Cation Control in Functional Helical Programming: Structures of a D,L-Peptide Ion Channel**

Hans-Dieter Arndt, Dirk Bockelmann, Andrea Knoll, Stefanie Lamberth, Christian Griesinger,* and Ulrich Koert*

The controlled folding of molecules into helical structures is a very important means to assemble functional entities. Helices can carry and process information (DNA double helix),[1] provide the basis for rigid materials (collagen triple helix),[2] can be used for transport purposes (single-stranded β-helix of D,L-peptides),[3] or for arranging photo-, electro-, or transport-active groups[4] in 3D space. The design of synthetic compounds with a predictable folding behavior (programmed folding) is actively pursued.[5] However, most of the known examples of helical folding remain on the structural level.

Our interest in functional folding of synthetic ion channels[6] led us to explore D,L-peptides, which we used as structural templates for ether–δ-peptide strands to yield ion channels with novel functions.[7] The pivotal D,L-peptide is the gramicidin A (gA) ion channel (Figure 1a).[3] gA itself can adopt a variety of conformations depending on the environment. In organic solvents, gA predominantly forms dimeric double helices, which vary in handedness and dimerization topology.[8] The binding of cations can force this double helix to increase its diameter.[9] Only in membranelike environments will gA form a unique head-to-head associated dimer of two right-handed, single-stranded β-helices.[10] This conformation, with 6.3 residues per turn, is generally regarded to be the "active" ion-channel structure,[11a–c] but as it has never been observed in isotropic solution or by X-ray crystallography, its nature has remained a source of debate.[11d]

We found that the fully synthetic 22mer "minigramicidin" **1** (Figure 1a) acts as an ion channel in phospholipid bilayers (Figure 1b). A remarkable dependence of the channel activity on the membrane thickness was observed,[12a] and preliminary

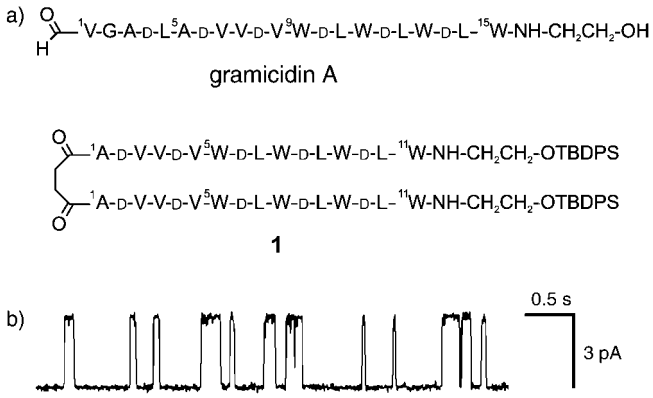

Figure 1. a) Primary sequence of **1** and gA; b) representative current trace of **1** in planar lipid bilayers (1M CsCl, 120 mV, soy-bean lecithin). Consult Supporting Information for details.

investigations indicated a more confined conformational space for **1** than for gA.[12b] With the goal of a detailed understanding of the ion-channel-active conformation of **1**, we focused on structural investigations in the absence and presence of Cs^+ ions.

Compound **1** features the terminal 11mer domain of gA, covalently dimerized by use of succinic acid in a head-to-head fashion. This linkage avoids the lateral dissociation equilibrium,[12b] which complicates structural studies of gA.[13,14] The *tert*-butyldiphenylsilyl (TBDPS) capping groups in **1** enhance its solubility in organic solvents and facilitate the structural studies. Single-channel current measurements in planar phospholipid bilayers revealed that **1** forms a highly active Cs^+-ion channel ($[\mathbf{1}] = 10^{-14}$ M in the aqueous bath solution, see Supporting Information). Only one type of channel is observed, which indicates a single channel-active conformation in the membrane (Figure 1b).

