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A METHOD OF MÖSSBAUER FOURIER SPECTROSCOPY FOR DETERMINATION OF THE BIOPOLIMER COORDINATE CORRELATION FUNCTIONS

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A method of Mössbauer Fourier spectroscopy is developed to determine the correlation function of coordinates of a macromolecular system. The method does not require the use of an a priori dynamic model. The application of the method to the analysis of RSMR data for human serum albumin has demonstrated considerable changes in the dynamic behavior of the protein globule when the temperature is changed from 270 to 310 K. The main conclusions of the present work is the simultaneous observation of low-frequency ($\tau \geqslant 10^{-9}$ sec) and high-frequency ($\tau \leqslant 10^{-9}$ sec) large-scaled motions, that is the two-humped distribution of correlation times of protein motions.

In recent years, Mössbauer spectroscopy has found wide application in studies of dynamic properties and functional activity of biopolymers [1–8] and synthetized polymers [9]. Containing an appreciable amount of solvent (water), a polymer system possesses conformational mobility owing to rotating isomers and bond-angle bending [10]. These motions are largely different from vibrations in usual solids. Mössbauer spectra of solvated polymer systems make it clear that conformational motions occur by local diffusion [9,11–18]. The overall amplitudes of the conformational motions are much greater than those of bond stretching and may be as high as a few Ångstroms as far as functionally important degrees of freedom are concerned [2,19–21]. The protein dynamics is convenient to describe with the help of temporal dependencies of the autocorrelation function of coordinates of different protein groups. So far, for the experimental determination of this function, models of polymer dynamics have been invoked. The approach using model-free analysis would enable investigators to consider the biomacromolecule dynamics and function on a qualitatively new level. In an attempt to achieve this goal, we propose a method based on Fourier analysis of Mössbauer spectra. Very informative in the domain $10^{-10} - 10^{-6}$ s, the Fourier analysis offers a time window in which our method works well. The use of the Fourier analysis for the derivation of the Van Hove correlation function permits one to avoid computational difficulties with non-Lorentzian spectra that have been encountered with other methods and which have not been surmounted entirely.

The shape of the gamma-emission (absorption) spectrum of a nucleus is known to be closely correlated with the dynamic behavior of the system and takes the form [23]:

$$g(\omega) = \frac{1}{\pi} \int_0^\infty \cos((\omega - \omega_e)t)\Psi(t) \exp(-\Gamma_0 t/2)\mathrm{d}t, \tag{1}$$

where Γ_0 is the natural line width and ω_e is the frequency of recoil-free emission.

Assuming that the motion of a nucleus is a classical random stationary Gaussian process [24], we have:

$$\Psi(t) = \exp\left\{- \frac{\langle(\Delta X(t))^2\rangle}{2\lambda\!\!\!^-{}^2}\right\} = \exp\left\{- \frac{\varphi(0) - \varphi(t)}{\lambda\!\!\!^-{}^2}\right\}, \tag{2}$$

where $\varphi(t) = \langle X(t)X(0)\rangle$ is the correlation function, $X(t)$ is the nucleus coordinate, and $2\pi\lambda\!\!\!^-$ is the emission wave length. The symbol $\langle \ldots \rangle$ denotes the averaging over the Gibb's ensemble. In calculating the spectrum of Rayleigh scattering of Mössbauer radiation (RSMR), the $\lambda\!\!\!^-{}^{-1}$ quantity is to be replaced by q – the momentum transfer of the γ-quantum [25].

Hence, in an approach using Mössbauer spectroscopy, information on the molecular dynamics is contained in the $\Psi(t)$ function. A method for determining $\Psi(t)$ from experimental Mössbauer spectra is proposed in the present paper.

Generally, the function $\Psi(t)$ in the integral of (1) decays monotonously from 1.0 to its ultimate value $\Psi(\infty)$ in a time which is of the order of the correlation time τ of the process. For convenience, in what follows we introduce the quantity $\Psi'(t)$ [26]:

$$\Psi(t) = \Psi(\infty) + \Psi'(t). \tag{3}$$

Substituting (3) into (1) gives two terms representing the so-called narrow and wide components of the spectrum:

$$\begin{aligned} g(\omega) &= \Psi(\infty)\ \frac{\Gamma_0/2\pi}{(\omega-\omega_e)^2+(\Gamma_0/2)^2} \\ &\quad + \frac{1}{\pi}\int_0^{\infty} \cos\left[(\omega-\omega_e)\cdot t\right]\, \exp\left(-\frac{\Gamma_0 t}{2}\right)\Psi'(t)\mathrm{d}t. \end{aligned} \tag{4}$$

