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Tetracyanoresorcin[4]arene ion channel shows pH dependent conductivity change†

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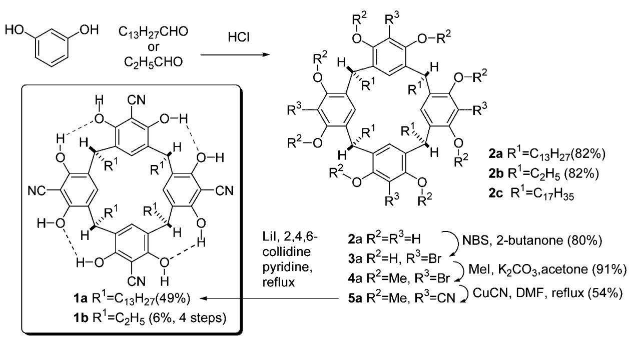
Received (in Cambridge, UK) 15th October 2003, Accepted 18th February 2004
First published as an Advance Article on the web 3rd March 2004

DOI: 10.1039/b312952g

A pH sensitive artificial ion channel, tetracyanoresorcin[4]arene, whose pK_{a1} to pK_{a3} are around pH 7, was synthesized. Conductivities for potassium ion were increased by the progress of dissociation at the channel mouth.

Ion channels are molecular devices which allow ions to pass through insulating bilayer lipid membranes[1] with extremely large flux. The ionic flux is controlled by the outer signal or membrane potential. The highly efficient design and function of natural ion channels provides directions for construction of artificial molecular machines and nanometer-scale devices. Specifically, the development of artificial ion channels[2] is directly connected with the design of ionic devices. Resorcin[4]arene, which shows only one conductivity state under normal conditions,[3] is one of the fundamental models for artificial ion channels. This allows the relationship between the conductivity and structure of ion channels to be understood. It is known that resorcin[4]arene forms a rigid bowl-like structure, in which all axial substituents are held together by a hydrogen bond network over four resorcin moieties,[4] and four protons can be dissociated under strongly basic conditions (> pH 12).[4a,5] Deprotonation of resorcin[4]arenes allows control of its ion channel properties. However, such highly basic conditions cannot be applied to ion channel measurement because lipid bilayers are unstable, or are not prepared, under such conditions. Here, we report the synthesis and ion channel properties of tetracyanoresorcin[4]arene, **1**, which has pK_{a1}, pK_{a2}, and pK_{a3} values at almost neutral pH. The conductivity of **1a** in a symmetric electrolyte (500 mM KCl) increased with pH in the range from 6.0 ≤ pH ≤ 8.5.[6]

Tetracyanoresorcin[4]arene, **1a**, was synthesized as described in Scheme 1. Condensation of resorcinol and tridecanal in the presence of HCl gave resorcin[4]arene, **2a**, in 82% yield. Bromination of **2a** with NBS in 2-butanone was carried out to give **3a** in 80% yield. The hydroxyl groups in **3a** were protected with methyl groups to give **4a**, and then cyanation of **4a** with copper cyanide gave **5a** in 54% yield. Finally, deprotection of the hydroxyl groups in **5a** with LiI in a mixture of 2,4,6-collidine and pyridine under reflux conditions afforded **1a** in 49% yield. The proton NMR spectrum of **1a** showed only one set of resorcin and alkyl moieties, indicating the formation of a symmetric all-axial conformer. MALDI-TOF mass spectroscopy also showed corresponding peaks at 1316 ([M]$^+$) and 1339 ([M + Na]$^+$).[7] Tetracyanoresorcin[4]arene, **1b**, which was soluble in H_2O (pH 7.2) at concentrations over 1.0 × 10^{-5} M, was also synthesized.



Scheme 1 Synthesis of tetracyanoresorcin[4]arenes.

