Porous inorganic capsules in action: modelling transmembrane cation-transport parameter-dependence based on water as vehicle†‡

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Insight into basic principles of cation transport through "molecular channels", and especially details of the related fundamental H_2O vehicle function, could be obtained *via* ^{7}Li NMR studies of the Li^+ uptake/release processes by the unique porous nanocapsule $[\{(Mo^{VI})Mo^{VI}_5O_{21}(H_2O)_6\}_{12} \{Mo^V_2O_4(SO_4)\}_{30}]^{72-}$ which behaves as a semi-permeable inorganic membrane open for H_2O and small cations; channel traffic as well as internal cavity distribution processes show a strong dependence on "environmental" effects such as exerted by solvent properties, the amount of water present, and competing complexing ligands, and end up in a complex equilibrium situation as in biological leak channels.

Can inorganic artificial cells be constructed for the study of cation uptake/release under changing environmental conditions, thus allowing insight into basic phenomena of related cation transport processes through channels as well as to model biologically relevant processes? Highly charged capsules of the type $\{(Mo)Mo_5\}_{12}\{Mo^V_2 (ligand)\}_{30}$ (ligand, *e.g.* sulfate), containing 20 pores connected to the tuneable inside cavity surface by channels[1,2] and which are according to Raman and [95]Mo NMR spectroscopy[2] in the absence of oxygen stable in solution, blaze the trail. The porous anionic capsule **1a** of $[Me_2NH_2]_{44}Li_n[Li_{28-n} \subset \{(Mo)Mo_5O_{21}(H_2O)_6\}_{12}\{Mo_2O_4(SO_4)\}_{30}]\cdot \approx 200\ H_2O$ **1**[2]§ shows Li^+ uptake and release between its interior and the surrounding medium; but until now details about that complex process could not be elucidated. Fig. 1 provides illustrative information on the topic, including a schematic notion of the different locations that can compete for cations. In this communication, detailed studies directed towards the factors which determine the exchange between different cation locations, the influence of environmental conditions and, in particular, the role of water as vehicle are presented. For this purpose, ^{7}Li NMR spectroscopy has been employed under varying conditions such as concentrations of **1** and of water, as well as of different solvents. Such a study provides model information for biological cation transport processes in the direction of the electrochemical gradient ("down hill"), especially
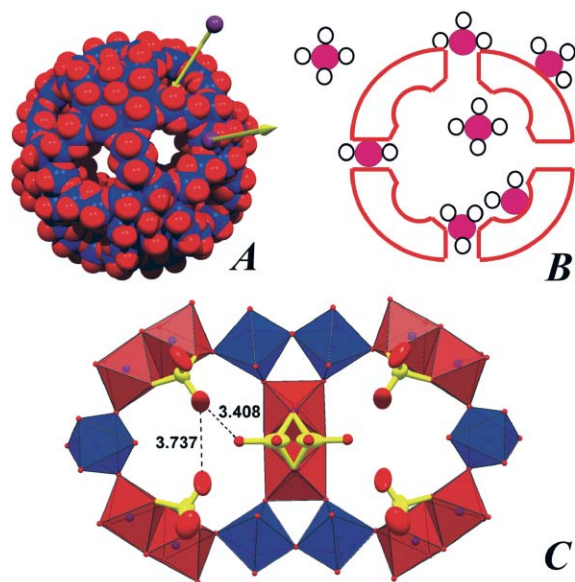
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Fig. 1 *A*: Space-filling representation, illustrating capsule cation uptake/release equilibrium. *B*: Schematic view of possible $\{Li^+(H_2O)_n\}$ sites associated with the capsule (outside, at the external surface, within the pores/rings, in and below the channels, and inside at a cavity functionality). *C*: Enlarged view of two capsule pores referring (indirectly) to encapsulated Li^+, which influence the shown SO_4^{2-} positions (see ref. 2). The pore size is determined by the three shown Mo^V_2 type linkers.[1] The final position of the cations within the capsule depends on the functionalities available in the pores, the channels, the interior of the cavities, *viz.* the polyoxomolybdate skeleton oxo groups, the sulfate ligands, and the encapsulated water molecules. Colour code: Mo, blue; Mo_2, red; O, red; S, yellow; Li, magenta; H_2O, open circles.

