Infrared evidence of a β-hairpin peptide structure in solution

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Abstract The IR spectrum of an 16-amino acid peptide corresponding, according to NMR studies, to a β-hairpin has been analysed. Two characteristic features distinguish its spectrum from that of an antiparallel β-sheet: the low-frequency band that in a β-sheet structure is located at ≈1632 cm^{-1} appears here at ≈1620 cm^{-1}, and the high-frequency component does not undergo the isotopic shift typical of β-sheet from 1690 to 1675 cm^{-1} when transferred to D_2O. The infrared characteristics associated with β-hairpins have been described so far in two proteins, in one of which, whose three-dimensional structure is known from X-ray diffraction, a β-hairpin has actually been detected.

Key words: Infrared; β-hairpin; Characterization; Conformational analysis

1. Introduction

A β-hairpin involves two connected consecutive β-strands that lie antiparallel and are linked by hydrogen bonds. They are prevalent in globular proteins, occurring as separate motifs or forming part of extended β-sheet structures, and have often been suggested as possible sites for nucleation in protein folding [1]. Observation of their solution structure is hampered because of the tendency of these peptides to aggregate. Recently, a 16-amino acid peptide corresponding to the second β-hairpin of the B1 domain of an immunoglobulin G-binding protein from group G *Streptococcus*, that folds in aqueous solution as a monomeric native β-hairpin, has been studied by NMR techniques [2]. Infrared studies have been carried out with this peptide with the two-fold aim of characterizing the spectrum obtained and of assessing the possibility of sorting out β-hairpins in protein structures.

The solution structure of a peptide can be obtained from the infrared spectrum because of the conformation-sensitive bands arising from the peptide bond. Most important in conformational studies is the amide I band, located between 1700 and 1600 cm^{-1}, and due mainly (≈80%) to the carbonyl stretching vibration. Dihedral angles and differences in hydrogen bonding make this vibrational band conformation-sensitive [3,4]. Decomposition of amide I into its constituents allows the assignment and quantitation of the band components. The number and position of the components is obtained by mathematical band-narrowing, and decomposition is achieved through iteration procedures. In order to improve the assignment of band components, spectra are taken in both H_2O and D_2O media to profit from the isotopic shifts that are known to occur in certain band constituents when transferred from one to another medium [5].

2. Materials and methods

Preparation of the fragment 41–56 of protein G B1 domain has been described elsewhere [6]. The peptide was analysed by IR at 2 mM concentration and pH 6.3 since it was found that under these conditions it was monomeric [2]. Solutions of the peptide were prepared in H_2O and D_2O media and measured in a Nicolet Magna 550 spectrometer equipped with a MCT detector. Pathlengths were 6 μm for H_2O and 50 μm for D_2O measurements. Typically, 1000 scans were averaged with a nominal resolution better than 2 cm^{-1}. Buffer contribution was subtracted and the spectra treated as described previously [5].

3. Results and discussion

Fig. 1 shows the decomposed amide I band contours of the hairpin peptide in H_2O and D_2O. Five bands are seen in H_2O buffer, located at 1693, 1686, 1659, 1642 and 1623 cm^{-1}. In D_2O buffer, the bands are seen at 1691, 1683, 1660, 1642 and 1620 cm^{-1}. Band position, percentage area and assignment of the spectral components (discussed below) are shown in Table 1. To obtain the peptide structure from these values our procedure, that takes advantage of the complementarity of information in the spectra in H_2O and D_2O, is followed. This method assumes that the intensity of the amide I band contributions in all secondary structures is the same per residue [4]. The secondary structure is then 62% β-strand, 15% unordered structure, and 24% turns (figures rounded off to the nearest integer). The presence of a population of unordered structure may be due either to intrinsic unordered components of the β-hairpin (i.e. the free N- and C-ends) or to the existence of a fast conformational equilibrium between the β-hairpin and unordered conformations. The previous NMR data [2] appear to support the latter hypothesis, although a peptide system may be observed as unordered by NMR, and yet give off IR and CD spectra corresponding to a structured conformation [7].

