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Comparative study on low resolution structures of apoferritin via SANS and SAXS

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Abstract. The results of small angle scattering investigation of protein apoferritin are presented. The sizes and shapes, including those determined by indirect Fourier transform method, are calculated. The pair-distance distribution function for both small angle neutron (SANS) and X-ray small angle scattering (SAXS) are obtained. It is shown that SANS and SAXS methods give similar shape (spherical shell with holes) of apoferritin. At the same time, fits of experimental data for SAXS and SANS curves give a little different sizes and volumes of the molecule. The reasons for these differences are discussed.

1. Introduction

The protein complex ferritin plays a key role in iron metabolism. Ferritin is a protein which resolves the oxide radicals and iron storage problems. Apoferritin protein or its analogues have been found in all living organisms [1-3].The technical applications of ferritin with modified central part are also discussed. As it is well known, ferritin is a large protein (about 120A in diameter), consists of 24 ferritin polypeptides and comprises central part containing iron based molecules. The apoferritin is a protein complex which is a ferritin shell (without iron core). Together with mineral nuclear apoferritin forms the ferritin. The name of "ferritin" was given by V.Laufberger due to protein containing over 20% (wt/wt) of iron according to [5, 6].

First investigations of iron metabolism were started in 1940-th by Granick [7], followed by studies on magnetic properties of ferrittin and ferric compounds [8] and the mechanisms of iron metabolism [9-11]. First X-ray diffraction experiments with the ferritin were performed in the 40-th of previous

century [12]. Nevertheless, at present, there is still quite big activity in the field. Recently, the structure of protein-protein complexes in native solution has received much attention [4]. Despite the fact that a lot of structures of these complexes are presented in PDB bank and the history of their studies is quite long there are still several open questions. In particular, recent structural studies are focused on interparticle correlations [13], and the protein complex appeared to be very useful as a test sample for SAS instruments [14].

The aim of this work is to apply recent SAS approaches to low resolution studies of the mentioned above protein complexes with a particular goal - to compare obtained in such way SAXS and SANS structures.

2. Materials and methods

Horse spleen apoferritin was purchased from Sigma-Aldrich Chemie GmbH (Product number A3660). Storage buffer (50% glycerol and 0.075 M NaCl) was replaced for buffer containing 150 mM NaCl and 5 mM Na2HPO4, pH=7.3. Buffer for neutron experiment was based on heavy water form best contrast and decreasing incoherent background. The buffer for x-ray experiments was based on light water. The buffer replacement was fulfilled by centrifugal ultrafiltration with filters Centricon Y-100 (Millipore corporation). The final concentration of protein was 25 mg/ml for SANS experiments and 50 mg/ml for SAXS.

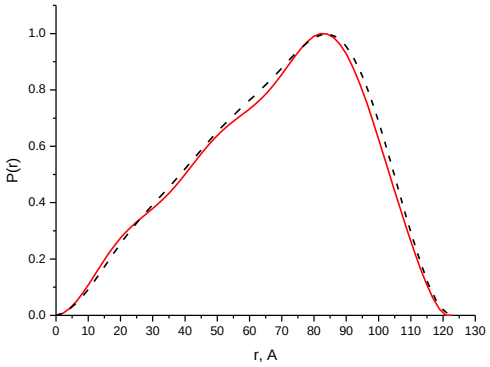
The neutron experiments were performed at YuMO spectrometer with two detectors system mode [16, 17]. The beam was collimated to a diameter of 14 mm on the sample. The obtained spectra were normalized and absolute values of the neutron scattering intensity obtained using a vanadium scatterer by the standard procedure with SAS software [18]. The investigations by small angle X-ray (SAXS) method were carried out on Brucker Nanostar instrument which is available at the Institute of Synthetic Polymer Materials RAS (Moscow, Russia) and in Prague, the experiments were performed using a pinhole camera of Molecular Metrology SAXS System at the Institute of Macromolecular Chemistry CAS (Prague, Czech republic).

A pinhole camera Molecular Metrology SAXS System is attached to a microfocused X-ray beam generator (Osmic MicroMax 002) that operates at 45 kV and 0.66 mA (30 W). The camera was equipped with a multiwire, gas-filled area detector with an active area diameter of 20 cm (Gabriel design). Two experimental setups were used to cover the q range of 0.0045 – 1.1 Å ($q=(4\pi/\lambda)\sin\Theta$, where λ is the wavelength and 2Θ is the scattering angle). The scattering intensities were put on absolute scale using a glassy carbon standard.

