$ and negatively stained with 1% uranyl acetate using the single-layer carbon technique [15]. Specimens were examined with a JEM-100C electron microscope at 80 kV and magnification of 80000 ×.

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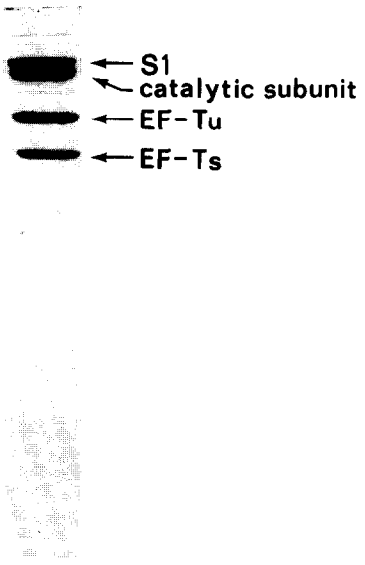

Fig.1. Electrophoretic analysis of the pure $Q\beta$ replicase preparation in SDS-polyacrylamide gel gradient (10–20%).

3. RESULTS AND DISCUSSION

Fig.2a represents a field of the preparation of $Q\beta$ replicase with homogeneously distributed compact rounded particles of 100 ± 10 Å size. A dense spot and a line separating the particle into two parts are observed on each of them. A close inspection of the images at a higher magnification shows that most of them can be referred to one of the three main types.

In the first type of images (fig.2b) the enzyme molecules appear to consist of two unequal subparticles, each one subdivided by a groove into two parts which could correspond to the subunits of $Q\beta$ replicase. It seems likely that the two bipartite subparticles represent the stable subcomplexes into which the enzyme readily dissociates at reduced ionic strength [16], namely the catalytic subunit·S1 protein subcomplex (126.5 kDa) and EF-Tu·EF-Ts subcomplex (73.5 kDa). This type of image occurs most frequently.

The second type of images (fig.2c) resembles the first one but differs by the presence of a clearly discernible protrusion at the interface edge of the larger subparticle.

In the third type of images (fig.2d) the replicase looks as if it consists of three symmetrically arranged subunits.

Assuming that these main types of images are different views of the same particle, we have constructed a three-dimensional model of the $Q\beta$ replicase molecule (fig.3). The formation of the enzyme from two unequal stable subcomplexes as well as the molecular mass ratios of the constituent subunits were taken into account. According to the model, $Q\beta$ replicase is assembled from two bipartite subparticles, each of a distinct shape. To obtain a better representation of the subparticle shapes, we analyzed the images of enzyme molecules undergoing dissociation and also those of the separated subparticles. Examples of such images are given in fig.2e. The smaller subparticle has a larger 'body' and a smaller 'head' which could be EF-Tu and EF-Ts, respectively. The larger subparticle is subdivided by a groove into two parts with more or less equal volumes. They seem to represent the phage-coded catalytic subunit and protein S1, but it is impossible to identify these subunits unambiguously. One of these two parts or subunits (the upper parts in fig.2e) has a boat-like shape with a hollow. It is perpendicular to the long axis of the subparticle.

Both the subparticles are somewhat bent elongated bodies with an axial ratio of about 2:1. They associate with each other by their concave sides so that the angle between their long axes is about 60° and the head is situated in the hollow. As a result, a compact three-dimensional structure with an all-round size of 100 Å is formed. From comparison of figs 3 and 2 one can see that different views of the model (fig.3a–c) fit the main electron microscopic images well (fig.2b–d). The first and second views of the model in fig.3 interconvert by rotation around the vertical axis by 180°. So we believe that the corresponding images (fig.2b and c) represent opposite sides of the $Q\beta$ replicase molecule. Indeed, these images have almost identical contours and their different fine structures can be the result of a different distribution of the stain in the molecule at its opposite facing on the carbon film. The rotation of the model around the horizontal axis at an angle of about 60° gives the third view.

Thus, all the observed $Q\beta$ replicase images can be readily interpreted in terms of the proposed model. Also, the volume of the enzyme molecule derived from the model (~300 nm^3) is close to that calculated from the enzyme molecular mass (290 nm^3).

