We emphasize that for an exact measurement of α we must use a single laser pulse. Such measurements are presently underway. The methodological error in the measurement of α is determined as shown above and can amount to less than several percent, i.e., the main measurement error is determined by the instrumental errors.

An exact measurement of α for a given material can be of practical importance for the following reasons. Knowing the constant α, we can use (16) to determine the duration of a rectangular laser pulse (or estimate the duration if the pulse is close to rectangular):

$$\tau = \frac{Q/\alpha d}{T_e - T_0}. \qquad (17)$$

It is essential here to measure the maximum value of the electron temperature T_e and the total absorbed energy Q.

Thus, to estimate the pulse duration there is no need for direct measurements with the streak camera. The proposed method of measuring the duration of ultrashort pulses, while subject to the shortcomings inherent in indirect measurement methods (e.g., the impossibility of resolving a fine temporal structure), has the important advantage that it can determine the durations of ultrashort laser pulses in the near and far infrared.

4. CONCLUSION

Inertialess glow of metals makes it possible to observe the onset of hot electrons, and can serve as the basis of a method for studying the properties of metals and for the conversion of IR laser radiation into visible light. This conversion offers prospects of expanding the spectral range of modern photoelectric recorders. This phenomenon is presently under study for various metals under the action of a single picosecond laser pulse. The purpose of further research is to determine the exact range in which the phenomenon is inertialess, to measure the constant α of various metals, to search for means of further expanding the power and laser-pulse-duration ranges of the observed phenomenon. For practical applications, this phenomenon is being investigated for the study of the properties of metals and for the measurement of the temporal parameters of picosecond IR laser pulses.

The authors are deeply grateful to S. I. Anisimov for a helpful discussion of the results.

[1)]The inequality (6), which is not contained in Ref. 2, corresponds to the condition of weak heating of the lattice, i.e., $T_l - T_0 \ll T_0$. It is due to the dependence of the coefficient of electronic thermal conductivity and of the quantity $q(\mathbf{r},t)$ on the lattice temperature, which is by itself subject to inertia. We note also that the constant α, the absorption coefficient , and the reflection coefficient R are practically independent of time and correspond to their values at $T_e = T_i = T_0$, inasmuch as thanks to (6) we have $T_i \approx T_0$, while the electron heating leads to relative corrections $\sim (T_e/T_F)^2 \ll 1$ (T_F is the Fermi temperature).

[1]M. B. Agranat, A. A. Benditskiĭ, G. M. Gandel'man, A. G. Devyatkov, P. S. Kondratenko, B. I. Makshantsev, G. I. Rukman, and B. M. Stepanov, Pis'ma Zh. Eksp. Teor. Fiz. **30**, 182 (1979) [JETP Lett. **30**, 167 (1979)].
[2]S. I. Anisimov, B. L. Kopelovich, and T. L. Perel'man, Zh. Eksp. Teor. Fiz. **66**, 76 (1974) [Sov. Phys. JETP **39**, 375 (1974)].
[3]M. Born and E. Wolf, Principles of Optics, Pergamon, 1970.

Translated by J. G. Adashko

Determination of the mean square displacement of the atomic vibrations in myoglobin molecules by measuring Rayleigh scattering of the Mössbauer radiation

Yu. F. Krupyanskiĭ, F. Parak, J. Hannon, E. É. Gaubman, V. I. Gol'danskiĭ, I. P. Suzdalev, and K. Hermes

Institute of Chemical Physics, USSR Academy of Sciences
(Submitted 10 January 1980)
Zh. Eksp. Teor. Fiz. **79**, 63–68 (July 1980)

Rayleigh scattering of Mössbauer radiation (RSMR) is employed to determine the mean square displacement $\langle x^2 \rangle$ of the oscillations of atoms in myoglobin molecules in the crystal state. A procedure is described which has been developed to separate $\langle x^2 \rangle$ of the Mb molecules in a crystalline sample consisting of a mixture of buffer-solution molecules and myoglobin molecules. The experimental value of $\langle x^2 \rangle$ obtained is found to exceed considerably that found by x-ray analysis. It is concluded that determination of $\langle x^2 \rangle$ by the latter method is greatly affected by thermal diffusive scattering, which appreciably lowers the value compared to the true value as determined by the RSMR technique.

