BRIEF COMMUNICATION

AN APPROACH FOR TIME-RESOLVED X-RAY SCATTERING

J. STAMATOFF, *Bell Laboratories, Murray Hill, New Jersey 07974*

ABSTRACT Recent biological optical spectroscopic studies have correlated discrete spectroscopic states with biological function in several systems. One of the challenges of molecular biophysics is to correlate structural changes with these spectroscopic states. From small-angle x-ray scattering one can obtain a key structural parameter, the radius of gyration of solubilized proteins. The method described in this paper would permit determination small changes in the radius using polychromatic synchrotron radiation. The high flux of the storage ring combined with an enhancement factor of $\sim 10^4$, obtained by removing the requirement for monochromatic radiation, will permit determining the radius on a millisecond time scale. Unlike energy-dispersive methods, this method would use all available energies over a wide range of angles.

Recently there have been many advances in correlating optical spectroscopic states with biological function (e.g., Lozier et al., 1975). one of the major challenges of molecular biophysics is to correlate structural changes with these spectroscopic states and, thus, with function.

Many systems show spectroscopic changes on a millisecond time scale. With the advent of synchrotron radiation one might consider x-ray scattering as a method of observing structural changes. Classical small-angle scattering (e.g., Sardet et al., 1976) can provide a great wealth of information including the radius of gyration which, in general, will be responsive to structural changes. However, even with such powerful sources as synchrotrons, the usual method of measuring the intensity as a function of angle for one energy proves to be too slow. The use of position-sensitive detectors has shortened the time required but not sufficiently.

A novel method of recording diffraction at one angle and a range of energies has been developed by Bordas et al., (1976). In this method the time scale is reduced but is limited due to saturation of the energy-dispersive detectors that are used. It is not readily apparent why collecting data at many energies and one angle should be intrinsically faster than collecting at several angles and one energy.

In a recent article, Stuhrmann (1978) suggests using polychromatic synchrotron radiation to provide rapid measurement of the radius. He points out that the intensity at zero angle is unaltered by the use of polychromatic radiation (i.e., it depends on the same parameters as monochromatic radiation). Further he shows that the measured radius is changed by only a small amount.

In this paper it is shown that exact measurement of the radius of gyration can be made. More importantly, small changes in the radius can be determined using polychromatic radiation. As previously stated, elimination of the monochromator will permit such determinations to be made with millisecond time resolution (Stuhrmann, 1978). As a result it may be possible to correlate changes in the radius with spectroscopic states and, thus, with function.

The following parameters are defined: t, time; θ, angle (2θ is the scattering angle); E, energy of the x rays; λ, wavelength of the x rays; f_k, atomic scattering factor of the K^{th} atom.

For a dilute homogeneous solution of M molecules, the recorded intensity is given by $I(\theta, t, E) = MI_m(\theta, t, E)$, where I_m is the scattering due to one molecule. Following Guinier (1939), $I_m \simeq n^2 \exp(-k^2R^2/3)$ where $k \equiv 4\pi \sin\theta/\lambda$, $n \equiv \sum_k f_k$ (the sum is over all atoms in the molecule), and $R^2 \equiv \sum_k f_k r_k^2 / \sum_k f_k$ (R is the radius of gyration). The origin within each molecule is chosen such that $\sum_k f_k \mathbf{r}_k = 0$. We are ignoring important effects due to the solvent's electron density. A detailed examination of solvent density-induced changes has been discussed by Luzzati et al. (1976). Thus for small angles I may be written as $I(\theta, t, E) \simeq Mn^2 \exp(-\alpha^2\theta^2E^2R^2(t)/3)$ where R is time dependent and $\alpha = 4\pi/hc$ (h and c are Planck's constant and the speed of light).

For a synchrotron with a spectral distribution $P(E)$, the intensity recorded for polychromatic incident radiation is given by $I(\theta, t) = \int_{E_0}^{E_1} I(\theta, t, E)P(E)dE$, where E_0 is the minimum and E_1 is the maximum energy. Expanding the exponential we have $I(\theta, t) = Mn^2 \int_{E_0}^{E_1} P(E)dE(1 - \alpha^2\theta^2\overline{E^2}R^2(t)/3)$, where $\overline{E^2} = \int_{E_0}^{E_1} E^2P(E)dE / (\int_{E_0}^{E_1} P(E)dE)$.

A plot of $I(\theta, t)$ vs. θ^2 will have an intercept at zero angle of $A = Mn^2 \int_{E_0}^{E_1} P(E)dE$ and a slope of $B = [-A\alpha^2/3]R^2(t)\overline{E^2}$. Standardization of the experiment with a sample of known radius would permit determination of the unknown experimental parameters. Thus exact determination of R is possible.

For fast biological reactions, one would like to record changes in $R(t)$. Following Simon (1971, 1972), consider the ratio of two patterns recorded at different times:

$$\frac{I(\theta, t_1)}{I(\theta, t_2)} \simeq \frac{\left(1 - \frac{\alpha^2\theta^2}{3}\overline{E^2}R^2(t_1) + \ldots\right)}{\left(1 - \frac{\alpha^2\theta^2}{3}\overline{E^2}R^2(t_2) + \ldots\right)}.$$

Expanding the denominator and neglecting terms of fourth order and higher, it follows that

$$\begin{aligned} I(\theta, t_1)/I(\theta, t_2) &\simeq 1 + \{(\alpha^2\theta^2/3)\overline{E^2}[R^2(t_2) - R^2(t_1)]\} \\ &\simeq 1 + \{(2\alpha^2\theta^2/3)\overline{E^2}[R(t_1) - \Delta R(t)]\} \end{aligned}$$

Comparing with previous results $I(\theta, t_1)/I(\theta, t_2) \simeq 1 - 2\theta^2(B/A)[\Delta R(t)/R(t_1)]$.

Therefore relative changes in radius may be precisely determined without the use of a standard.

The above equations are correct to second order in h(or θ). The exponential approximation developed by Guinier (1939) is also correct to second order. For higher orders in h, both expressions deviate from the exact form.

It is important to note that monitoring the incident polychromatic flux will eliminate time fluctuations in the incident beam. Thus the slope of the curve, $I(\theta, t_1)/I(\theta, t_2)$ vs. θ^2, will not be changed by small scaling errors. In addition, this method should eliminate many systematic errors in a fashion similar to the substitution method (Chonacky and Beeman, 1969). Recent measurements have shown that the ratio method provides ΔR accuracies of <0.2 Å in 4×10^4 s using 0.5 mM cytochrome *c*, an incident intensity of $\sim 10^7$ photons/s, and a linear position-sensitive detector.[1] The enhancement factors due to the use of a storage ring, polychromatic radiation, and larger molecules shorten the required time to ~ 4 ms for the same accuracy. The use of area x-ray detectors (e.g., Reynolds et al., 1978) further shortens the required time to the submillisecond time scale.

In summary, it seems possible with available synchrotron sources and integrating nonenergy sensitive area detectors, to measure small changes in the radius of gyration on a millisecond time scale. This may permit correlation of structural changes with function.

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