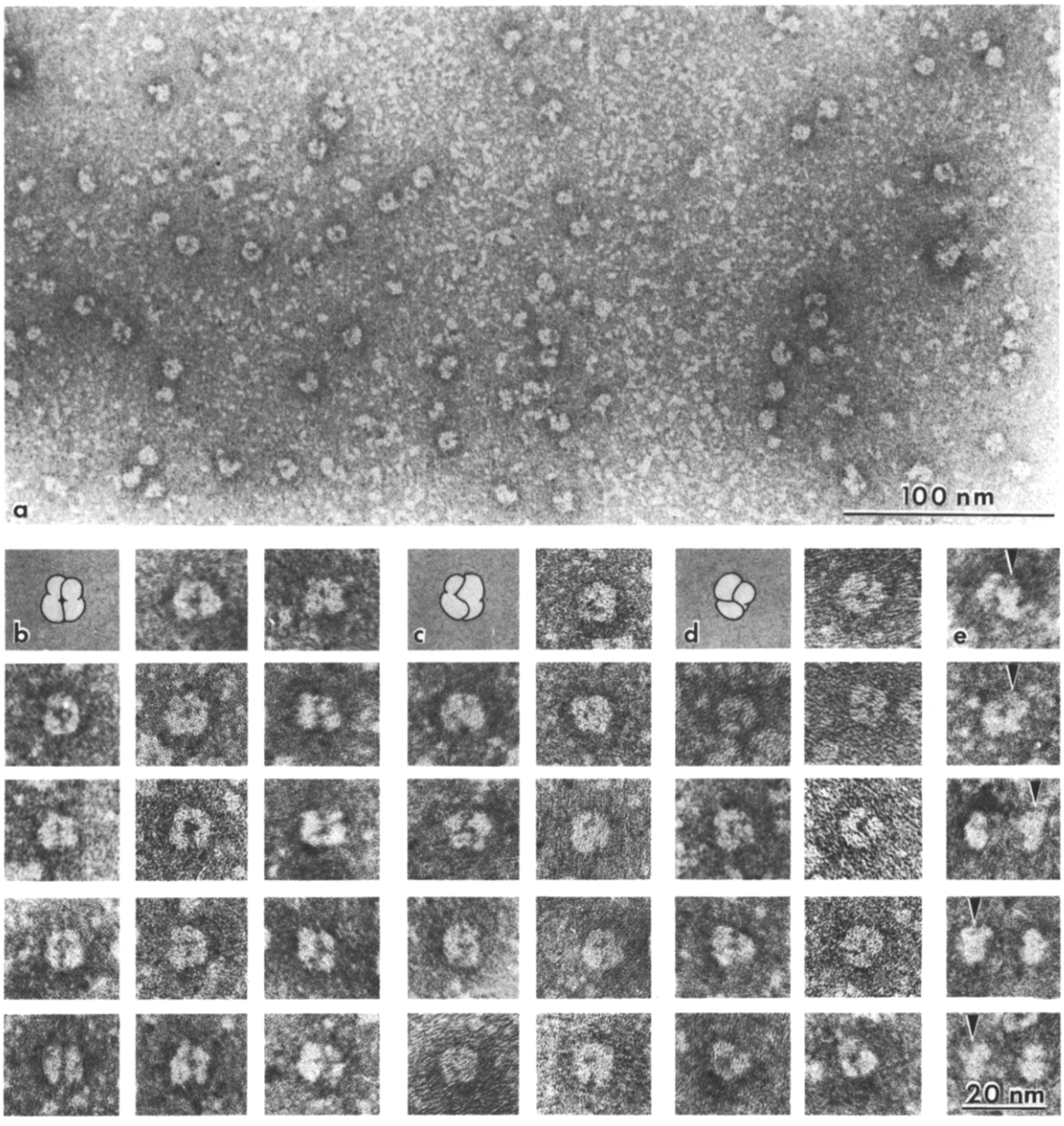

Fig.2. Electron micrographs of Q_β replicase. (a) Field of preparation. (b–d) Three main types of images of individual Q_β replicase molecules. They are schematically represented in the first frames. (e) Q_β replicase molecules partially dissociated into two subparticles as well as separate pairs of subparticles. Arrowheads show the larger subparticles.

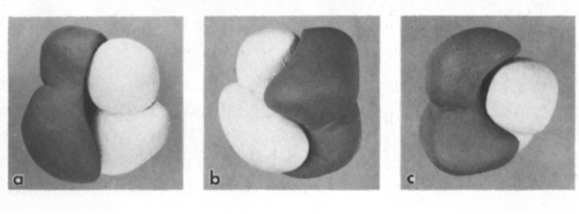

Fig.3. Three-dimensional model of the Q_β replicase molecule. Three views of the model correspond to the three main types of electron microscopy images in fig.2b–d.

It follows from the results obtained that just as the other subunits, S1 protein appears to have a globular conformation in the replicase complex. In any case, its maximal dimension cannot exceed that of the whole replicase (100 Å), which is much less than the values reported [8]. There were two preliminary reports on the localization of the S1 antigenic determinants in the regions of the 'platform' [17] and the head [18] of the 30 S ribosomal subunit which are not too distant from each other. We have attempted to localize the S1 protein in Q_β replicase using affinity-purified antibodies. The

