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Spatio – Temporal Features of Protein Specific Motions. The Influence of Hydration

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Abstract. The angular dependencies of inelastic intensities of Rayleigh scattering of Moessbauer radiation were measured for lysozyme and myoglobin (for different degrees of hydration: from h = 0.05 till h = 0.7). The treating of the data at h > 0.05 approves the existence of segmental motions (α-helices for myoglobin, α-helices and β-sheets for lysozyme) as well as of individual motions. Further hydration increase the mean-square displacements for both types of intraglobular motions for these proteins, while the motions of the globule as a whole remain nearly the same as for h = 0.05. Results of the study of the radial distribution function deduced by Fourier – transform from the diffuse x-ray measurements together with RSMR data allow to conclude that the water during hydration of proteins competes with the intramolecular hydrogen bonds, loosens the protein and increases the internal dynamics. At the same time water arranges the ordering of macromolecule from 'glassy' state at h $\approx$ 0.02 to the native state at h = 0.4–0.7. Different architecture of proteins leads to the different structural dynamics as in the case of lysozyme and myoglobin.

Key words: diffuse x-ray scattering, dynamics, hydration, lysozyme, myoglobin, Rayleigh scattering of Moessbauer radiation

The understanding of the fundamental principles of protein functioning requires the knowledge not only of the structure but the dynamics as well [1]. Among the modern physical methods of studying protein dynamics an important role is played by Rayleigh scattering of Moessbauer radiation (RSMR) technique [2, 3]. RSMR studies of protein dynamics were done with two different experimental version of this technique. The soft collimating conditions were used in the first version (the width of the angular resolution function is about 8 degrees) [2]. It allows to define the 'overall' mean square displacement of protein motions by measuring the elastic fraction of the scattered radiation [2] and correlation times of protein motions within the range of 10^{-7}–10^{-9} sec and less by measuring of RSMR spectra [2]. The second version with a strict collimating conditions (the width of the angular resolution function is about 2 degrees) allows to define the size (and the shape) of the moving parts of macromolecules by the study of the angular dependence of the inelastic scattering intensity [3].

This paper is devoted to investigations of protein specific motions in myoglobin and lysozyme with the help of second version of RSMR technique. Conclusions of the paper, however, will take into account the main results obtained by the first version as well [2].

