Infrared Amide I′ Band of the Coiled Coil†

William C. Reisdorf, Jr.,‡ and Samuel Krimm*

Biophysics Research Division and Department of Physics, University of Michigan, Ann Arbor, Michigan 48109

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ABSTRACT: The Fourier transform infrared (FTIR) spectra of several coiled-coil proteins have been shown to possess unusual features in the amide I′ region. Band maxima occur in the vicinity of 1630 cm^{-1}, with component bands at higher frequency. This is well below the observed band at 1650 cm^{-1} found in standard α-helical polypeptides such as poly-L-alanine. Normal mode calculations on models of the coiled-coil structure have been performed to investigate this issue. We find that the observed band profile can be reproduced with very small random variations on the ϕ,ψ of tropomyosin. We believe that the shift to lower frequency is due to additional hydrogen bonding of the solvent accessible backbone CO groups to water.

The coiled-coil structure, in which two parallel α-helices wrap around one another in a left-handed supercoil, was originally proposed as a means of explaining the diffraction patterns of keratin and other fibrous proteins (Crick, 1953; Pauling & Corey, 1953). Since then it has become evident that coiled coils are a motif in many proteins. Many of the muscle proteins, such as the myosin family and related proteins, have been shown to consist predominantly of coiled-coil domains. More recently coiled coils have been found in transcriptional activators that bind to DNA. One well-characterized example is the yeast GCN4 protein, whose structure has been solved by X-ray diffraction in the free (O'Shea et al., 1991) and DNA-bound (Ellenberger et al., 1992) states. This protein is the prototype of a family known as leucine zippers, so-called because they contain a 7-fold repeat of leucine residues. The role of coiled coils in protein structure and design has been reviewed recently (Cohen & Parry, 1990).

In this paper we are primarily interested in the muscle protein tropomyosin. It has been crystallized, but so far the structure is not known to high resolution (Phillips et al., 1986; Whitby et al., 1992). It is known to form a rod-like structure 420 Å in length with a coiled-coil pitch of 140 ± 10 Å. Fourier transform infrared (FTIR)[1] spectra of tropomyosin (and several other coiled-coil proteins) have been obtained by Heimburg et al. (1996; see accompanying paper). These spectra are unusual in that the amide I′ band peaks at a lower frequency (∼1630 cm^{-1}) than is normal for an α-helical molecule, and it shows a pronounced component at the normal frequency (∼1650 cm^{-1}). Since the lower frequency falls in a range associated with β-structure, we have undertaken normal mode calculations to gain a more fundamental understanding of this situation.

METHODS

A suite of programs called POLYPEP (Tasumi et al., 1982; Ataka & Tasumi, 1986) was used to compute the normal modes (eigenvectors) and frequencies (eigenvalues) for various models of a coiled-coil protein. We have used an empirical force field which has been shown to reproduce very well the experimental frequencies of synthetic polypeptides (Dwivedi & Krimm, 1984a). In addition, we have incorporated transition dipole coupling (TDC) (Krimm & Abe, 1972; Moore & Krimm, 1975) to obtain more accurate frequencies in the amide I region. *Ab initio* dipole derivatives are taken from an *N*-methylacetamide/formamide$_2$ system (Cheam, 1992) and are combined with the normal mode eigenvectors to obtain infrared intensity profiles (Reisdorf, 1994).

The calculations were carried out on a polypeptide chain of 140 residues, which, though only one-half the length of a tropomyosin molecule, was deemed large enough to be representative of a long coiled coil. All side chains have been approximated as point masses and assigned the mass of an alanine residue. This should have little effect on the backbone amide modes in general and no effect for the amide I′ region (which is mostly CO stretch). Although calculations have been done on the NH structure (Reisdorf, 1994), for ease in comparison with the experimental spectra of Heimburg et al. (see accompanying paper) we have replaced all the NH groups with ND.