through the open K^+ leak-type channels ubiquitous in eukaryotic cells and responsible for keeping the membrane potential constant. There is also a relevance for the Li^+/Na^+ counter transport, which plays a key role in the treatment of dipolar disorder (manic depression) and which is of interest for hypertension research.[3,4]

In the present case the driving force for cation uptake by the capsule is based on the high negative charge relative to the competing solvating abilities of the solvents, *i.e.* their complex formation abilities which can be correlated with donor numbers as defined by Gutmann.[5] Formally, the solvent molecules compete, through their affinity for the cation, with the capsule's functionalities. It is evident that the solvent molecules used here cannot, except for H_2O,[6] enter through the pores, exhibiting an average ring-aperture of 0.45 nm, Fig. 1, C.[1,6]¶ A perception of the different capsule sites, evidenced by high-field signals in addition to the $[Li(dmso)_n]^+$ peak, is provided by the study of the

concentration dependence of the ^{7}Li NMR spectra of a solution of **1** in dimethyl sulfoxide (DMSO) (see ESI‡ including Fig. S1 in connection with Fig. 2).[7]

The fundamental observation is that the spectral NMR pattern presented here for the DMSO case changes drastically on stepwise addition of increasing (small) amounts of water (Fig. 2), which gives insight into the type of the relevant processes in the equilibria $[Li(dmso)_n]^+ \rightleftharpoons \{Li^+(H_2O)_n \subset$ **1a**$\}$ (without referring to mixed ligand complexes) while proving the vehicle function of water for all transport processes involved. (Note: DMSO is hygroscopic and thus intrinsically contains some water, in the purchased solvent up to 0.2%, corresponding to $c(H_2O) \approx 0.12$ M.) In addition, *ca.* 200 crystal water molecules per capsule anion are present, which are released into the organic solvent resulting, at a capsule concentration of 1 mM, in an overall maximum water percentage of 0.53% ($c(H_2O)$ *ca.* 0.32 M). In trace a of Fig. 2, referring to a sample dissolved in absolute DMSO and kept under N$_2$, the low-field signal at −1.8 ppm exhibits the highest intensity. It is plausible to assign this signal to sites associated with the inner parts of the capsule **1a**, which are less easily accessible by water and thus do not readily exchange at low water contents in DMSO.‖ On increasing the amount of water (trace b), internal sites are increasingly mobilised and Li$^+$ ions are transported to peripheral capsule sites. Consequently, the high-field components ($\delta = -2.2$ to −2.6) gain in intensity. If a much larger amount of water is added stepwise, *i.e.* up to $c(H_2O) = 2.2$ M (trace c), overall exchange is accelerated to the extent that the signals for $\{Li^+ \subset$ **1a**$\}$ fuse to a single upfield signal at *ca.* −2.2 ppm and the signal for (formal) $[Li(solv)_n]^+$ moves downfield from −0.82 (*a*) to −0.67 ppm (*i.e.* in the direction of the peak of $[Li(H_2O)_n]^+$) (*c*); the situation reflects the increased exchange between $[Li(dmso)_n]^+$ and $[Li(H_2O)_n \subset$ **1a**$]^+$ as well as the mixed solvent complexes. The corresponding ^{7}Li,^{7}Li-EXSY spectrum (Fig. 2) for this situation proves nicely the exchange between $[Li(H_2O)_n \subset$ **1a**$]^+$ and external Li$^+$ in all types of environments. Further drastically increasing $c(H_2O)$ to 13 M results in a single low-field signal at −0.64 ppm (trace d), corresponding to a situation where all of the lithium is completely extract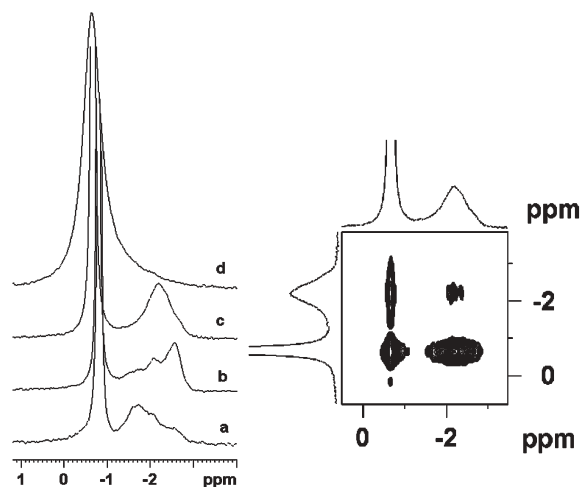ed into the solvent due to its strong affinity to water. Addition of cryptands with strong affinity towards Li ions shows a similar effect (see ESI‡).

