Published in final edited form as:
J Am Chem Soc. 2009 December 16; 131(49): 17777–17779. doi:10.1021/ja907918r.

Structural and functional characterization of the integral membrane protein VDAC-1 in lipid bilayer nanodiscs

Thomas Raschle[†], **Sebastian Hiller**[†], **Tsyr-Yan Yu**[†], **Amanda J. Rice**[‡], **Thomas Walz**[‡,§], and **Gerhard Wagner**[*,†]

[†] Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Ave., Boston, MA 02115, USA

[‡] Department of Cell Biology, Harvard Medical School, 240 Longwood Ave., Boston, MA 02115, USA

[§] Howard Hughes Medical Institute, Harvard Medical School, 240 Longwood Ave., Boston, MA 02115, USA

Abstract

Biophysical studies of membrane proteins are often impeded by the requirement for a membrane mimicking environment. Detergent micelles are the most common choice, but the denaturing properties make them unsatisfactory for studies of many membrane proteins and their interactions. In the present work, we explore phospholipid bilayer nanodiscs as membrane mimics and employ electron microscopy and solution NMR spectroscopy to characterize the structure and function of the human voltage dependent anion channel (VDAC-1) as an example of a polytopic integral membrane protein. Electron microscopy reveals the formation of VDAC-1 multimers, an observation that is consistent with results obtained in native mitochondrial outer membranes. High-resolution NMR spectroscopy demonstrates a well folded VDAC-1 protein and native NADH binding functionality. The observed chemical shift changes upon addition of the native ligand NADH to nanodisc-embedded VDAC-1 resemble those of micelle-embedded VDAC-1, indicating a similar structure and function in the two membrane-mimicking environments. Overall, the ability to study integral membrane proteins at atomic resolution with solution NMR in phospholipid bilayers, rather than in detergent micelles, offers exciting novel possibilities to approach the biophysical properties of membrane proteins under non-denaturing conditions, which makes this technology particular suitable for protein–protein interactions and other functional studies.

Membrane proteins are responsible for a wide range of essential physiological processes and are involved in various diseases. Most biophysical methods for studying membrane proteins *in vitro* require a membrane mimicking environment to stabilize the protein.[1] The most commonly used membrane mimics are detergents, but since they often have deteriorating effects on the structure and activity of a protein, the search for a suitable detergent is an empirical and thus time-consuming process. A better mimic are phospholipid bilayers, since their biophysical properties resemble more closely those of the native membrane and they are thus more likely to maintain membrane proteins in a stable and active state. Phospholipid bilayers in the form of bicelles have been used to study membrane proteins by high resolution biophysical methods such as NMR spectroscopy[2] and x-ray crystallography.[3] However, the morphology of bicelles is complex, depending on various sample conditions, such as

gerhard_wagner@hms.harvard.edu.

Supporting Information Available: Experimental procedures and data on the purification, refolding and reconstitution of VDAC-1 into nanodiscs. This material is available free of charge via the Internet at http://pubs.acs.org.

temperature and the ratio of long-chain lipid to short-chain lipid/detergent.[4] Furthermore, the selection of phospholipid/detergent combinations is limited and the presence of detergent may destabilize a membrane protein.

A promising new approach for studies of membrane proteins in a phospholipid bilayer uses high-density lipoprotein nanodiscs consisting of a central disk-shaped core of lipids, confined by two copies of an α-helical amphipathic protein.[5–7] These nanoscale lipid bilayers with reported diameters ranging from 9.5 to 12 nm provide a well-defined particle size and high stability in detergent-free, aqueous solution. The nanodisc technology has enabled biochemical studies of a number of membrane proteins, including cytochrome P450 monooxygenase,[8,9] bacteriorhodopsin,[10] rhodopsin,[11,12] the β2-adrenergic receptor,[13,14] the chemoreceptor Tar,[15] the glycolipid receptor G_{M1},[16] the membrane-active peptide antiamoebin-I,[17] and the membrane-spanning fragment of human CD4,[18] and it has also been used for the cell-free protein synthesis of membrane proteins.[19]

The use of atomic resolution biophysical methods is crucial for advancing our understanding of membrane protein structures and functions. Solution-state NMR has proven a valuable tool for investigating membrane proteins in a detergent solubilized form [20], and is expected to offer novel possibilities if combined with the nanodisc technology. The application of solution NMR for studying nanodisc-embedded proteins has so far been limited to a membrane-active peptide[17] and the single-helix membrane-spanning fragment of human CD4,[18] a coreceptor of the T cell receptor. So far, no polytopic integral membrane protein has been studied by solution NMR in a nanodisc-embedded form.

