Palladium(II)-gated ion channels†

Craig P. Wilson and Simon J. Webb*

Received (in Cambridge, UK) 29th May 2008, Accepted 2nd July 2008
First published as an Advance Article on the web 28th July 2008
DOI: 10.1039/b809087d

A simple ion channel has been developed that can be created or disassembled through the addition or removal of palladium(II).

Communication between cells in multicellular organisms depends upon the controlled release of messenger molecules. The influx and efflux of species into and out of cells is often facilitated by membrane-bound proteins, which are carefully controlled by sophisticated ''gating'' mechanisms. In particular, the controlled transport of ions through ion channels is a key cellular function, for example during the propagation of neural signals. The development of synthetic mimics of ion channels has progressed rapidly over the past 25 years, but the controlled ''gating'' of these biomimetic channels remains a significant challenge.[1] Elegant examples of enzyme-gated,[2] voltage-gated[3] and photochemically switchable ion channels[4] have been created, but despite being widespread in nature[5] reversible ligand-gating of ion channels has proved to be difficult to replicate.[6] Most synthetic ligand-gated ion channels use the ligands to block or prevent the assembly of channels,[7] and there are few examples of channels ''opened'' by ligand addition. Addition of dialkoxynaphthalenes or polyhistidine to oligo(*p*-phenylene)s gave ion channels,[8] but only the unblocking of a semi-synthetic alamethicin/leucine zipper channel by iron(III) has been shown to be reversible.[9]

To further our research on creating tissue-mimetic materials from vesicle aggregates,[10] we seek compounds that will reversibly form ligand-gated channels between adhering vesicles. These compounds must be easy to synthesise and modify, thereby allowing the creation of channels that can be opened *and* closed by different activating ligands (Fig. 1).

Taking inspiration from the work of Kobuke *et al.*[11] and Regen *et al.*,[12] we used cholic acid as the basis of our ligand-gated channels (Fig. 2). Kobuke and co-workers created covalently-linked head-to-head dimers of cholic acid that spanned phospholipid bilayers and self-assembled to form hydroxyl-lined membrane channels. These compounds gave good rates of alkali metal ion transport, showing selectivity for the transport of K^+ over Na^+ by up to 2.5 : 1; K^+ conductance through planar bilayers containing **3** was as high as 25 pS.[13] We hoped that replacement of the carbamate linker in Kobuke's channel-former **3** with a reversible palladium(II) bis(pyridyl) motif would give structurally-analogous complexes that would be switchable. These non-covalent complexes would span the membrane and facilitate ion transport, but subsequent addition of a palladium(II)-chelating agent would dissociate the channels and stop ion flow.

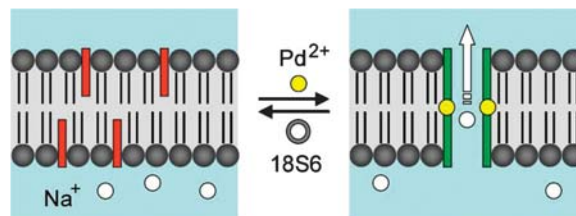

Fig. 1 Schematic representation showing the gating of ion channels formed by **1a** and **1b**; either opened (green) by the addition of $PdCl_2$ or closed (red) by the addition of hexathia-18-crown-6 (18S6).

In keeping with our desire for simple modular syntheses, cholate esters **1a** and **2** were synthesised in two steps from commercially available cholic acid *via* acylation of the less hindered 3α-hydroxyl group of methyl cholate. Similarly, acid **1b** was synthesised from benzyl cholate by acylation, followed by hydrogenolysis of the benzyl ester.†

Compounds **1a** to **3** were assessed for ion transport activity across phospholipid bilayers using the HPTS assay.[14] Compounds **1a** to **3** (20 μL, 1 mM in THF; final concentration 10 μM) were added to large unilamellar vesicles (800 nm diam, 20% cholesterol in egg yolk phosphatidylcholine (EYPC), 1 mM lipids, 2 mL) that encapsulated the pH sensitive dye HPTS (trisodium 8-hydroxypyrene-1,3,6-trisulfonate, internal concentration 100 μM). To create membrane-spanning Pd(II) complexes, aliquots of $PdCl_2$ (20 μL, 0.5 mM in MeOH; final concentration 5 μM) were added to vesicles containing **1a**, **1b** or **2**. Transport was indicated by the discharge of a 1 unit pH gradient induced by application of a ''base pulse'' (13 μL of 1 M NaOH or KOH) after sample incubation at 25 °C for 180 s. The increase in intravesicular pH caused by ion transport was reported as a ratiometric change in HPTS fluorescence.† Vesicle lysis after 2400 s with Triton-X 100 (25% v/v in MOPS buffer) gave maximum fluorescence values and allowed data normalisation.[15]

