RAMAN OPTICAL ACTIVITY OF THE CENTRAL PART OF HINGE PEPTIDE

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Central cyclic part of the hinge peptide (a parallel dimer of the pentapeptide Boc-Cys-Pro-Pro-Cys-Pro-$NHCH_3$ with two disulfide bonds) derived from the sequence of human IgG1 is a rather rigid structure having predominantly polyproline II helical conformation as shown by vibrational circular dichroism spectra. It exhibits significant Raman optical activity (ROA) signal due to vibrations associated with the disulfide bridges. We report positive ROA for the S–S stretching vibration at 510 cm^{-1} and for the C–S stretching vibration at 655 cm^{-1}. These signals can provide means to assess the conformation of disulfide bridges in proteins, otherwise difficult to investigate.

Keywords: Peptides; Conformation analysis; Chirality; Raman optical activity; Raman spectroscopy; Disulfide bridges; Vibrational circular dichroism; IgG1.

Investigation of peptide/protein conformation using chirality and circular dichroism is a well established procedure which is fast and fairly simple to execute[1,2]. Its success is based mainly on the very high sensitivity of chiroptical methods to even minor changes of chiral three-dimensional structure of systems under study and on the relative ease with which the experimental data in solution are taken. On the other hand, it is a low-resolution technique, affording only gross information on the relative proportion of characteristic secondary structure segments (helices, sheets, turns, etc.) which, in addition, is obtained in the indirect manner as spatial relations between electronic chromophores (electronic circular dichroism), functional groups and skeletal segments (vibrational optical activity)[3]. It seems,

however, that a concerted use of various chiroptical spectroscopies leads to a significant increase in the information content and also enhances reliability of the results in those cases where the analysis on the basis of a single technique is not unambiguous. Of the three established chiroptical spectroscopies (electronic circular dichroism[1,2] – ECD, vibrational circular dichroism[3,4] – VCD and Raman optical activity[5,6] – ROA), application of ROA to conformation of proteins and peptides appears the youngest[6]. However, the basic set of secondary structure fingerprints has already been established. The ROA spectroscopy also provides a possibility to record signals due to vibrations associated with the S–S bonds within disulfide bridges. Chirality of the disulfides is difficult to investigate by other means.

Very recently we investigated a family of peptides related to core of the human immunoglobulin IgG1[7]. The so-called hinge peptide, a parallel dimer of the octapeptide Thr-Cys-Pro-Pro-Cys-Pro-Ala-Pro with two disulfide bridges represents a fragment 225–232/225′–232′ of the parent molecule and acts as a swivel point crosslinking two rather heavy peptide chains[8]. The peptide itself has been suggested as a versatile general core for various active sequences and used as a carrier of antibodies against peptides related to gastrin[9,10]. The only published conformational analysis of the hinge peptide was based on a combined use of NMR experiments in dimethyl sulfoxide and on restricted molecular dynamics calculations[8,11,12]. This resulted in proposal of a rather compact turn-like structure, where at least in the cyclic part of the molecule the two peptide chains exhibit a right-handed helical conformation with all-trans peptide bonds. However, this is in contradiction with (i) the general tendency of small proline-rich peptides to adopt a polyproline II conformation (which is helical, but left-handed); (ii) the results of our electronic and vibrational CD investigation of this peptide and its models in water, D_2O and 2,2,2-trifluoroethan-1-ol[7] (TFE). The spectra (also supported by our NMR experiments in DMSO-d_6) strongly hint that the hinge peptide and its ring part (sequence 2–6/2′–6′) adopt in solution a polyproline II conformation, which is rather stable and can be partially unwound only in the strongly interacting solvent like TFE. It was therefore of interest to apply Raman optical activity spectroscopy to this problem and (i) to confront the spectra with the newly established ROA spectral pattern of the polyproline II conformation[13], and (ii) to try to detect the ROA signals that might be due to disulfide bridges. The fact, that the peptide is rather rigid and the molar percentage of cysteine residues in the molecule is quite high, suggests that such detection should be feasible. For the experiment we have chosen the peptide I which represents a model of the cyclic, most rigid part of the hinge peptide.

Boc-Cys-Pro-Pro-Cys-Pro-$NHCH_3$
| |
Boc-Cys-Pro-Pro-Cys-Pro-$NHCH_3$

I

The *tert*-butoxycarbonyl groups are used as weights simulating the attached continuation of the chain. We report here the data obtained with this model system.