Extended structures exhibit two components in the amide I band corresponding respectively to the (π,ϕ) and (ϕ,π) vibrational modes. Antiparallel β-sheet has a low frequency component around 1632 cm^{-1} irrespective of the medium in which the spectrum is measured, and a high frequency component located at 1690 cm^{-1} in H_2O, whereas in D_2O it is shifted to 1675 cm^{-1} [3,8]. The β-hairpin bands corresponding to the extended structure are seen to differ in two aspects from ordinary antiparallel β-sheets; first, the low frequency bands appear around 1620 cm^{-1} and second, in the high frequency component the expected isotopic shift is not produced. Both characteristic features have also been found in concanavalin A [9] and in apoB-100 from LDL [10], where bands from extended structure located below 1630 cm^{-1} have been dis-

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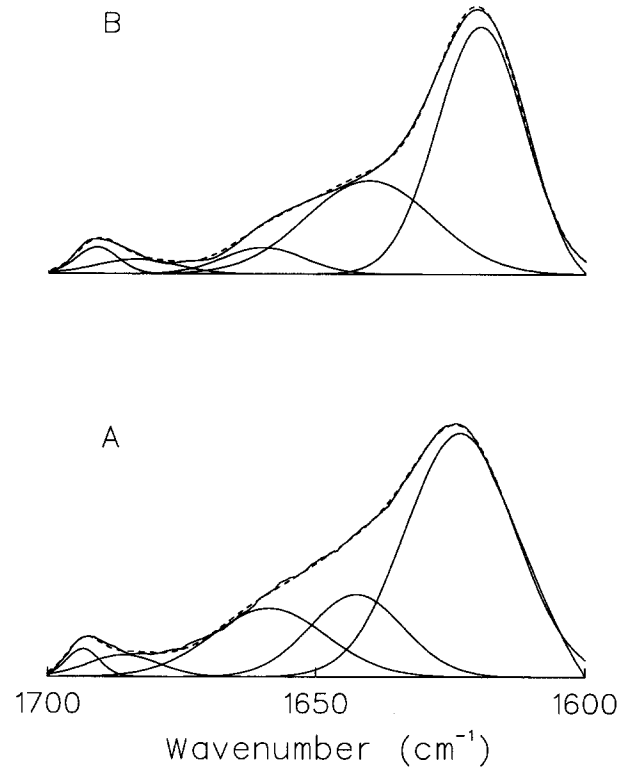

Fig. 1. Decomposition of the amide I band of the second β-hairpin of the B1 domain of an immunoglobulin G-binding protein from group G *Streptococcus* in (A) H_2O and (B) D_2O. Peptide concentration was 2 mM in sodium phosphate buffer (pH or pD 6.3). Spectral treatment has been described elsewhere [5].