The broad component has an effective width that exceeds the natural one by τ^{-1}. In the case of solids, the Mössbauer spectroscopy usually deals with processes for which $\tau^{-1} \gg \Gamma_0$ (for instance, lattice and intramolecular vibrations). The wide component in this case cannot be singled out from the spectrum measured, and the second term in (4) is neglected. Thus, one could observe a Lorentzian line of natural width (Mössbauer line) only. The area $\Psi(\infty)$ under this line is the probability of recoil-free processes. We denote it by f and f' for an emission and an absorption spectrum, respectively. The solution for $\Psi(\infty)$ of eq. (5) below, derived from (4),

$$g(\omega) = \Psi(\infty)\ \frac{\Gamma_0/2\pi}{(\omega-\omega_e)^2+(\Gamma_0/2)^2} \tag{5}$$

can be obtained by the conventional least-squares method. In the case of unrestricted diffusion of Mössbauer nuclei, that is, when $\langle(\mathrm{d}X(t))^2\rangle = 2.0\cdot D\cdot t$, with D the diffusion coefficient, no essential complications arise. For this,

$$\Psi(\infty) = 0, \quad \Psi'(t) = \Psi(t) = \exp\left\{-Dt/\bar{\lambda}^2\right\}.$$

Thus, (4) is reduced to the second term only, and integration yields an equation of type (5). In both cases, one deals with a Lorentzian spectral function, and searching for $\Psi(t)$ is quite a trivial problem.

From the early 1980's, however, a great deal of experimental work has been reported in which non-Lorentzian spectra were observed. The investigations were conducted on objects with conformational mobility constrained to local diffusion (proteins, polymers) [5–9]. If the correlation times of local diffusion are of the order of Γ_0^{-1}, the spectra are a superposition of narrow and wide non-Lorentzian components. Using specific models for local steric diffusion, one can interpret the experimental data in greater detail and obtain $\Psi(t)$ in an explicit form [11,14–17]. However, in such cases $\Psi(t)$ is restricted to a class of functions which rely upon the model used. Moreover, an analytically solvable expression for the integral of (1) cannot be obtained even for the simplest model such as a Brownian oscillator. This greatly complicates the choice of the optimum function, because the validity test of each tentative function requires the numerical calculation of the integral. This may also be expected to be the case with more complicated models.

Meanwhile, the correlation function of nucleus coordinates can be determined in a straightforward manner through the Fourier transform of the spectral function $g(\omega)$. The approach is the application to eq. (1) of methods used for solving ill-conditioned problems [27]. Using the experimental Mössbauer spectrum $g(\omega)$, it is possible to obtain through the Fourier transformation the experimental function $\Psi(t)$. It is then compared with the tentative model functions in order to arrive at the optimal one. It is nonetheless important in this approach, from the very onset, that the function $\Psi(t)$ is independent of choice of this or that dynamical model. The spectrum $g(\omega)$ contains information about system dynamics. This information becomes much more representative and clear when it is represented by the function $\Psi(t)$.

In dealing with the experimental spectrum, we have eq. (1) in the form:

$$N(\infty) - N(k) = C \int_0^\infty \cos(\omega_k t)\Psi(t)\exp(-\Gamma t/2)\mathrm{d}t, \tag{6}$$

where k is the channel number, $k \in [-K_\mathrm{B}, K_\mathrm{B}]$, $\omega_k = K \cdot \Delta\omega$, $\Delta\omega$ is the frequency spacing between the channels, K_B is the boundary channel number, $N(\infty)$ is the background, $N(k)$ is the set of observations in the kth channel, and C is the normalizing constant. For thin absorber $\Gamma = 2\Gamma_0$ (in fact, Γ is the line width for a low-temperature spectrum $T < 150$ K). According to methods dealing with ill-conditioned problems, in deriving $\Psi(t)$ the first step is to establish the regularizing operator (or another physically justificated procedure) used to generate tentative functions $R(t)$. The tentative function $\exp\{-\Gamma t/2\}\Psi(t)$ will be sought for a series with a finite-numbered set p_i :

$$R(t) = \sum_{i=1}^{N} S_i^\star \exp\{-p_i\Gamma t/2\} . \tag{7}$$

The adequacy of this formulation rests upon Müntz's theorem [28, p. 53], which states that if series $\Sigma_i\, 1/p_i$ is divergent, the series $\Sigma_i a_i X^{p_i}$ is convergent toward some predetermined continuous function which can be found by suitably selecting factors a_i (in our case, $X = \exp\{-\Gamma t/2\}$). After substituting (7) into (6), the right-hand side of the equation splits into N Lorentzian lines, each having areas $\{S_i\}$ and line width $\{p_i\Gamma\}$. The set $\{S_i\}$ and value $N(\infty)$ can be easily found by a linear regression technique. The resultant algorithm applied to the experimental function $N(k)$ leads to an approximation close to $\exp\{-\Gamma t/2\}\Psi(t)$ in the form of series $R(t)$. In fact, one speaks of the Fourier analysis of Mössbauer spectra.

