Communication

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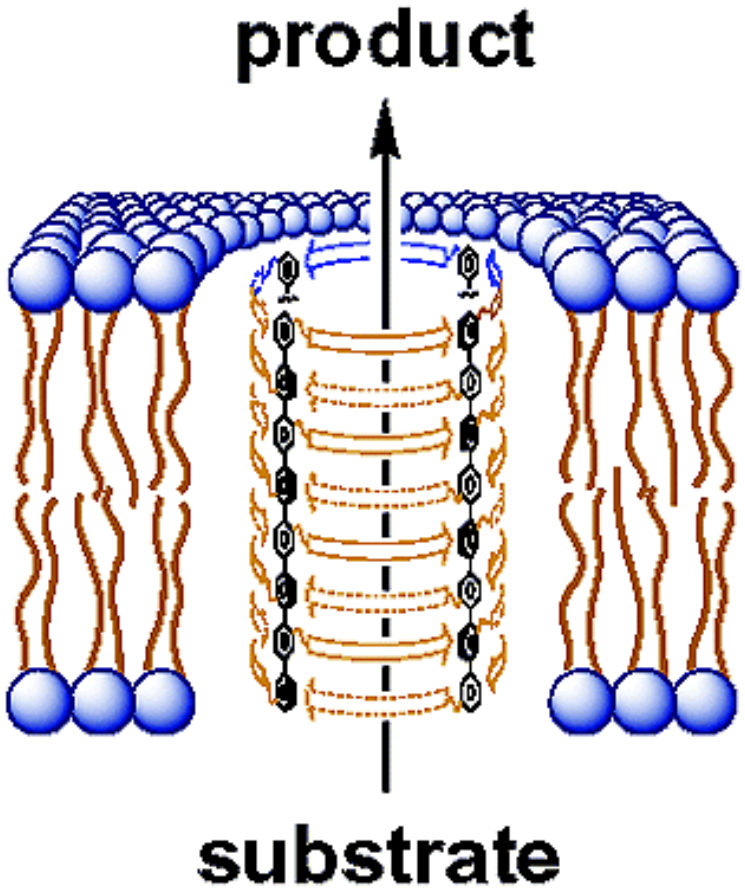

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Naomi Sakai,* Nathalie Sordé, and Stefan Matile*

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

Received December 20, 2002; E-mail: naomi.sakai@chiorg.unige.ch; stefan.matile@chiorg.unige.ch

In this communication, we report experimental evidence for the existence of synthetic catalytic pores (SCPs, Figure 1).[1,2] Substrate conversion during substrate translocation through a membrane-spanning SCPs has captivated our attention since the discovery of synthetic multifunctional pores (SMPs)[1] with esterase and ion channel activity.[2a] The scenario of substrate-loaded vesicles "spitting out" products formed on the way through an SCP was particularly attractive because it suggested that the rate of substrate binding (k_{on}) and substrate and/or product release (k_{off}) may be manipulated with, e.g., concentration gradients or membrane potentials.

To implement conditions appropriate to test this hypothesis, large unilamellar vesicles composed of egg yolk phosphatidylcholine (EYPC LUVs) were loaded with 8-acetoxypyrene-1,3,6-trisulfonate (AcPTS) as model substrate of choice.[2a−e] With the obtained EYPC-LUVs⊃AcPTS,[3,4] trans esterolysis[5] was initiated by extravesicular addition of **SCP$_1$**, a rigid-rod β-barrel with internal arginine-histidine dyads that forms highly stable, multifunctional ion channels.[6,7] Product formation after pore addition (Figure 2A, ii) was monitored continuously by following the increase in emission of product with time (Figure 2A, b).[2a,8,9] Nonlinear dependence of the initial velocity of product formation on substrate concentration (Figure 2B, ○) provided kinetic data for trans esterolysis in unpolarized spherical membranes (Table 1, entry 1).