Here we describe an effort to fully exploit the opportunities offered by the nanodisc technology in combination with solution NMR spectroscopy to investigate structure and function of a polytopic integral membrane protein. Furthermore, we would like to address the fundamental question whether membrane proteins adopt different conformations in micellar and phospholipid environments. In particular, we characterize structural and functional properties of the human mitochondrial voltage-dependent anion channel (VDAC-1) reconstituted into nanoscale lipid bilayers by solution NMR. The structure of this protein has recently been determined in three independent studies by NMR and X-ray crystallography using detergent micelles[21,22] and bicelles[23] as membrane mimics. All three studies revealed nearly identical folds with a 19-stranded β-barrel enclosing an N-terminal α-helix. VDAC-1 forms a voltage-gated channel, allowing the exchange of small molecules and ions across the outer mitochondrial membrane.[24] VDAC-1 is also a key player in mitochondrial apoptotic pathways by means of its interaction with members of the Bcl-2 protein family.[25–27]

VDAC-1 was expressed, purified and refolded into lauryldimethylamineoxide (LDAO) micelles as described[21,25] and then incorporated into 1,2-dimyristoyl-sn-glycero-3-phospho-choline (DMPC) nanoscale lipid bilayers;[6] see Supporting Information for details. The ratio of lipid to membrane scaffolding protein (MSP1) was optimized by analytical size exclusion chromatography to account for the displacement of lipid molecules by VDAC-1. A 1:4:200 ratio for VDAC-1:MSP1:DMPC lipid was found to yield the highest fraction of assembled nanodiscs, as monitored by size exclusion chromatography and SDS-PAGE analysis of the peak fractions (Figure S1). The refolding detergent LDAO was completely removed from the reconstituted VDAC-1 sample by treatment with Bio-Beads, as confirmed by the absence of LDAO signals in the 1D ^{1}H-NMR spectrum (Figure S2). Since VDAC-1 cannot stably fold in aqueous solution and since no LDAO molecules are present, all VDAC-1 molecules remaining in solution must have been transferred into the nanodiscs. His-tagged VDAC-1 in nanodiscs was separated from VDAC-1–free nanodiscs using immobilized metal affinity chromatography.

To assess the integrity and the oligomeric state of VDAC-1 reconstituted into nanodiscs, we acquired electron microscopy (EM) images of negatively stained samples. The images revealed nanodiscs containing one or more dark spots (Figure 1A). These spots were of relatively uniform size with a diameter of about 20 Å. This value is consistent with the open inner diameter of the VDAC-1 pore of 20–25 Å as determined by NMR and X-ray crystallography.[21–23] Since the EM sample was produced from highly purified components the spots are likely to represent stain-filled channels of the reconstituted VDAC-1 molecules. We selected 9243 nanodiscs from 55 images and classified them into 100 classes (Supplementary Figure S3). The resulting class averages showed that the nanodiscs have a variable size and shape. Many had an oval shape with dimensions of ~10 × 13 nm, but others were larger and/or had more irregular shapes (Figure 1B, classes 1–4).