The structure of **1** in the absence of metal ions was determined in [D_6]benzene/[D_6]acetone 10:1 and $CDCl_3$/[D_3]MeOH 1:1 (v/v) by using NMR spectroscopy. Both solvent mixtures mimic membranes in their polarity.[15] The same highly symmetric structure **A** was found in either case; this peptide dimer forms a left-handed double-β-helix with 5.7 residues per turn and a length of approximately 38 Å (Figure 2) with the succinate linker moiety in an antiperiplanar conformation. This type of secondary structure is adopted by gA only in the presence of $CaCl_2$.[8g]

The entire structure transforms into a new species upon addition of Cs^+ salts. After saturation with CsI, peptide **1** adopted the monomeric structure **B** in $CDCl_3$/[D_3]MeOH 1:1 or 3:1 (v/v, Figure 3). Each 11mer fragment forms a right-handed β-helix with approximately 6.3 residues per turn. The succinate moiety joins the two fragments symmetrically (C_2 axis) in an anticlinal fashion (mean structure) to yield a single helix with a total length of about 17 Å.

Structure **B** has all features of the presumed gA channel conformation—in contrast to the double β-helical structure **A** without Cs^+. The pore diameter rises from 1.2 Å in the dimer **A** to 4.5 Å in the monomeric $\beta^{6.3}$ helix **B**, wide enough to host Cs^+ ions. Structure **B** forms a continuous channel like the head-to-head dimer of gramicidin A in membranelike environments.[11] The amide carbonyl groups of the anticlinal

[*] Prof. Dr. C. Griesinger, Dipl.-Chem. D. Bockelmann, Dr. S. Lamberth
Max-Planck Institut für Biophysikalische Chemie
Am Fassberg 11, 37077 Göttingen (Germany)
Fax: (+49) 551-201-2201
E-mail: cigr@nmr.mpibpc.mpg.de
Prof. Dr. U. Koert
Philipps-Universität Marburg
Fachbereich Chemie
Hans-Meerwein-Strasse, 35032 Marburg/Lahn (Germany)
Fax: (+49) 6421-282-5677
E-mail: koert@chemie.uni-marburg.de
Dr. H.-D. Arndt, Dr. A. Knoll
Humboldt-Universität zu Berlin
Institut für Chemie
Brook-Taylor-Strasse 2, 12489 Berlin (Germany)

[**] This work was supported by the Volkswagen Foundation, the Max-Planck-Gesellschaft, the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and the Pinguin Foundation. We thank Dr. Clemens Mügge for NMR spectroscopic support as well as Dipl.-Chem. Jochen Pfeifer and MSc Loay Al-Momani for additional CD spectroscopic measurements.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

