STRUCTURE OF MEMBRANE OF SUBMITOCHONDRIAL PARTICLES STUDIED BY SMALL ANGLE NEUTRON SCATTERING

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Abstract
The radius of gyration of membrane thickness of submitochondrial particles (SMP) has been determined by small-angle neutron scattering (SANS). The asymmetry of SMP membrane was shown. The distance between geometrical centre of membrane and centre of mass of distribution of inhomogenities has been estimated to be 13.3 ± 0.7 Å. The lipid / protein volume ratio equals 0.7 / 0.3. The average scattering density of neutrons for SMP membrane has been determined as well. The average membrane thickness is 52.4 ± 0.7 Å.

Introduction

The mitochondrion is called the powerhouse of the cell which produces the energy that is needed to carry on all cellular processes. The intact mitochondrion is a complex object. More simpler and convenient objects for investigation of functional and structural features of mitochondrial membranes are submitochondrial particles (SMP). SMP can be prepared by ultrasonication. Obtained this way SMP represent vesicles with size about 40 nm [1] formed by the mitochondrial inner membrane. Submitochondrial particles have ATP-syntase and all enzymes of the respiratory chain. Samples of SMP have high enzymatic activity and are stable at experimental conditions. The aim of our experiments is to determine some structural parameters of SMP membranes (such as thickness, lipid-protein volume ratios) and use the obtained result for further analysis of influence of substrata of oxidative phosphorylation on structure of SMP.

Theory

The scattering intensity for randomly oriented membranes at low angles can be written as [2]:

$$I(q) \quad \frac{I(0)}{q^2}\exp(\ q^2 R_t^2\ ), \qquad (1)$$

where R_t is radius of gyration of membrane thickness, characterizing the thickness of the membrane, $I(0)$ is intensity of scattering into zero angle. Approximation (1) is valid at $2 \quad S < q < 1/R_t$, where S is the membrane area. The thickness of homogeneous lamella is given by:

$$T \quad \sqrt{12}R_t . \qquad (2)$$

It has been shown that this approximation applies also to curved lamellae such as hollow spheres or hollow cylinders [3].

For lamellar systems with homogeneous scattering density in the direction parallel to the lamella plane the scattering intensity at zero angle is a linear function of the scattering density of the solvent [4].

An important characteristic of a particle is the so called match point [4, 5], the composition of the solvent, at which contrast (difference in scattering densities of solvent and particle) equals zero, i.e. I(0) equals zero as well. At the match point the scattering density of the solvent equals an average coherent scattering density of the particle (hereinafter average scattering density). The definition of value of the average scattering density of a particle makes it possible to estimate the volume ratios of components in the particle. According to [6] for two-component system (in our case protein-lipid membrane) can be written as:

$$\quad {}_1V_1 \quad {}_2V_2 \quad {}_1(1\ V_2) \quad {}_2V_2,$$

where $_1$, $_2$ and V_1, V_2 are the densities of neutron scattering length and the partial volumes (taking into account that $V_1 + V_2 = 1$) for the first and second component respectively. Hence the volume fraction for the first component is:

$$V_1 \quad \frac{\quad}{\quad {}^2}. \qquad (3)$$
$$\quad {}_1 \quad {}_2$$

According to [5] the radius of gyration of heterogeneous particle depends on contrast:

$$R_t^2 \quad R_c^2 \quad \overline{\quad}(R_p^2 \quad L^2 \quad R_c^2) \quad \overline{\quad} \quad {}^2 L^2, \qquad (4)$$

where is contrast; R_c is the radius of gyration of the particle with constant scattering length density (radius of gyration of the shape); R_p is the radius of gyration of heterogeneous particle; L represents the distance between the geometrical centre of the particle and centre of mass of distribution of inhomogenities. For uniform particles $R_c = R_p$ and $L = 0$. If trinomial $R_p^2 + L^2 - R_c^2$ is positive then denser regions are on the periphery, and vice versa.

Materials and methods

Submitochondrial particles were prepared from bovine heart mitochondria as described in [7] and stored in liquid nitrogen. During experiments SMP were placed into incubation media composed of 0.25 M sucrose and 5 mM tris-HCl, pH = 7.4.