The number of VDAC-1 pores per nanodisc particle varied. A large fraction of the averages showed nanodiscs with two VDAC-1 molecules (Figure 1B, classes 5–12) or possibly even more additional VDAC-1 molecules (Figure 1B, classes 13–16). These additional VDAC-1 channels were either clearly visible (Figure 1B, classes 5–8 and 13–14) or less well defined (Figure 1B, classes 9–12 and 15–16). The weaker definition of some of the additional VDAC-1 channels may result from poor stain penetration or from slight differences in the VDAC-1 distribution in the averaged nanodiscs. Despite these ambiguities, we attempted to estimate the distribution of the number of VDAC-1 channels in the nanodisc population. We assigned each average into one of three classes with “two or less”, “exactly two” or “two or more” pores visible in a nanodisc (indicated by ≤2, 2 and ≥2 in Supplementary Figure S3). According to this classification, 4635 (~50%), 3250 (~35%), and 1358 (~15%) particles were found to have “two or less”, “exactly two” and “two or more” VDAC-1 channels per nanodiscs, respectively. The observation of different oligomeric states for VDAC-1 is in agreement with biochemical[25,28] and imaging studies.[29,30] In native membranes, VDAC-1 has been observed to adopt multimeric states from monomer to tetramer and hexamer, but no preferred oligomeric state has been noticed.[29] It has been speculated that the dynamic nature of the oligomeric state of VDAC-1 might modulate its function, such as its ability to form complexes with other proteins.[25]

In averaged images with two clearly revealed pores, the center-to-center distance between neighboring VDAC-1 channels was very similar (46 ± 4 Å, n = 28). This value closely relates to the outer diameter of the VDAC-1 channel of 45 Å as observed in the high resolution NMR- or X-ray structures[21–23] and thus suggests that the two molecules may be interacting with each other. Note, however, that the limited structural detail of the averaged images cannot resolve the site-specificity of this putative interaction.

To further characterize the state of VDAC-1 in nanodiscs 2D [^{15}N,^{1}H]-TROSY NMR spectra[31] were recorded at 750 MHz (Figure 2). The resonances are well dispersed, indicating the formation of extensive secondary structure. The qualitative comparison with the 2D [^{15}N,^{1}H]-TROSY spectra of detergent solubilized VDAC-1 shows a similar pattern of well-dispersed resonances in both spectra. These observations suggest that VDAC-1 in DMPC nanodiscs is well folded and the overall fold might be similar to VDAC-1 in detergent micelles; however, a definite structural characterization will require at least backbone assignments. The high quality of VDAC-1 spectra in nanodiscs shows the feasibility of a resonance assignment and structural studies by solution NMR.

The resonances of VDAC-1 in nanodiscs display slightly broader line widths in the proton dimension when compared to the protein–LDAO complex. The rotational correlation time, τ_c, of VDAC-1 in nanodiscs was estimated with the TRACT experiment[32] (Figure S4). The value of 93 ± 15 ns corresponds roughly to an overall molecular weight of 160–200 kDa, and

is in good agreement with the theoretical τ_c of 85 ns calculated for an empty nanodisc particle.[17] For comparison, the τ_c of VDAC-1 in LDAO micelles is about 70 ns.[33]

The activity of VDAC-1 reconstituted into nanodiscs was assessed by binding of the small ligand nicotinamide adenine dinucleotide (NADH). NADH is known to regulate the gating function for metabolites in the mitochondrial outer membrane by binding to VDAC-1.[34] NMR chemical shift perturbation mapping for VDAC-1 in LDAO has located the NADH-binding site at strands 17 and 18, comprising the segment of residues 235–245.[21] This experimental approach was employed here to evaluate the functionality of nanodisc-embedded VDAC-1. 2D [^{15}N,^{1}H]-TROSY correlation spectra were recorded before and after addition of NADH (Figure 3). The observation of several distinct chemical shift changes clearly indicates site-specific binding of NADH, and thus indicates that VDAC-1 in nanodiscs is functional. In addition, the positions of some of the affected resonances in nanodisc–VDAC-1 can be roughly correlated with assigned resonances that are affected upon binding of NADH in the detergent solubilized form of VDAC-1 (Figures 3). It thus seems that NADH binds similarly indicating that the VDAC-1 fold is preserved in both environments. VDAC-1weakly binds cholesterol in micelles[21]. To analyze similar small chemical shift changes in nanodiscs and generally pursue more detailed ligand binding studies will require sequence-specific resonance assignments of VDAC-1 in nanodiscs.

