Membrane Proteins

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The Native Conformation of the Human VDAC1 N Terminus**

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The voltage-dependent anion channel (VDAC) is located in the mitochondrial outer membrane and constitutes the major pathway for the transport of ADP, ATP, and other metabolites. It is also considered a key player in mitochondrial apoptosis.[1,2] Recently, the three-dimensional structure of the VDAC1 isoform was elucidated independently by three different experimental approaches.[3–5] All structures reveal a novel 19-stranded β-barrel architecture with an N-terminal α helix positioned horizontally inside the pore. Although all three structures are highly similar in terms of the β barrel, they exhibit clear differences in the functionally important N-terminal region (Figure 1 a). There is a general consensus that the N terminus is involved in the voltage-dependent gating process of the channel and that it may adopt different conformations depending on external factors.[1]

Based on these structural data, different models for voltage gating have been proposed. Ujwal et al. suggested that the whole helix may move towards the middle of the pore and thereby close the channel.[3] On the other hand, as residues 11–20 are difficult to observe in solution-state NMR,[4] Hiller and Wagner argued that they may be subject to conformational exchange and that movements in this part of the N terminus alone may explain the gating behavior.[6] Alternatively, larger conformational rearrangements upon voltage gating that also involve the β barrel have been suggested based on electron microscopy and electrophysiological data.[7–9]

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Additionally, some previous biophysical findings are at odds with all three published structures, and the question has been raised as to whether they really represent the native conformation in a natural membrane environment.[7] In particular, N-terminal truncation mutants of VDAC1 were found to exhibit lower conductance than the full-length channel,[10–12] which has been taken as an indication that the N-terminal helix may not lie inside the pore, but form part of the barrel wall.[7]

Solid-state NMR spectroscopy has proven to be a very useful method for structural investigations of membrane proteins in a natural lipid environment (see, for example, reference [13] for a recent review). We therefore investigated functional human VDAC1 in lipid bilayers using solid-state NMR spectroscopy, with a focus on the conformation of its N terminus. Functionality of our hVDAC1 preparation was confirmed by electrophysiological measurements in lipid bilayers (see the Supporting Information, Figure S1). Figure 1 b shows a ^{13}C–^{13}C proton-driven spin diffusion (PDSD) correlation spectrum of uniformly [^{13}C, ^{15}N] isotope-labeled hVDAC1 reconstituted into dimyristoylphosphocholine (DMPC) liposomes. The spectra exhibit excellent sensitivity and resolution. A prediction of the Cα–Cβ region of the spectrum based on the crystal structure of mouse VDAC1 (Supporting Information, Figure S2) agrees very well with the spectrum and indicates that the overall 19-stranded β-barrel fold is conserved in liposomes.

Comprising 283 amino acids, VDAC1 is a challenging protein for solid-state NMR spectroscopy. To identify the N-terminal residues, we made use of different isotope labeling schemes, involving a (Lys, Trp, Tyr, Val) reverse-labeled[14] and an (Ala, Asp, Leu, Val) forward-labeled protein variant along with the uniformly isotope-labeled sample. Furthermore, we studied an N-terminally truncated hVDAC1 variant (Δ(1-20)-hVDAC1). Figure 1 c illustrates how a combination of results from the different samples was used in the assignment process.

With a large set of homo- and heteronuclear correlation spectra of the different protein variants (see, for example, the Supporting Information, Figure S3) we were able to obtain unambiguous de novo sequential resonance assignments for residues Ala2–Val17 (Supporting Information, Figure S4 and Table S1). All of these residues give rise to distinct narrow cross-peaks in experiments based on dipolar transfer schemes at sample temperatures between +5 and +25 °C (Supporting Information, Figure S5 and S6). This result demonstrates that the hVDAC1 N terminus assumes a well-defined conformation in liposomes and does not exhibit sizable dynamics on the sub-millisecond timescale. No peak broadening or doubling owing to chemical exchange on slower timescales was