Vesicle polarization was achieved by means of a potassium diffusion potential as described previously, and verified by an increase in emission of potential-sensitive Safranin O (Figure 2A, c, v).[10,11] Taking advantage of differences in the pH profiles of

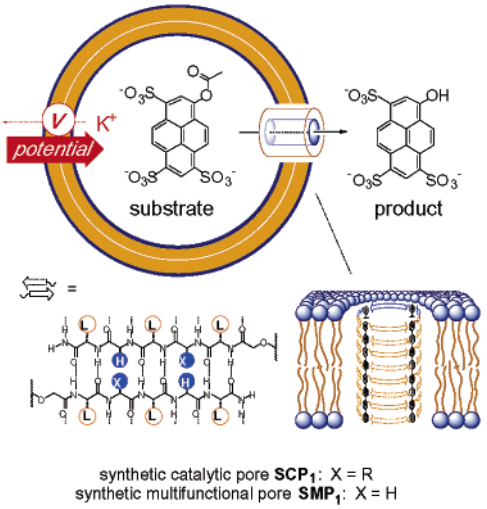

Figure 1. Trans catalysis in polarized membranes. The influence of inside-negative Nernst potentials (red) on the conversion of intravesicular anionic substrates (AcPTS) by externally added **SCP$_1$** into anionic products (HPTS) is assessed. **SCP$_1$**[6]/**SMP$_1$**[2a−e] in lipid bilayers (EYPC) are depicted in blue (hydrophilic), gold (hydrophobic), and black (rigid-rod and β-sheet scaffold), α-amino acid residues pointing exterior of the barrel are black on white, internal ones white on blue (single-letter abbreviations: L, Leu; H, His; R, Arg; *V*, valinomycin).

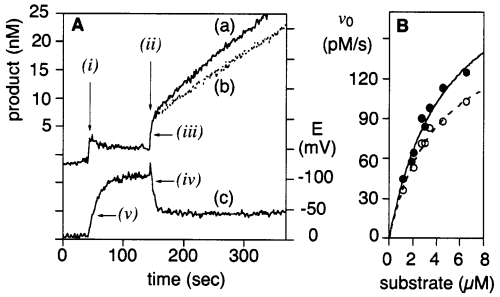

Figure 2. (A) Increase of product concentration with time after addition of (i) valinomycin and (ii) SCP$_1$ to EYPC-LUVs⊃AcPTS at (a) $E \approx -50$ mV and (b) $E = 0$ mV;[8] (c) membrane polarization during experiment a;[11] (iii−v): see text.[9,13] (B) Initial velocity of product formation as a function of substrate concentration at $E = 0$ mV (○) and $E \approx -50$ mV (●) with curve fit.

Table 1. Kinetic Data for Esterolysis of AcPTS by **SCP$_1$**

entry	cond.[a]	E (mV)[b]	K_M (μM)[c]	V_{max} (pM/s)[c]	f_{on}[d]	f_{off}[e]
1	trans	0	4.1 ± 0.7	170 ± 16[g]		
2	trans	−50	4.7 ± 1.0	222 ± 26[g]	1.10	1.30[h]
3	cis	0	6.1 ± 0.9[f]	202 ± 16[f]		
4	cis	−50	8.1 ± 1.9	213 ± 28	0.78	1.05

[a] Conditions: see text.[3−5,8] [b] Membrane potential. [c] Michaelis constant and maximal velocity calculated by fitting the experimental data to $v_0 = (V_{max} \times c_{AcPTS,total})/(K_M + c_{AcPTS,total})$. [d] $f_{on} = f_{off}\, K_M^{0\ mV}/K_M^{-50\ mV}$. [e] $f_{off} = V_{max}^{-50\ mV}/V_{max}^{0\ mV}$. [f] See ref 18. [g] Key data concerning the impact of polarization on trans catalysis. [h] Key data concerning eventual electrostatic steering (error ± 0.19).[15]

esterolysis (maximal at 5 < pH < 6), and pore formation (maximal at pH < 5) by **SCP$_1$**,[2a,c,12] it was possible to perform trans esterolysis in polarized membranes. The application of membrane potentials $E \approx -50$ mV[13] resulted in an increase of V_{max} beyond experimental error (+30%) together with an eventual minor increase of K_M (+15%, Table 1, entry 2 versus entry 1).[14]