To examine its dissociation behaviour, a pH titration of **1b** was carried out over the range 4 ≤ pH ≤ 14, with monitoring by UV absorption spectra (see ESI† S1 and S2). Initially, the completely protonated form, **1b**-$(OH)_8$, which had an absorption maximum at 320 nm, was observed at pH 4. When 1 eq. of NaOH was added, which resulted in a pH of 6, the first large spectral change was observed. Further addition of NaOH caused a gradual change of the spectrum without showing an isosbestic point until pH 11. Then, another large change was observed between pH 11 and 12. The spectral changes measured at 342 and 320 nm were plotted as a function of pH values (Fig. 1). The species obtained at pH 12 was assigned as the tetraanionic form, **1b**-$(OH)_4(O^-)_4$, by comparison with the spectrum of the tetraanion of **2b**, as well as by comparison with further dissociated states[8] of **1b** and **2b**. Based on these results, pK_{a1} and pK_{a4} of **1** were assigned as around 5 and 11.7, respectively. pK_{a2} and pK_{a3} were expected between 6 and 11, although they may have overlapped each other. These pK_a values indicated that partially dissociated species (from mono to trianionic forms) should co-exist at around pH 7.

Single ion channel currents of **1a** were measured by a planar bilayer lipid membrane method at pH = 6, 7.2, and 8.5 (in Tris-HEPES buffer).[9] In each experiment, predominantly stable open states were observed. However, the current–voltage plots for different experimental runs showed a somewhat unusual tendency. For example, two current–voltage plots recorded in a symmetric electrolyte (500 mM KCl solution) at pH 7.2 were superimposed to give Fig. 2A. The filled triangles show a linear relationship between current and voltage, while the filled circles formed a nonlinear curve. Such nonlinear behaviour was frequently observed for measurements taken at pH 8.5 (ESI† Fig. S3). This non-linear behaviour suggested that the structure of the ion channel was

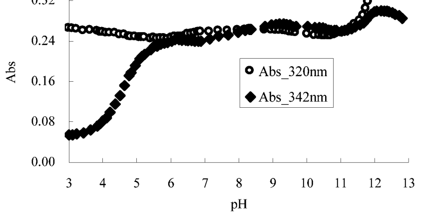


Fig. 1 pH Titration of tetracyanoresorcin[4]arene **1b** (2.80 × 10^{-5} M) in a water–methanol mixture (50/1, v/v) at 28 °C.

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† Electronic Supplementary Information (ESI) available: UV-Vis spectra; plot of equiv. NaOH *vs.* pH; *I–V* plots. See http://www.rsc.org/suppdata/cc/b3/b312952g/

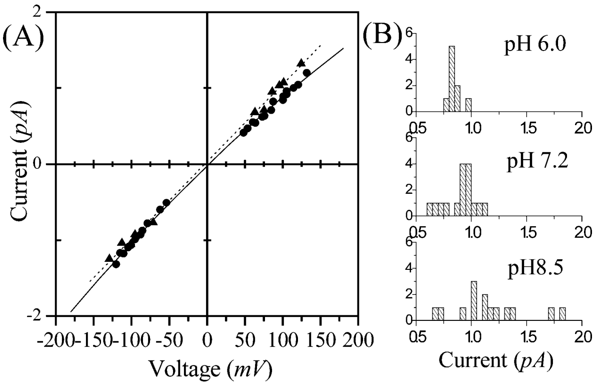

Fig. 2 (A) Current–voltage plots of **1a** in a symmetric electrolyte (500 mM KCl) at pH 7.2. (B) Histogram of single ion channel currents (pA) observed at ±100 mV at pH 6.0 (top), 7.2 (middle), and 8.5 (bottom).

asymmetric across the membrane. Since the resorcin[4]arene ion channel is believed to be composed of tail to tail dimers of **1a** (Fig. 3), the dissociation state at each terminal should be different. Possible structures will be discussed below. A histogram of single ion channel currents at ±100 mV obtained at pH 6.0, 7.2, and 8.5 is presented in Fig. 2B. At pH 6.0 and 7.2, the bars were relatively concentrated at 0.8 and 0.95 pA, respectively. At pH 8.5, on the other hand, the absolute value increased significantly, and those obtained in different experimental runs scattered over a relatively large range of *ca.* 0.7–1.8 pA.