The atomic coordinates for the coiled-coil models are generated from user-defined input values of the backbone dihedral angles (ϕ,ψ). The peptide groups are constrained to be planar, and the associated bond lengths and angles assume standard geometrical values (Krimm & Bandekar, 1986). However, unlike regular model helices, the backbone dihedral angles are allowed to vary. This results in intra-helical hydrogen bonds which are not identical throughout the molecule. To more accurately represent this perturbation, we allow the force constants associated with CO stretch (str) and O···H str to vary according to the hydrogen bond strength (Reisdorf, 1994). The hydrogen bond O···H distance was chosen as a convenient parameter to reflect the strength of the hydrogen bond. This choice, though arbitrary, is useful in its simplicity. Several studies (Baker & Hubbard, 1984; Ippolito et al., 1990; Stickle et al., 1992) have shown

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‡ Present address: Department of Biochemistry and Molecular Biophysics and Institute for Biomedical Computing, Washington University School of Medicine, St. Louis, MO 63110.

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[1] Abbreviations: FTIR, Fourier transform infrared; bR, bacteriorhodopsin; TDC, transition dipole coupling; str, stretch; α-PLA, α-helical poly-L-alanine; ND, amide group with ^1H replaced by ^2H; fwhm, full width at half-maximum.

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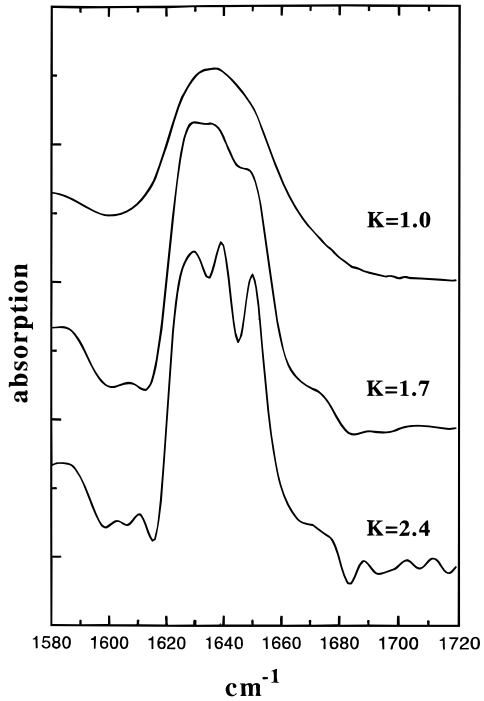

FIGURE 1: Amide I′ infrared spectra of tropomyosin in D_2O, pD = 7. Deconvoluted with an intrinsic line width of 17 cm^{-1}, triangular apodization, and varying resolution enhancement factors.

that the angular dependence of hydrogen bonding groups in proteins is also significant. However, modeling hydrogen bonding in a fully rigorous way is beyond the intent of this study.

All calculations were carried out on an ALPHA AXP 3000 Model 400 workstation (Digital Equipment Corporation).

RESULTS

Figure 1 shows the experimental spectrum of tropomyosin in the amide I′ region (the prime symbol indicates that the protein has been N-deuterated) as obtained by Heimburg et al. (see accompanying paper). Resolution enhancement and band decomposition reveal the presence of three component peaks centered near 1650, 1639, and 1630 cm^{-1}. In particular, the peak at lowest frequency is very unusual for a protein that is known to be α-helical. For deuterated α-helical poly-L-alanine (α-PLA-ND), the experimentally observed peak is at 1650 cm^{-1} (Dwivedi & Krimm, 1984b). Our efforts to account for the unusual spectrum of tropomyosin led us to examine several models for coiled coils.