EXPERIMENTAL

Peptide I has been synthesized by classical methods of solution phase synthesis. The two parallel chains were built simultaneously using preformed disulfide bridges, i.e. employing cystine derivatives. In this manner we avoid any improper closure of the S–S bonds. The procedure, which will be detailed separately[7], provided peptide I with 97% purity according to HPLC (Supelco HSF5 15 cm column, gradient 0–100% acetonitrile in 0.05% trifluoroacetic acid, elution time 19.5 min). The peptide was further characterized by FAB MS (M – 1253.2; M + Na – 1275.2; M – Boc – 1153.2; M – 2 Boc present).

VCD spectra were measured on a Bruker IFS66/S FTIR instrument equipped with a VCD/IRRAS module PMA 37. The spectra were measured in cells with CaF_2 windows of 50 μm path length and in general are based on 3200 FTIR scans. Sample concentrations for the VCD experiments were about 0.1–0.2 mol l^{-1} and the VCD intensities are expressed as $\Delta\varepsilon$ [l mol^{-1} cm^{-1}] per amino acid residue.

Raman optical activity and Raman scattering spectra were recorded on a newly rebuilt ROA spectrometer[14] that adopts the incident circular polarization (ICP) modulation scheme in backscattering geometry which is superior for biomolecular ROA [15]. The main experimental breakthrough that opens new research and application areas and makes peptide and protein studies possible has been made very recently. The spectrometer is now based on a fast stigmatic spectrograph Holospec HS-f/1.4 (Kaiser Optical Systems) equipped with a holographic transmission grating and a back-illuminated CCD detection system (Roper Scientific). It possesses excellent performance and long-term stability. The peptide solution was studied at nearly saturated concentration (≈35 mg ml^{-1}) in doubly distilled deionized water at ambient temperature (≈20 °C). The solution was filtered through a 0.22 μm Millipore filter into a quartz microfluorescence cell. Residual visible fluorescence from traces of impurities, which can give large and unstable background in Raman spectra, was quenched by leaving the sample to equilibrate in the laser beam for a few hours before acquiring ROA data. The experimental conditions were as follows: laser wavelength 514.5 nm, laser power at the sample ≈660 mW, spectral resolution ≈6.5 cm^{-1} and total acquisition time ≈29 h.

RESULTS AND DISCUSSION

Vibrational circular dichroism spectra of peptide I in the amide I and II regions (Fig. 1) show a distinct negative couplet for either amide I′ band (in

D_2O) centered at 1630 cm^{-1} or amide I band at 1640 cm^{-1} in TFE. This spectral pattern is recognized as a marker typical of the left-handed polyproline II helical conformation, which is sometimes ascribed to the so-called random coil in the spectra of proteins. This VCD pattern does not change much even after a rather dramatic solvent polarity change; we observe only a blue shift of about 10 cm^{-1} of the negative lobe and a slight intensity decrease in the positive band at 1660 cm^{-1}. Another weak negative couplet is visible for amide II′ at 1550 cm^{-1}. Similarly, the results of ECD and NMR investigation support the assumed prevalence of polyproline II conformation of peptide I in solution.

The back-scattered Raman and Raman optical activity spectra of peptide I are shown in Fig. 2. A rather low level of stray light within the newly reconstructed instrument[12] allowed to record experimental data below the usual limit of 600 cm^{-1}. As expected, using recently established ROA markers, we can detect polyproline II conformation[13,16]. Both the diagnostic bands (positive ROA at 1319 cm^{-1} in the extended amide III region and also positive ROA in the amide I region – centered at 1644 cm^{-1}) are present. Compared with model peptides, however, there is a significant difference: The positive ROA band at 1319 cm^{-1} is not a dominant feature of the ROA spectrum. There are several ROA bands in the same region which are even stronger. However, none of these bands corresponds to what might be a marker of right-handed helicity[5]. For the time being, the nature of the strong positive ROA at 1284 cm^{-1} and of the negative bands at 1271 and 1256 cm^{-1} remains uncertain. The negative band at 1256 cm^{-1} might indicate the pres-

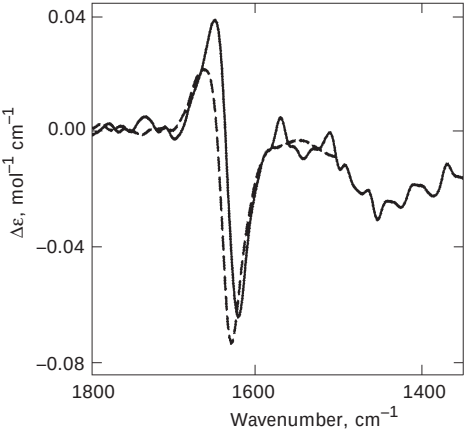

FIG. 1
Vibrational circular dichroism spectra of peptide I in D_2O (——) and TFE (– – –)

ence of the sheet conformation[6], but without further support. Moreover, this region (at 1250 and 1420 cm^{-1}) should contain deformation vibrations of CH_2–S groups of cysteine residues. It is to be noted that the assignments of bands due to polyproline II conformation are based on spectra of poly(L-lysine) and poly(L-glutamic acid) in the unordered conformation[16] and on spectra of alanine oligopeptides in aqueous solutions[13]. Therefore, with proline-rich peptides, the situation may be different and more model compounds are evidently needed. Similarly, for the strong ROA bands in the region of backbone skeletal vibrations, there is no structural interpretation.

