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Spectrographic Representation of Globular Protein Breathing Motions

Abstract. *Two useful ways of describing the frequency composition of the breathing motions of globular proteins are the spectrogram and three-dimensional power spectrum, representations similar to those frequently used in speech analysis. In this report "low-frequency" vibrations of globular proteins, corresponding to the collective oscillations of atoms from many different residues, are considered. Radii of gyration fluctuations provide a sensitive way to characterize such concerted motions.*

A complete description of a globular protein requires not only a static three-dimensional x-ray structure but also an understanding of its flexibility and the role that structural fluctuations play in the protein's function. Globular proteins in solution exhibit a large variety of motions (*1, 2*). Evidence from a number of different experiments suggests that a globular protein is a fluid, dynamic structure involving rapid conformational fluctuations that allow relatively easy access of interior groups to various molecular probes (*3*). It has been hypothesized that the motions of globular proteins play an essential role in their function and may affect important processes such as binding of ligands, enzyme catalysis, hemoglobin cooperativity, immunoglobin action, electron transfer, and the assembly of supramolecular structures like viruses (*2, 4*).

This report addresses the "low-frequency" vibrations of globular proteins (*2, 5, 6*) that correspond to the collective oscillations of atoms from many different residues. In previous studies these breathing motions were investigated by representing the globular protein as a vibrating sphere of homogeneous elastic material (*5, 7*). In the research described here, protein breathing was investigated by determining the radius of gyration of 650 consecutive 0.147-psec atomic coordinate frames from the dynamic model of bovine pancreatic trypsin inhibitor (BPTI). The fluctuations in the equilibrium structure of BPTI have been examined in several molecular dynamics simulations (*4, 8, 9*). BPTI, a small globular protein (molecular weight, 6500), consists of one polypeptide chain with 58 amino acids and three disulfide bonds. The radius of gyration of BPTI, derived from atomic coordinates, was calculated from the relation

$$R_g = \sqrt{\frac{\Sigma Z_i a_i^2}{\Sigma Z_i}}$$

where Z_i is the atomic number for atom i increased by the number of attached hydrogens, the sums are over all atoms; R_g is the radius of gyration calculated about the electronic center of gravity for BPTI's dynamic structure; and a_i is the distance of atom i from the electronic center of gravity.

A useful way of describing the frequency composition of the R_g values through time is the three-dimensional power spectrum and spectrogram. In 1947 the search for an effective way to display and examine speech wave forms in natural speech led to the development of the sound spectrograph at Bell Laboratories. This spectrograph, which is in common use today, displays a trivariate representation of speech energy with abscissal time and ordinal frequency; relative intensity is indicated by darkness on the graph. In the present research a system for the computation of digital spectrograms and topographic spectral distribution functions of protein breathing motions was developed (*10*). In the topographic power spectrum (amplitude versus frequency versus time) shown in this report, hillocks are indicative of prominent periodicities in the concerted radial motions within the protein.

A three-dimensional power spectrum computed for the R_g fluctuations (Fig. 1) indicates most of the frequency power below 1 psec with a particularly prominent breathing mode centered at 3 psec. Higher frequencies (25 to 113 cm^{-1}) are evident to a lesser degree. The high ridge close to zero frequency may correspond to a slower radial oscillation or an "infrequent process" (*1*) that is not being observed long enough for adequate characterization in this conventional molecular dynamics simulation. Longer simulations would be required to determine the significance of such a slower oscillation.

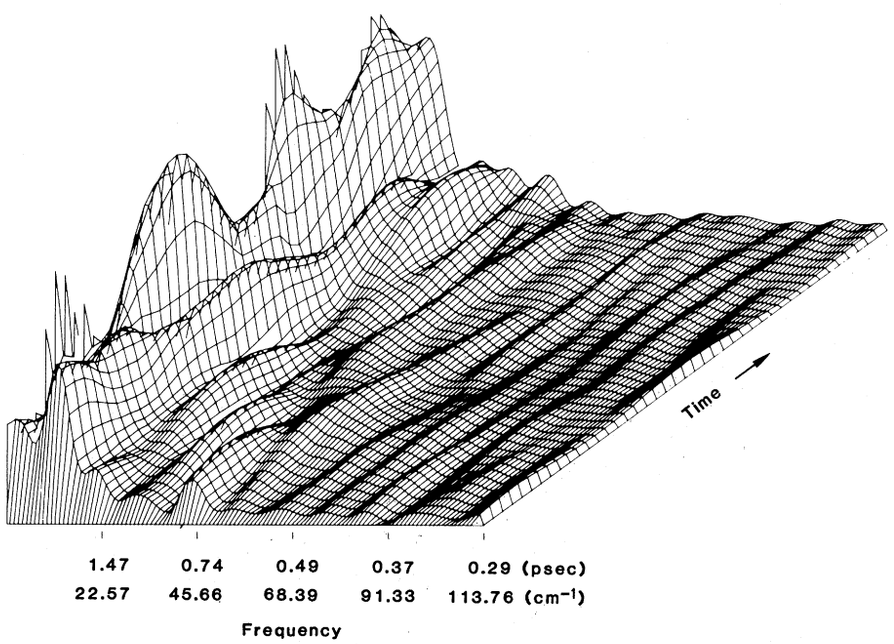

Fig. 1. Topographic spectral distribution function (power spectrum) calculated for the fluctuations in the radius of gyration of BPTI through time (96 psec of data are represented).