The first model is based on the ϕ,ψ angles given by Parry and Suzuki (1969) in their study of the energetics of the coiled coil versus the α-helix. Their dihedral angles repeat with a period of seven residues (−51.42°, −52.52°; −51.77°, −52.46°; −51.14°, −53.01°; −51.23°, −52.90°; −51.80°, −52.27°; −51.46°, −52.78°; −50.98°, −53.16°). This is reasonable because the amino acid sequence of coiled-coil proteins displays a heptad repeat, which is diagnostic of the coiled-coil structure. The intrachain hydrogen bonds thus also repeat every seven residues. Surprisingly, the hydrogen bond geometries are very similar to those of a standard α-helix, whose ϕ,ψ are −57.37° and −47.49°, respectively. Figure 2 shows the results of our calculations on a 140-residue ND-polypeptide chain with these values of ϕ,ψ. There are 139 (n - 1) individual modes, each modeled as a Gaussian−Lorentzian band with specific position and intensity and summed to produce the band envelopes shown. The three different curves assume different values for the full width at half-maximum (fwhm) of the individual modes (1, 5, and 10 cm^{-} from bottom to top). We see that the modes cluster together to form three distinct bands at 1646, 1652, and 1660.5 cm^{-1}. Although the band positions do not correspond exactly to those observed in Figure 1, this is not so surprising. Our force field was refined to fit the experimental data for α-PLA, and the variation in hydrogen bond force constants is parameterized from the variation between the α and β forms of PLA. The specific values for these force constants might be somewhat different in a typical protein. We will discuss this problem at greater length below. The frequency separations between components in our calculations are 6 and 8.5 cm^{-1}, compared to 8 and 11 cm^{-1} as observed. The ratio of band intensities differs somewhat from the observed tropomyosin spectrum (Figure 1), but this only reflects possible inadequacies in the *ab initio* dipole derivatives due to a limited basis set (Chem, 1992).

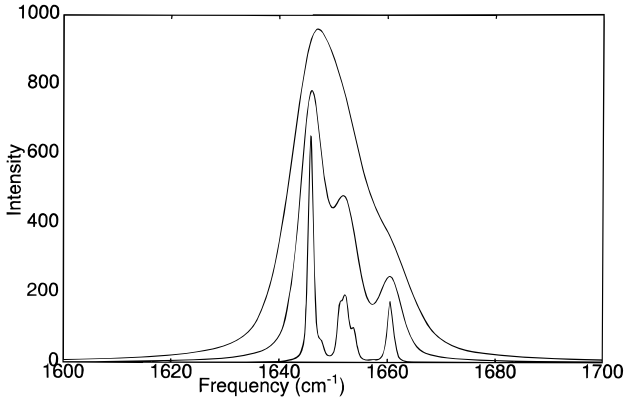

FIGURE 2: Calculated amide I′ infrared band profile for a 140-residue model coiled coil. Backbone dihedral angles are taken from Parry and Suzuki (1969). The three curves represent assumed individual CO line widths of 1, 5, and 10 cm^{-1} fwhm, from bottom to top.

We chose our second model to be in closer agreement with what is currently known of the atomic structure of tropomyosin. X-ray diffraction studies have determined the structure of tropomyosin to a resolution of 15 Å (Phillips et al., 1988) and 9 Å (Whitby et al., 1992), but at that level only approximate positions for the C^{α} atoms are available. In an appendix to the former article, there is a discussion about constructing an atomic model of tropomyosin. The authors determined that a structure with average values for ϕ,ψ of −58 and −45°, respectively, gave a good fit to the observed rise per residue and number of residues per turn. We adopted these values for the dihedral angles. However, rather than developing and refining our own model using the rough C^{α} positions in the Protein Data Bank file 2TMA, we chose instead to add various levels of random variation to the ϕ,ψ. This was done by generating a set of random numbers that were uniformly distributed over an interval of, e.g., ±1°. These were then scaled to whatever particular range was desired and added to the mean values (−58°,−45°). Ranges of 0.6, 1.2, 2, 3, and 4° were tried, and the results for ±1.2, ±2, and ±3° are shown in Figure 3.