It has remained elusive as to what extent the 3D structure of VDAC-1 in detergent micelles represents the physiologically active structure found in biological membranes. The current study further corroborates the physiological relevance of the published micelle-bound VDAC-1 structure by the high similarity of the NMR spectra in micelles and DMPC nanodiscs, and by the similarity of NADH binding properties. Furthermore, the observation of multimeric assemblies of VDAC-1 in nanodiscs by EM indicates that the nanoscale lipid bilayers provide a native-like membrane environment. Additional evidence has also been gained by the independent determination of an essentially identical structure by x-ray crystallography in bicelles.[23]

In summary, we have demonstrated that the human integral membrane protein VDAC-1 can be embedded into lipid bilayer nanodiscs in a folded and functional form. Electron microscopy revealed that nanodiscs can harbor between one and three copies of VDAC-1. The current data do not define whether there are well-defined oligomeric states or whether VDAC-1 associates in a random orientation. Furthermore, the ability to study VDAC-1 in lipid bilayers at atomic resolution with solution NMR, rather than in detergent micelles, offers novel possibilities to approach its biophysical properties: The absence of detergent will enable protein–protein interaction studies between VDAC-1 and its soluble interaction partners; this is crucial for cases where the soluble protein would be denatured by the detergent when using micelle-embedded protein. Of particular interest are the highly relevant associations with members of the Bcl-2 family, including Bcl-x_L 25,27, Bax and Bak,[26] as well as hexokinase.[35] Furthermore, the effects of different lipids and lipid compositions on structure and function of the VDAC-1 protein can now be investigated.

In a broader context, we anticipate the nanodisc technology to provide a more general system for the study of high molecular weight integral membrane proteins by solution NMR spectroscopy. Whereas for smaller systems, such as peptides, the size difference between the micelle system and the nanodisc complex are dramatic, for integral membrane proteins of the size of VDAC, the effective molecular size and thus the NMR relaxation properties are similar. Moreover, for even larger membrane proteins, the nanodiscs might even be advantageous compared to detergent micelles, since the effective molecular weight is dominated by the size of the nanodisc particle and thus largely independent of the molecular weight of the membrane protein under study. With the additional benefit that the nanodiscs provide a substantially more

native-like environment than micelles, they represent a highly valuable tool for functional studies with solution NMR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Bernd W. Koenig and Julian M. Glück are kindly acknowledged for providing the plasmid for expression of the Apolipoprotein. This work was supported by NIH Grants GM075879, GM047467 and EB002026. T.R. and S.H. were supported in part by the Swiss National Science Foundation and T.R. also by the Roche Research Foundation. T.W. is an investigator of the Howard Hughes Medical Institute.