3. Results and discussion

3.1. The pair-distance distribution function

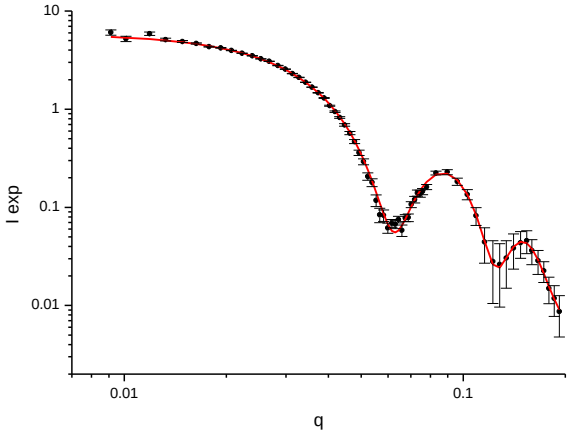
The pair-distance distribution function P(r) was obtained using indirect Fourier transformation of experimental small angle neutron scattering data treatments for apoferritin. The function P(r) was reconstructed using program GNOM [19] of ATSAS program package [20]. The pair-distance distribution function (P(r)) obtained by this procedure and its approximation of experimental curves are shown in Figure.1. Functions are bell-shaped, the maxima of this function are shifted from the center position, which indicates the displacement of the scattering density from the center (unlike for spherical shell) to the periphery of the molecule. The maximum of the P(r) function corresponds to the most probable distance between two points in a protein molecule. This distance is about 85 Å. For the obtained pair-distance distribution functions P (r) the values of the radii of gyration, according to the [21], are 51.3 and 51.73 Å for the SANS and SAXS, respectively. The values of the radius of gyration obtained from the indirect transform are equal to those obtained previously from fitting the experimental curves by the model of spherical shell scattering function. From the indirect transform, we can also estimate the maximum size of protein molecule, which is about 120 Å.



A

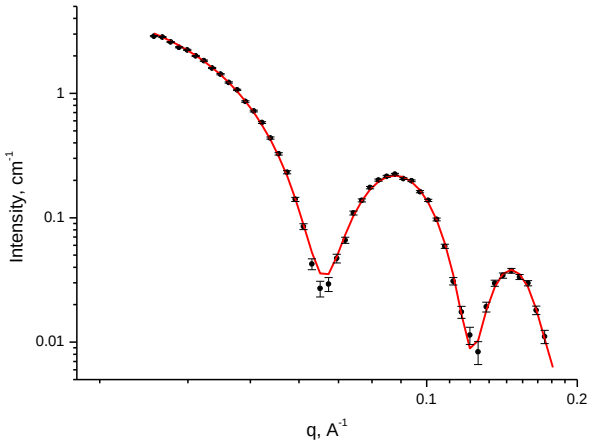
Figure 1. The result of the pair-distance distribution function reconstruction for the apoferritin molecule for small-angle neutron scattering (SANS) and X-ray (SAXS)

(A) Pair-distance distribution function P(r) SANS (continuous line) and X-ray (SAXS) (dash line).



B

(B) Fit of SAXS experimental data by the scattering curve calculated from P(r)



C

(C) Fit of SANS experimental data by the scattering curve calculated from P(r)

3.2. Determination of the volume of the molecules by Porod approximation

The Porod invariant [22] characterizes the integral scattering from object and it is calculated from Eq.(1):

$$Q = \int_0^\infty q^2 I(q) dq \tag{1}$$

The volume of the object is calculated from Eq.(2).

$$Q = 2\pi^2 \gamma(0) = 2\pi^2 \int_V \rho^2(r) dr \tag{2}$$

In particular for homogenous particle the volume is related to Porod invariant by:

$$V = 2\pi^2 I(0) / Q = 2\pi^2 / Q_0 \tag{3}$$

Where

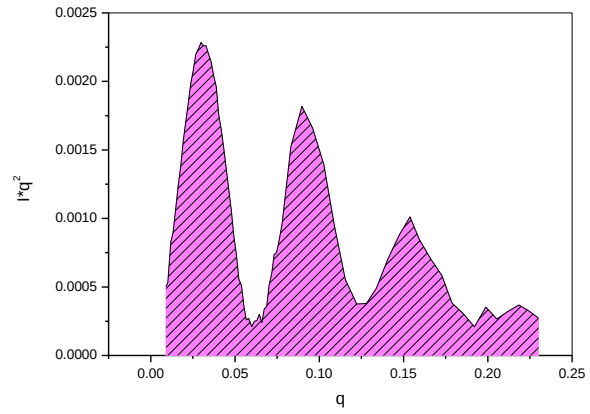
$$Q_0 = \int_0^\infty i(q) q^2 dq$$

and (4)