Because of the boundedness and discreteness of the domain of definition of $N(k)$ and because an error may arise in determining $N(\infty)$, valid information on the behavior of $\Psi(t)$ is available only for the time domain

$$\left[\left(\frac{K_B \Delta\omega}{4}\right)^{-1}, (2\Delta\omega)^{-1}\right].$$

For each set $\{p_i\}$, there is its own tentative function and model spectrum $G(k)$. We investigated the natural series $\{p_i\} = \{d \cdot i\}$, which obeys Müntz's theorem, the power series $\{p_i\} = \{C \cdot i^b\}$, and the exponential series $\{p_i\} = \{d \cdot m^i\}$. Although not obeying Müntz's theorem, the use of the last two series is physically justified, since series composed of steeply broadening Lorentzian lines have a general appearance resembling the function $(N(\infty) - N(k))$ which descends steeply with k. That the class of functions described by series $R(t)$ is consequently limited leads to stable wings of the spectral function $G(k)$. The selection of an optimal set $\{p_i\}$ involves two steps. First, we find $\{p_i\}$ for which $G(k)$ has the value of chi-square χ^2 close to 1.0:

$$\chi^2 = \frac{1}{2K_B} \sum_{k=-K_B}^{K_B} \{G(k) - (1 - N(k)/N(\infty))\}^2/G(k).$$

(The true spectral function satisfies the condition $\chi^2 = 1.0$ because the standard deviation of observation in the channel of interest is equal to the square root of $N(k)$.) The condition $\chi^2 = 1.0$ was satisfied by choosing a suitable number of terms in series (7). It is important that the involvement of exponential lines for which $p_i\Gamma > 2K_B\Delta\omega$ caused no change in χ^2. Moreover, with regard to $R(0)$, the parameter equal to the area under the model spectrum, the deviation of $R(0)$ from $\Psi(0)$ increases for series $\{p_i\}$ extended beyond the domain bounded to $2K_B\Delta\omega$ from above.

Among the series $\{p_i\}$ leading to spectra for which $\chi^2 = 1.0$, the optimal set was chosen from consideration of best smoothness of $R(t)$. In practice, this reduces to the minimization of the stabilizing functional [27]:

$$\Omega(\{R(t)\}) = \int_0^\infty (a(\mathrm{d}R/\mathrm{d}t)^2 + bR^2)\mathrm{d}t, \tag{8}$$

where a and b are constants.

For our case, the relation corresponding to (8) is

$$\Omega(\{R(t)\}) = \sum_{i,j=1}^{N} \frac{S_i \cdot S_j(1 + p_i p_j)}{p_i + p_j}.$$

As a result of the foregoing treatment, the exponential series $\{p_i\}$ below with radix 2.0 was obtained as the most suitable:

$$p_i = 1 + 2^{i-M}, \quad i = 1, \ldots, N, \ M = 4, \tag{9}$$

where N is the number of terms by which the series can be extended without loss of the condition $2^{N-M} < 2K_B \Delta\omega/\Gamma$.

After the model spectrum $G(k)$ has been constructed, i.e. the set $\{p_i\}$ and value of $N(\infty)$ specified, it is possible to find the error with which the function $\Psi(t)$ was found. To do this, a Gaussian noise with variance $\sigma^2 = G(k)$ is superposed upon the model spectrum, and a new function $R(t)$ is constructed. The scatter between the $R(t)$ functions derived is the statistical error sought. It should be emphasized that the resultant error does not arise from the method used. It depends on the noise-to-signal ratio of the experimental spectrum.

In the foregoing, it has been assumed that low-temperature spectra ($T < 150$ K) are Lorentzian. The determination of $\Psi(t)$ by the proposed procedure involves the determination of the width of low-temperature Lorentzian lines. In reality, the low-temperature spectra are not Lorentzian because of the effects of the equipment and other associated effects. At higher temperature, this may misrepresent information. The problem is solved by deriving $R(t, T_0)$ for one of the lower-temperature spectra ($T = T_0$). Then one has:

$$\Psi(t, T) = R(t, T) f'(T_0)/R(t, T_0). \tag{10}$$

If $f'(T_0)$ is known, $\Psi(t, T)$, when calculated by (10), is automatically normalized.