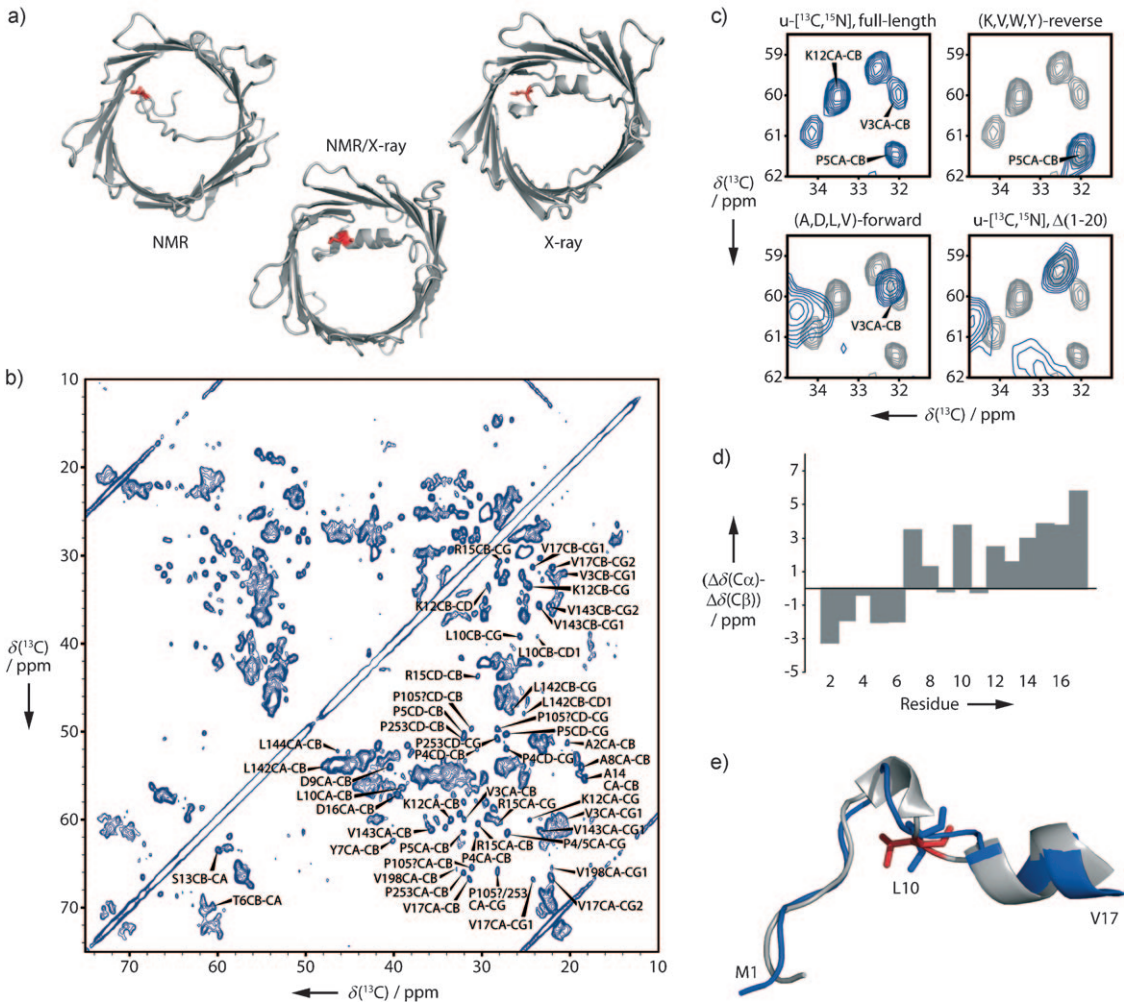

Figure 1. Assignment and conformation of the hVDAC1 N terminus in lipid bilayers. a) Three existing VDAC1 structures with Leu10 (red) in stick representation, illustrating the different conformations of the N-terminal helix. Left: hVDAC1 NMR structure (PDB: 2K4T); center: hVDAC1 combined NMR/X-ray structure (PDB: 2JK4); right: mVDAC1 X-ray structure (PDB: 3EMN). b) ^{13}C–^{13}C PDSD spectrum (15 ms mixing) of full-length, u-[^{13}C,^{15}N]-hVDAC1 in lipid bilayers recorded on an 850 MHz spectrometer. De novo resonance assignments are indicated. c) Regions from ^{13}C–^{13}C PDSD spectra of hVDAC1 samples with different lengths and labeling schemes as indicated above the panels. For comparison, peaks from the u-[^{13}C,^{15}N]-hVDAC1 sample are reproduced in the other panels in gray. Higher centrifugation speeds were used in final preparation steps of the samples in the lower two panels, resulting in broader lines and some additional signals from loop residues. d) (Cα,Cβ) secondary chemical shifts of hVDAC1 residues 2–17 in lipid bilayers. e) Model of the hVDAC1 N terminus in lipid bilayers as derived from ssNMR data (blue; see Supporting Information for details) aligned with the mVDAC1 crystal structure (PDB: 3EMN, gray/red), with Leu10 in stick representation.