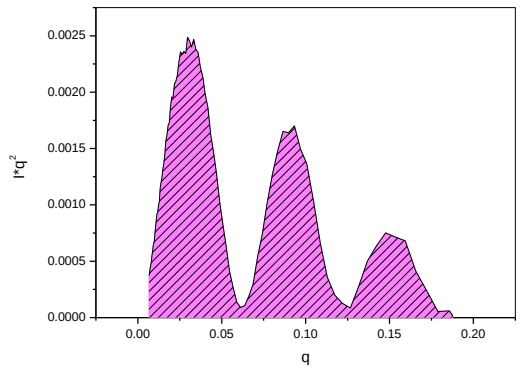
$$i(q) = \frac{I(q)}{I(0)}$$

The apoferritin molecule volume was estimated from Porod invariant Q. It is equal to the area under q-dependence of the curve $I(q)q^2$. The calculation was performed by the Primus program [23] from the ATSAS software package [20]. The $I(q)q^2$ vs q is shown in Figure. 2.

The volume values are 560 x10^3 and 600 x10^3 Å^3 for SANS and SAXS, respectively.



A



B

Figure 2. The Porod dependence Iq^2 vs q obtained from SANS **(A)** and SAXS **(B)** data of apoferritin. The hatched area corresponds to the Porod invariant Q (1).

3.3. Three-dimensional model of apoferritin molecule, constructed by Monte-Carlo method

Often, three-dimensional representation of the object's shape can be obtained by fitting the experimental scattering curve by theoretical one, which corresponds to the selected model. The model search without any *a priori* information about the object structure could be done using small-angle scattering data, for example, by an algorithm, implemented in the program DAMMIF [24]. This algorithm uses the Monte Carlo method and represents a model of an object as a set of small spheres (dummy-atoms). The resulting model is a low resolution mode, because SANS method is a representative of low-resolution techniques, which give structural information in the range 10-1000 Å. The scattering intensity for the dummy-model is calculated as follows:

$$I(q) = 2\pi 2 \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \left| A_{lm}(q) \right|^2, \tag{4}$$

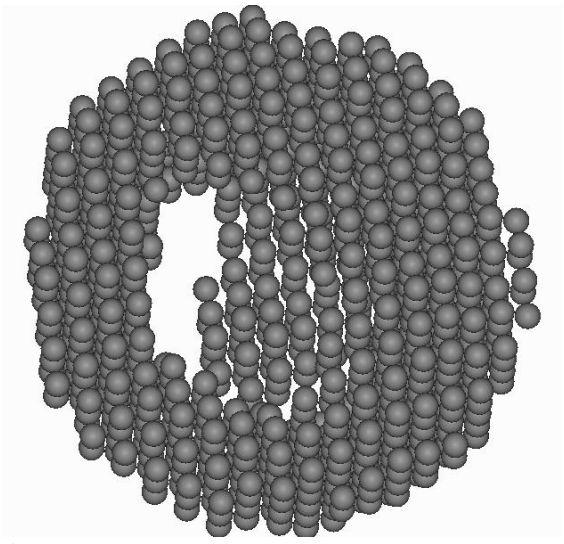
where A_{lm} amplitude is calculated as:

$$A_{lm}(q) = i^l (2/\pi)^{1/2} \nu_a \sum_{\substack{j=1 \\ X(j)=1}}^{M} j_l(qr_j) Y^*_{lm}(\omega_j) \tag{5}$$