References

1. Seddon AM, Curnow P, Booth PJ. Biochim Biophys Acta 2004;1666:105–17. [PubMed: 15519311]
2. Prosser RS, Evanics F, Kitevski JL, Al-Abdul-Wahid MS. Biochemistry 2006;45:8453–65. [PubMed: 16834319]
3. Caffrey M. J Struct Biol 2003;142:108–32. [PubMed: 12718924]
4. Marcotte I, Auger M. Concepts Magn Reson Part A 2005;24A:17–37.
5. Jonas A. Methods Enzymol 1986;128:553–82. [PubMed: 3724523]
6. Bayburt TH, Grinkova YV, Sligar SG. Nano Lett 2002;2:853–856.
7. Denisov IG, Grinkova YV, Lazarides AA, Sligar SG. J Am Chem Soc 2004;126:3477–87. [PubMed: 15025475]
8. Kijac AZ, Li Y, Sligar SG, Rienstra CM. Biochemistry 2007;46:13696–703. [PubMed: 17985934]
9. Bayburt TH, Carlson JW, Sligar SG. J Struct Biol 1998;123:37–44. [PubMed: 9774543]
10. Bayburt TH, Grinkova YV, Sligar SG. Arch Biochem Biophys 2006;450:215–22. [PubMed: 16620766]
11. Bayburt TH, Leitz AJ, Xie G, Oprian DD, Sligar SG. J Biol Chem 2007;282:14875–81. [PubMed: 17395586]
12. Whorton MR, Jastrzebska B, Park PS, Fotiadis D, Engel A, Palczewski K, Sunahara RK. J Biol Chem 2008;283:4387–94. [PubMed: 18033822]
13. Leitz AJ, Bayburt TH, Barnakov AN, Springer BA, Sligar SG. Biotechniques 2006;40:601–2. 604, 606. [PubMed: 16708760]
14. Whorton MR, Bokoch MP, Rasmussen SG, Huang B, Zare RN, Kobilka B, Sunahara RK. Proc Natl Acad Sci U S A 2007;104:7682–7. [PubMed: 17452637]
15. Boldog T, Grimme S, Li M, Sligar SG, Hazelbauer GL. Proc Natl Acad Sci U S A 2006;103:11509–14. [PubMed: 16864771]
16. Borch J, Torta F, Sligar SG, Roepstorff P. Anal Chem 2008;80:6245–52. [PubMed: 18616345]
17. Lyukmanova EN, Shenkarev ZO, Paramonov AS, Sobol AG, Ovchinnikova TV, Chupin VV, Kirpichnikov MP, Blommers MJ, Arseniev AS. J Am Chem Soc 2008;130:2140–1. [PubMed: 18229924]
18. Gluck JM, Wittlich M, Feuerstein S, Hoffmann S, Willbold D, Koenig BW. J Am Chem Soc. 2009
19. Katzen F, Fletcher JE, Yang JP, Kang D, Peterson TC, Cappuccio JA, Blanchette CD, Sulchek T, Chromy BA, Hoeprich PD, Coleman MA, Kudlicki W. J Proteome Res 2008;7:3535–42. [PubMed: 18557639]
20. Sanders CR, Sonnichsen F. Magn Reson Chem 2006;44:S24–40. [PubMed: 16826539]Spec No
21. Hiller S, Garces RG, Malia TJ, Orekhov VY, Colombini M, Wagner G. Science 2008;321:1206–10. [PubMed: 18755977]
22. Bayrhuber M, Meins T, Habeck M, Becker S, Giller K, Villinger S, Vonrhein C, Griesinger C, Zweckstetter M, Zeth K. Proc Natl Acad Sci U S A 2008;105:15370–5. [PubMed: 18832158]

23. Ujwal R, Cascio D, Colletier JP, Faham S, Zhang J, Toro L, Ping P, Abramson J. Proc Natl Acad Sci U S A 2008;105:17742–7. [PubMed: 18988731]
24. Lemasters JJ, Holmuhamedov E. Biochim Biophys Acta 2006;1762:181–90. [PubMed: 16307870]
25. Malia TJ, Wagner G. Biochemistry 2007;46:514–25. [PubMed: 17209561]
26. Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ. Science 2003;301:513–7. [PubMed: 12881569]
27. Shimizu S, Narita M, Tsujimoto Y. Nature 1999;399:483–7. [PubMed: 10365962]
28. Zalk R, Israelson A, Garty ES, Azoulay-Zohar H, Shoshan-Barmatz V. Biochem J 2005;386:73–83. [PubMed: 15456403]
29. Hoogenboom BW, Suda K, Engel A, Fotiadis D. J Mol Biol 2007;370:246–55. [PubMed: 17524423]
30. Goncalves RP, Buzhynskyy N, Prima V, Sturgis JN, Scheuring S. J Mol Biol 2007;369:413–8. [PubMed: 17439818]
31. Pervushin K, Riek R, Wider G, Wuthrich K. Proc Natl Acad Sci U S A 1997;94:12366–71. [PubMed: 9356455]
32. Lee D, Hilty C, Wider G, Wuthrich K. J Magn Reson 2006;178:72–6. [PubMed: 16188473]
33. Hiller S, Ibraghimov I, Wagner G, Orekhov VY. J Am Chem Soc 2009;131:12970–8. [PubMed: 19737017]
34. Zizi M, Forte M, Blachly-Dyson E, Colombini M. J Biol Chem 1994;269:1614–6. [PubMed: 7507479]
35. Linden M, Gellerfors P, Nelson BD. FEBS Lett 1982;141:189–92. [PubMed: 6178620]

