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Anion transport across phospholipid membranes mediated by a diphosphine–Pd(II) complex†

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The [Pd(dppp)(OTf)$_2$] complex acts as an efficient transporter of halide anions, in particular the biologically relevant chloride anion, across a phospholipid bilayer.

In recent years, there has been growing interest in the potential biological activity of synthetic ionophores[1] and, in particular, in transmembrane anion transporters.[2] This interest is in large part motivated by the awareness that defects in anion-transport proteins can lead to a number of diseases known as "channelopathies",[3] the best known being cystic fibrosis (CF), a severe illness caused by impairment of chloride transport through the CFTR anion channel in epithelial cell membranes. It has been proposed that synthetic ionophores may facilitate chloride transport in ill cells and seminal work on artificial peptide based chloride channels,[4,5] on steroid based chloride transporters,[6,7] and on isophthalamide derivatives[8] has shown promising results in *in vitro* biological tests. Moreover, prodiginines,[9] a family of naturally occurring low molecular weight chloride transporters, and some synthetic structurally related[10] or unrelated[11] transmembrane anion carriers are under investigation because of their promising anticancer activity. Supramolecular chemists have developed a large number of systems able to promote transmembrane anion transport[12] and among them, low molecular weight molecules are very appealing targets since their physicochemical properties can be more easily implemented and optimized[13] in order to obtain a real usable drug.[14] This has led to the investigation of several organic ligands, ranging from steroid based cholapods[12c] to simple acylthioureas,[15] which bind chloride, mainly through hydrogen-bond interactions, forming complexes that efficiently transport the anion across the phospholipid membrane. Surprisingly and despite their well-documented use as recognition elements for anions,[16] studies on metal complexes as anion carriers through lipid bilayers are virtually absent in the literature.

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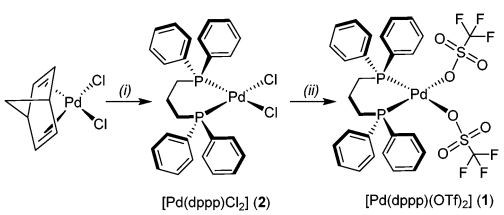
Following our interest in synthetic ionophores[17] we recently reported the ionophoric activity of a 4 + 4 metallacycle obtained by the metal-mediated self-assembly of *trans*-di(4-pyridyl)-porphyrins and Re(I) complexes.[18] A parallel investigation of porphyrin metallacycles connected by Pd(II) fragments clearly indicated that this metal ion is not inert enough to form or maintain such metallacycles in a phospholipid membrane environment, as also observed by Fyles[19] and by Webb.[20] Nevertheless, and in analogy with Fyles,[19] we measured some ionophoric activity which was reasonably due to the Pd(II) fragment itself. Indeed, at least one report in the literature showed that Pd(II) and Pt(II) complexes are able to transport amino acids across a bulk chloroform membrane.[21] However, the ability of Pd(II) complexes to transport anions across lipid membranes has apparently not been investigated. This is yet more surprising as, in analogy with the well-known Pt(II) complexes, the biological and in particular the anticancer activity of Pd(II) complexes is intensely investigated.[22] We therefore decided to undertake a systematic investigation of the ionophoric activity of a series of Pd(II) complexes, and we report here our first results showing that a neutral square planar Pd(II) complex, bearing as ligands one chelating diphosphine and two trifluoromethanesulphonate ions, efficiently transports chloride across phospholipid membranes.