PACS numbers: 61.65. + d, 33.20.Fb

The investigation of the dynamic properties of proteins takes on great importance in connection with the strong influence of these properties on the functional activity of proteins.[1] One promising method for the study of protein dynamics is x-ray diffraction, which yields a map of the mean square displacements $\langle x^2 \rangle$ of the atoms in the molecules under study.[2,3] However, the time scale in this technique is $\tau_x \sim 10^{-15}$ sec, and consequently this method is insensitive to conformational transition frequencies. Static disorder in the arrangement of the molecules in the crystals gives the same contribution to $\langle x^2 \rangle$ as real motions, which may be rather slow ($\sim 10^7$–10^{10} Hz) for protein molecules. Moreover, with an energy resolution to several electron-volts in protein x-ray diffraction, it is difficult to separate elastic scattering from inelastic thermal dif-

 0038-5646/80/070031-04$02.40

fusive scattering; this may give inaccurate values for $\langle x^2\rangle$.

In a number of papers a new technique has been developed which has considerably greater energy resolution ($\sim 10^{-8}$–10^{-9} eV). We refer to the method of Rayleigh scattering of the Mössbauer radiation (RSMR), in which Mössbauer nuclei are used as the source of radiation and as the analyzer of the scattered radiation.[4-6] This method makes it possible to also describe slow motions, since the time scale here is the lifetime of the nuclear level τ_M; for example, τ_M for ^{57}Fe is equal to $1.4\cdot 10^{-7}$ sec. In the RSMR technique, only real atomic motions contribute to $\langle x^2\rangle$, and the high energy resolution makes it possible to easily separate the elastic and the inelastic scattering components.

However, the investigation of complex substances such as proteins requires that we take into account the contribution of various background components to $\langle x^2\rangle$, e.g., the contribution of the atomic vibrations of the buffer solution in which the protein molecules are in the native state. Accordingly, we undertook an investigation in which we worked out a system to determine $\langle x^2\rangle$ for a rather well-studied protein—myoglobin—in the polycrystalline state, and we compare the results with data from x-ray diffraction in order to clarify the influence of thermal diffusive scattering on the determination of $\langle x^2\rangle$.

PRINCIPLES OF TREATMENT OF RESULTS

The RSMR technique is described in Refs. 4–6. Let us recall that the fraction of elastically scattered γ quanta is determined by the following equation[4]:

$$f=\gamma\exp\{-Q^2\langle x^2\rangle\}, \tag{1}$$

where $\langle x^2\rangle$ is the mean square amplitude of the atomic vibrations for the scatterer, $Q=4\pi\sin\theta/\lambda$ is the scattering factor, the factor γ is equal to the ratio of the Rayleigh scattering intensity to the summed Rayleigh and Compton scattering intensity, 2θ is the scattering angle, λ is the wavelength of the Mössbauer γ quantum.

The myoglobin (Mb) crystal consists of myoglobin molecules arranged in an orderly fashion in a buffer solution of $(NH_4)_2SO_4$. Thus, Rayleigh scattering occurs both at the myoglobin molecules themselves and at the molecules of the buffer solution. In order to isolate the motion of the myoglobin molecule in a background of motion of buffer solution molecules from the experimental data, we resorted to the following procedure. It is known that Bragg peaks occur very close to one another in crystalline myoglobin (30′ separation).[7] Under our experimental conditions, when the receiving angle of the detector is equal to ~5°, about 100 Bragg peaks occur within the limits of receiving angle. In this case, with a good degree of accuracy, we may consider the elastic Rayleigh scattering to be incoherent. Then the intensity of the Rayleigh scattered γ quanta is equal to the sum of the intensities of the γ quanta scattered from the myoglobin molecule and the γ quanta scattered from the buffer solution molecules:

$$P_\Sigma=P_{Mb}+P_B. \tag{2}$$

The parameter determined in the experiment (the fraction of elastic scattering) is

$$f=P_{\Sigma,el}/(P_{\Sigma,tot}+I_C), \tag{3}$$

where $P_{\Sigma,el}$ is the elastic part of the Rayleigh scattering intensity, $P_{\Sigma,tot}$ is the Rayleigh scattering intensity (including the inelastic component), and I_C is the Compton scattering intensity.

Eq. (3) may be rewritten in the following form:

$$f=\frac{xf_{0M}^2\exp(-2W_M)+(1-x)f_{0B}^2\exp(-2W_B)}{xf_{0M}^2+(1-x)f_{0B}^2+xF_M+(1-x)F_B}, \tag{4}$$

where x is the fraction of myoglobin molecules relative to the total number of molecules contained in the scatterer; f_{0M}^2, f_{0B}^2, F_M, F_B are the Rayleigh and Compton scattering intensities from molecules of myoglobin and the buffer solution, which may be expressed in terms of the corresponding atomic form factors and the Compton scattering intensities at the atoms for the given molecules; $\exp(-2W_i)$ are the Debye-Waller factors for myoglobin ($i=M$) and the buffer-solution ($i=B$) molecules.

