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NOVEL TENDENCIES IN DEVELOPING SMALL-ANGLE NEUTRON SCATTERING METHODS FOR STUDYING THE STRUCTURE OF BIOLOGICAL MACROMOLECULES

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ABSTRACT

In recent 20 years thermal neutron scattering has been acknowledged an important instrument for structural studies in molecular biology. The methods of neutron diffraction of high resolution, which are not discussed in this paper, have already permitted to obtain a detailed representation of the course of proteolytic reactions and have arisen a number of new problems connected with the localization of water molecules and the H-D exchange. The methods of low resolution widely used due to a relative simplicity of the experiment have been successfully applied for both solving structural problems per se and investigating the changes in the structure when macromolecules perform their biological functions. The most promising are novel experimental approaches: the triple isotopic substitution method and the method of spin dynamic polarization. These methods ensure solving structural problems at a higher resolution than the dimensions of the macromolecules studied. Installation of new experimental instruments makes neutron measurements more accessible, and development of direct methods for interpretation of experimental data using the apparatus of spherical harmonics opens new possibilities for small-angle neutron scattering making it a necessary element for interpretation of diffraction data of monocrystals of intricate biological macromolecules.

The paper presents a brief account of the tendencies in theoretical development and practical use of small-angle scattering for studying biological macromolecules. Special attention is given to the studies carried out in the Laboratory of Neutron Physics on a unique pulse IBR-2 reactor.

1. Modern Small-angle Spectrometers

The development of experimental methods and possibilities in neutron physics have always been determined by the accessible power of neutron sources. By 1969 these possibilities had been developed to such an extent that it became possible to use neutrons for investigation of real biological macromolecules. In 1972 such an attempt was successfully realized on hemoglobin on a FRJ-2 reactor by a group of German physicists (Schelten, Schlecht, Schmatz and Mayer) in Julich [1]. The interest to the

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studies was explained by a number of advantages of neutron investigations over X-ray ones: much higher "sensitivity" of neutrons to the presence of light atoms in the structure and, in the first place, of hydrogen atoms, and unique isotopic and spin "sensitivity" of neutrons. At the same time, the neutron method has certain disadvantages. They are a high level of incoherent neutron scattering in hydrogen-containing molecules and appreciable stiff neutron scattering. However, the main disadvantage of neutrons is low intensity and limited accessibility of neutron beams which is explained by the technical possibilities of the existing neutron sources and their high cost. Therefore the first studies of hemoglobin were made at very high concentrations of the protein (up to 144 mg/ml) [1].

The usage and accessibility of neutron sources has noticeably increased. First of all this can be said about Europe where the International Laue-Langevin Institute in Grenoble (France) is the leading center of neutron studies. The installation of a new small-angle D22 spectrometer and modernizing of the small-angle D11 spectrometer by substituting the wave rate selector have essentially enhanced the capabilities of neutron diffraction for studying biological macromolecules. The recently adopted decision to construct a new powerful reactor in the suburbs of Munich will make such measurements far more accessible. Now the concentrations of biomacromolecules required for measurements vary from 2 to 20 mg/ml at the volume of the measuring cell 0.2-0.3 ml.

At present a small-angle "YUMO" spectrometer located in the fourth canal of the pulse IBR-2 reactor is successfully operating in Dubna [2]. The average heat flux of neutrons in the sample is about $4 \cdot 10^7$ neutrons/cm$^2 \cdot$s that is only two times lower than the flux in the sample of the D11 spectrometer (prior to modernizing). The low pulse frequency of the IBR-2 reactor (5 Hz) makes it possible to apply the time-flight method with high efficiency. Eight angular detectors provide for automated radial averaging. A comparative study of particles of large molecular dimensions (ribosomes with a molecular weight of about $2 \cdot 10^6$ and the radius of gyration of 90 Å) and particles of average molecular dimensions (the protein of the translation apparatus with a molecular weight of about $43 \cdot 10^3$ and the radius of gyration 24 Å) has shown that the pulse IBR-2 reactor with a heating retarder (Dubna) and the stationary HFR reactor with a cooling retarder (Grenoble) allows to obtain results comparable in the following parameters: the absolute values of the scattering intensity and the calculated radii of gyration coincide within the limits of statistical errors [3]. It was shown on tetramethyl urea that the YUMO spectrometer is highly efficient for studying weakly scattering samples ($=0.05$ cm^{-1}) [4]. The high transmission of the spectrometer is combined with a good three-dimensional resolution which is illustrated by the scattering curve for a protein particle possessing a spherical symmetry (apoferritin) [3].

The installation of a new sliding reflector and improvement of the infrastructure of the YUMO spectrometer allow us to expect that in the nearest years the spectrometer will be the main instrument for carrying out neutron studies of biological macromolecules for users from the states of the CIS, East Europe and Germany.