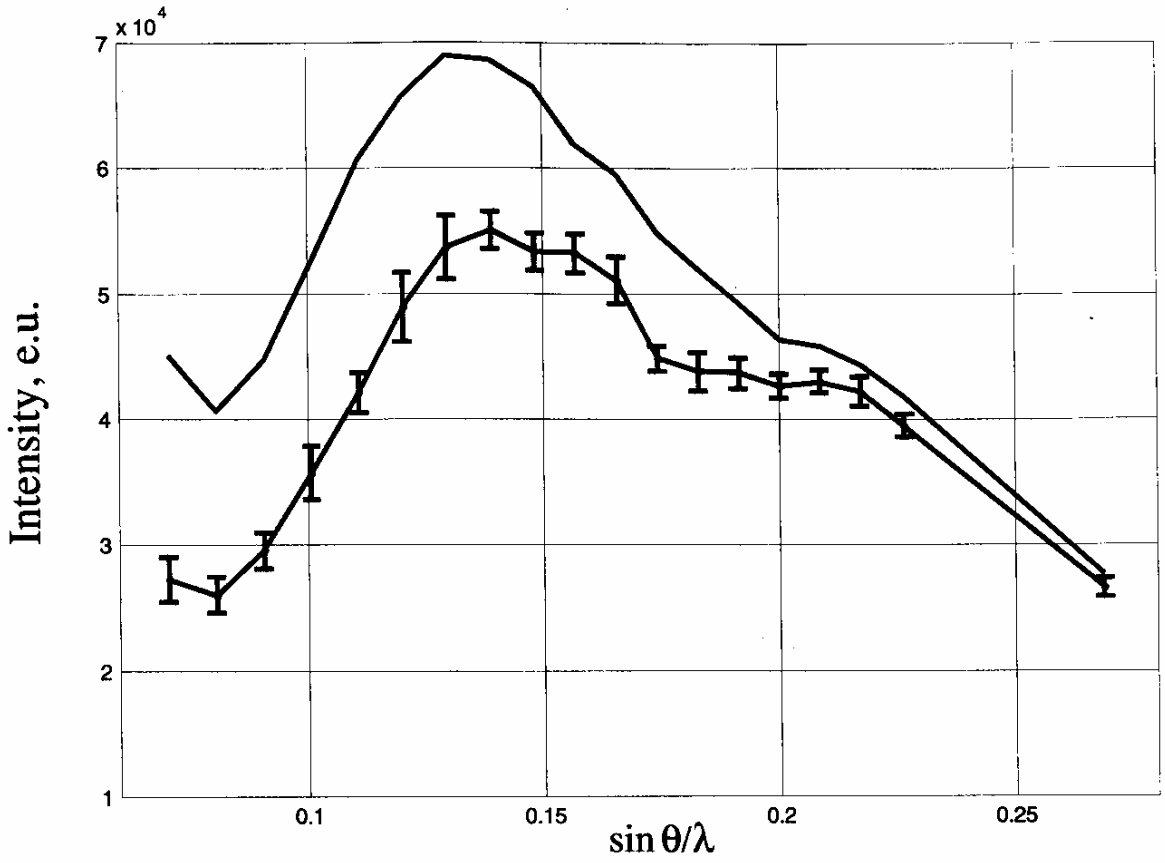

Figure 1. Angular dependencies of total and inelastic (in error bars) intensities of RSMR for myoglobin (h = 0.44).

Let us consider the main approximations and equations which were used for the interpretation of the angular dependencies of the inelastic scattering intensity. It is well known that the scattering functions $S_{mn}(\vec{Q}, \omega)$ contain all the structural and dynamic information on the system under investigation and by a simple way connected with an intermediate scattering function $I_{mn}(\vec{Q}, t)$ [4] Here $\vec{Q}$ is the momentum transfer ($|\vec{Q}| = \frac{4\pi \sin\theta}{\lambda}$, 2θ is the scattering angle, $\lambda = 0.86$ Å is the wavelength of incident radiation). The total scattering intensity is defined by $I(\vec{Q}, 0)$ and is composed from the elastic $I(\vec{Q}, \infty)$ and the inelastic parts. It is suggested in the model used that the motions of the protein macromolecule as a whole are independent on the intramolecular motions. Intramolecular motions consist of the segmental motions, the size of the segments may vary from the individual atoms till the elements of the secondary structure or domain. All atoms within a segments moves cooperatively and these motions are independent on the motions of atoms in another segment.

In such a case the inelastic scattering intensity is given by the following expression:

$$I_{inel}(\vec{Q}) = S_{glob}(\vec{Q})(1 - e^{-Q^2\langle x^2\rangle_g}) + \sum_s S_{seg}(\vec{Q})(1 - e^{-Q^2\langle x^2\rangle_s}) \qquad (1)$$