Samples for contrast variation method contained the following D_2O / (H_2O+D_2O) ratios: 0, 0.08, 0.12, 0.2, 0.35, 0.42, 0.55, 0.7, 0.8, 0.9 and 0.95. The protein concentration was 3 - 17 mg / ml (known for each sample).

SANS experiments were carried out on the YuMO spectrometer at the pulsed reactor IBR-2 (JINR, Dubna) [8].

Results and discussion

Figure 1 represents scattering curves for submitochondrial particles in media containing 95 % and 55 % D_2O (curves for others compositions are not shown). In the inset of figure 1 the Kratky-Porod plot for these samples is shown. Presence of linear region in such plot displays the lamellar structure of investigated object. By means of the Kratky-Porod approximation (1) the value of I(0) was determined for each kind of solvent in the range 0.02 Å^{-1} < q < 0.08 Å^{-1}.

The dependence of on the scattering density of solvent is presented on figure 2. The SMP membrane cannot be completely contrast matched. Only a minimum in contrast is reached at 25.5 % D_2O, corresponding to $\bar{}$ = (1.08 ± 0.13) · 10^{10} cm^{-2}. This finding is an indication of a non-random distribution of lipids and proteins in the plane of the membrane. However, the minimum of I(0) is close to zero, so for the subsequent analysis we assume that the mixture of lipids and proteins is homogeneous.

According to (3) the partial volume of the protein in membrane equals:

$$V_p \quad \frac{\bar{} \quad L}{P \quad L},$$

where $_L$ and $_P$ are scattering densities of lipids and proteins correspondingly. Substituting the values of $_L$ = 0.7·10^{10} cm^{-2} and $_P$ = 1.9·10^{10} cm^{-2} [5], we have V_P = 0.32, V_L = 0.68.

The value of radius of gyration was determined at the D_2O / (H_2O + D_2O) ratios of 0.95, 0.9, 0.8, 0.55, 0.12, and 0.

Figure 2 represents the plot of R_t^2 against 1/ (= $\bar{}$ - $_{solvent}$). The data were fitted by equation (4) using a least-squares method. The best fit between function (4) and