  0044-8249/02/4121-4062 $ 20.00+.50/0

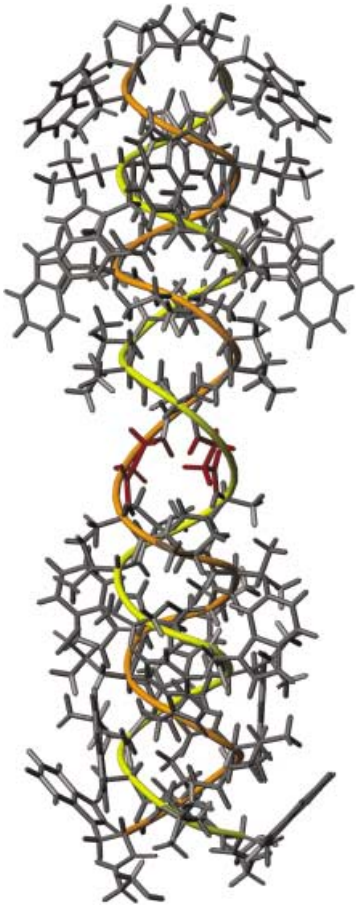

Figure 2. Averaged mean structure **A** (PDB = protein database accession code 1 KQE; calculated from the sixteen energetically favored structures) of **1** in [D_6]benzene/[D_6]acetone 10:1. The TBDPS protecting groups were omitted from the structure determination because of overlapping signals. The linker region is colored in red. NMR spectra were acquired at 600 MHz (Bruker AMX). Assignments were obtained with the program Felix 2000 (Accelrys, San Diego, CA) by a combination of DQF–COSY and TOCSY experiments according to standard procedures.[16] H–H distance restraints were extracted from NOESY spectra at 150 and 300 ms. 688 NOE cross-peaks (330 intraresidual, 138 sequential, and 220 medium-range) were used. For all amino acid residues $^3J(H^N,H^\alpha)$ coupling constants could be measured. They are all in the range typical for a β-sheet. The NOE restraints and the H^N,H^α couplings were used in a simulated annealing protocol[17] with the program X-PLOR[18] to calculate sixteen structures that have a root-mean-square deviation (rmsd) of the backbone positions of 0.61 Å.

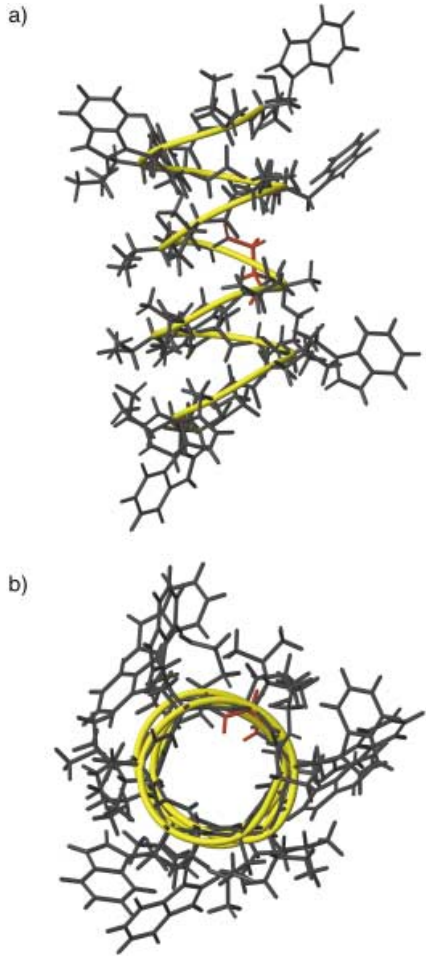

Figure 3. Averaged mean structure **B** of **1** in $CDCl_3$/[D_3]MeOH 3:1 saturated with CsI, a) side view, b) top view. The structure was determined in the same way as described for **2**. In this case, 213 NOE cross-peaks (97 intraresidual, 51 sequential, and 65 medium-range) were used. The rmsd of the backbone positions of the ten best structures was 0.91 Å.

succinate linker fit well into the overall pattern of the channel carbonyl groups, completing the coordination sphere of the Cs^+ ions which are putatively bound in symmetrical positions.[19]

The conformational switch from **A** to **B** was monitored by NMR spectroscopic titration in $CDCl_3$/[D_3]MeOH 1:1 with CsI (Figure 4a, see Supporting Information). A nonlinear least-squares fit for several scenarios (1–2 ions, no aggregation, dimer, trimer, or tetramer) revealed that only the scenario shown in Figure 4b could reproduce the data well: The dimer **A** dissociates into a monomeric state **C** first (K_D), which takes up two Cs^+ ions consecutively (K_1, K_2). It was not possible to extract a value for K_D reliably from the data, because K_D and K_1 are strongly coupled. With an approximated value of $K_D = 0.00126\,M^{0.5}$ (98% dimer at $[Cs^+]=0$), binding constants of $K_1 = (63 \pm 5) \times 10^3\,M^{-1}$ and $K_2 = (36 \pm 20)\,M^{-1}$ were obtained (see Supporting Information). The much stronger binding of the first Cs^+ ion ($\Delta G_1 = -28\,kJ\,mol^{-1}$) than the second one ($\Delta G_2 = -9\,kJ\,mol^{-1}$) indicates that the conformational reorganization of the entire structure on binding of the first Cs^+ ion is less energetically costly than the coulomb repulsion encountered on the second binding event.