observed. Whilst other, highly dynamic conformations not accessible to dipolar-based experiments may exist in equilibrium, our data thus strongly argue against a flexible N-terminus as proposed by Hiller and Wagner.[6] Furthermore, comparative data for hVDAC1 reconstituted into DMPC, DOPE, and DOPC liposomes show that the N terminus, in the absence of transmembrane voltage, is unaffected by the associated changes in membrane thickness and lateral pressure (Supporting Information, Figure S7).

Secondary chemical shifts for hVDAC1 in liposomes that report on secondary structure[15] are depicted in Figure 1 d. In agreement with backbone dihedral angles predicted from our chemical shift assignments by the program TALOS,[16] they show that residues 7–8 and 12–17 are in helical conformation, whereas Asp9, Leu10, and Gly11 form a kink and residues 2–6 are in extended conformation. The results agree well with the mVDAC1 crystal structure (PDB: 3EMN; Figure 1 e; Supporting Information, Tables S2 and S3 and Supporting Methods).

Despite considerable signal overlap in the spectra, it was possible to obtain resonance assignments for additional residues in β-strand and loop conformations as well (Supporting Information, Table S1). In particular, we could unambiguously assign residues Leu142, Val143, and Leu144 in β strand 9, as they form the only Leu-Val sequential pairs in hVDAC1 (Supporting Information, Figure S8). Notably, Val143 and Leu150 form a hydrophobic patch that points into the interior of the barrel. Long-range correlations consistent with a contact between the Leu-Val-Leu motif and the N-terminal helix (Leu10–Val143) could be identified