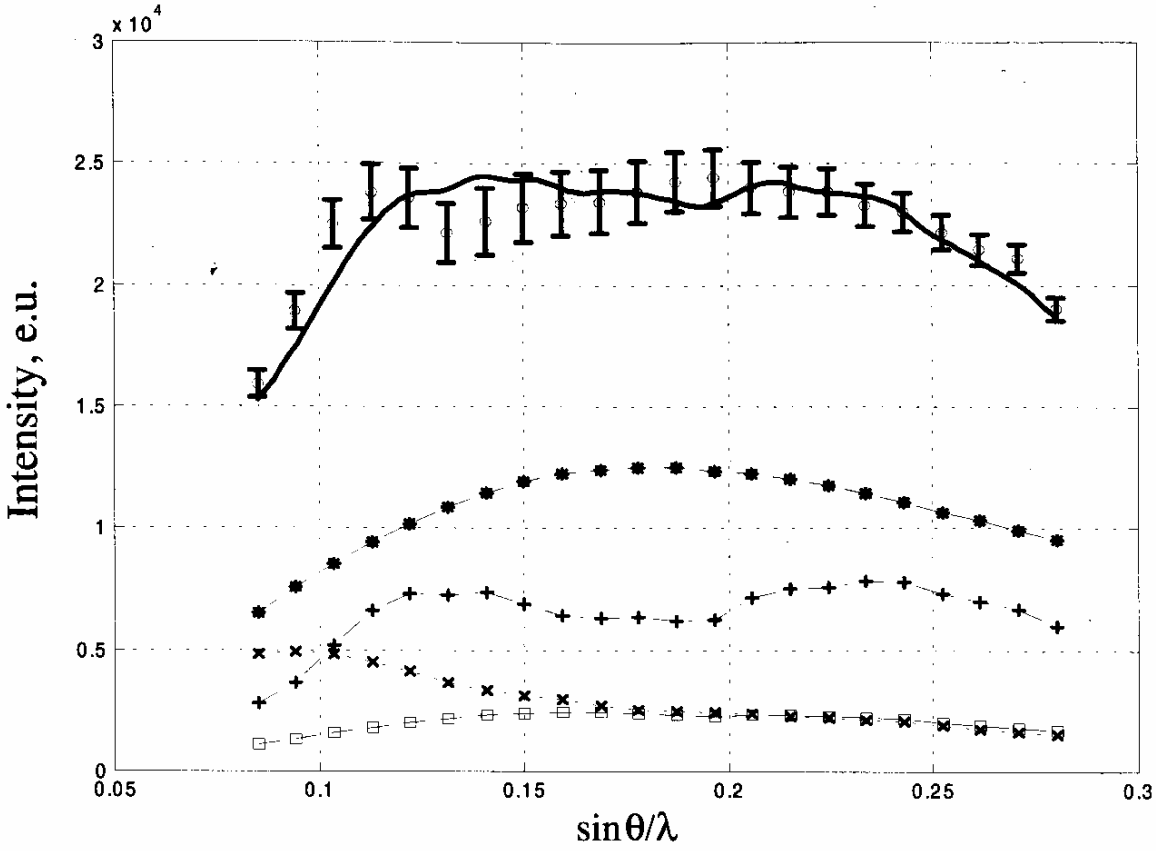

Figure 2. The angular dependence of inelastic scattered intensity (in error bars) of RSMR for lysozyme (h = 0.2) and the best fit to this curve (solid line) which consist from the following components: –*– individual motions; –+– motions of macromolecule as a whole; –x– segmental motions of α-helices and β-sheets; –□– motions of the bound water.

Here $S_{seg}(\vec{Q})$ describes the diffraction pattern from a collectively moving segment of given size (and shape).

Commercial preparates of myoglobin (from horse skeletal muscle, Sigma) and lysozyme (from chicken egg white, Sigma) were hydrated in an atmosphere with a certain relative humidity. Hydration degrees h were determined gravimetrically.

The angular dependencies of total, elastic and inelastic intensities of RSMR were measured for α-helical plus β-sheet lysozyme (three degrees of hydration: h = 0.05, h = 0.2 and h = 0.45) and α-helical myoglobin (three degrees of hydration: h = 0.05, h = 0.44 and h = 0.7). Figure 1 represent as an example these dependencies for the case of myoglobin (h = 0.44). The room of the article restrict us to show all the dependencies. Theoretical diffraction pattern were calculated for α-helices, β-sheets of lysozyme, α-helices for myoglobin and for independent motions of atoms as well. The angular dependence of inelastic scattered intensity of RSMR (lysozyme, h = 0.2) and the best fit to this curve (α-helices and β-sheets as a moving segments and individual motions of atoms are taken into account) are displayed on Figure 2. Figure 3 show the angular dependence of inelastic scattered intensity of RSMR (myoglobin, h = 0.7) and the best fit to this curve (α-helices as a moving segments and individual motions of atoms are taken into account). Another attempt to fit this angular dependence (myoglobin, h = 0.7) when only the