To relate these findings further to gA,[8f,12b] circular dichroism (CD) spectra of these secondary structures were recorded (Figure 5). In organic solvents such as CH_3CN or $C_2H_4Cl_2$/MeOH, compound **1** shows two *negative* Cotton effects at $\lambda =$ 228 and 208 nm (Figure 5a, spectra c and d),[12b] in full accordance with the left-handed double-*β*-helical structure **A**.[12b,20] The Cs^+ complexes **B** in $C_2H_4Cl_2$/MeOH or pure MeOH instead display a single *positive* Cotton effect at $\lambda =$ 230 nm (Figure 5a; spectra a and b), which indicates right-handedness of the β-helix. This spectrum differs significantly from the spectrum of **1** in 1,2-di-myristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) vesicles,[20] which nearly coincides with that of gA (Figure 5b). The latter spectrum is normally assigned to the right-handed, single-stranded $\beta^{6.3}$-

  0044-8249/02/4121-4063 $ 20.00+.50/0

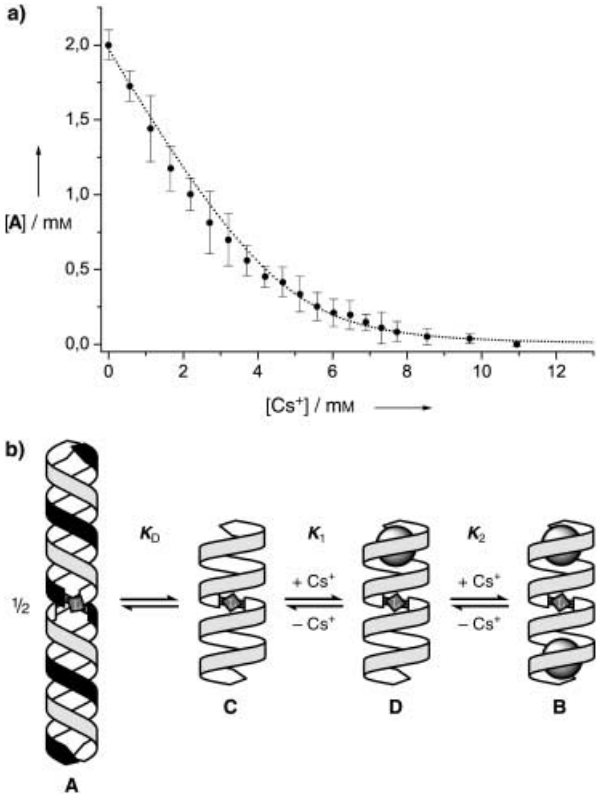

Figure 4. a) NMR spectroscopic titration of **1** with CsI in $CDCl_3$/$[D_3]$MeOH 1:1, dotted line refers to the fitting; b) schematic representation of the equilibra involved in the conformational transition from **A** to **B** via the putative monomer **C** and the 1:1 complex **D** upon addition of Cs^+ ions.

helix,[8f] which we found for the Cs^+ complex **B** in organic solvents. The origin of the different spectra remains unclear at this stage. We reason, however, that the differences in the CD spectra report subtle changes in local conformations of the gramicidines, which do not affect the global structure (to be published elsewhere).

To correlate the Cs^+ binding found for **1** in organic solvents with the ion-transport process, we studied the dependence of the channel conductance Λ on the concentration of Cs^+ ions in the solution (Figure 6a).[21] The data displayed three remarkable features: 1) Conductance can be measured at very low concentrations of Cs^+ ions, 2) the curve is supralinear (shows an over-proportional increase) in the medium-concentration range (10–100 mM) and 3) a maximum is observed at approximately 1.5 M. These features indicate a multiple-binding-site transport process.[21b] A kinetic rate model with two symmetrical binding sites and four states[22] reproduces the data well (Figure 6b). The apparent rate constants thus obtained ($\pm 30\,\%$) are $A = 5.2 \times 10^8\,\mathrm{L\,mol^{-1}\,s^{-1}}$, $B = 4.9 \times 10^6\,\mathrm{s^{-1}}$, $K = 5.1 \times 10^7\,\mathrm{s^{-1}}$, $D = 4.4 \times 10^7\,\mathrm{L\,mol^{-1}\,s^{-1}}$, and $E = 5.4 \times 10^7\,\mathrm{s^{-1}}$. Clearly, a doubly occupied state becomes transiently populated during the ion-transport process mediated by minigramicidin **1** at higher concentrations of Cs^+ ions.