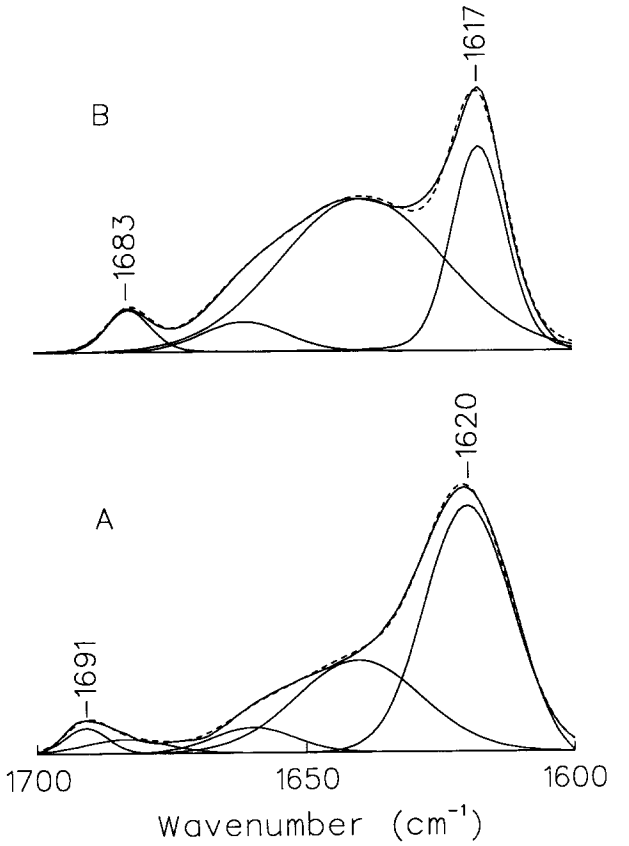

Fig. 2. Peptide amide I band decomposition in D_2O buffer at (A) 2 mM (β-hairpin) and (B) 10 mM (intermolecular β-sheet).

cussed. Although the three-dimensional structure of apoB-100 is not yet known, the existence of a β-hairpin has been characterized using X-ray diffraction data in concanavalin A [11,12]. The area ratio 1620/1690 of ≥10 in the β-hairpin (Table 1) is similar to what was predicted earlier for extended structures [13], and can be of help in quantitating the high frequency component in a protein, often mixed with contributions from β-turns.

Three bands are seen in the β-hairpin spectrum that are assignable to the turn. It is not clear whether in a protein each type of turn gives rise to more than one band or else different bands indicate distinguishable populations of β-turns. The results obtained with the peptide cannot be entirely conclusive, but they would rather point to more than one band for a single turn. This approach is consistent with theoretical studies on β-turn structure [3]. A remarkable feature is the band at 1642 cm^{-1} in H_2O buffer. This band has been attributed in proteins to different structures [5]. In our case however the contribution can only arise from the turn, a similar component having been assigned to a turn in a cyclic peptide [14]. The existence of this band comparable to that found in cyclic peptides would involve a rigidity of the structure that would account for the differences observed in the infrared spectrum between β-hairpins and antiparallel β-sheets.

The hypothesis that high frequency components resistant to isotopic shift are characteristic of β-hairpins has been tested by increasing the concentration of the peptide, a condition known to produce peptide aggregation [2]. It is also known that peptide aggregation produces intermolecular contacts that mimick in the infrared spectrum the antiparallel β-sheet signal, with the strong band component of the extended chain appearing at frequencies lower than 1630 cm^{-1} [4]. Fig. 2 shows the decomposition of the peptide amide I band at concentrations at which the β-hairpin occurs, as well as under aggregation conditions. It is clear that the aggregated form undergoes the isotopic shift, as occurs in the antiparallel β-sheet, but not in the hairpin.

Table 1
Parameters corresponding to the band decomposition shown in Fig. 1.

H_2O			D_2O		
Band position (cm^{-1})	% area	Assignment	Band position (cm^{-1})	% area	Assignment
1693	2	β-sheet	1691	3	β-sheet
1686	3	β-turn	1683	3	β-turn
1659	18	β-turn+unordered	1660	3	β-turn
1642	18	β-turn	1642	33	β-turn+unordered
1623	60	β-sheet	1620	59	β-sheet

Assignment of the bands is described in the text. Figures are rounded off to the nearest integer.