The first aspect one notices is that in each case the overall band peak is at or above 1660 cm^{-1}, which is much higher than for the observed spectrum. The reason is that when one uses −58°,−45° and *no* random fluctuations about the

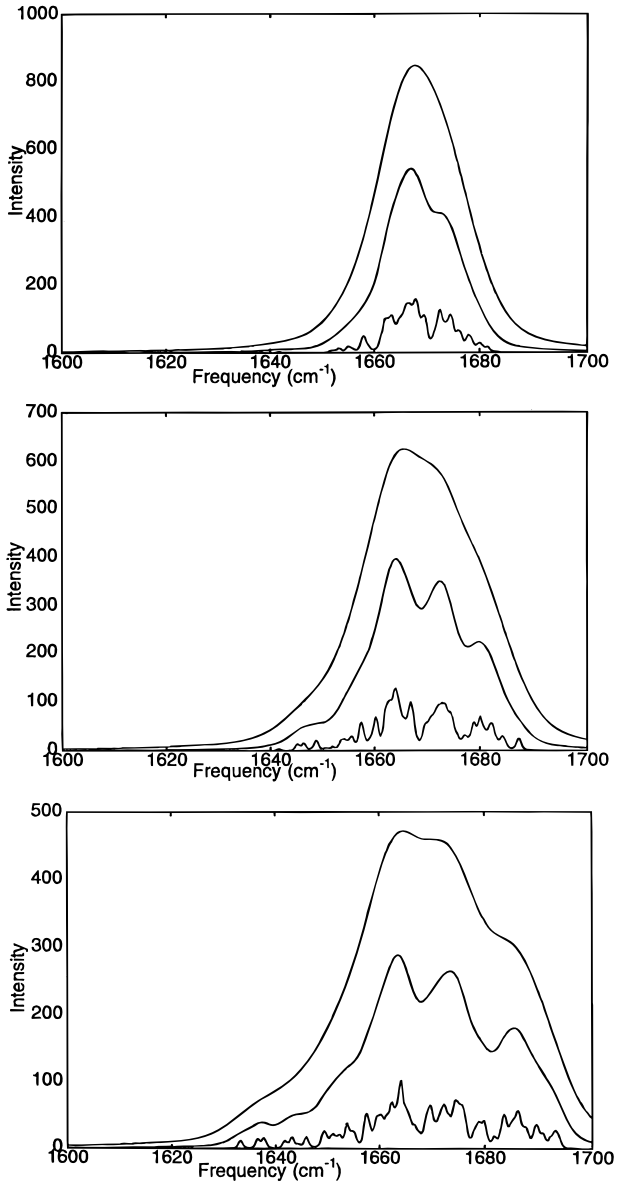

FIGURE 3: Calculated amide I′ infrared band profiles for a 140-residue model coiled coil with $\phi,\psi = -58°,-45°$ plus random variations: (top) ±1.2° range of random variation; (middle) ±2.0° range of variation; (bottom) ±3.0° range of variation.

ϕ,ψ, the standard peptide group geometry in POLYPEP yields a structure with a somewhat longer O···H distance and hence *weaker* hydrogen bond than in a standard α-helix (O···H distances are 1.989 and 1.838 Å for this structure and the standard α-helix, respectively). This leads to a larger CO str force constant and a shift to higher frequency. Therefore all of the trials with random variations in ϕ,ψ about these mean values will result in ands that are at *higher* rather than *lower* frequency than that of a standard deuterated helix (viz. 1650 cm^{-1}). Any mechanism that leads to an increase in the strength of the hydrogen bond would put our calculated model frequencies into good agreement with the observed spectrum. In the Discussion below we will propose such a mechanism.

Figure 3 also shows that the larger the range of random fluctuations, the broader the overall band becomes. This overall band fwhm (for 10 cm^{-1} fwhm modes) increases from approximately 20 cm^{-1} for the ±1.2° trial to about 27 cm^{-1} for the ±2° case and to about 35 cm^{-1} for the ±3° case. This is simply a reflection of the distribution of hydrogen bond lengths in the structure. Finally, we observe that in the ±2° trial, the three component bands are separated from one another by about 8 cm^{-1}, which is also close to that for the experimental spectrum (Figure 1).

DISCUSSION

There are two main features of the observed spectrum (Figure 1) that need particular explanation: (1) the underlying three-component structure of the amide I′ mode and (2) the unusually low frequency of the band position.