In summary, the present study shows that the minigramicidin **1** forms a distinct double-helical rod **A** in media of low polarity. Its disaggregation into the channel-active conformation is triggered by membrane insertion or by Cs^+-ion binding. The latter allowed us to study the right-handed β-helix of the D,L-peptides in solution for the first time. The structure **B** probably reflects the ion-channel-active conformation of **1** in the membrane—occupied by two Cs^+ ions.

Received: January 9, 2002
Revised: July 29, 2002 [Z18500]

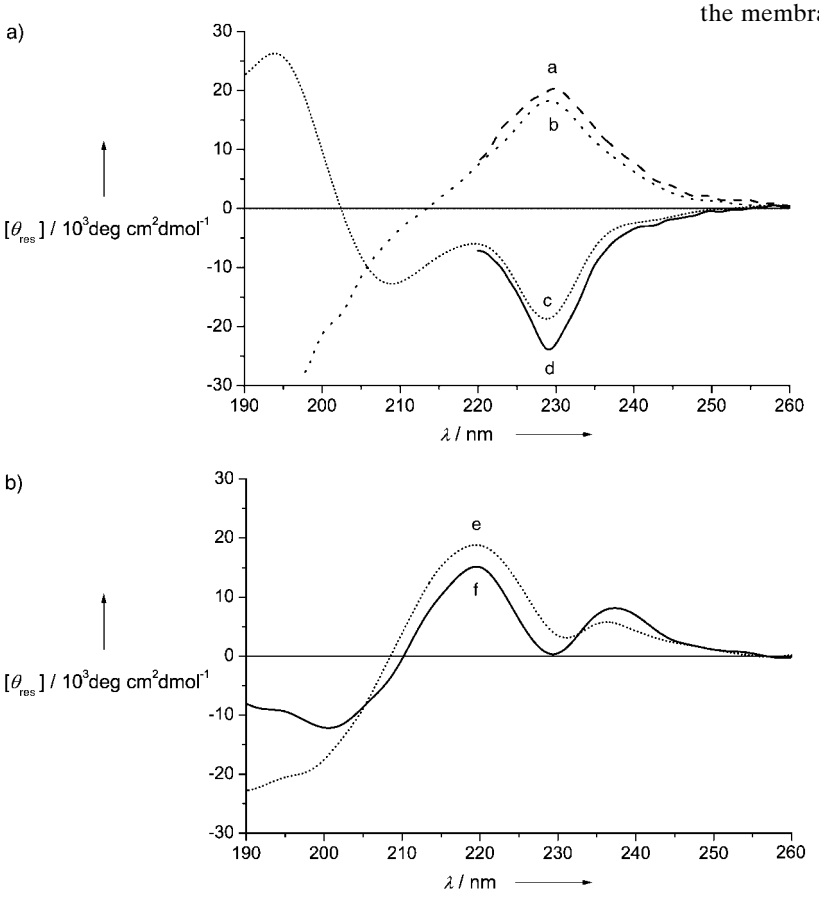

Figure 5. a) CD measurements of **1** in organic solvents ($T = 22\,^\circ\mathrm{C}$, $[\mathbf{1}] = 10^{-5}$ M): Spectrum a: $C_2H_4Cl_2$/MeOH 1:1, saturated with CsCl (– – –); b: 50 mM CsCl in MeOH (- - - -); c: CH_3CN (••••); d: $C_2H_4Cl_2$/MeOH 1:1 (——); b) CD measurements in DMPC vesicles[20] ($T = 22\,^\circ\mathrm{C}$, [gA] or $[\mathbf{1}] = 10^{-5}$ M): spectrum e: gA (••••); f: **1** (——). The shape of the spectra a and d was unchanged at $[\mathbf{1}^+] = 10^{-3}$ M (not shown). $[\theta_{res}]$ = mean molar ellipticity per residue.