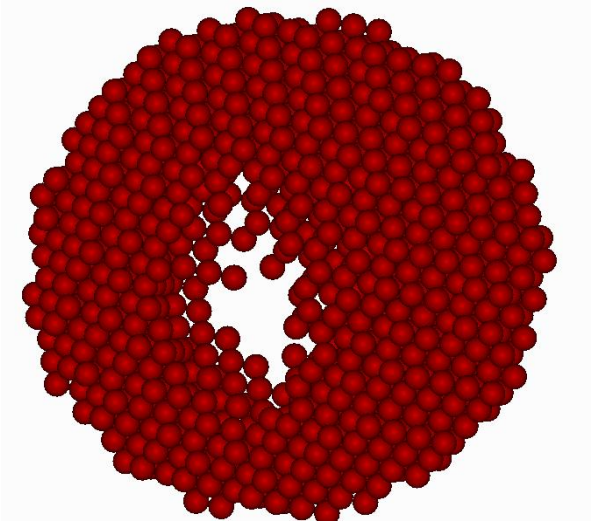
(r_j, ω_j) – polar coordinates, $\nu_a = \frac{4\pi r_0^3}{3} \frac{1}{0.74}$ – dummy-atom volume, $Y^*_{lm}(\omega_j)$ – corresponding spherical harmonic, $j_l(qr_j)$ – Bessel function. For the optimal model searching the following function f (X) should be minimized:

$$f(X) = R^2(I, X) + \sum_k \alpha_k P_k(X), \tag{6}$$

The first term is the model intensity deviation from the experiment, the second term is the sum of penalties imposed on the solution, with their weights. The simulation result is a compact model of the balls that approximates the experimental data of small-angle scattering in the best way according to (7).



A



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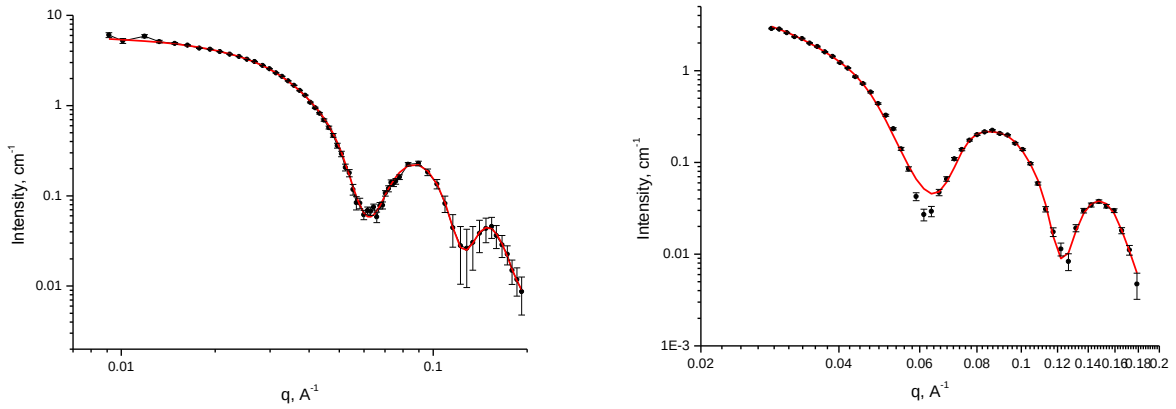


C **D**

Figure 3. The projection of apoferritin 3D model obtained by DAMMIF program for SANS **(A)** and for SAXS **(B)**. Approximations of experimental curves for each 3D model for SANS(**C**) and SAXS (**D**) correspondently.

SANS and SAXS data for the apoferritin solution were treated by the DAMMIF program. The projections of the obtained structures are shown in Figure.3. The model is a spherical shell with outer and inner diameters of about 120 Å and 75 Å respectively. The volume of this structure is 510 x10^3Å^3 for SANS and 640 x10^3Å^3 for SAXS. There are "holes" in the shell, which could appear due to the problem of instability solution due to the small thickness of the protein shell (20 Å) or by the presence of regions in the protein shell with the same scattering density as for the solvent. Last assumption requires further analysis.

3.4. The scattering curves approximation by spherical shell function

According to the crystallographic data (Protein Data Bank, PDB) and data obtained by authors using DAMMIF program the apoferritin molecule could be defined by a spherical shell. The neutron and x-ray scattering curves for apoferritin were approximated by the scattering function for a spherical shell (8):

$$I_{sph}(q) = \left[V_1 \Phi(qR_1) - V_2 \Phi(qR_2) \right]^2, \tag{7}$$

where $V_1 = \frac{4}{3}\pi R_1^3$, $V_2 = \frac{4}{3}\pi R_2^3$, $\Phi(t) = 3\frac{\sin t - t\cos t}{t^3}$, R_1 and R_2 – outer and inner spherical shell radius correspondingly.