Since we may independently measure the fraction of elastic scattering in the buffer solution, Eq. (4) may be transformed to the following form:

$$\exp(-2W_M)=C_1f_\Sigma-C_2f_B, \tag{5}$$

where C_1 and C_2 are coefficients which are respectively equal to

$$\begin{gathered}C_1=1+\gamma_M'+kF_{BM}\frac{\gamma_M'}{\gamma_B'}(1+\gamma_B'),\quad C_2=kF_{BM}\frac{\gamma_M'}{\gamma_B'}(1+\gamma_B'),\\ \gamma_i'=(1-\gamma_i)/\gamma_i,\quad i=B,\ M,\quad k=(1-x)/x,\quad F_{BM}=F_B/F_M;\end{gathered} \tag{6}$$

C_1 and C_2 may be calculated on the basis of data in Ref. 8 for a known concentration x; f_Σ and f_B are experimentally measured values.

Thus, using the described procedure of two measurements on crystalline myoglobin and the buffer solution, we may obtain for the dynamics of Mb molecules in the crystalline state information impossible to obtain directly from experiment.

EXPERIMENTAL TECHNIQUE

The scattering geometry in our work is essentially the same as that used earlier.[4-6] We used ^{57}Co in Rd with an active area of 2 mm diameter and 120 mCi activity as the source, and a krypton proportional counter as the detector for the scattered radiation. In order to determine the fraction of elastic scattering, we used a "black absorber," $Li_3FeF_6+(NH_4)F_3(FeF_3)_2$, containing 7.0 mg/cm^2 of ^{57}Fe. The RSMR spectra were analyzed with the aid of the compound $K_4^{57}Fe(CN)_6\cdot 3H_2O$, containing 2.0 mg/cm^2 ^{57}Fe.

The scattering geometry was optimized in order to decrease the relative error in the determination of the mean square vibrational amplitude. The scattering angle was accordingly chosen to be $2\theta=14.5°$. The receiving angle, determined by the collimating system, was $\pm 2.5°$.

One of the greatest sources of error in the analysis of

the experimental results is the averaging of the data over the receiving angle. One possible variant is to determine the average scattering angle $\langle 2\theta \rangle$.[9] Then $\langle x^2 \rangle$ should be determined from the following expression:

$$f=\gamma(\bar{\theta})\exp\{-(4\pi\sin\bar{\theta}/\lambda)^2\langle x^2\rangle\}. \quad (7)$$

It is more correct to directly average the fraction of elastic scattering over the receiving angle. In this case, $\langle x^2 \rangle$ will be determined from an expression such as:

$$f=\sum\frac{S(\theta_i)}{S}\gamma(\theta_i)\exp\left\{-\left(\frac{4\pi\sin\theta_i}{\lambda}\right)^2\langle x^2\rangle\right\}, \quad (8)$$

where $S(\theta_i)$ is the fraction of the area of the detector with a specified value of the scattering angle θ_i; consequently, $\sum S(\theta_i)=S$, where S is the receiving area for the radiation.

We carried out both of these procedures, and in both cases the values of $\langle x^2 \rangle$ turned out to be the same. This indicates that determination of the average scattering angle $\langle 2\theta \rangle$ is an entirely proper procedure. In our case, $\langle 2\theta \rangle = 15.3 \pm 1.5°$.

The concentration of myoglobin in the Mb crystal was determined spectrophotometrically and proved to be equal to 46%.

EXPERIMENTAL RESULTS AND DISCUSSION

We measured the fraction of elastic scattering for four substances at room temperature and present the results in Table I. Since the temperature dependence $f(T)$ was not measured, we took the calculated value for γ. Thus, for example, for an Mb molecule containing 823 C atoms, 214 N atoms, 221 O atoms, 1268 H atoms, 2 S atoms and one Fe atom, γ_M was determined from the following expression:

$$\frac{\gamma_M}{1-\gamma_M}=\frac{823f_C^2+214f_N^2+221f_O^2+1268f_H^2+2f_S^2+f_{Fe}^2}{823F_C+214F_N+221F_O+1268F_H+2F_S+F_{Fe}},$$

and proved to be $\gamma_M = 0.93$. Since crystalline Mb is a mixture of Mb molecules and buffer-solution molecules, we do not give a value for γ in the table for this case.

Using Eqs. (2)–(5) and selecting data from Table I for f_C and f_B, we determined the mean square displacement of the atoms of the Mb molecule in the crystalline state at room temperature by the RSMR method: 0.22 ± 0.02 Å^2. The mean square vibrational amplitude for atoms of the Mb molecules, as determined by x-ray diffraction, has the value 0.108 ± 0.003 Å^2.

