J|A|C|S
COMMUNICATIONS

Published on Web 01/26/2002

Recognition of Polarized Lipid Bilayers by *p*-Oligophenyl Ion Channels: From Push−Pull Rods to Push−Pull Barrels

Naomi Sakai* and Stefan Matile*

Department of Organic Chemistry, University of Geneva, CH-1211 Geneva, Switzerland

Received November 9, 2001

The rational design of synthetic ion channels has seen remarkable progress during the last two decades.[1] The construction of synthetic ion channels that act selectively, however, remains a central challenge in bioorganic chemistry. Recognition of bilayer membranes by specific characteristics such as thickness,[2] charge,[3] specific components,[4] and potential[5] is an aspect of ion-channel selectivity with medicinal relevance. To elucidate the importance of dipole-potential interactions in the recognition of highly polarized membranes (e.g., bacterial plasma membranes) by electronically asymmetric "rods" (e.g., α-helical peptides), we have recently developed a strategy to turn the axial dipole of rigid *p*-octiphenyl scaffolds "on" and "off" without major changes in global structure.[6] Although the expected increase in activity was observed with push−pull ionophores **1** and **2** in polarized bilayer membranes, their selectivity and, in particular, their activity were quite modest (Figure 1). In a different line of research, we found that *p*-octiphenyls equipped with eight lateral peptide strands of the sequence *G*LKL-NH_2 can self-assemble into hexameric rigid-rod β-barrels **3** with high ion-channel activity.[7] In this report, we unify the activity of synthetic rigid-rod β-barrels and the selectivity of push−pull rods in push−pull β-barrel $\mathbf{4}^{\uparrow\uparrow\uparrow\uparrow}$.

Push−pull hexa(*G*LKL-NH_2)-*p*-octiphenyl **4** was synthesized from hexa(*GO*^{t}Bu)-*p*-sexiphenyl **5** (Scheme 1).[6a] Suzuki-coupling with methyl ether **6** and methyl sulfide **7** gave *p*-octiphenyls **8**, **9** and **10**. The axial dipole in rod **9** was "turned on" by oxidation of the sulfide π donor into the sulfone π acceptor in **11**. After deprotection, lateral peptide strands were introduced by the coupling with tripeptide **12**,[7a] and removal of the Boc groups in *p*-octiphenyl **13** provided the desired push−pull rod **4**. The symmetric push−push rod **14** was prepared from *p*-octiphenyl **10** along the same route.[6b]

Activity and selectivity of push−pull rod **4** were assessed with small unilamellar vesicles (SUVs) composed of fresh egg yolk phosphatidylcholine (EYPC). In the employed assay, changes in transmembrane pH gradient and potential were simultaneously monitored by entrapped pH-sensitive fluorophore HPTS, and potential-sensitive dye safranin O, respectively (Figure 2a).[6a] An inside negative Nernst potential was applied by addition of potassium carrier valinomycin to vesicles with a transmembrane potassium concentration gradient and quantified by comparing the emission intensity of safranin O with calibration curves (see Supporting Information). Application of a pH gradient by extravesicular addition of base completed preparation of the system for the evaluation of push−pull rod **4**.

Figure 2b shows the differential changes in HPTS emission after addition of push−pull rod **4** to polarized spherical bilayers. The molecular origin of these changes (i.e., HPTS efflux, ion selectivity, etc.) was not determined; absence of lysis was, however, demonstrated by the unchanged membrane potential (see Supporting Information). The activity of push−pull rod **4** in the presence of increasingly polarized membranes exhibited an exponential increase (Figure 2c, ●). Application of eq 1[8]

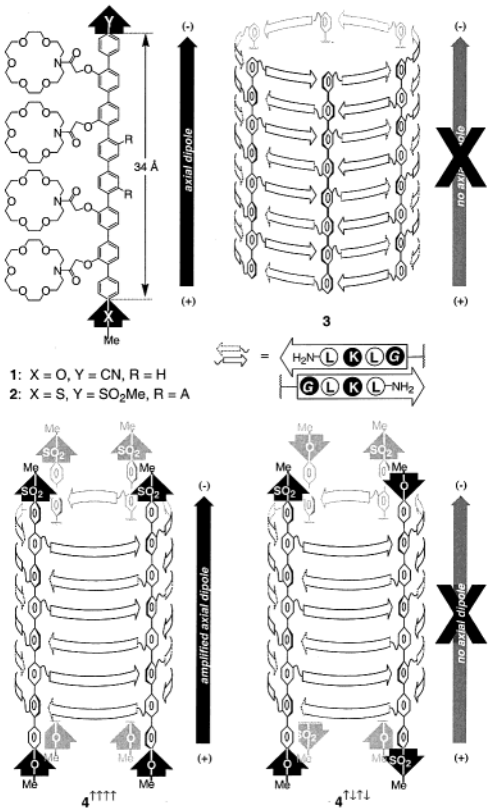


Figure 1. Push−pull rods**1** and **2**, rigid-rod β-barrel **3**, and parallel/antiparallel push−pull β-barrels $\mathbf{4}^{\uparrow\uparrow\uparrow\uparrow}$/$\mathbf{4}^{\uparrow\uparrow\downarrow\downarrow}$. β-Sheets are given as arrows pointing to the C terminus, amino acid residues (one-letter abbreviation, *G* = −OCH_2CO−) pointing toward the barrel exterior are black on white, internal residues white on black.