Complex **1** has been widely employed as a metal precursor for the construction of metal-mediated 2D and 3D supramolecular architectures,[23] and was here efficiently prepared by a modified literature procedure (Scheme 1 and ESI†).[24] The choice of the dppp ancillary ligand was crucial owing to its lipophilic nature and, more



Scheme 1 (*i*) MeOH, dppp, 3 h, 90%; (*ii*) CH_2Cl_2, AgOTf, reflux, 24 h, 75%.

importantly, to its inertness towards ligand exchange even in the presence of water. These characteristics ensure the partition of **1** in the phospholipid membrane.‡ On the other hand, the two OTf^- ligands are labile and can, therefore, be exchanged with other anions thus ensuring the anion transport across the membrane.§

The ionophoric behaviour of complex **1** was studied using liposomes made of a 95 : 5 phosphatidylcholine (PC) and phosphatidylglycerol (PG) blend prepared in HEPES buffer (pH 7.0) containing 100 mM NaCl and loaded with the pH sensitive HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid) fluorescent dye. After addition of the ionophore, a pH gradient of 0.6 pH units is applied by external addition of NaOH and the fluorescence emission of HPTS is recorded. The collapse of the transmembrane pH-gradient implies basification of the liposome inner water pool which is signalled by an increase of the HPTS fluorescence emission. Therefore, the rate of the pH gradient collapse gives direct information on the transportation of OH^- (influx) or H^+ (efflux) and indirect information on the correlated symport/antiport of counterions. Fig. 1a shows the typical kinetic profiles obtained upon addition of NaOH at 100 s and lysis of the liposomes by addition of Triton X-100 at 700 s. Complex **1** is considerably effective in promoting the pH-gradient collapse which is complete after less than 120 s at a 0.1% concentration of ionophore. As controls the more hydrophilic $PdCl_2$ and the dppp ligand alone were tested, each at a concentration 10 times higher, and were found to be almost inactive, thus underlining the importance of a lipophilic portion in the metal complex in order to achieve activity. Interestingly, complex **2** shows a very similar activity to **1** suggesting that the Cl^- and the OTf^- ligands in the two metal complexes are both exchanged under the experimental conditions, leading to a similar ionophoric Pd(II) species. However, to avoid the presence of an additional exogenous chloride ion, complex **1** was selected as a candidate for further investigation. Fitting of the kinetic traces affords the first-order rate constants (k_t, s^{-1}) for the transport process, those of **1** are reported as a function of the complex concentration in Fig. 1b. The profile shows an upward curvature, and interpolation of the experimental points with the equation proposed by Regen[25] gives $n = 1.7$ (Fig. 1b and ESI†). This suggests that, at high concentration of **1**, dimeric Pd-complexes, probably formed by μ-OH^- bridges,[26] participate in the transport process.¶

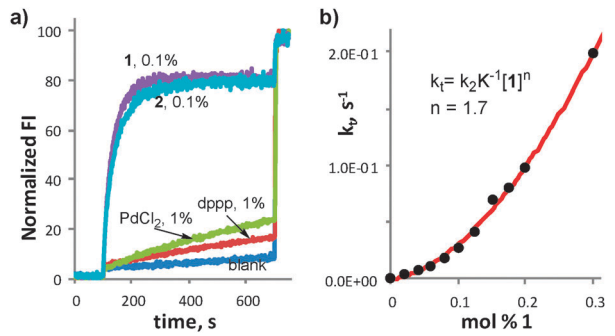


Fig. 1 (a) Normalized fluorescence change in HPTS emission as a function of time after addition of the base (50 μL of 0.5 M NaOH) to 95 : 5 PC/PG LUVs (100 nm diameter) loaded with HPTS (0.1 mM HPTS, 0.17 mM total lipid concentration, 25 mM HEPES, 100 mM NaCl, pH 7.0), in the presence of Pd(II) complexes **1** and **2**, $PdCl_2$ and dppp. The concentrations of ionophores, given in percent with respect to the total concentration of lipids, are indicated in the figure. (b) Dependence of the observed rate constant for the transport process (k_t, s^{-1}) *vs.* concentration of **1**. The red line corresponds to the fitting of the data with Regen's equation reported in the graph (see ESI†).