To illustrate how the proposed method works, we shall analyze the RSMR data for human serum albumin HSA [23]. Series $\{p_i\}$ was taken in the form of (9) for $N = 9$. The temporal course of correlator $\langle [\mathrm{d}X(t)]^2 \rangle$ for temperatures 270, 280, 310 K are shown in fig. 1 (lines 1, 2, 3, respectively).

To describe the experimental data theoretically, we used a model incorporating three types of motion: high-frequency motion with frequences $\omega \gg \Gamma$, local motion with frequencies $\omega \leqslant \Gamma$, and quasi-diffusion. The latter is similar to unrestricted diffusion on a time scale around 10^{-8} s. Local motion was considered on a time scale of correlation times of overdamped Brownian oscillators. The density distribution function for inversal correlation time ω_c of Brownian oscillators was displayed as:

$$\rho(\omega_c) = d \cdot \omega_c^{\alpha-1} \exp(-\beta\omega_c), \tag{11}$$

where ω_c is the reciprocal correlation time, d is the normalizing factor, and α, β are parameters of the model. Thus, the model correlator is composed of three terms representing three types of motion:

$$\langle [\mathrm{d}X(t)]^2 \rangle = X_h^2 + q^2\left(1 - \left(\frac{\beta}{t+\beta}\right)^{\alpha}\right) + 2 \cdot D \cdot t, \tag{12}$$

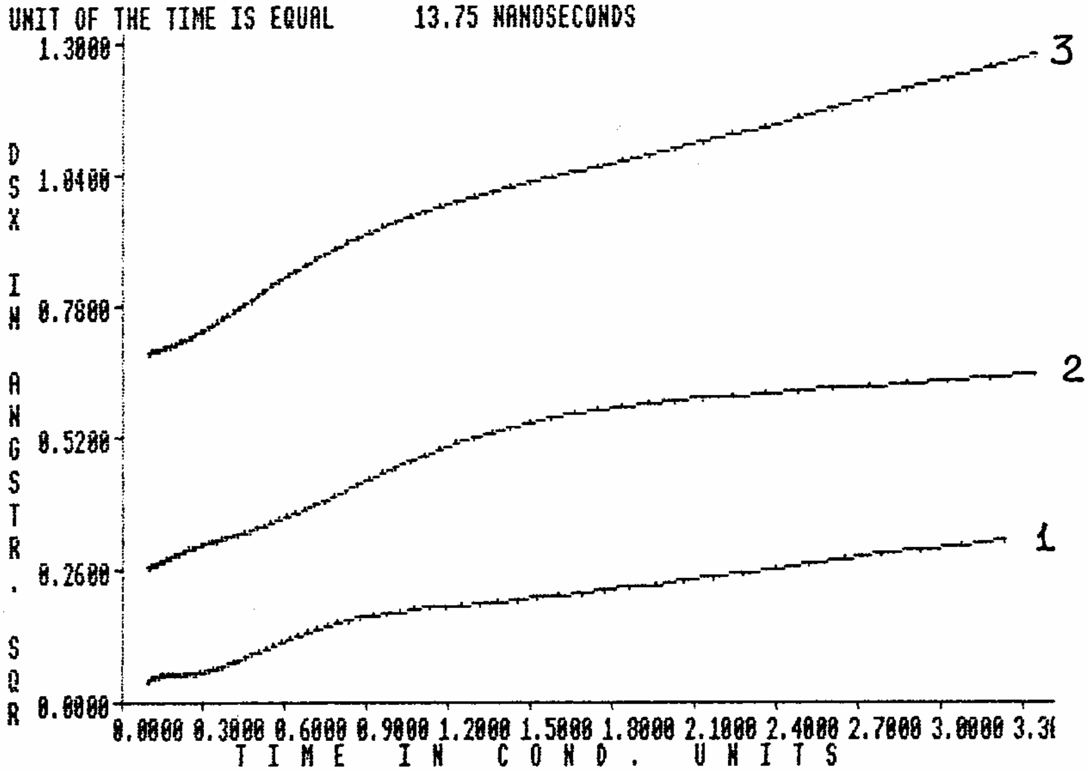

Fig. 1. The temporal dependencies of the correlation function $\langle [\mathrm{d}X(t)]^2 \rangle$ for coordinates of HSA atoms for T = 270, 280, 310 K (lines 1, 2, 3, respectively).

where X_h^2 is the total mean-square displacement due to high-frequency motions with $\omega > 10^9\ \mathrm{s}^{-1}$, q^2 is the total mean-square displacement due to all the oscillators in distribution (11), and $2 \cdot D \cdot t$ is the contribution due to the quasi-diffusion.