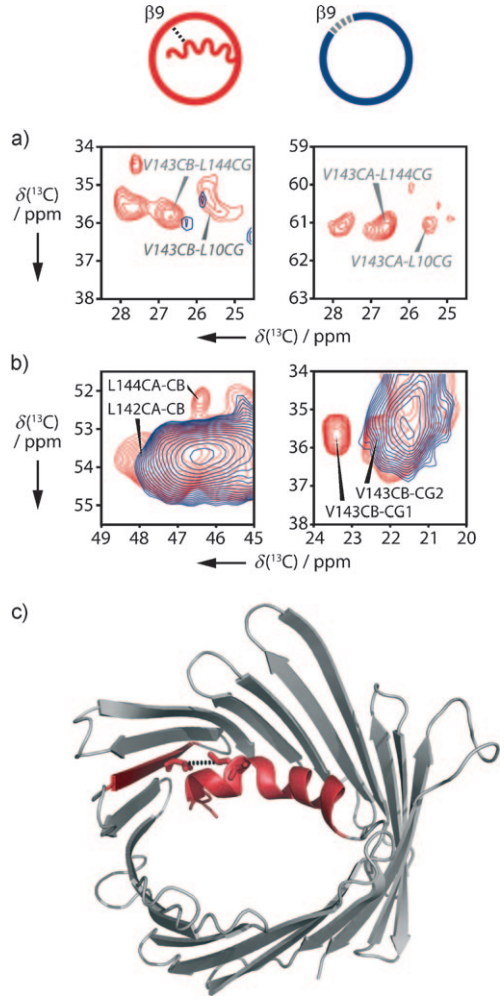

Figure 2. Comparison of spectra from wild-type (wt-)hVDAC1 (red) and Δ(1-20)-hVDAC1 (blue). a) Overlaid regions from 300 ms ^{13}C–^{13}C PDSD spectra on (Ala, Asp, Leu, Val) forward-labeled samples. Correlations consistent with Val143 sequential and long-range contacts are only visible in wt-hVDAC1. Labels in gray italics indicate ambiguous assignments. b) Overlaid regions from ^{13}C–^{13}C DREAM (left) and 15 ms ^{13}C–^{13}C PDSD spectra (right) on u-[^{13}C,^{15}N]-labeled samples, showing intraresidue correlations from the hydrophobic patch around Val143 that vanish in Δ(1-20)-hVDAC1. c) View of the mVDAC1 crystal structure (PDB: 3EMN) with N terminus and β strand 9 shown in red. The hydrophobic contact between residues Leu10 and Val143 (in stick representation) is indicated by a dotted line.

(Figure 2a). Similar contacts were also observed in the previous NMR study[4] and are in line with the mVDAC1 crystal structure.

Signals assigned to the Leu-Val-Leu motif in β strand 9 are strongly affected upon truncation of the N terminus, as seen in spectra of Δ(1-20)-hVDAC1 (Figure 2b). This result further confirms the contact between N terminus and β strand 9. Furthermore, it also suggests that the helix plays a role in maintaining β-barrel integrity. Our data indicate that signals from β strand 9 disappear rather than shift their position (Supporting Information, Figure S8), which points to a sizable increase in local molecular plasticity upon removal of the N terminus.

In summary, we have shown that the N terminus of functional hVDAC1 in a lipid environment assumes a well-defined rigid conformation, in agreement with the crystal structure of mVDAC1. Changes in the β barrel upon truncation of the N terminus as observed herein may explain why N-terminally truncated hVDAC1 exhibits lower conductance than the full-length channel.[10–12] Further experiments will be needed to delineate the exact nature of the conformational rearrangement involved. However, since the N terminus appears to play a role in stabilizing the β barrel, its removal could lead to a reduced pore diameter. It is tempting to speculate that such conformational changes could also play a role in VDAC1 voltage gating, as postulated by earlier models.[7–9] Our data for the first time provide structural evidence that the conformation of the barrel can indeed be influenced by the N terminus.

Experimental Section

hVDAC1 was expressed, refolded, and purified according to the procedure described in reference [17]. For solid-state NMR measurements, the protein was reconstituted into DMPC, DOPC, or DOPE liposomes at a protein/lipid ratio of 1:50 (mol/mol). Solid-state NMR experiments were conducted using 3.2 mm or 4 mm triple-resonance (^1H,^{13}C,^{15}N) magic-angle spinning (MAS) probeheads at static magnetic fields of 18.8 T and 20.0 T (Bruker Biospin, Karlsruhe, Germany). Sample temperatures were +5 °C for ^{13}C–^{13}C correlation experiments and +5 °C or −15 °C for ^{15}N–^{13}C spectra. Initial cross-polarization (CP) times were set to 600 μs for ^1H–^{13}C and 400 μs for ^1H–^{15}N transfers. Typical proton field strength for 90° pulses and SPINAL64[18] decoupling was 83 kHz. ^{13}C–^{13}C mixing was accomplished by proton-driven spin diffusion (PDSD) for 15 ms, 150 ms, and 300 ms to obtain intra-residue, sequential, and long-range correlations, respectively. At a static field of 20.0 T, a MAS frequency of 10.6 kHz was used, except for 150 ms PDSD mixing, where weak coupling conditions[19] (PDSD-WC) were set by using a MAS frequency of 13.28 kHz. ^{13}C–^{13}C DREAM[20] correlation spectra were recorded at 18 kHz MAS, using a tangential ramp of 2.5 ms duration. ^{15}N–^{13}C correlation experiments involved 1.5 to 3.5 ms of SPECIFIC-CP[21] transfer. For obtaining side-chain correlations, subsequent homonuclear ^{13}C–^{13}C DARR[22] mixing of 20 to 50 ms was used.

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