EVIDENCE FOR THE ABSENCE OF A CENTRAL CORE IN PARTICLES AGGREGATED FROM PROTEIN SUBUNITS OF BACTERIOPHAGE FR

P. ZIPPER and O. KRATKY
Institut für Physikalische Chemie der Universität Graz, Austria

and

D. SCHUBERT
II. Physiologisches Institut der Universität des Saarlandes, Homburg (Saar), Germany

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1. Introduction

A method has been described for the formation of virus-like protein particles from isolated protein subunits of the icosahedral bacteriophage fr [1]. This demonstrated for the first time that self-assembly is possible for the coat protein of icosahedral viruses in the absence of nucleic acid. The structure of these virus-like particles is still controversial. It was first assumed that they were empty shells corresponding to the protein shell of the virus [1]. This was supported by electron microscopic observations [2–4]. On the contrary, a model for these particles was derived from sedimentation analysis and also from electron microscopic observations which showed a virus-like shell surrounding a central protein core. This core was thought to consist of 60 subunits [5, 6]. It seems that the latter model has been widely accepted [7–9].

Since small angle X-ray scattering should be particularly capable of clearing up these conflicting results and hypotheses, we have performed a study on the reaggregated particles by this technique. By comparison of the scattering curves for these particles with those for empty protein shells derived from the phage by alkaline degradation of the RNA [10] and with theoretical scattering curves for the different models, we are now able to show that the reaggregated virus-like particles are indeed empty shells which are very similar to the protein shell of the virus.

2. Materials and methods

2.1. *Preparation of fr protein particles*

Reaggregated virus-like particles were prepared according to the methods described earlier [4, 11]. Reaggregation was performed by dialysis of the protein dissolved at pH 7–8, $I < 10^{-3}$ against SP-buffer (1.0 M NaCl, 0.02 M sodium phosphate buffer pH 7.3) [4]. The sample was then centrifuged for 4 hr at 120,000 *g* in a sucrose gradient (5–20% sucrose in SP-buffer, underlayed with 60% sucrose in the same buffer) in order to remove low molecular weight aggregates. It was concentrated by dialysis against a solution of 30% polyethyleneglycol in SP-buffer and finally dialyzed extensively against SP-buffer.

Artificial top component (ATC) was prepared similar to the method of Samuelson and Kaesberg [10]. Removal of degraded RNA and of low molecular weight aggregates from ATC and the concentration of the samples was performed as described above.

2.2. *Small angle X-ray scattering measurements*

Small angle X-ray scattering measurements were performed using the experimental techniques and the theory described previously [12, 13]. The experimental scattering curves were corrected only for the influence of the K_β-line and for collimation effects due to the width of the primary beam, but not for collimation effects due to the length of the

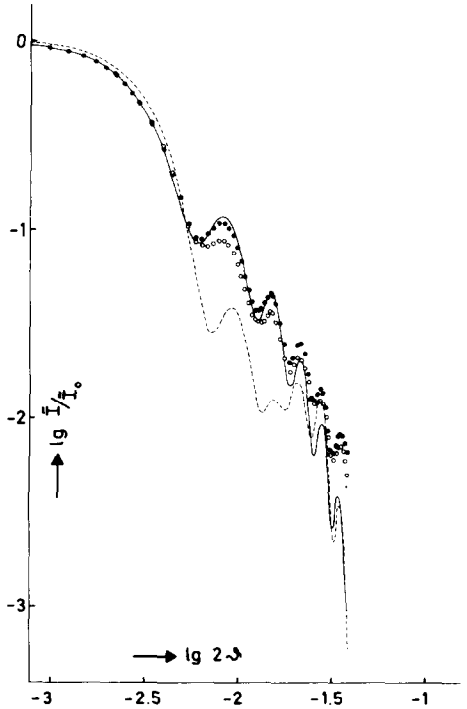

Fig. 1. Log-log plot of the experimental scattering curves of the reaggregated particles (○) and of ATC (●) and of theoretical scattering curves calculated for different models. Solid line: single shell model; broken line: shell-core model. All curves are afflicted with the collimation error due to the length of the primary beam and normalized to an intensity at zero angle $\tilde{I}_0 = 1$.

primary beam. To compare the experimental curves with the curves calculated for the different models the theoretical curves were smeared by means of a computer program [14, 15] assuming an infinitely long primary beam.