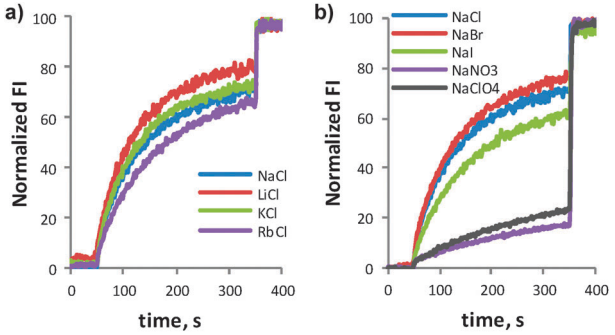


Fig. 2 (a) Cation selectivity of **1** (0.05%) by using the HPTS assay (MCl (100 mM), pH 7.0, base pulse by addition of MOH (50 μL, 0.5 M)). (b) Anion selectivity of **1** (0.05%) by using the HPTS assay (NaX (100 mm), pH 7.0, base pulse by addition of NaOH (50 μL, 0.5 M)). Other conditions as specified in Fig. 1.

Selectivity in ion transport was investigated using the HPTS assay and using the protocol described above and in the presence of only the cation or the anion under investigation added as the MCl or NaX salt, respectively. The results show similar transport rates in the presence of the first group alkali metals (Fig. 2a), as well as halide anions (Fig. 2b). In contrast, oxygenated anions such as NO_3^- and ClO_4^- inhibit the transport process (Fig. 2b). The observation that the transport rate is independent of the nature of the cation and dependent on the nature of the anion suggests that the inner vesicular pH change signalled by HPTS correlates with the OH^-/X^- antiport (or with the kinetically equivalent H^+/X^- symport).

Anion selectivity was further investigated with an experiment in which liposomes with entrapped HPTS and prepared in HEPES buffer (25 mM, pH 7) were exposed to an anion gradient by external addition of the anion (NaX). If the anion is transported from outside to inside the liposome, a compensative OH^- antiport (or the H^+ symport) should be observed leading to acidification of the liposome inner water pool and consequent decrease of the HPTS emission. The results shown in Fig. 3a confirm the selectivity sequence observed with the pH-jump experiment with halides activating a transport process much more efficiently than nitrate or perchlorate. In this case the difference in activity between halides and oxygenated anions is lower, probably because of the high anion gradient applied (33 mM outside, 0 mM inside) that enhances the transport process. However, the trend is similar, thus confirming the anion transporting ability of the Pd(II) complex. Finally, anion transport was confirmed with the chloride sensitive lucigenin dye trapped inside liposomes, prepared in HEPES (25 mM, pH 7) containing 100 mM $NaNO_3$, and by applying a NaCl pulse from outside (Fig. 3b). The decrease in the lucigenin fluorescence emission confirms that **1** transports chloride across the

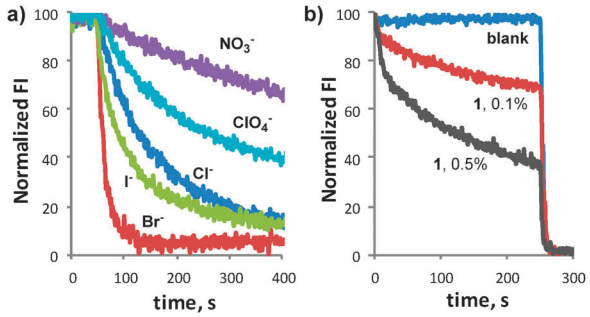


Fig. 3 (a) Normalized fluorescence change in HPTS emission as a function of time in the presence of **1** (0.1%) after the external addition of anions. Conditions: 25 mM HEPES, pH 7.0, anion pulse by addition of 50 μL of 2 M NaX. The final external concentration of anion is 33 mM. (b) Normalized fluorescence change in lucigenin emission in the presence of different concentrations of complex **1** after the addition of NaCl (50 μL of 2 M solution, final external concentration 33 mM) to PC LUVs loaded with lucigenin (1 mM lucigenin, 0.1 mM total lipid concentration, 25 mM HEPES, 100 mM $NaNO_3$, pH 7.0). The mother solution of **1** was prepared in methanol.