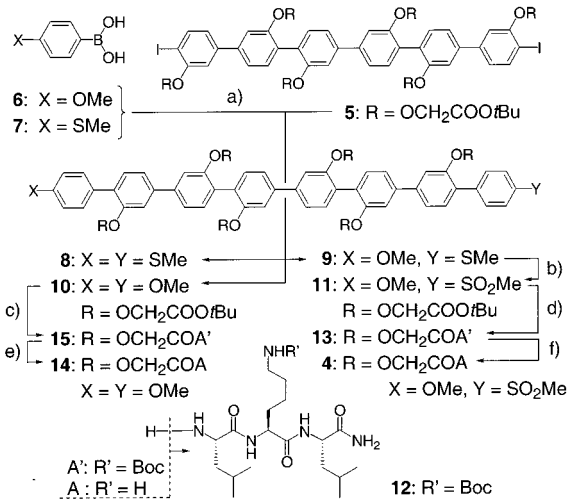
$$G_{vd} = g_0 \exp(z_g eE/kT) \qquad (1)$$

where G_{vd} is the observed activity, *e* the elementary charge, *k* the Boltzmann constant, and *T* the absolute temperature, gave a gating charge $z_g = 0.85$/channel for push−pull rod **4**. A gating charge $z_g = 1.2$/channel reported for melittin[8b,c] suggested that cell membrane recognition by push−pull rods may compare well with that by α-helix bundles which comprise invariable axial dipoles due to uniform orientation of the backbone amides.[5]

This substantial potential dependence was consistent with quantitative amplification of the axial dipole of push−pull rod **4** by self-assembly into parallel push−pull β-barrel $\mathbf{4}^{\uparrow\uparrow\uparrow\uparrow}$ in the presence of polarized bilayer membranes (Figure 1). Self-assembly into

10.1021/ja017497c CCC: $22.00

Scheme 1 [a]



[a] (a) $Pd(PPh)_3$, Na_2CO_3, 24% (**8**), 51% (**9**), 20% (**10**). (b) 1. *m*CPBA, 2. $KMnO_4$, $MgSO_4$, 65%. (c) 1. TFA, 2. **12**, HBTU, TEA, 47%. (d) 1. TFA, 2. **12**, HBTU, TEA, 58%. (e) TFA, 94%. (f) TFA, 78%.

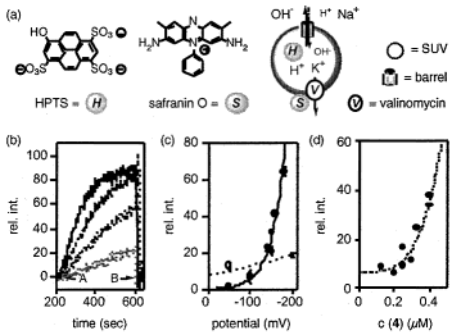


Figure 2. Activity of *p*-octiphenyl **4** in polarized EYPC−SUVs. (a) Schematic presentation of the assay (see text). (b) Representative differential changes in fluorescence intensity I of HPTS (λ_{em} = 510 nm, λ_{ex} = 450/405 nm) within EYPC-SUVs (pH 6.4, inside: 10 mM $K_mH_nPO_4$, 100 mM KCl, 90 μM HPTS; outside: n/10 mM $K_mH_nPO_4$, 10 − n/10 mM $Na_mH_nPO_4$, n mM KCl, 100 − n mM NaCl, n = 2.02 (−100 mV), 0.425 (−140 mV), 0.195 (−160 mV), 0.09 (−180 mV)) as a function of time during addition of safranin O (6 nM), NaOH (5 mM), valinomycin (0.6 μM), **4** (A; 1 μM) and excess melittin (B; I_∞). Curves are obtained at (from top to bottom) −180, −160, −140, and −100 mV. (c) Dependence of activity (e.g., % emission change 100 sec after rod addition) of **4** (1 μM, ●) and **14** (1 μM, ○) on membrane potential with exponential (solid) and linear (dotted) curve fit. (d) Dependence of activity on the concentration of **4** at E = −180 mV. Experimental details: see Supporting Information.

antiparallel tetramers $\mathbf{4}^{\uparrow\downarrow\uparrow\downarrow}$ without axial dipole, expected to be favored in isotropic media, can thus be excluded in polarized membranes. The near independence of the activity of push−push rod **14** on membrane polarization confirmed that the dipole moments of peptide backbone amides are canceled out in rigid-rod β-barrels as expected for β-sheet conformation (Figure 2c, ○).