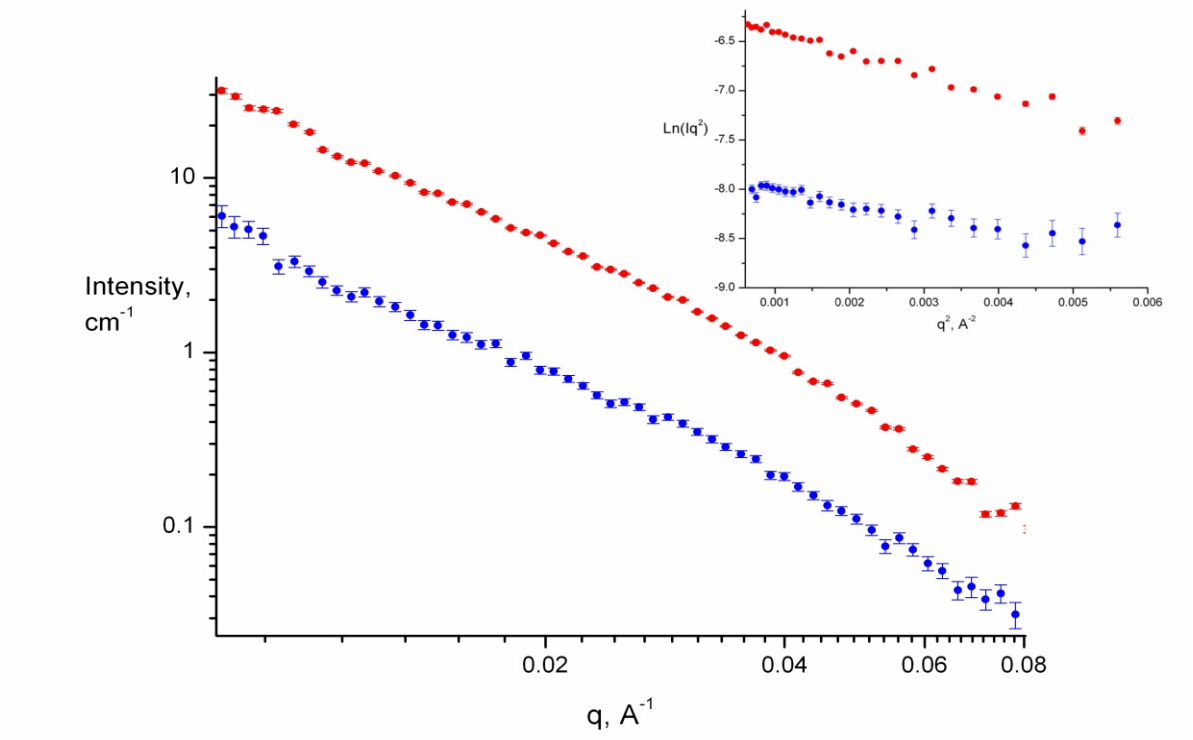

Fig. 1. Intensity scattered by SMP in media with 95 % D_2O (upper points) and 55 % D_2O (lower points). The Kratky-Porod plot for these samples is given in the inset.

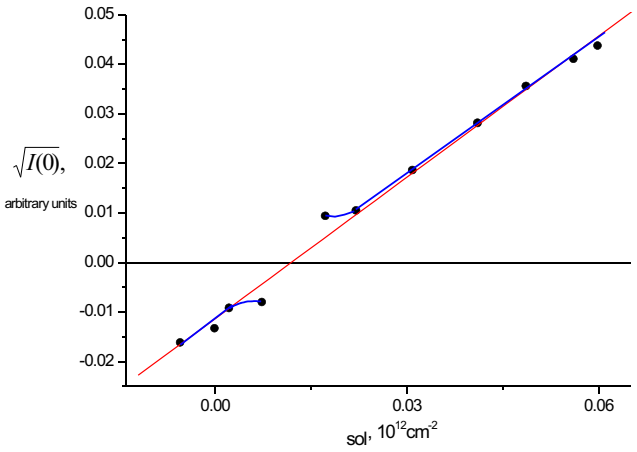

Fig. 2. Square root of zero-angle scattering of submitochondrial particles as a function of the solvent scattering density.

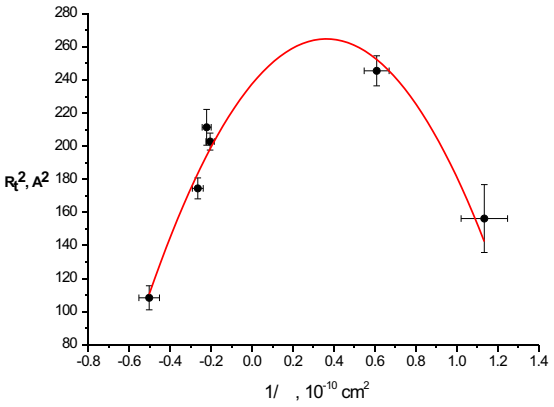

Fig. 3. R_t^2 versus 1/ of submitochondrial particles.

the experimental data correspond to the parameters: R_c = 15.4 ± 0.2 Å, L = 13.3 ± 0.7 Å, R_p = 14.1 ± 0.4 Å. Supposing lamella to be homogeneous we estimate the effective thickness of SMP membrane (see formula 2) to be equal 53.4 ± 0.7 Å. The positive value of trinomial $R_p^2 + L^2 - R_c^2$ indicates that the high scattering density is placed at the periphery of the membrane. In other words the proteins are located on the outside of the lipid bilayer.

Conclusions

The membrane of submitochondrial particles is heterogeneous. It is formed by a lipid bilayer including proteins. The fractions of proteins and lipids are V_P = 0.3, V_L = 0.7, respectively. The radius of gyration of SMP membrane is approximately 15.4 ± 0.2 Å. The membrane is asymmetric, the distance between geometrical centre of membrane and centre of mass of distribution of inhomogenities is about 13.3 ± 0.7 Å. The estimated effective average membrane thickness is equal to 53.4 ± 0.7 Å.

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