In addition to Raman and ROA bands related to molecular skeleton and amide groups within the molecule of I, we observe also two bands in the low-wavenumber region, which are associated with disulfide bonds[17,18]. The positive ROA intensity centered at 520 cm^{-1} and the corresponding sharp Raman scattering band at 510 cm^{-1} are due to the S–S stretching vibration[19,20] and the intense positive ROA band at 658 cm^{-1} is ascribed to the C–S stretching motion[21,22]. As far as we know, the positive ROA at 520 cm^{-1} is the first report on the ROA signal due to the S–S stretching vibration. The

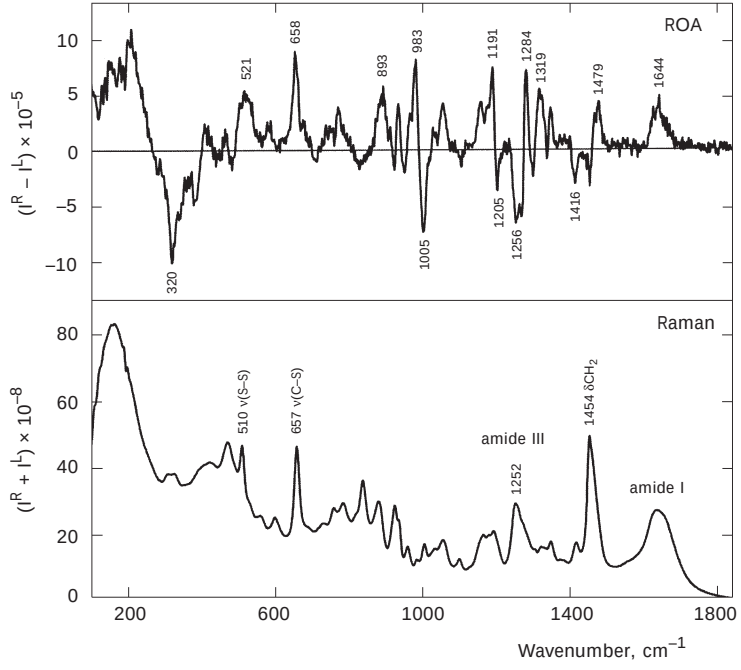

FIG. 2
Back-scattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of peptide I in H_2O

Raman band at 510 cm^{-1} indicates the gauche–gauche–gauche conformation of the C_α–C_β–S–S′–C′$_\beta$–C′$_\alpha$ segment if we assume that the C–S–S–C dihedral angle is in the proximity of ±90° (generally assumed as the low-energy conformation of the S–S bridge)[17]. More information including the sign of the relevant dihedral angles is probably hidden in the positive sign of the associated ROA signal at 520 cm^{-1}, but such a detailed interpretation needs more tailor-made model compounds and is beyond the scope of this paper. The C–S stretching vibration at 658 cm^{-1} also correlates with a distinct conformation of the cysteine residue, namely with the conformation of the C_α–C_β bond[17]. It is compatible with the so-called P_H conformation, in which the H–C_α bond is oriented *trans* with respect to the adjacent C_β–S bond. Again there is a corresponding significant ROA signal. We will use further models to investigate the potential of ROA signals for the detection and chirality assignment of the S–S group. There are yet two very strong ROA bands in the low-energy region. They form a high-intensity couplet centered at about 270 cm^{-1}. The origin of this couplet is unknown, but we cannot exclude its relation to the –S–S– torsional motion and hence a direct connection to the S–S group chirality.

CONCLUSION

The Raman and ROA spectra confirm prevailing polyproline II conformation in the cyclic part of the hinge peptide in aqueous solution. Quite interestingly, it seems that a diagnostic positive ROA band at 1319 cm^{-1} remains present even in the case of a proline-rich peptide. The spectrum of peptide I clearly shows ROA bands due to vibrations of the S–S bridges, which might probably be used for conformational analysis at least for the rigid molecules.

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