Formation of a tetrameric supramolecule $\mathbf{4}^{\uparrow\uparrow\uparrow\uparrow}$ was indicated at −180 mV by the nonlinear dependence of activity on the concentration of monomeric push−pull rod **4** (Figure 2d). The best curve fit to eq 2[2a,3a,4a]

$$k_{obs} = k_0 + k_{int}\,[\text{monomer}]^n/K_D \qquad (2)$$

where K_D is the dissociation constant, k_{obs} the observed transport rate, k_0 the rate without pore, and k_{int} an intrinsic rate constant, suggested that n = 3.9 ± 1.6 monomers **4** are required for thermodynamically unfavorable self-assembly of an active pore $\mathbf{4}^n$. Since push−pull rod **2** exhibited linear concentration dependence[6a] in agreement with a monomeric active structure, it was not possible to compare the activity of tetramer $\mathbf{4}^{\uparrow\uparrow\uparrow\uparrow}$ and monomer **2** quantitatively. The different concentrations needed to detect substantial activity, i.e., 100 nM *tetramer* $\mathbf{4}^{\uparrow\uparrow\uparrow\uparrow}$ (Figure 2d) versus 10 μM monomer **2**, however, may illustrate an increase that extends clearly beyond 2 orders of magnitude.

In summary, we show that push−pull β-barrels recognize polarized bilayer membranes at nanomolar concentrations with a gating charge of z_g = 0.85 per channel. Together with fourth-order dependence of activity on monomer concentration, these findings identify parallel self-assembly in polarized membranes as a powerful strategy to amplify the selectivity and activity of ion channels formed by rigid push−pull rods. Comprehensive, ongoing studies on structure and function of push−pull β-barrels in planar and spherical bilayer membranes and in solution support the conclusions made in this communication and will be reported in due course.

Acknowledgment. We thank A. Pinto, J.-P. Saulnier, and the group of F. Gülaçar for NMR and MS, respectively, and the Swiss NSF (21-57059.99 and 2000-064818.01) for financial support.

Supporting Information Available: Preparation of new compounds and depolarization assays (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Recent reviews: (a) Gokel, G. W.; Mukhopadhyay, A. *Chem. Soc. Rev.* **2001**, *30*, 274−286. (b) Kirkovits, G. J.; Hall, C. D. *Adv. Supramol. Chem.* **2000**, *7*, 1−47.

(2) (a) Janout, V.; Staina, I. V.; Bandyopadhyay, P.; Regen, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 9926−9927. (b) Ghebremariam, B.; Sidorov, V.; Matile, S. *Tetrahedron Lett.* **1999**, *40*, 1445−1449.

(3) (a) Merritt, M.; Lanier, M.; Deng, G.; Regen, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 8494−8501. (b) Liu, D.; DeGrado, W. F. *J. Am. Chem. Soc.* **2001**, *123*, 7553−7559. (c) Porter, E. A.; Wang, X.; Lee, H.-S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565−565.

(4) (a) DiGiorgio, A. F.; Otto, S.; Bandyopadhyay, P.; Regen, S. L. *J. Am. Chem. Soc.* **2000**, 11029−11030. (b) Kakio, A.; Nishimoto, S. I.; Yanagisawa, K.; Kozutsumi, Y.; Matsuzaki, K. *J. Biol. Chem.* **2001**, *276*, 24985−24990. (c) Breukink, E.; Wiedemann, I.; van Kraaij, C.; Kuipers, O. P.; Sahl, H.; de Kruijff, B. *Science* **1999**, *286*, 2361−2364.

(5) (a) Review: Sakai, N.; Matile, S. *Chem. Eur. J.* **2000**, *6*, 1731−1737. (b) Recent publication: Goto, C.; Yamamura, M.; Satake, A.; Kobuke, Y. *J. Am. Chem. Soc.* **2001**, *123*, 12152−12159.

(6) (a) Sakai, N.; Gerard, D.; Matile, S. *J. Am. Chem. Soc.* **2001**, *123*, 2517−2524. (b) Push−push (**14**) rather than pull−pull rods were selected because of problematic properties of previous $1^4,8^4$-disulfone-*p*-octiphenyls; controls with pull−pull $1^4,8^4$-dicyano-*p*-octiphenyls have been reported.[5a,6a]

(7) (a) Baumeister, B.; Sakai, N.; Matile, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 1955−1958. (b) Sakai, N.; Baumeister, B.; Matile, S. *ChemBioChem* **2000**, *1*, 123−125.

(8) (a) Hille, B. *Ionic Channels of Excitable Membrane*, 2nd ed.; Sinauer: Sunderland, MA, 1992. (b) Pawlak, M.; Stankowski, S.; Schwarz, G. *Biochim. Biophys. Acta* **1991**, *1062*, 94−102. (c) $z_g \approx 4$ was reported for melittin in unbuffered solution: Tosteson, M. T.; Tosteson, D. C. *Biophys. J.* **1981**, *36*, 109−116.

JA017497C