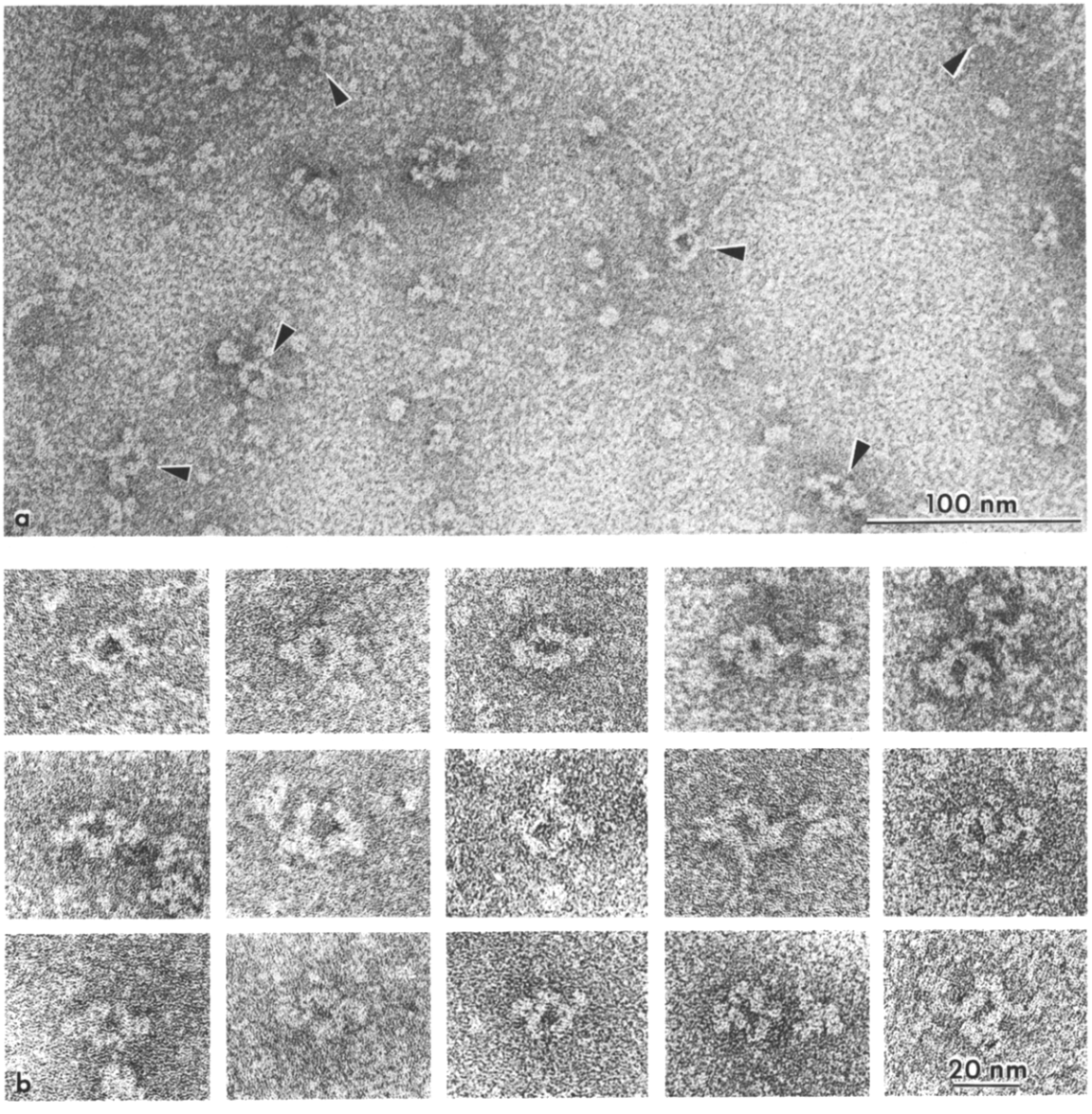

Fig.4. Electron micrographs of Q_β replicase after its interaction with anti-S1-IgG. (a) Field of preparation. Arrowheads show circular structures produced by pairs of the antibody molecules. Evidently, the antigen-to-antibody ratio and their concentrations are optimal for the circular structure formation. (b) Gallery of circular structures at higher magnification. No intact Q_β replicase molecules are seen in the complexes. It is likely that the protein material interacting with antibodies is unstructured. An aggregate of five antibody molecules bound to filamentous material is shown in the upper frame at the right.

addition of an equimolar amount of anti-S1-IgG to the enzyme preparation results in a drastic change of the electron microscopy pattern (fig.4a). The expected enzyme-antibody complexes are not found. Instead, one can see sparsely distributed unreacted enzyme and antibody molecules. Most of the protein material represents aggregates of two or more antibody molecules either associated by their F_{ab} fragments to produce circular structures or bound to low-ordered filamentous material consisting presumably of partially unfolded S1 molecules. Particles smaller than the whole enzyme molecules are also present (fig.4b).

Thus, S1 protein exists in a globular conformation (or at least in a much less elongated one than that in the isolated state) when bound to the Q_β replicase molecule, and loses this conformation upon removal from the enzyme as a result of its interaction with the antibodies. Bearing in mind that the interactions of S1 protein with the Q_β replicase and with the ribosome have many common features including the close values of the equilibrium dissociation constants ($\sim 10^{-8}\ M^{-1}$) and the similar functions of S1 protein in each of these cases (review [8]), it would be reasonable to suppose that protein S1 has a globular conformation within the native ribosomes as well.

Acknowledgements: We thank Professor A.S. Spirin for valuable discussions and O.M. Selivanova, S.N. Ryazantsev, E.E. Maximov, A.A. Vepritsky and E.V. Chetverina for their help. We are indebted to Drs A. Palmenberg from the Wisconsin University and N.I. Matvienko from this Institute for the bacterial and phage strains used in growing the infected cells.

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