Similar low-frequency values have been obtained by less sophisticated models of protein breathing motions. A globular protein possesses a number of vibrational motions that may be represented by a vibrating homogeneous elastic sphere. Suezaki and Go (*7*) calculated the fundamental vibration of such a sphere, assuming a radius of 20 Å, a density of 1 g/cm^3, and a Young's modulus of 10^{11} dyne/cm^2. For this simple elastic sphere the frequency of vibration for the radial pulsating mode was calculated to be 26 cm^{-1} (1.2 psec). deGennes and Papoular (*11*) used a spherical Bessel function of order 1 in analyzing the radial pulsations of a vibrating elastic material and reported similar values, varying with certain assumptions of the protein's elasticity (1.9, 1.3, and 0.44 psec; 18, 26, and 76 cm^{-1}). Longitudinal acoustic modes found in chain molecules with lengths comparable to that of BPTI also have frequencies on the picosecond time scale (*6*).

There is experimental evidence for the existence of such low-frequency breathing vibrations in other proteins. Strong Raman-active modes have been found at 29 cm^{-1} in films and crystals of native α-chymotrypsin (*12*). The 29 cm^{-1} mode in α-chymotrypsin was assigned to an elastic breathing mode of this globular protein, and the mode disappears when the protein is denatured. A 25 cm^{-1} mode in lysozyme is also probably due to an internal mode of the lysozyme molecule (*6*).

Since both the spectrogram and three-dimensional power spectrum present breathing motion data in a way that is easily understood by the biophysicist, characterization of the dynamic richness of proteins is greatly facilitated. The interactive nature of the research station allows rapid generation of these functions with a variety of input data scaling and windowing parameters. From this and other studies it is clear that a compact, rigid view of globular proteins is incomplete. Aside from the relatively fast processes, including collisions between neighboring atoms and localized group vibrations, proteins may undergo somewhat regular low-frequency breathing motions of varying complexity. These motions involve the collective movement of many different atoms. The functional importance of such breathing modes, and protein motion in general, has begun to attract the interest of an increasing number of physical chemists, as evidenced by proliferating spectroscopic, kinetic, and theoretical studies of protein dynamics (*1, 2*). It is hoped that these spectrographic methods will provide a useful tool for future investigation into the breathing motions of globular proteins and other internal motions of biomolecules.

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10. The user console consists of a vector graphics display (Tektronix 618) and a standard cathode-ray tube terminal (IBM 3277 GA). The user-written support software is implemented in PL/I. The Graphics Compatibility System (U.S. Army Engineer Waterways Experiment Station, 803F3-R0200), a package of general-purpose, device-independent computer graphics routines, is used to produce the plots. In both the three-dimensional power spectrum and the spectrogram, Hamming cosine tapers are first applied to each window of data before fast Fourier transformation. In the three-dimensional plot, a far-infrared filter is used to smooth the output display data. A system for converting the breathing motion data to sound has also been developed, enabling the biophysicist to "listen" to the R_g fluctuations.
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Microbial Transformation of Sulfate in Forest Soils

Abstract. *Incubation of forest soils containing sulfate labeled with sulfur-35 showed rapid conversion of the added sulfate to organic sulfur forms by microbial populations. Activity rates were highest in the forest floor, but significant activity was observed throughout the soil profile. The annual potential sulfur incorporation for forest floor and soil combined is estimated to be 30 kilograms per hectare. The metabolism of inorganic sulfate to organic forms can be a major process in the sulfur cycle, influencing sulfate accumulation and mobility in forest ecosystems.*

Sulfate has been identified as a major constituent of acid precipitation in several regions of the United States (*1, 2*). However, the fate of exogenous sulfate in forest ecosystems is not well known. Studies have shown that soil adsorption of sulfate can be an important component of the sulfur cycle for some forests (*3, 4*). In agreement with results obtained by Freney *et al.* (*5*) for agricultural soils, our research has suggested that the metabolism of sulfate to organic sulfur forms may also act as a major pathway in the sulfur cycle of forest ecosystems (*6*). In this report we present quantitative evidence substantiating the importance of biological transformations of sulfate in these ecosystems.

The study site is located at the Coweeta Hydrologic Laboratory in western North Carolina. The U.S. Department of Agriculture Forest Service facility is a site of long-term hydrologic and ecological research with collaboration between university and government scientists. During the past 14 years, the program has emphasized research on biogeochemical processes important in forest ecosystems, with the gaged watershed used as the unit of ecosystem investigation. On the basis of 80 gage-years (eight gages in operation for 10 years) of bulk precipitation chemistry measurements taken over the 2185-ha basin, sulfate makes up 68 percent of precipitation anions and the average annual *p*H of bulk precipitation is 4.45. During the period 1973 to 1982, the mean annual input of sulfate-sulfur in bulk precipitation was 10.0 kg ha^{-1} year^{-1} and export in streamflow for mixed hardwood-cover watersheds was 1.5 kg ha^{-1} year^{-1}; thus there was a large apparent accumulation of sulfate-sulfur of 8.5 kg ha^{-1} year^{-1}.

Our preliminary research with surface soil from four Coweeta watersheds indicated that exogenous $^{35}SO_4^{2-}$ was rapidly converted into nonsalt-extractable ester sulfate and carbon-bonded sulfur (*6*). More intensive sampling was initiated on watershed 18 in May 1981 to quantitatively assess the importance of transformations relative to sulfate accumulation within a watershed. Watershed 18 is a 12.5-ha north-facing catchment that has been a primary site of long-term research on nutrient cycling. Overstory vegetation is dominated by *Quercus*, *Carya*, and *Acer* species with *Rhododendron* and *Kalmia* in the understory. Several soil types occur over the watershed, but the dominant soil is a sandy loam Ashe, a