As anticipated, monomeric cholate **1a** was relatively ineffective at transporting Na^+ across EYPC bilayers, the HPTS assay revealing a Na^+ transport rate only just above the rate of background leakage through the vesicle membrane (Fig. 3). However if $PdCl_2$ had been incubated with vesicles containing **1a** for 3 min, the ''base pulse'' led to a rapid increase in HPTS fluorescence and discharge of half of the ion gradient within 20 min. Given that incubation with $PdCl_2$ in the absence of **1a** gave only a negligible increase in HPTS

Manchester Interdisciplinary Biocentre (MIB) and School of Chemistry, University of Manchester, 131 Princess St, Manchester, UK M1 7DN. E-mail: s.webb@manchester.ac.uk

† Electronic supplementary information (ESI) available: Experimental details of syntheses, ion transport experiments and titrations. See DOI: 10.1039/b809087d

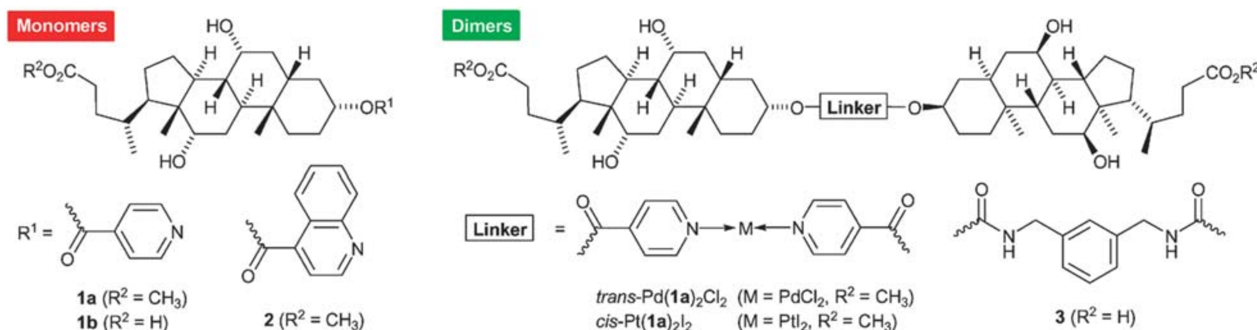

Fig. 2 Channel-forming compounds **1a** and **1b**; fluorescent analogue **2**; complexes *trans*-Pd(**1a**)$_2$Cl$_2$ and *cis*-Pt(**1a**)$_2$I$_2$, and control compound **3**.

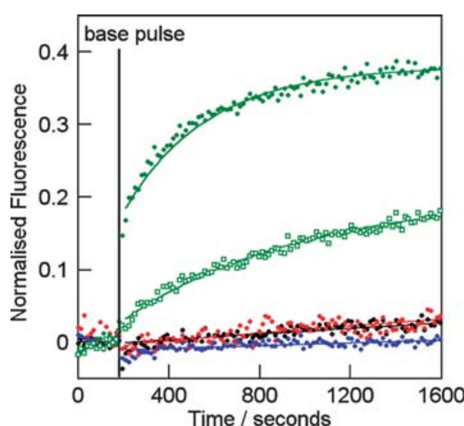

Fig. 3 Normalised change in HPTS emission after NaOH addition to vesicles containing HPTS (base pulse at 180 s). Vesicles mixed with: PdCl$_2$ (●); **1a** (●); **3** (●); **1a** and PdCl$_2$ (●); *trans*-Pd(**1a**)$_2$Cl$_2$ (□). The background rate has been subtracted and first order curve fits are shown.

fluorescence, these observations suggested synergistic transport of Na$^+$ by the *in situ* combination of **1a** and PdCl$_2$.