  0044-8249/02/4121-4064 $ 20.00+.50/0

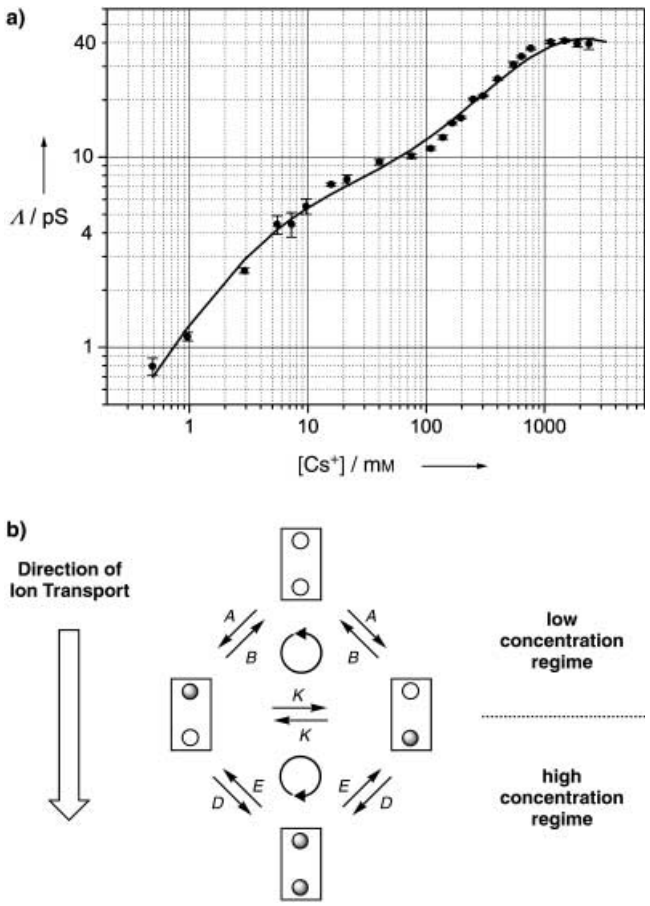

Figure 6. a) Concentration dependence of the channel Cs^+-ion conductance of **1** at $T = 22\,^{\circ}C$ in soybean lecithin ($n > 50$ in each case); b) kinetic rate model (two binding sites—four states) used to model the ion transport through the channel **1**.[22]

[1] *Nucleic Acids in Chemistry and Biology*, 2nd ed. (Eds.: G. M. Blackburn, M. J. Gait), Oxford University Press, Oxford, **1996**.

[2] D. R. Eyre, *Science* **1980**, *207*, 1315 – 1322.

[3] *Gramicidin And Related Ion Channel-Forming Peptides* (Eds.: D. J. Chadwick, G. Cardew), Wiley, Chichester, **1999**b) (Novartis Foundation Symposium 225); R. E. Koeppe II, O. S. Andersen, *Annu. Rev. Biophys. Biomol. Struct.* **1996**, *25*, 231 – 258; c) P. Läuger, *Angew. Chem.* **1985**, *97*, 939 – 959; *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 905 – 925.

[4] a) T. Katz, *Angew. Chem.* **2000**, *112*, 1997 – 1999; *Angew. Chem. Int. Ed.* **2000**, *39*, 1921 – 1923; b) J.-C. Meillon, N. Voyer, *Angew. Chem.* **1997**, *109*, 1004 – 1006; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 967 – 969; c) U. Koert, M. M. Harding, J.-M. Lehn, *Nature* **1990**, *346*, 339 – 342.

