COLLAGEN

An Inelastic Neutron-Scattering Study of Low-Frequency Vibrational Modes

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INTRODUCTION

A number of previous vibrational spectroscopic studies have focused on the structural aspects of collagen to elucidate the relationship between its molecular structure and function (see Yannas, 1972; Doyle et al., 1975; Diem et al, 1984; Lararev et al., 1985). Recently, progress has been made in calculational treatments of the vibrational properties of complex biological molecules (Tasumi et al., 1982; Tidor et al., 1983). The lower-lying vibrational frequencies are important constraints on the calculation studies of these systems, and inelastic neutron scattering (Berney and Yip, 1980) is a technique well suited for observing them. Very low frequency modes of proteins, which involve a collective motion of a large number of atoms, provide valuable information on molecular dynamics of proteins in the solid phase (Peticolas, 1979). Low-frequency modes of α-chymotrypsin (Brown et al., 1972) and lysozyme (Genzel et al., 1976) have been reported in the literature, and in the case of lysozyme an attempt was made to relate the molecular dynamics with its enzymatic activity (McCammon et al., 1976). More recently, a molecular dynamics study of bovine pancreatic trypsin inhibitor (BPTI) was undertaken by the Karplus group (McCammon et al., 1977), which has provided low-frequency modes for this protein. Longitudinal acoustical modes (LAM) of collagen were measured using laser Brillouin scattering (Harney et al., 1977). A low-frequency Raman study of collagen in the region 200–600 cm^{-1} was recently reported (Renugopalakrishnan et al., 1985). Unfortunately, the structural complexity of collagen makes it a difficult subject for molecular dynamic calculations; its triple helical structure consists of three chains of heterogeneous composition. Phonon dispersion curves have been calculated for helical polyglycine II (Small et al., 1970) and an incoherent inelastic neutron-scattering study of polyglycine I and II has been reported by Gupta et al. (1968). In this report, we present the results of an inelastic neutron-scattering study of collagen at ambient and low temperatures.

MATERIALS AND METHODS

Calf collagen (Type I) was obtained from Sigma Chemical Co. (St. Louis, MO) and used as received. The neutron-scattering spectrum was obtained on the crystal analyzer spectrometer (CAS) of the Intense Pulsed Neutron Source (IPNS) facility at Argonne National Laboratory (this instrument is similar to the one described in Chen et al., 1978). Approximately 1 g of collagen was placed in an aluminum cell which was then placed in a cryogenic refrigerator serving as the sample cell of the CAS. Data were accumulated at room temperature for 24 h. The sample was then cooled for 12 h and data were taken at a stable minimum temperature (110 K) for another 10 h.

The raw data consisted of neutron counts for each of 116 time channels separated by 100 μs and of monitor counts for each channel. The energy transfer ΔE in cm^{-1} associated with each channel was calculated from the expression

$$\Delta E(n) = 8065.5\left(\frac{723}{100n + 2{,}795}\right)^2 - 29.3,$$

where n represents channel number. Neutron counts were divided by monitor counts, and the spectrum of the blank cell was subtracted. The collagen proved to be a weak scatterer and the data were quite noisy; accordingly they were subjected to a seven-point Pascal smooth in which each point is replaced by a weighted average of itself and adjacent points, the weights being derived from binomial coefficients. The resulting spectra are shown in Fig. 1.

RESULTS AND DISCUSSION

The low-frequency region of the ambient collagen spectrum (Fig. 1 *a*) contains four peaks (17, 27, 40, and 57 cm^{-1}), each presumably representing a normal mode (or group of normal modes) of the collagen chain. Five peaks (22, 31, 40, 68, and 101 cm^{-1}) can be discerned in the spectrum of the cooled sample (Fig. 1 *b*). It is probable that significant structural changes accompany the cooling, vitiating the validity of a peak-by-peak comparison of the two spectra.