The species formed from **1a** and PdCl$_2$ was more active at transporting Na$^+$ than Kobuke's carbamate-linked dimer **3**; in our hands the HPTS assay did not show **3** to appreciably transport Na$^+$. Similarly, the weaker binding quinoline analogue **2** was found to be ineffective for Na$^+$ transport, either with or without PdCl$_2$. There was a similar synergistic increase in the rate of K$^+$ transport by the combination of PdCl$_2$ and **1a**, although in contrast to **3**, the rate of K$^+$ transport was slower than that of Na$^+$ (Table 1). The corresponding acid **1b** was found to exhibit very similar behaviour to **1a** with rapid Na$^+$ and K$^+$ ion transport after the addition of palladium, but both the rate of release and total response was lower than for the analogous methyl ester **1a** (normalised HPTS emission at 1600 s: 0.48 (Na$^+$) and 0.24 (K$^+$) for **1b** *vs*. 0.55 (Na$^+$) and 0.37 (K$^+$) for **1a**).† U-tube ion transport experiments showed ion transport by PdCl$_2$ + **1a** did not proceed by a carrier mechanism; unlike dibenzo-18-crown-6, neither **1a** nor *trans*-Pd(**1a**)$_2$Cl$_2$ transported Na$^+$ or K$^+$ through a chloroform phase over 24 h (<1% the rate of dibenzo-18-crown-6).† In addition, PdCl$_2$ + **1a** rapidly transported Na$^+$ through the membranes of gel-phase DPPC vesicles, which is consistent with a channel mechanism.†

Analysis of the changes in HPTS fluorescence showed an initial 30 second burst phase, followed by the smooth first order release of Na$^+$ or K$^+$. This biphasic behaviour has been attributed to either vesicle-adsorbed HPTS[16] or rapid ion transport through channels preformed during the incubation period;[17] adding the base pulse before addition of PdCl$_2$ + **1a** removed this burst phase† and is consistent with the latter explanation. With the background rate of ion leakage subtracted and the burst phase excluded, these data were fitted to first order kinetics. (Table 1). To ensure that only the synergistic effect of **1a** with PdCl$_2$ was assessed, the ion transport rates for the separated components **1a** and PdCl$_2$ were also subtracted from the data for **1a** + PdCl$_2$. This conservative data analysis revealed that the combination of **1a** and PdCl$_2$ gave at least a 9-fold enhancement in the rate of Na$^+$ release and a 5-fold increase in the rate of K$^+$ release.

Table 1 Rate constants for ion transport[a]

	k for Na$^+$/×10^{-4} s^{-1}	k for K$^+$/×10^{-4} s^{-1}
1a	3.0 ± 1.5	0.4 ± 0.1
PdCl$_2$	1.5 ± 0.5	1.5 ± 1.0
1a + PdCl$_2$	27.0 ± 1.0	13.0 ± 2.0
3	0.3 ± 0.1	2.3 ± 1.3

[a] Background rate subtracted.

Having shown that Na$^+$ transport could be switched on by the palladium-induced self-assembly of channels in vesicle membranes, the next task was to "close" these channels. Addition of a chelating ligand would extract the palladium from the presumed active species, a PdCl$_2$–**1a** complex, disassembling and closing the membrane-spanning channel. This ligand had to be lipophilic enough to partition into the membrane, yet not disrupt the membrane or discharge the pH gradient. Hexathia-18-crown-6 (18S6) appeared to be ideal, with low basicity and high extraction efficiency for Pd(II).[18] Hexathia-18-crown-6 (18S6) was injected one minute after the base pulse was applied (4 min after start). A small drop in HPTS fluorescence[19] was followed by a slow increase in fluorescence comparable to that observed with **1a** only. Vesicle lysis with Triton-X 100, which gave the typical maximum fluorescence intensity, showed that Na$^+$ transport had been attenuated close to the background rate (Fig. 4). Nonetheless, for these to be true ligand-gated channels it had to be possible to "re-open" the channels by adding further PdCl$_2$. The "closing" experiment was repeated, but after the addition of 18S6 (4 min), a fresh aliquot of PdCl$_2$ was added (8 min). Gratifyingly, the addition of further palladium(II) resulted in a

 This journal is © The Royal Society of Chemistry 2008

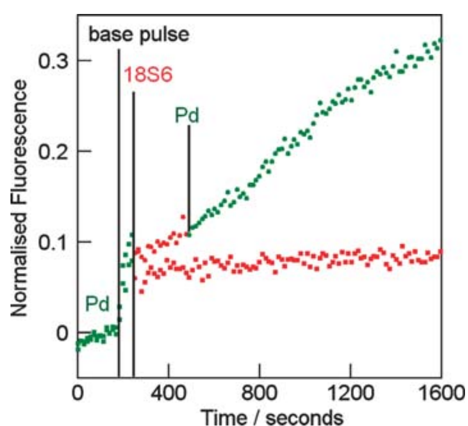

Fig. 4 Change in fractional HPTS emission intensity after the addition of NaOH (base pulse at 180 s) to HPTS vesicles mixed with **1a** and $PdCl_2$ (■, ●). 18S6 was added to the samples (indicated by 18S6 at 240 s) resulting in closed channels (■, ●). To one sample, addition of further $PdCl_2$ (at 480 s, indicated by Pd) re-opened the channels (●).

smooth increase in the Na^+ transport rate, giving a sigmoidal profile to the Na^+ transport rate (Fig. 4).

