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Short communication

# Unusual structural transition of antimicrobial VP1 peptide

Ganesh Shanmugam [a], Nsoki Phambu [b], Prasad L. Polavarapu [a,*]

[a] Department of Chemistry Vanderbilt University, Nashville, TN 37235, USA
[b] Department of Chemistry, Tennessee State University, Nashville, TN 37209, USA

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ABSTRACT

VP1 peptide, an active domain of m-calpain enzyme with antimicrobial activity is found to undergo an unusual conformational transition in trifluoroethanol (TFE) solvent. The nature of, and time dependent variations in, circular dichroism associated with the amide I vibrations, suggest that VP1 undergoes self-aggregation forming anti-parallel β-sheet structure in TFE. Transmission electron micrograph (TEM) images revealed that β-sheet aggregates formed by VP1 possess fibril-like assemblies.



## 1. Introduction

Calpains [1] belong to the superfamily of calcium dependent intracellular cysteine proteases that are expressed ubiquitously in mammals [2,3]. These enzymes are believed to play important roles in many processes such as cytoskeletal remodeling, cell differentiation, embryonic development, signal transduction and apoptosis [4–6]. Calpain 2 (or m-calpain) is the most studied member of the calpain superfamily. The larger subunit (80 kDa) of m-calpain consists of four domains (I–IV), while smaller subunit (30 kDa) consists of two domains (V and VI) [7]. Domain V is largely cleaved from m-calpain during autolysis [8]. It has been demonstrated that a C-terminal sequence of domain V, GTAMRILGG, is required for lipid interaction of the protease [9]. Further, domain V derived from four different mammalian (porcine, human, bovine and rabbit) m-calpains showed that each of them hold a common segment, GTAMRILGGVI, referred to as the VP1 peptide [10]. Later studies, using peptides homologous to various regions of m-calpain's domain V, showed that although the presence of TAMRIL sequence is required for m-calpain–lipid interaction, the presence of glycine units is also necessary for such interaction [11].

The conformational preferences of this important VP1 peptide are not well established. Infrared (IR) spectroscopic results on films were interpreted to suggest that VP1 peptide adopts β-sheet structure in aqueous buffer solution and varying amounts of α-helical and β-sheet structures in the presence of different anionic lipids [12]. In addition, VP1 was suggested to have ~45% helical and ~25% β-sheet structures in the presence of mixed lipid vesicles [13]. Based on IR spectra, a similar level of α-helicity was proposed in 90% trifluoroethanol (TFE) [13]. Although the crystal structure was reported for m-calpain [14,15], it did not include domain V, so the three-dimensional structure for domain V are not currently available.

Nuclear magnetic resonance (NMR) and X-ray crystallography are generally used to determine the three-dimensional structures of proteins and peptides. However it is not easy to utilize NMR when a protein/peptide aggregates lead to fibril formation. In such situations, electronic circular dichroism (ECD) and IR spectroscopy are often used for the elucidation of secondary structures and transitions between them in solution state. The spectra–structure correlations in ECD depend on observing characteristic ECD bands associated with a particular type of structure. For proteins and peptides containing substantial number of aromatic amino acids, particularly phenylalanine, such correlation can sometimes be misleading because of the overlap of ECD bands originating from the peptide back bone with those from aromatic groups [16]. Furthermore, in some cases, spectra–structure correlation deduced from ECD spectra may not be consistent with the structure determined from other methods [17,18]. The spectra–structure correlation from IR spectra depends predominantly on observing the characteristic amide carbonyl group stretching (amide I) band in the 1700–1600 $cm^{-1}$ region. Even though the resolution enhancement methods, such as Fourier self deconvolution and second derivatives, have been used to separate the

* Corresponding author. Tel.: +1 615 322 2836; fax: +1 615 343 1234.
*E-mail address:* Prasad.L.Polavarapu@Vanderbilt.edu (P.L. Polavarapu).

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overlapping bands, the structural deductions based on IR spectra alone are not unambiguous. The combined application of multiple spectroscopic methods is useful to avoid such uncertainties.

In recent years, vibrational circular dichroism (VCD) and Raman optical activity (ROA) have emerged as powerful tools to determine the secondary structure of proteins and peptides [19,20]. The ability to measure VCD spectra under a broad range of sample conditions (including dried films, protein aggregates, protein fibrils, gel, and adsorbed to different solid surfaces) makes VCD a useful tool for probing the protein structure [21–24].

The structure of VP1 in TFE solvent is investigated here using multiple spectroscopic methods: VCD, IR, and ECD. TFE solvent has been used as a membrane mimicking environment for studying proteins and peptides. Since the m-calpain activity [12,13] and antimicrobial property of VP1 peptide [25] are often correlated with VP1–lipid interaction, the choice of TFE solvent is practically relevant for VP1 structural study.

## 2. Experimental

VP1 peptide was obtained from Genscript Inc. with >95% purity. HPLC and Mass spectrometry were used to assess the purity of the peptide. TFE was purchased from Acros (USA). Phosphotungstic acid was obtained from Aldrich (USA). The fixed path length IR cell (100 μm) with $BaF_2$ windows was purchased from International Crystal Labs (USA).

### 2.1. VCD and FTIR spectroscopy

All VCD and IR absorption spectra were recorded on a commercial Chiralir spectrometer (BioTools, USA). This instrument was modified to minimize the spectral artifacts. IR absorption spectra were acquired simultaneously with VCD measurements. All spectra were recorded at room temperature. For time dependent VCD spectra, 1 mg of VP1 peptide was dissolved in 100 μL of TFE solvent. This solution was left at room temperature for 0.5 h before VCD measurements. VP1 solution was loaded into the 100 μm fixed path length cell containing $BaF_2$ windows. The VCD spectra were collected over a period of four days, each with 1 h data collection time at resolution of 8 $cm^{-1}$. The VCD spectrum of solvent (TFE) itself was recorded under similar conditions to obtain the VCD baseline and subtracted from the VCD spectra of VP1. The IR absorption spectra of VP1 are also solvent subtracted.