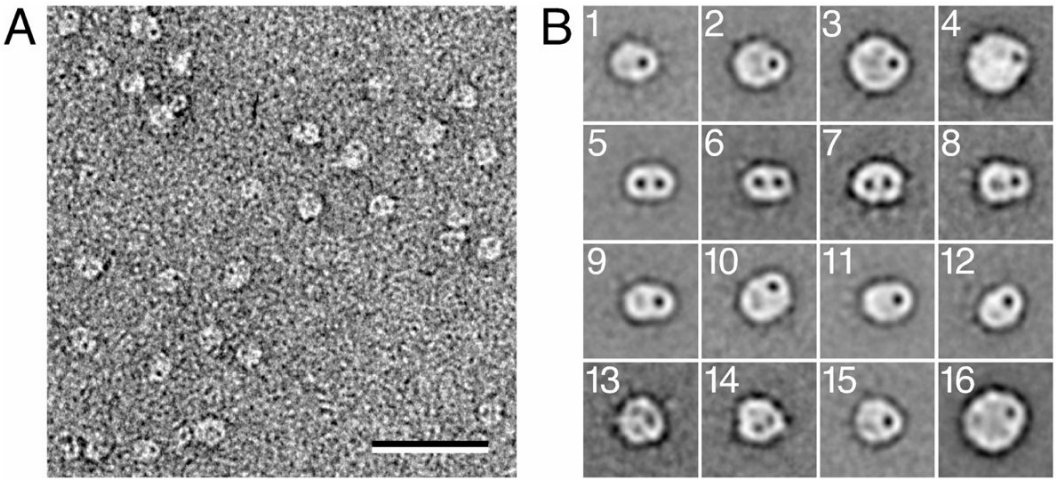

Figure 1.

Electron microscopy of VDAC-1 in DMPC nanodiscs. (A) Representative image of negatively stained nanodiscs containing VDAC channels. The stain-filled channels appear as dark spots in the brighter nanodiscs. The scale bar is 50 nm. (B) Representative class averages showing the variation of the nanodisc population in size (averages 1 to 4) and in the number of VDAC channels per nanodisc (averages 5 to 16). The side length of the individual panels is 28.8 nm.