The structure of the active species formed by the combination of $PdCl_2$ and membrane-bound **1a** can only be speculated upon given the lack of good spectroscopic handles in **1a**. The *in situ* combination of $PdCl_2$ and **1a** should give *cis*-Pd(**1a**)$_2$Cl$_2$ initially, which should convert to the *trans* isomer over a period of a few minutes.[20] A kinetically stable analogue of the *cis* complex, *cis*-Pt(**1a**)$_2$I$_2$, was tested for ion channel activity, but was found to be completely inactive.† Therefore the thermodynamic product from complexation, *trans*-Pd(**1a**)$_2$Cl$_2$, was synthesised and tested for ion channel activity. The *trans* complex was more active than **1a** or $PdCl_2$, giving $k = (12 \pm 2) \times 10^{-4}\ s^{-1}$ for Na^+ and $(6 \pm 2) \times 10^{-4}\ s^{-1}$ for K^+, yet it discharged less of the M^+/H^+ gradient than *in situ* mixing of $PdCl_2$ and **1a**.

To then estimate the amount of *trans*-Pd(**1a**)$_2$Cl$_2$ that may be formed during the ion transport assays, we used fluorescent derivative **2**. Although **2** did not transport ions when mixed with $PdCl_2$, it had a spectroscopic handle that allowed binding to $PdCl_2$ to be directly measured. Fluorescence quenching titrations of vesicle-bound **2** with $PdCl_2$ provided stepwise equilibrium constants of *ca.* 2200 M^{-1} and 1100 M^{-1} for the formation of Pd(**2**)Cl$_2$ and Pd(**2**)$_2$Cl$_2$, respectively. Subsequent ligand competition experiments between Pd(**1a**)$_2$Cl$_2$ and **2** in $CDCl_3$, a solvent with polarity akin to the centre of the bilayer, showed **1a** bound palladium(II) 23 ± 3 times more strongly than **2**.† Speciation profiles calculated from these approximate stepwise binding constants predicted that a mixture of 10 μM **1a** and 5 μM $PdCl_2$ (as used during the ion transport assays) would form ~0.3 μM of Pd(**1a**)$_2$Cl$_2$ but only ~0.001 μM of the quinoline analogue Pd(**2**)$_2$Cl$_2$. The predicted concentration of Pd(**1a**)$_2$Cl$_2$ is comparable to some established channel-forming systems,[21] while the low concentration of Pd(**2**)$_2$Cl$_2$ predicted correlates with the lack of activity of **2**.

These observations suggest a membrane-spanning *trans* complex is a potential active species. The lower activity of preformed *trans*-Pd(**1a**)$_2$Cl$_2$, which does not exhibit a "burst" phase,† may be due to slow insertion into the membrane or dissociation of the complex before membrane insertion.

In summary, we have developed new cholate-based ion channels that can be gated "open" or "closed" by the addition or removal of palladium(II). The simple methodology developed to synthesise these cholate ion channels lends itself to the creation of multifunctional channels that could be gated by other ligands, such a biochemical messengers. Work is ongoing to create new ligand-gated channels that will also mediate targeted vesicle–vesicle and vesicle–cell adhesion.