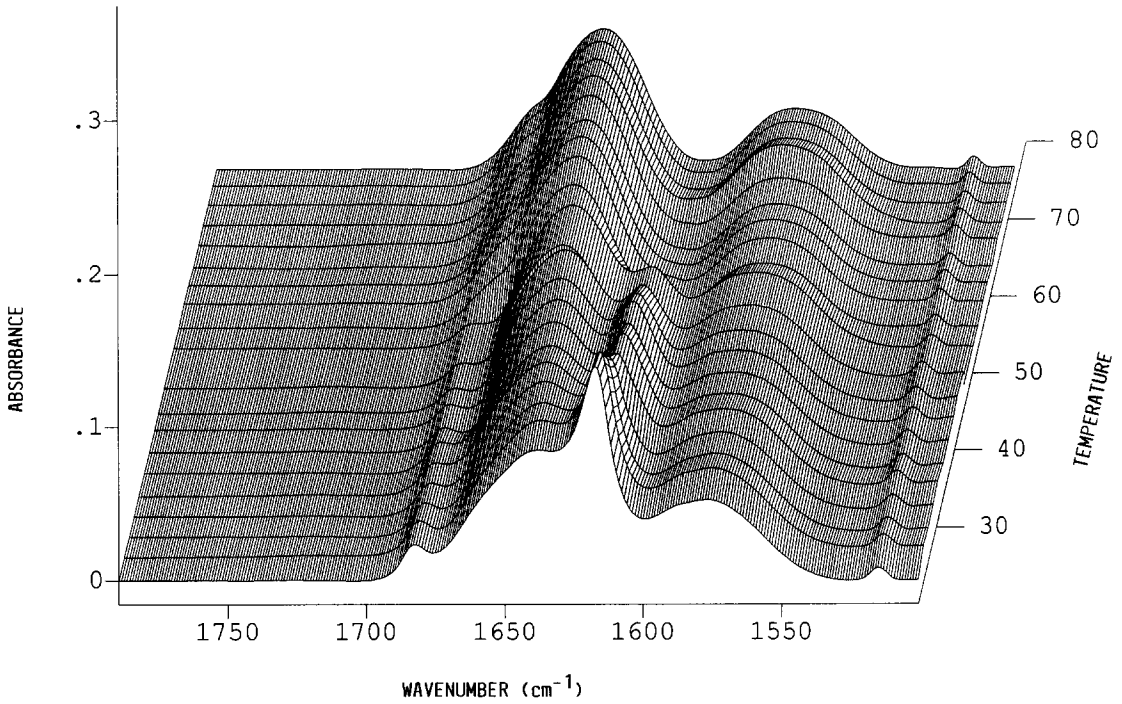

Fig. 3. Three-dimensional representation of the peptide infrared spectrum in the region 1750–1500 cm^{-1}.

Sample preparation for infrared measurements has been suspected to influence the results obtained [15]. Thus, the presence of bands corresponding to extended structures at non-expected frequencies might not be a consequence of true conformational features. In order to test the stability of the band components, the thermal behaviour of the peptide has been studied. Most of the proteins studied by infrared spectroscopy underwent irreversible thermal denaturation exhibiting a pattern with two bands attributed to protein-protein contacts located in D_2O buffer below 1630 cm^{-1} and above 1675 cm^{-1} [5]. The result of increasing the temperature in the peptide spectrum is shown in Fig. 3. The disappearance of the bands characteristic of the extended structure is observed above 55°C together with the presence of an amide I band centred at around 1643 cm^{-1}, typical of unordered structure. For technical reasons it has not been possible to collect the spectra while cooling the sample at the same rate as in the heating runs, but after the peptide is cooled down, the process is seen to be reversible (not shown). The infrared result has been confirmed by CD, where after heating at 80°C, the same spectrum is obtained after cooling down to room temperature (F. Blanco, unpublished results). This equilibrium is also concentration-dependent. At values close to the aggregation limit, after cooling down the sample, the peptide becomes aggregated, with a spectrum as shown in Fig. 2.

In summary, a β-hairpin gives off a characteristic infrared pattern described by two bands at around 1620 and 1690 cm^{-1} with the latter not undergoing isotopic shift when measured in a D_2O buffer. The turn region gives rise to several bands, one of them suggesting a rigid structure that would account for the spectral difference observed between the β-hairpin strands and the β-sheet. The characteristic hairpin bands have been so far found in two proteins, the presence of a β-hairpin having been confirmed in the one whose three-dimensional structure is known.

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