[5] a) D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, 2015 – 2022; b) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173 – 180; c) V. Berl, I. Huc, R. G. Khoury, M. J. Krische, J.-M. Lehn, *Nature* **2000**, *407*, 720 – 723; reviews: d) A. E. Rowan, R. J. M. Nolte, *Angew. Chem.* **1998**, *110*, 65; *Angew. Chem. Int. Ed.* **1998**, *37*, 63; e) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219 – 3232; f) D. Seebach, M. Albert, P. I. Arvidsson, M. Rueping, J. V. Schreiber, *Chimia* **2001**, *55*, 345 – 353.

[6] Reviews: a) Y. Kobuke in *Advances in Supramolecular Chemistry, Vol. 4* (Ed.: G. W. Gokel), JAI, Greenwich, **1997**, pp. 163 – 210; b) N. Voyer, *Top. Curr. Chem.* **1996**, *184*, 1 – 37; c) G. W. Gokel, O. Murillo, *Acc. Chem. Res.* **1996**, *29*, 425 – 432; recent contributions: d) B. Baumeister, N. Sakai, S. Matile, *Angew. Chem.* **2000**, *112*, 2031 – 2034; *Angew. Chem. Int. Ed.* **2000**, *39*, 1955 – 1958; e) N. Yoshino, A. Satake, Y. Kobuke, *Angew. Chem.* **2001**, *113*, 471 – 473; *Angew. Chem. Int. Ed.* **2001**, *40*, 457 – 459, and citations in ref. [7b]

[7] a) H.-D. Arndt, A. Knoll, U. Koert, *Angew. Chem.* **2001**, *113*, 2137 – 2140; *Angew. Chem. Int. Ed.* **2001**, *40*, 2076 – 2078; b) A. Schrey, A. Vescovi, A. Knoll, C. Rickert, U. Koert, *Angew. Chem.* **2000**, *112*, 928 – 931; *Angew. Chem. Int. Ed.* **2000**, *39*, 900 – 902.

[8] a) W. R. Veatch, E. T. Fossel, E. R. Blout, *Biochemistry* **1974**, *13*, 5249 – 5257; b) V. F. Bystrov, A. S. Arseniev, *Tetrahedron* **1988**, *44*, 925 – 949; c) D. A. Langs, *Science* **1988**, *241*, 182 – 187; d) Z. Zhang, S. M. Pascal, T. A. Cross, *Biochemistry* **1992**, *31*, 8822 – 8828; e) S. M. Pascal, T. A. Cross, *J. Mol. Biol.* **1992**, *226*, 1101 – 1109; f) S. M. Pascal, T. A. Cross, *J. Biomol. NMR* **1993**, *3*, 495 – 513; g) Y. Chen, A. Tucker, B. A. Wallace, *J. Mol. Biol.* **1996**, *264*, 757 – 769; h) D. A. Doyle, B. A. Wallace, *J. Mol. Biol.* **1997**, *266*, 963 – 977.

[9] a) A. S. Arseniev, I. L. Barsukov, V. F. Bystrov, *FEBS Lett.* **1985**, *180*, 33 – 39; b) A. N. Nekrasov, A. V. Stepanov, V. P. Timofeev, *FEBS Lett.* **1995**, *371*, 35 – 38; c) V. F. Bystrov, A. S. Arseniev, I. L. Barsukov, A. L. Lomize, *Bull. Magn. Reson.* **1987**, *8*, 84 – 94.

[10] a) A. S. Arseniev, I. L. Barsukov, V. F. Bystrov, A. L. Lomize, Y. A. Ovchinnikov, *FEBS Lett.* **1985**, *186*, 168 – 174; b) R. R. Ketchem, W. Hu, T. A. Cross, *Science* **1993**, *261*, 1457 – 1460; c) F. Tian, T. A. Cross, *J. Mol. Biol.* **1999**, *285*, 1993 – 2003; d) R. Fu, M. Cotton, T. A. Cross, *J. Biomol. NMR* **2000**, *16*, 261 – 268.