Notes and references

1 (*a*) B. A. McNally, W. M. Leevy and B. D. Smith, *Supramol. Chem.*, 2007, **19**, 29; (*b*) J. H. Fuhrhop, U. Liman and V. Koesling, *J. Am. Chem. Soc.*, 1988, **110**, 6840; (*c*) T. M. Fyles, T. D. James and K. C. Kaye, *J. Am. Chem. Soc.*, 1993, **115**, 12315; (*d*) K. S. J. Iqbal, M. C. Allen, F. Fucassi and P. J. Cragg, *Chem. Commun.*, 2007, 3951.
2 S. Litvinchuk, H. Tanaka, T. Miyatake, D. Pasini, T. Tanaka, G. Bollot, J. Mareda and S. Matile, *Nat. Mater.*, 2007, **6**, 576.
3 (*a*) T. M. Fyles, D. Loock and X. Zhou, *J. Am. Chem. Soc.*, 1998, **120**, 2997; (*b*) N. Sakai, D. Houdebert and S. Matile, *Chem.–Eur. J.*, 2003, **9**, 223; (*c*) P. H. Schlesinger, R. Ferdani, J. Liu, J. Pajewska, R. Pajewski, M. Saito, H. Shabany and G. W. Gokel, *J. Am. Chem. Soc.*, 2002, **124**, 1848.
4 (*a*) A. Koçer, M. Walko, W. Meijberg and B. L. Feringa, *Science*, 2005, **309**, 755; (*b*) S. Loudwig and H. Bayley, *J. Am. Chem. Soc.*, 2006, **128**, 12404.
5 G. A. Woolley and T. Lougheed, *Curr. Opin. Chem. Biol.*, 2003, **7**, 710.
6 R. S. Hector and M. S. Gin, *Supramol. Chem.*, 2005, **17**, 129.
7 (*a*) B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek and R. J. Pace, *Nature*, 1997, **387**, 580; (*b*) S. Terrettaz, W.-P. Ulrich, R. Guerrini, A. Verdini and H. Vogel, *Angew. Chem., Int. Ed.*, 2001, **40**, 1740; (*c*) V. Gorteau, F. Perret, G. Bollot, J. Mareda, A. N. Lazar, A. W. Coleman, D.-H. Tran, N. Sakai and S. Matile, *J. Am. Chem. Soc.*, 2004, **126**, 13592; (*d*) M. Mayer, V. Semetey, I. Gitlin, J. Yang and G. M. Whitesides, *J. Am. Chem. Soc.*, 2008, **130**, 1453.
8 (*a*) P. Talukdar, G. Bollot, J. Mareda, N. Sakai and S. Matile, *J. Am. Chem. Soc.*, 2005, **127**, 6528; (*b*) M. M. Tedesco, B. Ghebremariam, N. Sakai and S. Matile, *Angew. Chem., Int. Ed.*, 1999, **38**, 540.
9 T. Kiwada, K. Sonomura, Y. Sugiura, K. Asami and S. Futaki, *J. Am. Chem. Soc.*, 2006, **128**, 6010.
10 (*a*) R. J. Mart, K. P. Liem, X. Wang and S. J. Webb, *J. Am. Chem. Soc.*, 2006, **128**, 14462; (*b*) X. Wang, R. J. Mart and S. J. Webb, *Org. Biomol. Chem.*, 2007, **5**, 2498; (*c*) K. P. Liem, R. J. Mart and S. J. Webb, *J. Am. Chem. Soc.*, 2007, **129**, 12080.
11 Y. Kobuke and T. Nagatani, *J. Org. Chem.*, 2001, **66**, 5094.
12 W.-H. Chen, X.-B. Shao and S. L. Regen, *J. Am. Chem. Soc.*, 2005, **127**, 12727.
13 M. Yoshii, M. Yamamura, A. Satake and Y. Kobuke, *Org. Biomol. Chem.*, 2004, **2**, 2619.
14 K. Kano and J. H. Fendler, *Biochim. Biophys. Acta*, 1978, **589**, 289.
15 C. A. Winschel, A. Kalidindi, I. Zgani, J. L. Magruder and V. Sidorov, *J. Am. Chem. Soc.*, 2005, **127**, 14704.
16 M. Merritt, M. Lanier, G. Deng and S. L. Regen, *J. Am. Chem. Soc.*, 1998, **120**, 8494.
17 V. Gorteau, G. Bollot, J. Mareda and S. Matile, *Org. Biomol. Chem.*, 2007, **5**, 3000.
18 M. Draye, A. Favre-Réguillon, G. Le Buzit, J. Foos, M. Lemaire and A. Guy, *J. Radioanal. Nucl. Chem.*, 1997, **220**, 105.
19 UV and fluorescence experiments revealed this drop was due to a slight decrease in turbidity of the vesicle suspension combined with a small amount of HPTS fluorescence quenching by 18S6.
20 L. Cattalini and M. Martelli, *J. Am. Chem. Soc.*, 1969, **91**, 312.
21 0.19 μM of an aminocyclodextrin ion channel transported Na^+ with a rate constant of $20 \times 10^{-4}\ s^{-1}$. See: N. Madhavan, E. C. Robert and M. S. Gin, *Angew. Chem., Int. Ed.*, 2005, **44**, 7584.

This journal is © The Royal Society of Chemistry 2008