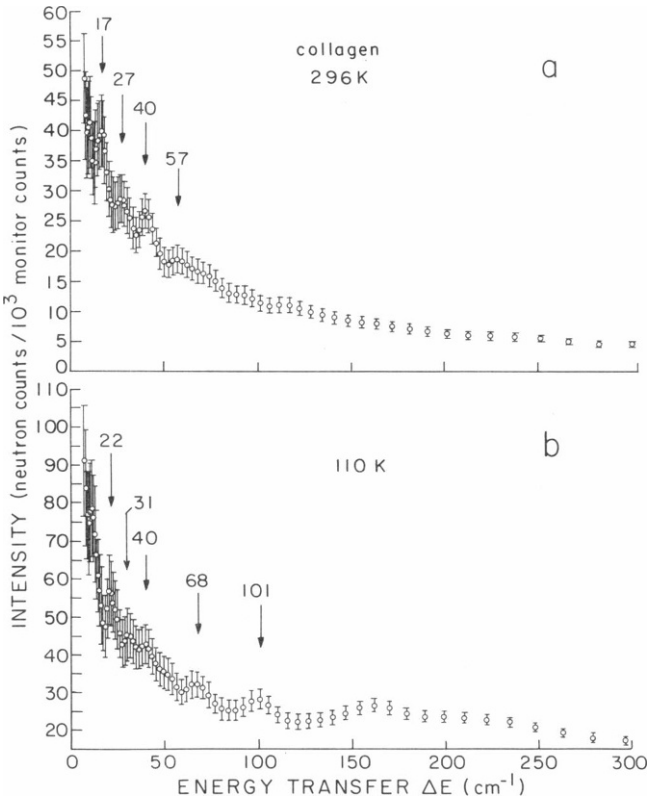

FIGURE 1 Inelastic neutron-scattering spectrum of collagen (*a*) at ambient temperature (296 K) and (*b*) cooled to 110 K. Error bars represent one standard deviation. A seven-point Pascal smoothing routine has been applied to both spectra.

Globular proteins, α-chymotrypsin (Brown et al., 1972), and lysozyme (Genzel et al., 1976) exhibit strong Raman active modes at 30 and 25 cm^{-1}, respectively. Brown et al. (1972) assigned the 30-cm^{-1} band in α-chymotripsin to an internal breathing mode, whereas Genzel et al. (1976) assigned the 25-cm^{-1} band in lysozyme to an intermolecular crystalline mode, based on its extinction in solution phase. The 27-cm^{-1} band observed in collagen at room temperature is reminiscent of the 25- and 30-cm^{-1} features in lysozyme and α-chymotrypsin and is interestingly close to the 29-cm^{-1} band predicted from theoretical vibrational analysis of polyglycine II (Dwivedi and Krimm, 1982). Collagen bears similarities in structure to polyglycine II, and therefore in the absence of any reported data on low-frequency vibrational modes in the literature on collagen, polyglycine II offers itself as a candidate for comparison.

The 40- and 57-cm^{-1} bands in collagen at room temperature also bear similarities to the 43-, 50-, and 63-cm^{-1} bands theoretically calculated for polyglycine II. A weak Raman-active band at 50 cm^{-1} has been experimentally reported for polyglycine II (see Krimm and Dwivedi, 1982). Gupta et al. (1968) have also observed regions of high density of states in the range 50–75 cm^{-1} in frequency distributions obtained from their neutron scattering study of polyglycine I and II. Low-frequency Raman and far-IR data on collagen or collagen-like polypeptides have so far not been reported in the literature and hence a direct comparison with the results of the present study is unfortunately not possible.

The cooling of collagen to 110 K has been little investigated from the standpoint of structural changes. However, the cooling process should be expected to modify the molecular dynamics of proteins. Sub-zero NMR, FT-IR, Raman, and x-ray diffraction studies have been suggested as techniques to investigate a series of "stop-action" pictures of biological processes (see Douzou and Petsko, 1984). The cooling of collagen causes substantial shifts toward higher frequencies except for an intermediate peak of 40 cm^{-1}. Qualitatively, these shifts reflect the stiffening of the collagen chain expected as a result of thermal contraction and closer packing. More detailed studies are necessary to obtain quantitative information.

To our knowledge, this is the first investigation of very low-frequency vibrational modes of collagen. Structurally, collagen resembles both polyglycine II and polyproline II conformations, primarily because of the presence of a larger number of imino residues than in any other protein, and the presence of glycine in every third residue. Thus each chain of collagen exists in a polyproline conformation and the supercoiling of three such chains is facilitated by the presence of glycine in every third residue. The stereochemical constraints imposed on the backbone by the presence of the abundant imino residues result in a rather rigid conformation, however, since imino residues are not evenly distributed axially, but are present in clusters separated by segments lacking in these residues. In collagen (Piez, 1976) considerable regional differences in intra- and interchain mobility can be expected. Extensive NMR studies have demonstrated intermolecular mobility in collagen fibers (Torchia et al., 1983); however, the mode of intramolecular motion is not fully understood. Although it is not possible to make definitive conclusions from the present study, it may be speculated that the very low-frequency modes observed at 17, 27, and 40 cm^{-1} may represent internal breathing modes resulting from the flexibility of the imino-deficient regions of collagen, which nominally resemble noncollagenous proteins in their behavior. When collagen is cooled to 110 K, the attenuation of intrachain motion resulting from more general effects on the entire chain (including the mobile imino-deficient segments) may result in a relative stiffening of the chains to conform more to the polyglycine II–polyproline II type behavior, as suggested by the appearance of the 68-cm^{-1} band, reflecting constrained segmental motion in the chain. This mode suggests that the cooled collagen chain may behave more like a polyglycine chain than collagen chains at ambient temperature. The 101-cm^{-1} band may reflect interchain motion as a harmonic of the new mode of segmental motion.

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