If we assume that the dissociation behaviour of **1a** in lipids is similar to that of **1b** in bulk water, mono-anionic **1a**$^{1-}$ was expected to form at pH 6.0, and dianionic **1a**$^{2-}$ and trianionic **1a**$^{3-}$ species increase gradually at pH 7.2 and 8.5, while compound **2c** was believed to remain undissociated in these pH regions. In fact, all the conductivities of **2c** under the same salt conditions at pH 6.0, 7.2,[3a] and 8.5 existed between 5.6–6.1 pS at currents of 0.56–0.61 pA at ±100 mV. Only linear voltage–current relationships were observed. Therefore, the increase in conductivity observed for **1a** should correspond to the extent of dissociation. Generally speaking, the channel conductivity should increase with the hydrophilicity of the pore and introduction of a strong binding site near the ion channel pathway. In the system under consideration, the pore, which is formed by four alkyl chains, should be insensitive to the dissociation state, and the latter should be the main factor to consider. It is hypothesised that the increase in anionic charge caused a concentration of cationic species by electrostatic interaction. Further, the increase of electron density of the aromatic moiety induced stronger cation–π interactions between the resorcin[4]arene and potassium ions. As a result, effectively dehydrated potassium ions were supplied to the ion channel pathway. The reason why the conductance values varied significantly at pH 8.5 is not clear. It may be speculated that this behaviour arose from the asymmetric disposition of dissociated species of resorcin[4]arene. The environment around the ion channel may differ because of interactions with the lipid components. The more dissociated species, *e.g.* trianionic-**1a**, is expected to be more influenced by this effect.

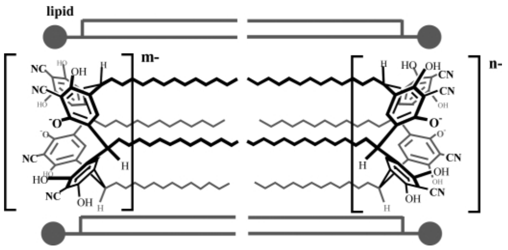

Fig. 3 Expected ion channel structure of **1a**. *m*, *n* = 1–3.

In conclusion, tetracyanoresorcin[4]arene, which showed $\mathrm{p}K_{a1}$ $\mathrm{p}K_{a2}$ and $\mathrm{p}K_{a3}$ values at physiological pH, was successfully synthesised. The conductivities of potassium ions elucidated by single ion channel measurements increased with the progress of dissociation at the channel mouth. A pH-sensitive artificial ion channel such as this may have applications in the design of pH-controlled ionic devices.

WHC thanks NSFC (20242009) and SRF for ROCS, SEM for financial support.

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7 **1a**; ^{1}HNMR (270 MHz, $CDCl_3/CD_3OD = 5/1$, rt, TMS): $\delta = 7.19$ (s, 4H, ArH), 4.25 (t, $J = 7.6$ Hz, 4H, CH), 2.10–2.00 (m, 8H, CH_2), 1.10–1.13 (88H, CH_2), 0.80 (t, $J = 6.8$ Hz, 12H, CH_3); ^{13}CNMR (67.7 MHz, $CDCl_3/CD_3OD = 5/1$, rt, TMS): $\delta = 154.2$, 127.7, 124.1, 114.4, 90.90, 33.17, 32.87, 31.67, 29.49, 29.47, 29.42, 29.11, 27.64, 22.42, 13.72; MALDI-TOF mass calcd. for $C_{84}H_{124}N_4O_8$: 1316.94; found: 1316.36 ($[M]^+$), 1338.96 ($[M + Na]^+$); analysis calcd. for $C_{84}H_{124}N_4O_8\cdot 6H_2O$: C 70.75, H 9.61, N 3.93; found: C 70.39, H 9.45, N 3.22%.

8 When **1b** was dissolved in 4 N NaOH, the absorption peak at 342 nm turned into a shoulder peak, and two strong absorption peaks were observed at *ca.* 310 and 320 nm. This was assigned as a further dissociated state (> 4 anion). The spectrum of **2b** in 4 N NaOH also showed similar peak shifts. This shorter wavelength shift is explained by the collapse of hydrogen bond networks.

9 Details for the planar bilayer lipid membrane method can be seen in references cited in ref. 2e.