One possible explanation of these changes is that inside-negative potentials guide internal, anionic substrates into the **SCP$_1$** and the anionic products into the external media.[15] Assuming a Briggs−Haldane mechanism,[16] factors $f_{on} = k_{on}^{-50mV}/k_{on}^{0mV}$ and $f_{off} = k_{off}^{-50mV}/k_{off}^{0mV}$ could be calculated (Table 1, entry 2).[3] The obtained values supported acceleration of substrate/product binding/release (i.e., $f_{off} \geq f_{on} \geq 1$) by constructive electrostatic steering in trans catalysis, with $f_{off} > 1$ as key factor beyond experimental error.[17]

The key observation in control experiments with cis esterolysis[3] was the absence of comparably strong changes upon polarization. This difference supported validity of the changes seen with trans esterolysis.[5,18] Within experimental error, v_0 decreased only slightly because K_M (+33%) increased more than V_{max} (+5%, Table 1, entry 4 versus entry 3). Hindered substrate binding by interfering electrostatic steering was one possible explanation for these less relevant changes ($f_{on} = 0.78$, Table 1, entry 4).[19,15] These complementary trends with cis and trans esterolysis in polarized membranes hinted at the attractive scope of a novel approach toward

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vectorial control of catalysis and, most importantly, corroborated the existence of catalytic pores.

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Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Synthetic catalytic pores (SCPs): pores constructed from abiotic scaffolds that catalyze substrate conversion *during* substrate translocation across the same pore; "synthetic multifunctional pores" (SMPs): pores constructed from abiotic scaffolds with additional function(s); all SCPs are SMPs, but not all SMPs are SCPs.

(2) Catalytic pores have not been reported previously. SMPs with ion channel and catalytic activity: (a) Baumeister, B.; Sakai, N.; Matile, S. *Org. Lett.* **2001**, *3*, 4229−4232. (b) Som, A.; Matile, S. *Eur. J. Org. Chem.* **2002**, 3874−3883. (c) Baumeister, B.; Som, A.; Das, G.; Sakai, N.; Vilbois, F.; Gerard, D.; Shahi, S. P.; Matile, S. *Helv. Chim. Acta* **2002**, *85*, 2740−2753. (d) Baumeister, B.; Matile, S. *Macromolecules* **2002**, *35*, 1549−1555. (e) Som, A.; Sakai, N.; Matile, S. *Bioorg. Med. Chem.* **2003**, *11*, 1363−1369. Molecular recognition within pores: (f) Bayley, H.; Cremer, P. S. *Nature* **2001**, *413*, 226−230. (g) Das, G.; Talukdar, P.; Matile, S. *Science* **2002**, *298*, 1600−1602. Covalent capture within pores: (h) Mindell, J. A.; Zhan, H.; Huynh, P. D.; Collier, R. J.; Finkelstein, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5272−5276. (i) Shin, S.; Luchian, T.; Cheley, S.; Braha, O.; Bayley, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3707−3709. Reactions within vesicles: (j) Walde, P.; Ichikawa, S. *Biomol. Eng.* **2001**, *18*, 143−177. Synthetic ion channels/pores: (k) Gokel, G. W.; Mukhopadhyay, A. *Chem. Soc. Rev.* **2001**, *30*, 274−286.

(3) See Supporting Information.

(4) Osmotic stress from intravesicular AcPTS was compensated by sucrose. EYPC-LUVs⊃AcPTS, trans catalysis, $E = 0$ mV: *Inside*; *x* mM AcPTS, 100−3.2 *x* mM sucrose, 100 mM KCl, 5 mM TES, pH 7.0, $x = 3-18$ mM, *outside*; 100 mM sucrose, 100 mM KCl, 10 mM MES, pH 5.5.[2a,c,5,8] EYPC-LUVs, cis catalysis, $E = 0$ mV: *Inside*; 100 mM KCl, 5 mM TES, pH 7.0, *outside*; 100 mM KCl, 10 mM MES, pH 5.5.[2a,c,5,8] For $E = -50$ mV: NaCl instead of KCl was used for outside buffer in cis and trans esterolysis.