2. New Experimental Approach

Recently two additional experimental schemes have been added to the existing three ones traditionally used in SANS studies: contrast variation by changing the scattering properties of the solvent or particle (isotopic substitution [5]), contrast variation by using a mixture of protonated and deuterated particles (double isotopic substitution [6]), the method allowing us to determine the distance between the marked sites (the triangulation method). The first is based on using three types of particles and is called a triple isotopic substitution method (the TIS-method) [7, 8]. In this scheme, the difference between the scattering of two solutions is considered. One solution contains a mixture of normal (undeuterated) and deuterium labelled particles, and the other contains intermediately deuterated particles only. A necessary condition for the application of the TIS-method is that the two solutions are identical in all respects except for the extent of the deuterium label. The main properties of the TIS-method are elimination of interparticle effects, "invisibility" of the isotopically non-substituted parts of the particle and independence of the TIS scattering curve on the buffer isotopic content (D_2O:H_2O ratio). Recently unique possibilities of the TIS-method were illustrated in the study of the polypeptide elongation factor Tu (EF-Tu) from *E.coli* associated with GDP and within the ternary complex EF-Tu·GTP·aminoacyl-tRNA [9]. The minimal part of the macromolecular assembly whose structure can be obtained by the TIS-method is close to several percent. It was demonstrated that the radius of gyration of protein S4 within the 30S subunit can be really measured by the TIS-method [10]].]

The second experimental scheme called spin contrast variation is based on a great change in the scattering amplitude of hydrogen when polarized neutrons are scattered by polarized protons. The scattering length of hydrogen varies from $-18.3 \cdot 10^{-12}$ cm, when the polarization direction of the neutron beam is antiparallel to that of the proton spins, to $+10.82 \cdot 10^{-12}$ cm, when the interacting spins have the same direction with respect to the external magnetic field. In a strong magnetic field (B=2.5 T) and at low temperatures (T < 1 K), irradiation of 4 mm microwaves will align the nuclear spin system parallel (or antiparallel) to the polarized electronic spins of added paramagnetic centers [11].

Recently this idea has been used for determining the structure of ribosomal proteins from the large (50S) ribosomal subparticle. The position and shape of the two proteins, L3 and L4, were determined [12].

3. Small-angle Scattering in Solution and Diffraction in Crystal

Between an ideal solution and an ideal monocrystal there exists an intermediate region of structural arrangement from which it would be possible to obtain, in a general case, structural information with a resolution intermediate between the dimensions of the macromolecule and the atom. Great ambiguity of the shapes of the structural arrangement in this region makes it impossible to obtain a general solution of the structural task. However, in the cases when this ambiguity is removed, the task becomes structurally sensitive. In particular, such an approach can be used not only for studying the distance and position of the particles

in specifically bound dimers and more complicated macromolecular complexes, but also for obtaining information on their shape and internal structure. The practical realization of such an approach is complicated due to the fact that the scattering curve of a certain biological particle always consists of three contributions: the contribution of the shape, the contribution of the internal structure and the contribution of the intersecting member. To isolate each of the contributions, the neutron scattering curve is measured at different contrasts (it is evident that the minimal number of contrasts is three), and the scattering curve of a "homogeneous" particle is obtained by extrapolation to infinite contrast. The envelope of a globular particle can be described by the angular function $F(\omega)$ which is parametrized using the multipole expansion

$$F(\omega) = \sum_{l=0}^{L} \sum_{m=-1}^{1} f_{lm} Y_{lm}(\omega).$$

In this formula, the highest value of L determines the resolution (the angular resolution is $\pi/(L+1)$ and the spatial resolution is $R_o\pi/(L+1)$, where R_o is the radius of the equivalent sphere), f_{lm} are complex numbers, $Y_{lm}(\omega)$ are spherical harmonics, and (r,ω) are spherical coordinates. The partial amplitudes $A_{lm}(Q)$ in the corresponding scattering intensity

$$I(Q) = 2\pi^2 \sum_{l=0}^{L} \sum_{m=-1}^{1} |A_{lm}(Q)|^2$$

are expressed in terms of a power series with coefficients that are non-linear combinations of the shape coefficients f_{lm}. Recently this approach has been successfully applied to study the tertiary structure of protein (pyruvate decarbozylaze [13]) and the 50S subunits of *E.coli* [14]. The model for the 50S subunit (the spatial resolution being about 40 Å) is in good agreement with electron microscopic studies.

The possibility to construct such models opens principally new perspectives for a joint use of the data of small-angle neutron scattering and X-ray diffraction. Let us define these possibilities for the 50S ribosomal subparticle whose crystals diffracting up to 10 Å are obtained in some laboratories, but the phase problem is far from its solution. To construct a model of the 50S ribosomal subparticle at a resolution of ~20 Å, it is necessary to get scattering curves in a maximally possible range of scattering vectors (from 0.007 to 0.30-0.35 Å). This can be done only in conditions when the contribution of incoherent scattering of the solvent is minimal, in other words, all measurements should be done in 100% D_2O. In this case, the contrast can be varied by reverse contrast using particles with a different contribution to the scattering of the RNA and protein components. Such particles are isolated from the bacterial mass grown on a specific combination of protonated and deuterated substrates. The developed three-dimensional model of the 50S subunit with a resolution of 40 Å will be used for obtaining the function of packing in the crystal, and the resolution will be increased using a diffraction set. The data in the range of transmitted pulses from 0.15 to 0.35 Å will serve as a base for refinement and interpretation of reflexes corresponding to the resolution of 40-20 Å. A group of physicists from our laboratory jointly with representatives from a number of foreign laboratories have been working on realization of this project in which for the first time small-angle scattering in solution is combined with diffraction in crystal.

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