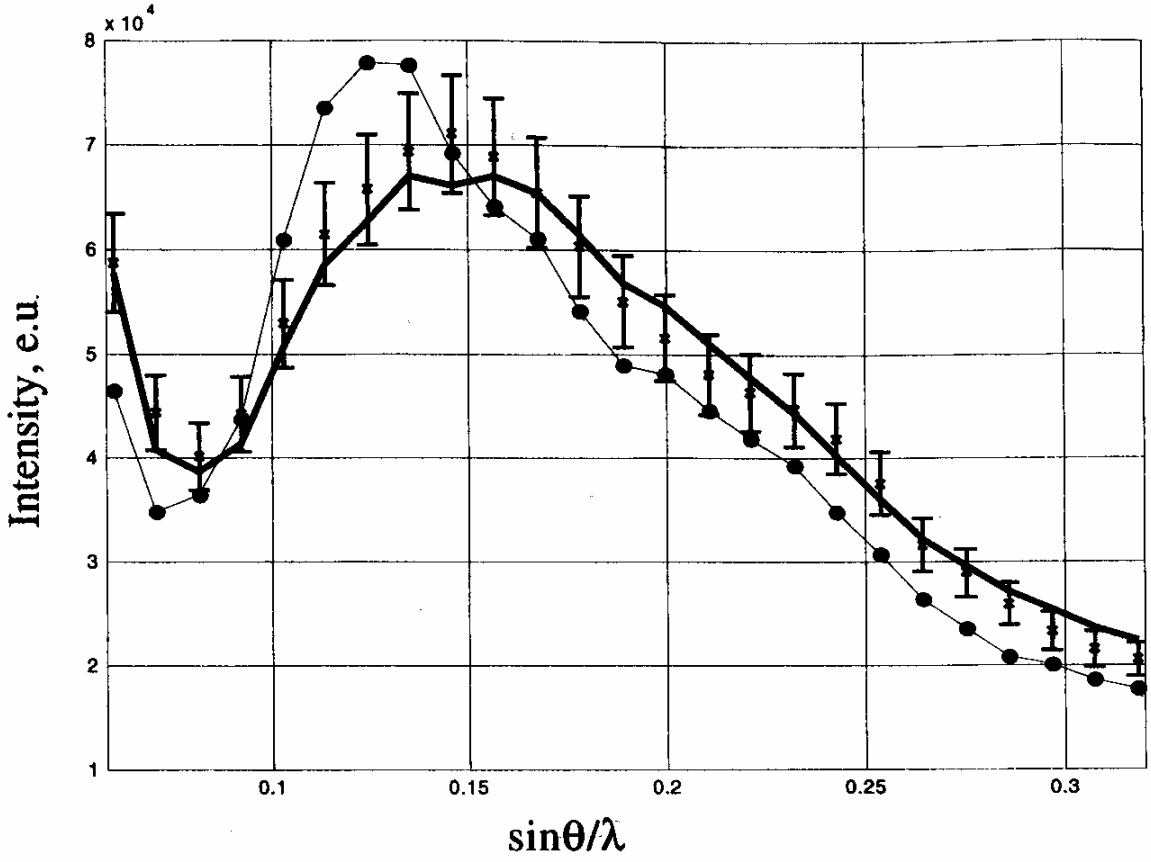

Figure 3. The angular dependence of inelastic scattered intensity (in error bars) of RSMR for myoglobin (h = 0.7) and two fits: solid line (best fit) – α-helices and individual motions are taken as a moving segments; –●– motion of macromolecule as a whole.

motion of macromolecule as a whole considered as a moving segment is displayed on Figure 3 as well. It is evident that the intraglobular motions of α-helices and individual atoms with a minor contribution of the motion of macromolecule as a whole much better fit experimental data. This result is in a good agreement with our previous considerations of the range of hydration degree ($h \leq 0.7$–0.8) where the effects observed by RSMR can be attributed mostly to intramolecular mobility rather than to translational and rotational diffusion of macromolecule as a whole [5]. These conclusions are not valid for the case of x-ray analysis [6], since RSMR technique is sensitive to the motions with a correlation times less than 10^{-7} sec only, while x-ray is sensitive even to static disorder. Table I displays the summary of RSMR results. Long-range correlated motions (globule as a whole) are essential at low hydration degree for both proteins.