(5) "Trans catalysis": addition of substrate (here: intravesicular) and catalyst (here: extravesicular) from opposite sides of the membrane; "cis catalysis": addition of substrate and catalyst from the same side of the membrane (here: extravesicular).[2c]

(6) (a) Sordé, N.; Matile, S. *J. Supramol. Chem.* **2003**. In press. (b) Sakai, N.; Sordé, N.; Das, G.; Perrottet, P.; Gerard, D.; Matile, S. *Org. Biomol. Chem.* **2003**, *1*, 1226−1231.

(7) As in previous reports on *p*-octiphenyl β-barrels, we reiterate that the depicted suprastructures (as in Figure 1) can be viewed as, at worst, a productive working hypothesis supported by all data on structure and function available today; all concentrations indicated for **SCP$_1$**/**SMP$_1$** refer to tetramers.

(8) Continuous detection of AcPTS esterolysis (representative original data: Figure 2A (a and b): Safranin O (60 nM) and AcPTS (0 μM for trans, 1−8 μM for cis esterolysis) were added to EYPC-LUVs⊃AcPTS (95 ± 15 μM PC, cis esterolysis: 0 mM AcPTS.; trans: 3−18 mM AcPTS) in a stirred and thermostated fluorescence cuvette (2 mL).[4] Changes in fluorescence intensity *I* of HPTS ($\lambda_{em} = 510$ nm, $\lambda_{ex} = 415.5$ nm) were then recorded as a function of time during addition of valinomycin (Figure 2A, i; for a: 600 nM, b: 0 nM) and **SCP$_1$** (Figure 2A, ii; 50 nM). Concentration of HPTS was determined from HPTS emission intensities using calibration curves.[3]

(9) The origin of initial "burst" in fluorescence emission of HPTS after the addition of **SCP$_1$** is unknown (Figure 2A, iii). However, clear dependence on substrate concentration suggested that these "bursts" do not originate from accumulation of reactive intermediates.[3]

(10) (a) Sakai, N.; Matile, S. *J. Am. Chem. Soc.* **2002**, *124*, 1184−1185. (b) Sakai, N.; Houdebert, D.; Matile, S. *Chem.—Eur. J.* **2003**, *9*, 223−232.

(11) Continuous detection of membrane potential (representative original data: Figure 2Ac): Fluorescence intensity *I* of Safranin O (*c*; $\lambda_{em} = 581$ nm, $\lambda_{ex} = 522$ nm) was followed as a function of time simultaneously to the continuous detection of trans AcPTS esterolysis in polarized LUVs (Figure 2A, a).[8] Potentials were quantified from Safranin O emission intensities using calibration curves.[3, 10, 13]

(12) Data not shown.

(13) We were unable to find conditions where an "initial burst" of depolarization from $E \approx -100$ mM to $E \approx -50$ mM can be avoided (Figure 2A, iv). This observation was rationalized by initially poor selectivity between AcPTS efflux and Na^+ influx (i.e., depolarization by compensating, valinomycin-mediated K^+ antiport) until each pore is loaded with anionic AcPTS blockers,[6b] "hopping" toward the exterior and inhibiting concurrent cation exchange. (Note that, given the near absence of anion/cation selectivity of **SCP$_1$** at pH 5.5,[6b] about 50% initial depolarization is consistent with this explanation.) Decrease of Safranin O emission to the original value for $E \approx 0$ mV upon addition of excess melittin confirmed existence of partial polarization ($E \approx -50$ mV, Figure 2A, c) during esterolysis (Figure 2Aa).