Distribution (11) was chosen for two reasons. First, we wanted a distribution with the minimum possible number of parameters that can model a large variety of "bell-shaped" distributions. Secondly, a distribution of this kind permits the physical interpretation in terms of the concept of a pencil of path for a conformational transition [30]. The domain of permissible values in the phase space of the five parameters of the model function (12) for each temperature can be defined by minimizing the deviation of the model function (12) from the experimental by a method of conjugated gradients. Within this domain, the variation of the parameter X_h^2 was found to fall within 20% around the mean. For T = 280 K, a pronounced minimum was found to exist. It corresponds to nearly zero D and to a narrow $\rho(\omega_\mathrm{c})$ distribution that resembles the δ-function, that is, the case of a single Brownian oscillator. Because at T = 280 K D is equal to zero, one may expect it to be zero at a lower temperature too (i.e. at T = 270 K). On this assumption, the $\rho(\omega_\mathrm{c})$ distribution was derived for T = 270 K and appeared to be broad. Probably, a distribution of this kind is a consequence of the microheterogeneity of the system in the melting temperature range for a water-protein system. Melting of bound water clusters that occur at temperatures within this range changes the protein dynamics [31]. The statistics for the RSMR spectrum for T = 310 K are insufficient to arrive at an unambiguous conclusion as to whether or not the distribution is broad. At this temperature, however, there is a vast phase space of the admissible values of the parameters, where there is a distinct minimum corresponding to a narrow $\rho(\omega_c)$ distribution (this may also be the case with a single Brownian oscillator) and to a relatively large diffusion coefficient. Assuming that for T = 280 K and 310 K spatial motion can be modelled by a single Brownian oscillator, we calculated the parameters D, X_a^2, and τ_c (X_a^2 is the mean-square displacement and τ_c is the correlation time of the Brownian oscillator) (table 1). The values of the parameters of model (12) for T = 270 K and D = 0.0 are represented in table 1.

Table 1

Result of processing experimental correlation function $\langle[\mathrm{d}X(t)]^2\rangle$ for human serum albumin for different temperatures (designation in text)

T (K)	α	β (nsec)	q^2 (Å^2)	X_h^2 (Å^2)	$D \times 10^{10}$ (cm^2/s)	τ_c (nsec)	X_a^2 (Å^2)
270	0.1	13.4	2.1	0.04	0.0	-	-
280	-	-	-	0.23	0.0	21.2	0.49
310	-	-	-	0.63	3.8	11.5	0.29

Conclusions

A method of Mössbauer Fourier spectroscopy has been developed to determine the correlation function of coordinates of a macromolecular system. The error in determining the correlation function is not higher than the noise-to-signal ratio, i.e. in the order of the product of amplitude and square root of the number of counts per channel. The method requires no a priori dynamic model. This may, in turn, facilitate the construction and analysis of dynamic models because of no necessity of calculating the integral of (1) (most frequently by numerical methods).

The application of the method to the analysis of RSMR data for human serum albumin (HSA) has demonstrated considerable changes in the dynamic behavior of the protein globule when the temperature is changed from 270 K to 310 K. At T = 270 K, the HSE dynamic behavior is characterized by a broad range of correlation times. The heterogeneity of the system within the melting temperature range for the water-protein system is most likely responsible for this. At $T > 280$ K, the dynamics of the globule may be interpreted, within the error of experiment, in terms of a single Brownian oscillator model and unrestricted quasi-diffusion.

With increasing temperature, the correlation time of the Brownian oscillator decreased and the diffusion coefficient increased from nearly zero at T = 280 K to

$3.8 \cdot 10^{-10}$ cm^2 s^{-1} at $T = 310$ K. For a limited time interval it is, unfortunately, impossible to conclusively clarify the notion of unrestricted quasi-diffusion to be able to distinguish between diffusion of the globule as a whole and local diffusion with large correlations times.

Noticeably, inherent in the system are motions with correlation times of $\tau \ll 10^{-9}$ s whose amplitudes increase with T, reaching $X_{\mathrm{h}}^2 = 0.63$ Å^2 at $T = 310$ K. Thus, the main conclusion of the present work, which is of fundamental significance, is the simultaneous observation of low-frequency ($\tau^{-1} \sim \Gamma$) and high-frequency ($\tau^{-1} \gg \Gamma$) large-scale motions, that is the two-humped distribution of correlation times of protein motions. As follows from [18], the quasi-diffusion observed in the preparation under study is most likely to be due to the local diffusion of protein fragments having relaxation times of $\tau \sim 10^{-7}$ s. Note in conclusion that the proposed method works best of all with spectra having a low noise-to-signal ratio.

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