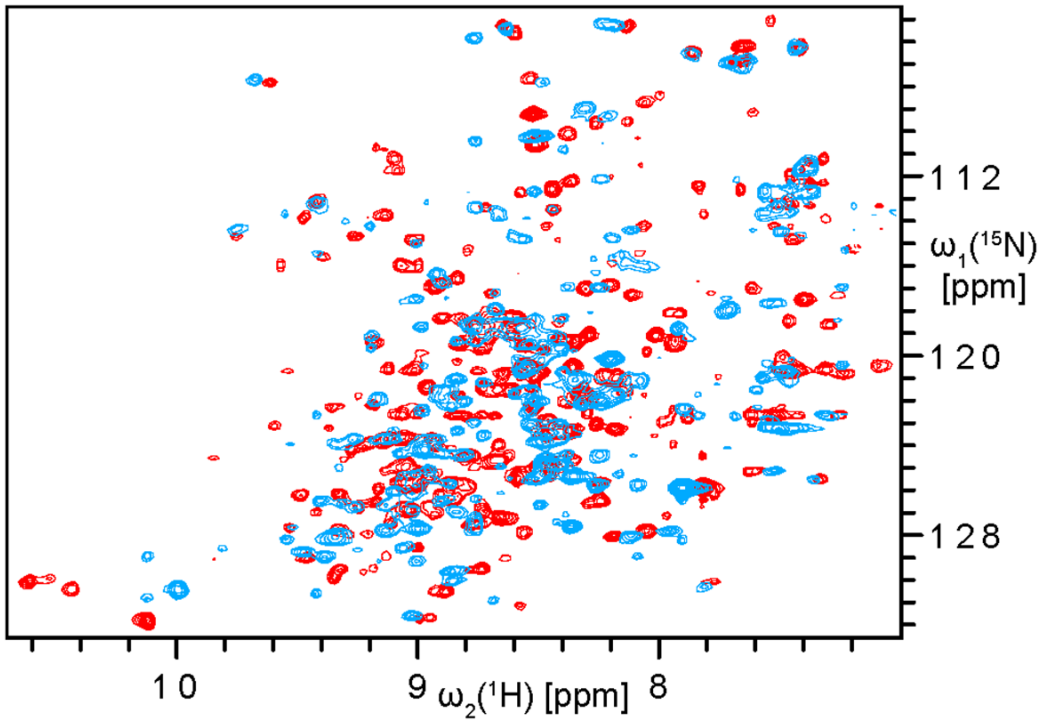

Figure 2.
2D [^{15}N,^{1}H]-TROSY spectrum of [*U*-^{2}H,^{15}N]-VDAC-1 in DMPC nanodiscs (blue) and in LDAO micelles (red).

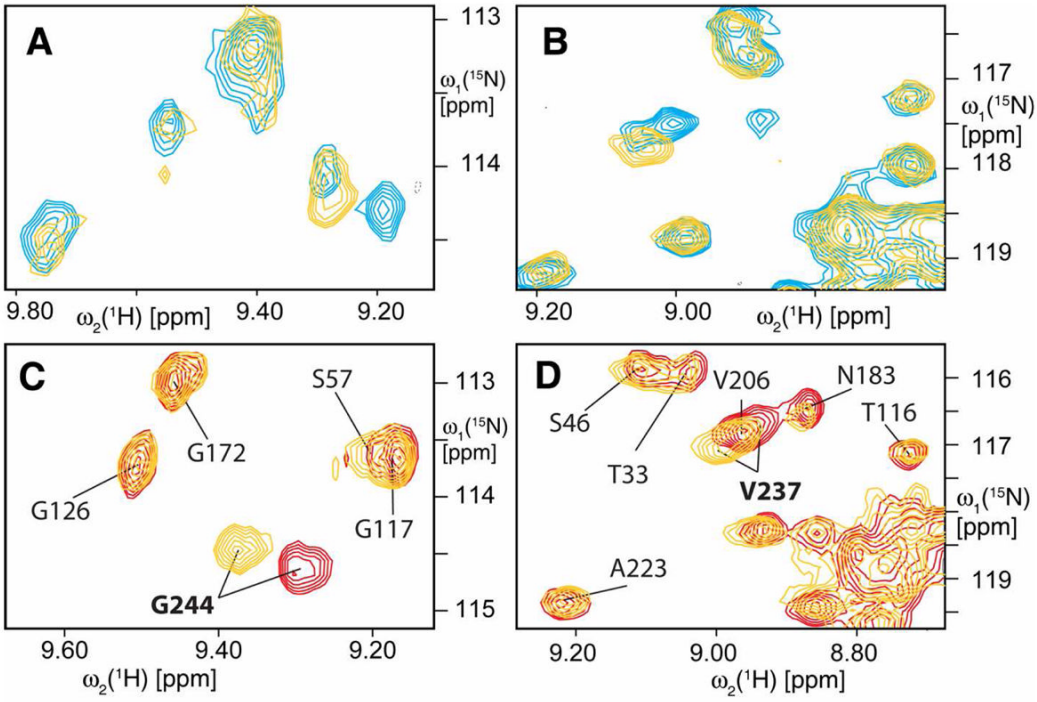

Figure 3.

NADH binding of VDAC-1. Selected regions of 2D [^{15}N,^{1}H]-TROSY spectra of VDAC-1 in (A,B) nanodiscs and (C,D) LDAO micelles. The blue and red spectra correspond to Figure 2, recorded in the absence of NADH. The orange spectra were recorded after addition of NADH. Sequence-specific resonance assignments of VDAC-1 in LDAO micelles are indicated. Residues G244 and V237, which are part of the NADH binding site, are printed bold.