[11] a) F. Kovacs, J. Quine, T. A. Cross, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 7910 – 7915; b) O. S. Andersen, H.-J. Apell, E. Bamberg, D. D. Busath, R. E. Koeppe II, F. J. Sigworth, G. Szabo, D. W. Urry, A. Woolley, *Nat. Struct. Biol.* **1999**, *6*, 609; c) T. A. Cross, A. S. Arseniev, B. A. Cornell, J. H. Davis, J. A. Kilian, R. E. Koeppe II, L. K. Nicholson, F. Separovic, B. A. Wallace, *Nat. Struct. Biol.* **1999**, *6*, 610 – 611; d) B. M. Burkhart, W. L. Duax, *Nat. Struct. Biol.* **1999**, *6*, 611 – 612.

[12] a) H.-D. Arndt, A. Knoll, U. Koert, *ChemBioChem* **2001**, *221*–223; b) H.-D. Arndt, A. Vescovi, A. Schrey, J. R. Pfeifer, U. Koert, *Tetrahedron* **2002**, *58*, 2789 – 2801.

[13] a) M. C. Goodall, *Arch. Biochem. Biophys.* **1973**, *157*, 514; b) D. W. Urry, C. M. Venkatachalam, A. Spisni, R. J. Bradley, T. L. Trapane, K. U. Prasad, *J. Membr. Biol.* **1980**, *55*, 29 – 51; c) C. J. Stankovic, S. H. Heinemann, J. M. Delfino, F. J. Sigworth, S. L. Schreiber, *Science* **1989**, *244*, 813 – 817; d) D. Lelievre, Y. Trudelle, F. Heitz, G. Spach, *Int. J. Pept. Protein Res.* **1989**, *33*, 379 – 385; e) C. J. Stankovic, S. H. Heinemann, S. L. Schreiber, *J. Am. Chem. Soc.* **1990**, *112*, 3702 – 3704; f) S. Crouzy, T. B. Woolf, B. Roux, *Biophys. J.* **1994**, *67*, 1370 – 1386.

[14] I. L. Barsukov, A. L. Lomize, A. S. Arseniev, V. F. Bystrov, *Biol. Membr. (USSR)* **1987**, *4*, 171 – 193.

[15] R. Gratias, H. Kessler, *J. Phys. Chem. B* **1998**, *102*, 2027 – 2031.

[16] K. Wüthrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, **1986**.

[17] M. Nilges, S. I. O'Donoghue, *Prog. Nucl. Magn. Reson. Spectrosc.* **1998**, *32*, 107 – 139.

[18] A. T. Brünger, *X-PLOR: A System for X-ray Crystallography and NMR*, Yale University Press, New Haven, **1992**.

[19] The symmetrical structure **B** indicates an even number of bound Cs^+ ions. The NMR spectroscopic titration of **1** with CsI suggests that two Cs^+ ions are present, as does the ESI mass spectrum of the complex, which is dominated by the $[\mathbf{1}+2\,Cs]^{2+}$ ion peak. Presumably, the Cs^+ ions reside in the respective upper and lower thirds of the channel pore. But as the positions of the Cs^+ ions in the complex could not be deduced from the present NMR spectroscopic data, they were not taken into account during the calculation of structure **B**.

[20] a) J. A. Killian, K. U. Prasad, D. Hains, D. W. Urry, *Biochemistry* **1988**, 27, 4848 – 4855; b) D. V. Greathouse, J. F. Hinton, K. S. Kim, R. E. Koeppe II, *Biochemistry* **1994**, *33*, 4291 – 4299.

[21] a) B. W. Urban, S. B. Hladky, D. A. Haydon, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1978**, *37*, 2628 – 2632; b) B. Hille, *Ionic Channels of Excitable Membranes*, 2nd ed., Sinauer, Sunderland, **1992**.

[22] S. B. Hladky, D. A. Haydon in *Current Topics in Membranes and Transport, Vol. 21* (Ed.: F. Bronner), Academic Press, New York, **1984**, pp. 327 – 372.

  0044-8249/02/4121-4065 $ 20.00+.50/0