### 2.2. ECD spectroscopy

All ECD spectra were recorded on a Jasco J720 spectropolarimeter at room temperature. The instrument was calibrated with ammonium D-camphor-10-sulfonate as described by the instrument manufacturer. For time dependent ECD spectra, similar to VCD, 1 mg of VP1 peptide was dissolved in 100 μL of TFE. This solution was left at room temperature for 0.5 h before ECD measurements. About 20 μL of the VP1 solution was loaded into the 0.01 cm circular quartz cell. All ECD spectra are averages of three individual scans. A scan speed of 50 nm/min, time constant of 0.125 s, resolution of 1 nm, and sensitivity of 100 mdeg were used. The ECD spectrum of solvent was subtracted from those of VP1 solution. The ECD spectra were collected over a period of three days.

### 2.3. Electron microscopy

Aged solution (96 h) of VP1 in TFE (1 mg/100 μL) was examined by transmission electron microcopy. A 5 μL sample was placed on carbon-coated grids and left there for 1 min. The sample grids were stained with 1% (w/v) phosphotungstic acid for 20 s, washed, and air dried. Grids were imaged using a Philips CM20 Transmission Electron Microscope operating at 200 kV and a magnification of 20,000×.

## 3. Results and discussion

The time dependent IR and VCD spectra of VP1 in TFE are shown in Fig. 1. The IR spectrum at 30 min shows a broad amide I band centered at 1674 $cm^{-1}$ and the corresponding VCD spectrum shows a negative band at 1670 $cm^{-1}$, both characteristic of a β-turn structure. The IR spectrum at 6.5 h shows decrease in intensity at 1674 $cm^{-1}$ (Fig. 2) and a new amide I band developing at 1620 $cm^{-1}$ and this later band is characteristic of β-sheet structure. The corresponding VCD spectrum, accordingly, showed a negative couplet, negative at 1609 $cm^{-1}$ and positive at 1624 $cm^{-1}$, and two additional negative VCD bands at 1682 and 1640 $cm^{-1}$. It should be noted that the VCD intensities at 1609 and 1624 $cm^{-1}$are significantly enhanced when compared to the initial VCD (note that IR absorption intensity at 1620 $cm^{-1}$ is only ~0.1 at 6.5 h). The dissymmetry factor, ΔA/A, at 1624 $cm^{-1}$ is ~5 times larger compared to that in the initial spectra. Enhanced VCD intensities were also observed previously for protein fibrils that are enriched with β-sheet structure [21]. Enhanced VCD is also characteristic of supramolecular chirality as observed in insulin fibrils [22]. Furthermore, the fibrils formed from a shorter octapeptide also showed enhanced VCD intensities in the amide I region [26]. Theoretical models for VCD intensities of amyloid-like fibrils rationalizing the different levels of intensity enhancements, starting from a factor of 2 and higher, have also been proposed recently [27]. Thus, the current time dependent VCD

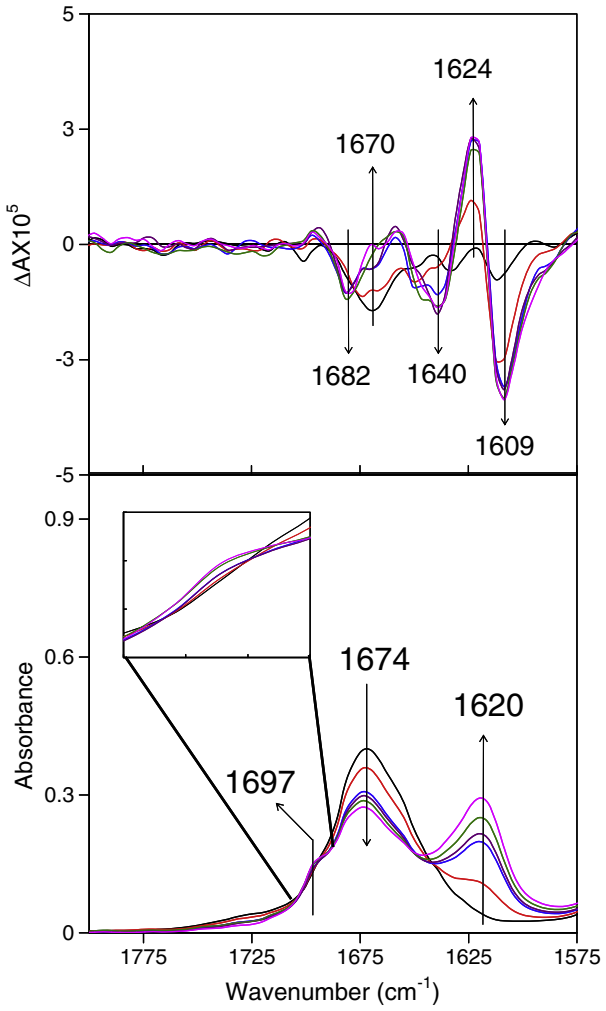


**Fig. 1.** Time dependent VCD (top) and IR absorption (bottom) spectra of VP1 peptide in TFE (concentration 9.2 mM, path length 100 μm, and resolution 8 $cm^{-1}$) measured at 0.5 h (black), 6.5 h (red), 23.5 h (blue), 29.5 h (violet), 50.5 h (green) and 96 h (pink). Inset: expanded region of 1697 $cm^{-1}$ band.