The observed three-component structure is well reproduced by our calculations, including the frequency separation between the components (cf. Figures 2 and 3). These components probably correspond roughly to the A, E_1, and E_2 species modes of a helix (Krimm & Bandekar, 1986), with the E_2 species (normally IR-inactive) gaining intensity because of the absence of translational symmetry.

With respect to the second point, all of the long coiled-coil proteins investigated thus far have shown an amide I′ band with components at uncharacteristically low frequency. This is initially puzzling because we know from X-ray diffraction that tropomyosin, for example, is a coiled-coil α-helix with the exception of a few residues near the molecular termini. In the absence of this structural information, one might infer that tropomyosin contained a significant portion of other secondary structures. Using the results of Byler and Susi (1986) to assign secondary structure on the basis of FTIR spectra, one would predict that tropomyosin was composed of a substantial fraction of extended chain or unordered structure. Clearly this is not the case, unless the conformation of tropomyosin changes drastically in going from the crystalline environment to solution.

We favor another possibility. Parrish and Blout (1972) studied α-PLA in the strongly hydrogen bonding solvent hexafluoroisopropyl alcohol and observed a similar downshift in the amide I band. All other spectroscopic indications were that the polypeptide remained significantly helical. Their explanation was that a slight distortion occurs in the normal helix geometry in which the CO groups point away from the helix axis while the NH groups point toward the axis. This would enable the backbone CO groups to form strong hydrogen bonds with the solvent in addition to the intrahelical hydrogen bonds. This in turn would reduce the CO str force constant and lead to a shift to lower frequency. In fact, the authors estimated that values of ϕ,ψ near −65 and −40°, respectively, would be favorable for formation of the dual or bifurcated hydrogen bonds. Interestingly, those dihedral angles are very close to the average values of ϕ,ψ found in recent surveys of helices in globular protein structures (Blundell et al., 1983; Barlow & Thornton, 1988). They are also quite close to the average values used in our second model.

Others have also proposed that unusually low amide I′ frequencies in proteins are caused by distorted peptide groups that can hydrogen bond to the solvent. Trewhella et al. (1989) made a similar observation in their FTIR spectra of the calcium-binding proteins calmodulin and troponin C. Both molecules are very similar structurally; they are composed of two globular domains at the N- and C-termini connected by a long, solvent-exposed helix. This unusual "dumbbell" shape gives rise to a significantly larger surface to volume ratio compared to a more or less spherical globule

of similar volume. This would greatly favor the potential for stronger interaction with the solvent than in a typical globular protein.

It is of interest to ask how common is this situation in which distorted helices give rise to unusual FTIR spectra. Another well-known case of anomalous amide I frequencies is found in bacteriorhodopsin (bR). Rothschild and Clark (1979) found an unusually *high* amide I band in bR. Krimm and Dwivedi (1982) suggested that in this case a weaker than average hydrogen bond is responsible, and that this is caused by a conformational change from an α_I to an α_{II} type helix. The α_{II}-helix has different ϕ,ψ than α_I but does have a very similar helical rise per residue and number of residues per turn [for $\phi,\psi = -70.47°,-35.75°$, the helical parameters are identical to those of the α_I-helix (Dwivedi & Krimm, 1984b)]. It also has peptide CO groups that point slightly away from the axis and NH groups that point toward the axis. This results in a lengthening of the hydrogen bond distance from 1.838 Å in the α_I-helix to 2.081 Å in the α_{II}-helix. But because these helices are sequestered within the phospholipid membrane, there are no solvent molecules available to form bifurcated hydrogen bonds with the backbone CO acceptors, and thus the CO str force constant is larger than in the α_I-helix. Further investigation is needed to confirm or refute the hypothesis that α_{II}-helices exist in bR. Recently, infrared linear dichroism has been proposed as a possible method to distinguish between the two conformations (Reisdorf & Krimm, 1995).

This study emphasizes the fact that, while infrared spectroscopy is a powerful tool for studying the conformation of proteins, occasionally their spectra can be difficult to interpret. However, normal mode analysis can be of considerable help in guiding us toward a deeper understanding of the structural basis of the observed spectra.

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