(14) Initial velocities of product formation were nearly identical to that at $E = 0$ mV (Table 1, entry 1) in absence of either valinomycin or K^+/Na^+ gradient.[3] The error level in Table 1 is affected by experimental inaccessibility of data points at high substrate concentration; clear differences in v_0 with/without polarization were reproducibly observed in trans catalysis with >3 μM substrate, measured always in parallel at fixed substrate concentration (Figure 2B, filled versus empty circles).[3]

(15) Origins of the voltage dependence of esterolysis other than electrostatic steering were considered. (a) Contributions from potential-induced asymmetric changes of the pK_a's of internal histidines:[2c] Relevance of this interesting potential expression of remote control in catalysis is not supported by ohmic ion-channel behavior,[6b] relatively flat pH profiles around pH 5.5, and increase (rather than decrease) of v_0 in trans catalysis upon polarization.[2a,c,12] (b) Changes in **SCP$_1$** concentration/ conformation upon polarization are not supported by potential-independence of membrane-bound-**SCP$_1$** concentration found by fluorescence depth quenching experiments[3] and ohmic ion-channel behavior.[6b] (c) Relevant contributions from substrate conversion in the external media are not supported by the found increase in V_{max} and K_M upon polarization (rather than unchanged V_{max} and reduced K_M expected for accelerated efflux, i.e., increased external substrate concentration, upon polarization) and experimental evidence for blockage of cation exchange[6b] during 1,3,6-pyrenetrisulfonate efflux.[2a,c,e] under relevant conditions.[2a,6b,12] (d) Exclusive substrate conversion in the internal media is excluded by the found dependence of trans catalysis on substrate concentration.

(16) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman and company: New York, 1985. Here, k_{cat} is assumed to be k_{off} of product, and effects of membrane polarization on k_{off} of substrate and product to be the same.[3]

(17) Electrostatic steering in enzymes, e.g.: Wade, R.; Gabdoulline, R. R.; Lüdemann, S. K.; Lounnas, V. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5942−5949.

(18) The influence of internal H→R mutation on catalyst characteristics was as follows (cis esterolysis, $E = 0$ mV):[a]

pore	k_{cat} [min^{-1}]	K_M[b] [μM]	$(k_{cat}/K_M)/k_{MeIm}$[c]	k_{cat}/k_{uncat}[d]	$(k_{cat}/K_M)/k_{uncat}$[d,e] [M^{-1}]
SMP$_1$	0.13[f]	0.7[f]	9.6×10^5 [f]	5.0×10^3	7.1×10^9
SCP$_1$	0.24	6.1	2.0×10^5	9.2×10^3	1.5×10^9

[a] Conditions, see refs 2a, 3, and 8. [b] $\Delta\Delta G_{ES}^0 = \Delta G_{ES}^0$ (**SCP$_1$**) (−29.6 kJ/mol) − ΔG_{ES}^0 (**SMP$_1$**) (−35.0 kJ/mol) = +5.4 kJ/mol [assuming $K_M = K_D$(substrate)]. [c] Catalysis by 4(5)-methylimidazole: $k_{MeIm} = 0.0032$ M^{-1} s^{-1}.[2a] [d] Autohydrolysis: $k_{uncat} = 4.34 \times 10^{-7}$ s^{-1} (pH 5.5). [e] $\Delta\Delta G_{TS}^0 = \Delta G_{TS}^\circ$ (**SCP$_1$**) (−52.2 kJ/mol) − ΔG_{TS}^0 (**SMP$_1$**) (−56.0 kJ/mol) = +3.8 kJ/mol. [f] Data from 2a.

(19) It was intriguing to note that the effect of membrane polarization on f_{on} was more pronounced in cis esterolysis than in trans esterolysis. These results might suggest that the rate-limiting step in cis esterolysis is binding of the substrate, while that in trans esterolysis is dissociation of the product. This difference may originate from an increased binding rate in *trans* esterolysis due to the locally increased substrate concentration at the entrance of **SCP$_1$** (about 3000×).

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