Let us consider the data for lysozyme. The best agreement with the experiment at h = 0.2 was obtained when individual motions of small groups (B = 0.62–0.8) together with the motions of α-helices and β-sheets (as cooperative segments) were considered (A = 0.2–0.38). At further hydration (h = 0.45) these both types of intraglobular motions only increase theirs mean-square displacements up to 0.3 Å^2. The mean-square displacements of the motions of the globule as a whole remain approximately on the same level as for h = 0.05. The study of RSMR spectra [2] for lysozyme indicates that correlation times of intraglobular motions are of the

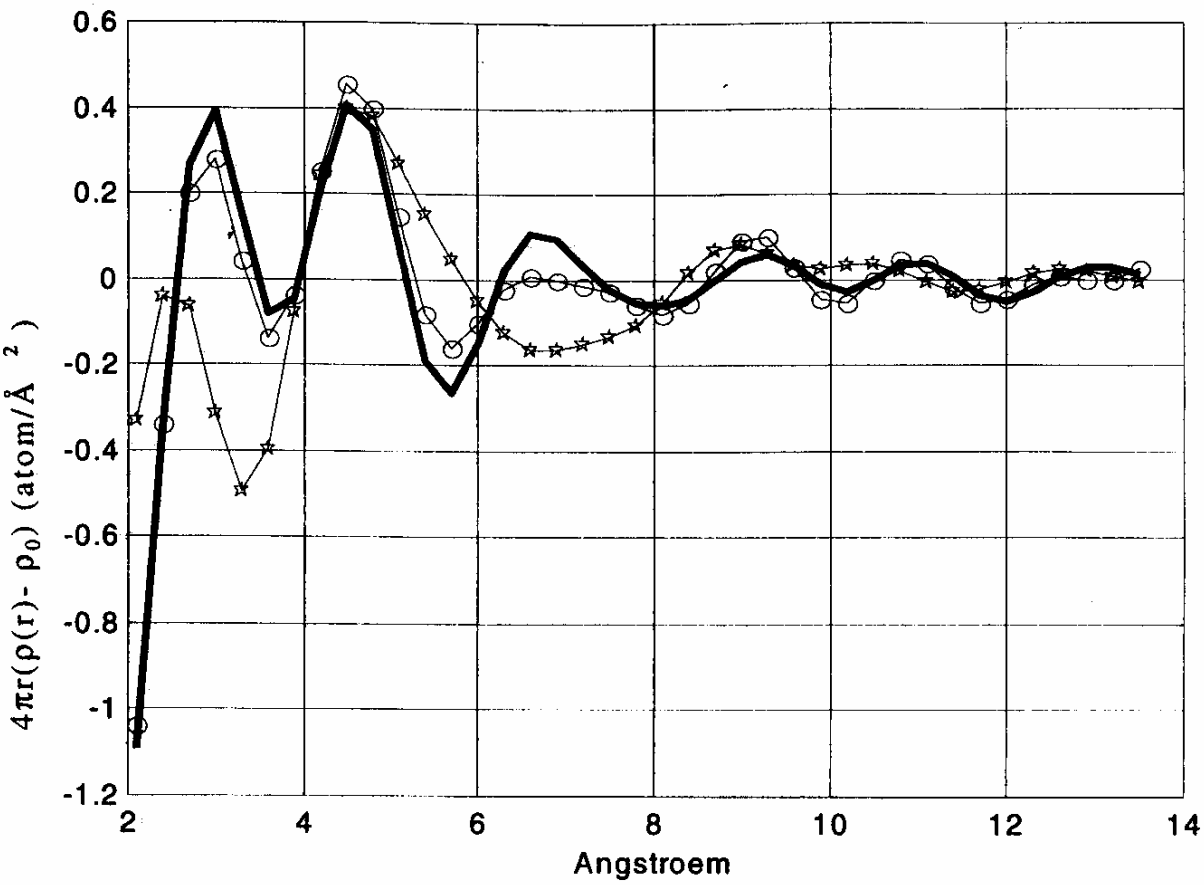

Figure 4. Radial distribution function for lysozyme at different hydration degrees: * – h = 0.05; o – h = 0.45; solid line – h = 1.0.