3. Results and discussion

The small angle scattering curves of the reaggregated particles and of ATC are shown in fig. 1. For both measurements protein concentration was about 15 mg/ml. The scattering curves are normalized to an intensity at zero angle $\tilde{I}_0 = 1$.

Obviously the two experimental curves for the different kinds of particles are very similar to each other. The angular positions of the maxima and minima are almost identical in both curves and the same is true for the intensities in the central maximum region. In the outer part of the curves the intensities measured for the reaggregated particles are only slightly lower than those obtained with ATC. Since ATC is a hollow protein shell similar in structure to the protein portion of the virus [10], this result strongly favours the conception that the reaggregated virus-like particles are empty shells.

The conclusion drawn above is corroborated by a comparison of the experimental curves with theoretical curves calculated for the different models. This is also demonstrated in fig. 1. The solid line in the figure is the calculated scattering curve for the protein shell of the virus, the broken line represents the theoretical curve for a particle consisting of a viral protein shell and of a central core. In both cases a value of 104.9 Å was used for the mean inner and of 131.7 Å for the mean outer radius of the viral shell [13]. The dimensions of the core – 54.6 Å and 81.4 Å as mean inner and outer radius, respectively – correspond to an arrangement of 60 protein subunits in a T = 1 shell according to Hohn [5]. They were calculated assuming the integral thickness and electron density of this shell equal to that of the outer protein shell. The outer radius obtained in this way does not differ much from the value of 76 Å given by other authors [16]. As is clearly shown by fig. 1 the curve for the single shell model fits both experimental curves very well. On the other hand, the curve calculated for the shell-core model is obviously not consistent with the experimental data.

Further and even more convincing evidence for the absence of a central core in the reaggregated particles comes from a comparison of the radii of gyration. For their determination we made use of the fact that for spherical particles the product of their radius of gyration and of the abscissa of the first submaximum in the scattering curve is approximately constant [17]. The constant was determined from the smeared theoretical curves for the different models and from their known radii of gyration. Division of this constant by the abscissa of the first submaximum in the experimental curves yielded the radii of gyration of both kinds of particles with an accuracy of 1–2%. In this way the radius of gyration of the reaggregated particles was found to be 123.5 Å, the corresponding value for ATC is 124.3 Å. These values agree within the limits of errors.

On the other hand, the radius of gyration of a hollow sphere containing a core inside is smaller than that of the corresponding hollow sphere without a core. For example, the radius of gyration of the single shell model was calculated as 119.6 Å, whereas the radius of gyration of the shell-core model is 109.3 Å. A similar value, namely 111 Å, resulted if it was assumed that the additional 60 subunits were not regularly arranged in a T = 1 core but randomly distributed within a sphere having a radius of 104.9 Å. Furthermore the assumption of the 60 subunits intimately bound to the inner surface of the outer shell led to a radius of gyration of about 113 Å.

As can be seen the radius of gyration of ATC is slightly larger than the value calculated for the single shell model. This could be explained by the assumption that the mean diameter of ATC is a little larger or the integral thickness of its shell is a little smaller than the values obtained for the phage by the previous measurements [13]. Taking this into account for the calculation of the different models the radii of gyration of the shell-core models would be only slightly larger than those calculated above and still considerably smaller than the radius of gyration obtained with the reaggregated particles.

Obviously these calculations not only show that the reaggregated particles do not contain a T = 1 core but also exclude the presence of any considerable amount of protein inside the virus-like shell.

It must be noted that with a few samples of reaggregated particles scattering curves were obtained which showed considerably lower submaxima than the corresponding curve in fig. 1. The same effect was observed with one sample of ATC. However, during preparation all these samples suffered from substantial loss of material by precipitation. Furthermore, the yield of high molecular weight aggregates from the reaggregation process was very low (less than 20%). This indicates that the virus used for the preparation of these samples was partially degraded. The use of partially degraded virus for protein preparation normally leads to solutions containing unspecifically aggregated material, so we conclude that the results obtained with the samples prepared from such degraded virus are affected by the presence of unspecific aggregates and are therefore not representative.

Thus we believe that our studies clearly demonstrate that the virus-like particles reaggregated from protein subunits of bacteriophage fr are hollow spheres which are very similar to ATC and to the protein shell of the virus, in agreement with the results of Schubert and Frank [2–4].

Further investigations are being carried out in order to determine the exact radial dimensions of the reaggregated particles and of ATC and to obtain an insight in the structural differences between both kinds of particles.

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