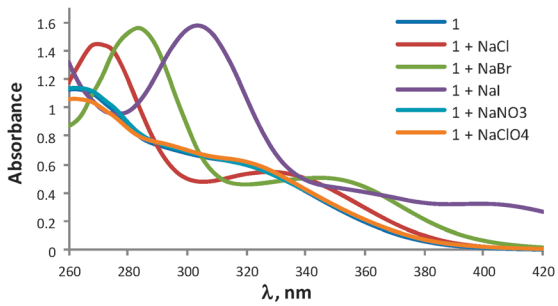


Fig. 4 UV-Vis spectra of **1** (60 μM) in 1 : 1 DMSO/HEPES buffer (25 mM, pH 7.0) alone and in the presence of different anions (25 mM). The spectrum of $NaNO_3$ is corrected for the absorbance of NO_3^-.

bilayer although, probably for an unfavourable NO_3^-/Cl^- antiport, the efficiency observed with this assay is somewhat lower.

The selectivity in anion transport correlates well with the anion binding ability of the Pd(II) complex as disclosed by UV-Vis titrations (Fig. 4). Indeed, addition of halides to a solution of **1** in DMSO/HEPES 1 : 1 generates a new band centred respectively at 270 (Cl^-), 283 (Br^-) and 303 (I^-) nm with a shoulder at longer wavelengths. In contrast, addition of NO_3^- and ClO_4^- does not induce any change in the spectra of **1** suggesting that these anions are not or only weakly coordinated to the metal complex. Interestingly, analysis of the titration curves shows the stepwise formation of a $[Pd(dppp)X_2]$ complex with $\log K_1$ in the range 3.7–5.1 for the insertion of the first halide and $\log K_2$ in the range 1.9–3.5 for the second one, with I^- being the most tightly bound anion (see ESI†). The values obtained for Cl^- are in good agreement with those reported for $[Pd(en)(H_2O)_2]^{2+}$ (en = ethylenediamine).[26]

Taking together all the experimental evidence and in analogy with the mechanism proposed by Feringa for the transport of amino acids across a bulk membrane,[21] we propose that the $[Pd(dppp)]^{2+}$ fragment, as a monomer or at higher concentration as a μ-OH^- dimer, acts as a carrier residing in the membrane and shuttling the ions across the phospholipid bilayer, by exchanging halides with OH^-. The feasibility of this mechanism is also supported by the observation that **1** is able to transport chloride across a bulk chloroform membrane in a classical U-tube experiment (see ESI†).

In conclusion we have demonstrated for the first time that a simple Pd(II) complex is able to efficiently transport halides, in particular the biologically relevant chloride ion, across a phospholipid bilayer thus representing a prototype of a new class of low molecular weight 'drug-like' anion transporters. The transport efficiency is high although not yet at the level of the best hydrogen-bonding anion carriers. Studies are in progress to better understand the mechanism of transport in order to implement the Pd(II) complex characteristics and ultimately improve its performance.

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Notes and references

‡ Preliminary experiments using 4,4′-di-*tert*-butyl-2,2′-dipyridyl as an ancillary ligand showed a very complex behaviour probably due to the partial detachment of the ligand from the metal ion. On the other hand the hydrophilic $PdCl_2$ does not show any ionophoric activity.
§ ^{19}F-NMR shows that OTf^- is fully dissociated from **1**. Moreover, UV-Vis studies in 1 : 1 DMSO/H_2O suggest the formation of a $[Pd(dppp)(H_2O)_2]^{2+}$ complex which dissociates to $[Pd(dppp)(H_2O)OH]^+$ with pK_a = 6.1. See ESI.†
¶ Experiments on calcein release excluded that the observed transport processes are related to dye leakage induced by **1**, see ESI.†

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