order of nanoseconds or less. It was surprising that we were unable to fit the data by the plain picture of hinge-bending motions of domains, which should play an important role in the functioning of lysozyme [6]. Therefore we suggest that hinge-bending motions have more complicated character, they consist of the motions of α-helices, β-sheets and individual motions of small groups, but complete space – time correlations are absent within domain during its motion and, for example, small groups, connected with loops, come independently and later on a place than α-helices.

Now to the data for myoglobin. The treating of the data at h = 0.44 approves the existence of individual motions (B = 0.6) as well as segmental motions of α-helices (A = 0.4). Further hydration (h = 0.7) increase the mean-square displacements for both types of intraglobular motions up to 0.64 $Å^2$, while the motions of the globule as a whole remain nearly on the same level as for h = 0.05. Correlation times of segmental motions, which are deduced from RSMR spectra for myoglobin [2], become, during hydration, of the order of hundreds of nanoseconds.

The study of the radial distribution function (see Figure 4) deduced by Fourier – transform from the diffuse x-ray measurements allows observing the clear correlation between the shift of the peak corresponding to the intraglobular hydrogen bonds or the weakening of intraglobular hydrogen bonds and the increase of intra-

Table I. The values of parameters of expression (1) which are best fitted experimental data

Lysozyme							
h	A	B	$<x^2>_g$ (Å^2)	$<x^2>_s=$ $<x^2>_i$ (Å^2)	$<x^2>_{bw}$ (Å^2)	Nb	Nf
0.05	–	–	0.05	–	–	–	–
0.2	0.2	0.8	0.03	0.16	0.5	180	–
0.45	0.2	0.8	0.03	0.3	0.78	346	54

Myoglobin							
h	A	B	$<x^2>_g$ (Å^2)	$<x^2>_s=$ $<x^2>_i$ (Å^2)	$<x^2>_{bw}$ (Å^2)	Nb	Nf
0.1	–	–	0.1	–	–	–	–
0.44	0.4	0.6	0.04	0.39	0.56	250	200
0.7	0.4	0.6	0.04	0.64	0.64	350	350

Here A = Ns/N, B = Ni/N, where Ns – number of atoms participating in segmental motions, Ni – number of atoms participating in individual motions, N – number of atoms in a macromolecule, Nb – number of bound water molecules and Nf – number of free water molecules. During fitting it was suggested that $<x^2>_s=<x^2>_i$.

globular mobility for both proteins. This peak (in the case of lysozyme) changes its position from 2.5 Å (h = 0.05) to 2.9 Å (h = 0.45). At further hydration of lysozyme its position does not change. The similar peak (case of myoglobin) gradually changes its position from 2.4 Å (h = 0.05) up to 2.9 Å (h = 0.89) in the whole range of hydration degree. The so called 'second' peak does not change its position at 4.6 Å during hydration (case of lysozyme) or slightly shifts to the smaller values from 4.8 Å to 4.6 Å (at h = 0.44, case of myoglobin). Inhomogeneous broadening of the 'second' peak is strongly decreased during hydration. We consider that it is connected with the ordering of the macromolecule structure during hydration.

Thus the water during hydration of proteins competes with the intramolecular hydrogen bonds, loosens the protein and increases the internal dynamics. At the same time water arranges the ordering of macromolecule from 'glassy' state at h $\approx$ 0.02 to the native state at h = 0.4–07. Different architecture of proteins leads to the different structural dynamics as in the case of lysozyme and myoglobin.

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