Using the data in Table I, it is easy to show that the assumption that hydration shells of water with the viscosity of glycerine surround the Mb molecules in the crystal[1] does not affect the final determination of $\langle x^2 \rangle$ by the RSMR method. The values of γ_M were also varied, assuming in the calculation that the carbon atoms occur in the C^+ states, the nitrogen in the N^+ and N^- states, and the oxygen in the O^+ and O^- states. The change in γ_M in this case proved to be insignificant, and $\langle x^2 \rangle$ remained within the limits of measurement error.

TABLE I. Values of the fraction of elastic scattering at room temperature and of the parameter γ for the substances measured.

Substance	f	γ
Water	0.014±0.001	0.96
Buffer solution	0.014±0.001	0.96
37% glycerine	0.017±0.001	0.98
Mb crystal	0.159±0.008	–

There is an obvious discrepancy between the values of $\langle x^2 \rangle$ determined by the RSMR method and by x-ray diffraction. We measured the RSMR spectra to test whether the difference in the time scales τ_x and τ_M (which may affect the line broadening) affects the determination of $\langle x^2 \rangle$ with RSMR. Line broadening was not noticeable. The reason for such a large discrepancy may be the influence of the thermal diffusive scattering (TDS) on the value of $\langle x^2 \rangle$ obtained from x-ray diffraction. Indeed, in x-ray diffraction, in contrast to RSMR, thermal diffusive scattering is detected in the Bragg reflection. For biological crystals, the Bragg peaks are situated very close to one another (30′ separation). As a consequence of the extreme closeness of the Bragg peaks, the entire one-phonon scattering lies in a very narrow angular interval relative to the Bragg peak, since all the phonon vectors are contained in the first Brillouin zone. Consequently, even high-resolution x-ray diffraction will detect a large part of the TDS. The situation will be similar with multiphonon processes, and also will lead to detection of a large part of the TDS. A slight divergence in the primary beam also leads to detection of a large fraction of the TDS. Despite the fact that the TDS intensity may be rather low, even in this case TDS will give a large error in the determination of $\langle x^2 \rangle$ in x-ray diffraction.

Let us write the intensity of the scattered x-ray beam detected in the Bragg reflection as follows:

$$P=P_{coh,\,el}+P_{coh,\,inel}. \quad (9)$$

Here the intensity of coherent elastic scattering is equal to[10]

$$P_{coh,\,el}\approx A_Q^2\exp(-Q^2\langle x^2\rangle), \quad (10)$$

where A_Q is the scattering amplitude for the Bragg reflection in the Q direction, and the intensity of the coherent inelastic scattering is given approximately by the following expression:

$$P_{coh,\,inel}\approx A_Q^2\exp(-Q^2\langle x^2\rangle)Q^2\langle x^2\rangle f_\Omega, \quad (11)$$

where f_Ω is approximately equal to the fraction of one-phonon scattered x-ray beams detected in the experiment; f_Ω depends on the structure of the detector and on the experimental apparatus.

We estimate $f_\Omega \approx (d/\lambda)^3 d\Omega\delta\Phi$, where d characterizes the unit cell of the crystal, λ is the wavelength of the radiation, $d\Omega$ is the receiving angle of the detector, and $\delta\Phi$ is the initial divergence. For the case of an ordinary crystal with a small unit cell, $d \leq 10$ Å, $f_\Omega \ll 1$. For a crystal comprised of large biomolecules ($d \approx 50$–100 Å), with quite realistic assumptions concerning the divergence of the primary beam and the

angle of resolution of the detector, $f_\Omega \lesssim 1$. In this case we should point out that in an experiment carried out on silicon crystals,[11] the TDS intensity may amount to up to 40% of the overall scattering intensity in the Bragg reflection. Judging from the estimate given above, we can only expect this fraction to increase for protein crystals.

TDS may significantly influence the determination of $\langle x^2 \rangle$, if $f_\Omega \sim 1$. In fact, $\langle x^2 \rangle$ is extracted from the experimental data by determining the dependence of $\log P$ on Q^2. From Eqs. (9)–(11) we obtain

$$\log P = -(1-f_\Omega)\langle x^2 \rangle Q^2 + \text{const}, \qquad (12)$$

if $Q^2\langle x^2 \rangle \ll 1$ (for the first Bragg peak, $Q^2\langle x^2 \rangle \sim 10^{-3}$), so that the experimental slope proves to be $-(1-f_\Omega)\langle x^2 \rangle$, and not $-\langle x^2 \rangle$.