For a spherical shell the radius of gyration, inner and outer radii are related as:

$$R_g = \frac{3}{5}\frac{R_1^5 - R_2^5}{R_1^3 - R_2^3} \tag{8}$$

Experimental curves approximation was carried out by program Fitter 2.1.2 [25] and presented in Figure. 4. The maxima size of molecule is $2R_1$ and the volume of the spherical shell is:

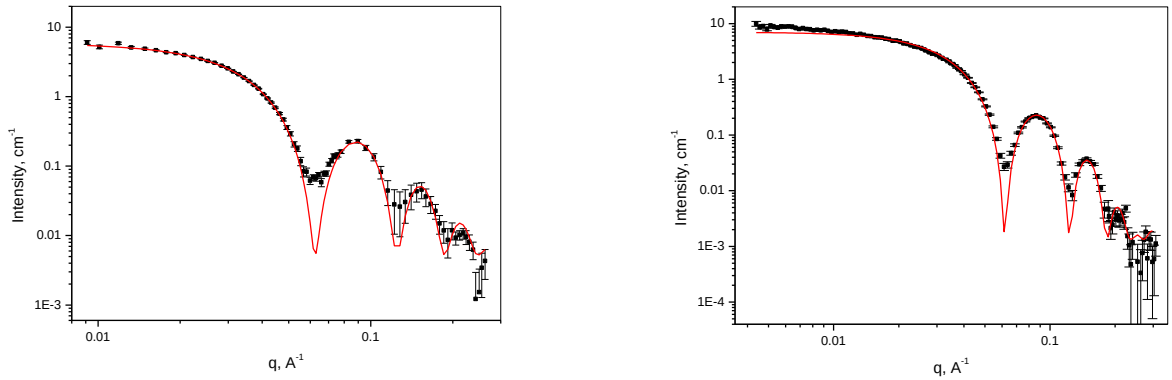
$$V_{shell} = \frac{4}{3}\pi(R_1^3 - R_2^3) \tag{9}$$

Table 1. The main parameters of fitting obtained for spherical shell model. R1 and R2 values were calculated by program Fitter, other parameters were calculated according to Eqs.(9,10).

№	Parameter	SANS	SAXS
1	Outer radius, R_1, Å	59.1±0.3	62.0 ±0.1
2	Inner radius R_2, Å	40.1±0.4	37.5 ±0.1

3	Radius of Giration R_g,Å	51.1±0.3	52.2 ±0.1
4	Volume of Shell, Vp, x10^3 Å^3	594.2±23	776 ±18
5	Volume (from Porod approximation) Vp x10^3 Å^3	560	600
6	Maximum size, Å	118.2±0.3	124±0.1

The parameters for the spherical shell, obtained by Fitter [25] and formula (9,10) are given in Table 1. These parameters (inner and outer radii, radius of gyration) are self-consistent. Nevertheless, there are small systematic differences in the parameters obtained by SANS and SAXS. The calculated volumes of the molecules for the models calculated by program DAMMIF is 510x10^3Å^3 and 640x10^3Å^3 for SANS and SAXS data respectively. In comparison, the difference between SANS and SAXS volumes obtained from Porod approximation (560 x10^3Å^3 and 600 x10^3 Å^3) is much smaller. This can be explained by the following: (1) the shape of apoferritin is not exactly spherical; (2) the scattering density distribution of molecules are slightly different; (3) the values, obtained by Porod approximation are quite sensitive to the background level; (4) the difference between volumes and inner and outer radius obtained by SANS and SAXS could be also be explained by H-D exchange of protein and solvent in SANS curves case.



A **B**

Figure 4. SANS (A) and SAXS (B) curves for apoferritin solution. Experimental data shown by points and spherical shell model by line.

4. Conclusion

Obtained SANS and SAXS data for the apoferritin in solution were treated by the DAMMIF program. The structural models correspond to spherical shell with outer and inner diameter of about 120 Å and 75 Å respectively. The shape and 3D model obtained by the low resolution method is consistent with the known results (PDB bank data). The calculated volumes for the models of the molecules is 510x10^3Å^3 and 640x10^3Å^3 for SANS and SAXS data respectively, however, the apoferritin molecule volumes determined from Porod approximation are 560 x10^3Å^3 and 600 x10^3Å^3 for SANS and SAXS, respectively. We have observed a slight systematic difference of structural parameters obtained for SAXS and SANS data. We suggest that this should be taken into account in the interpretation of the results of low resolution studies of proteins.

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