Thus, for a crystal comprised of large biomolecules, f_Ω may be ~1; and consequently the experimentally determined $\langle x^2 \rangle$ in x-ray diffraction will be significantly less than the true value.

In the case of RSMR, TDS is easily separated from the elastic scattering by the Mössbauer detector, and consequently the determination of $\langle x^2 \rangle$ by this technique should be more accurate.

CONCLUSION

Thus, in our work we determined the amplitude of the mean square atomic displacement $\langle x^2 \rangle$ for myoglobin in the crystalline state by the technique of measuring the Rayleigh scattering of the Mössbauer radiation (RSMR). We have taken the RSMR spectra and determined the fraction of elastic scattering for crystalline myoglobin, the buffer, glycerine, and water. We developed a technique to separate $\langle x^2 \rangle$ for the Mb molecules from a crystalline sample comprised of molecules of buffer solution and myoglobin. The value of $\langle x^2 \rangle$ experimentally measured using RSMR proved to be significantly greater than the $\langle x^2 \rangle$ measured by x-ray diffraction. We show that in the case of crystals comprised of large biomolecules, thermal diffusive scattering (TDS) strongly affects the value of $\langle x^2 \rangle$ experimentally determined from x-ray diffraction, leading to a significant under-estimation of these values compared with the true ones determined by RSMR.

In conclusion, the authors express their thanks to V. A. Namiot for useful discussions.

The collaboration of different institutes was supported by the Academy of Sciences of the USSR and the German Scientific Research Society.

[1]Ravnovesnaya dinamika nativnoĭ struktury belka (Equilibrium Dynamics of the Native Protein Structure), Pushchino (1977).
[2]H. Frauenfelder, G. A. Petsko, and D. Tsernoglou, Nature **280**, 558 (1979).
[3]P. J. Artymiuk, C. C. F. Blake, D. E. P. Grace, S. J. Oatley, D. C. Phillips, and M. J. E. Sternberg, Nature **280**, 563 (1979).
[4]D. C. Champeney and G. W. D. Woodhams, J. Phys. B **1**, 620 (1968).
[5]D. C. Champeney and G. W. Dean, J. Phys. C **8**, 1276 (1975).
[6]E. É. Gaubman, Yu. F. Krupyanskiĭ, V. I. Gol'danskiĭ, T. V. Zhuravleva, and I. P. Suzdalev, Zh. Éksp. Teor. Fiz. **72**, 2172 (1977). [Soviet Physics JETP **45**, 1141 (1977)].
[7]F. Parak and H. Formanek, Acta Cryst., Sect. A **27**, 573 (1971).
[8]International Table for X-Ray Crystallography, Kynoch, London (1962).
[9]D. C. Champeney and D. F. Sedgwick, J. Phys. C **5**, 1903 (1972).
[10]C. Kittel, Introduction to Solid State Physics, Wiley, New York, 1971. (Russ. Transl., Nauka, 1978, p. 76).
[11]G. Albanese, C. Ghezzi, A. Merlini, and S. Pace, Phys. Rev. B **5**, 1746 (1972).

Translated by Cathy Flick

Nonresonant many-photon ionization of atoms in a strong stochastic field

V. P. Kraĭnov and S. S. Todirashku[1)]

Moscow Institute of Engineering Physics
(Submitted 23 January 1980)
Zh. Eksp. Teor. Fiz. **79**, 69–74 (July 1980)

The probability of nonresonant ionization is calculated for the case of atoms in a strong stochastic low-frequency electromagnetic field. The calculations are made with accuracy up to the coefficient of an exponential function. It is found that, as in the case of a monochromatic field, the probability of ionization by a stochastic field is determined by a single parameter; it is called the stochastic adiabatic parameter. The well-known limiting cases of a stepwise many-photon process and of tunnel ionization are discussed. A general expression is derived for the statistical factor which characterizes the ratio of the ionization probabilities in stochastic and monochromatic fields with the same radiation intensity. The known experimental results agree well with the calculations.

PACS numbers: 32.80.Fb, 32.80.Kf

The study of the features of the process of ionization of atoms by a field of stochastic radiation is a subject of present importance in theoretical and experimental spectroscopy. After the fundamental work of Keldysh,[1] who investigated the basic laws of the ionization of quantum systems by a strong coherent electromagnetic field, the development of powerful lasers made it possible to study this process experimentally for specific atomic systems (see, for example, Refs. 2 and 3 and the literature they cite). However, since present-

 0038-5646/80/070034-04$02.40