To get information about further details regarding the environment-influence on the process, the following solvents with different dielectric constants ε, donor numbers DN** and dipole moments μ[D] having a varying affinity to Li$^+$ were employed:†† water (ε 80.1, DN 18.0, μ 1.84), acetonitrile (ε 36.6, DN 14.1, μ 3.92), nitromethane (ε 37.3, DN 2.7, μ 3.46), methanol (ε 33.6, DN 19, μ 1.70), dimethyl sulfoxide (ε 47.2, DN 29.8, μ 3.96), *N,N*-dimethylformamide (DMF, ε 38.3, DN 26.6, μ 3.82) and 1-methyl-2-pyrrolidone (NMP, ε 32.6, DN 27.3, μ 4.1). As the solubility of **1** in MeOH, MeCN and nitromethane is limited, saturated solutions were studied while for the remaining solvents, concentrations were adjusted to c(**1**) = 1 mM (c(Li) = 28 mM). The ^{7}Li NMR spectra are depicted in Fig. 3.

From the rough spectral patterns, the solvents can be divided into two classes. Class I solvents show only a single and comparatively sharp signal corresponding to $[Li(solv)_n]^+$, or to a fast exchange between solvated Li$^+$ and Li$^+$ sites in the capsule. (The exchange – if any – could not be quenched down to temperatures as low as −78 °C in the case of CD$_3$OD‡‡; in all cases, the observed chemical shifts correspond to those for $[Li(solv)_n]^+$.[8,9]) For class II, *viz.* DMSO, DMF and NMP, with donor numbers larger than those of class I *and* rather large dipole moments, the above mentioned broad high-field signals representing Li$^+$ ions at internal capsule sites are found as in case of the DMSO system in addition to the relatively narrow low-field signal for $[Li(solv)_n]^+$. The donor number of the solvent apparently influences the situation/equilibria, while the dielectric properties do not seem to have a systematic effect.

As the solvents belonging to class II readily dissolve **1**, it is correspondingly evident that, along with the $[Li(solv)_n]^+$ complexes present, the capsule **1a** is solvated effectively. The "barrier" thus formed slows down the Li$^+$ exchange between $[Li(solv)_n]^+$ and $\{Li^+(H_2O)_n \subset$ **1a**$\}$ to the extent where the equilibrium processes involved can be detected on the NMR time scale, allowing an observation of those occupied internal capsule sites which have a sufficiently long lifetime. (In addition, the relatively large molecular volumes of the class II solvents, compared to class I, should, because of their limited mobility, also slow down the exchange.)

Fig. 2 ^{7}Li NMR spectra of **1** dissolved in DMSO (c(**1**) = 0.8 mM), dependent on the amount of water: Trace **a**, dry DMSO under N$_2$, $c(H_2O)$ ≈ 0.16 M; trace **b**, $c(H_2O)$ ≈ 0.26 M; trace **c**, $c(H_2O)$ ≈ 2.5 M; trace **d**, $c(H_2O)$ ≈ 13 M. Right: ^{7}Li,^{7}Li EXSY spectrum (1.5 s mixing time) of the situation represented by trace **c**.
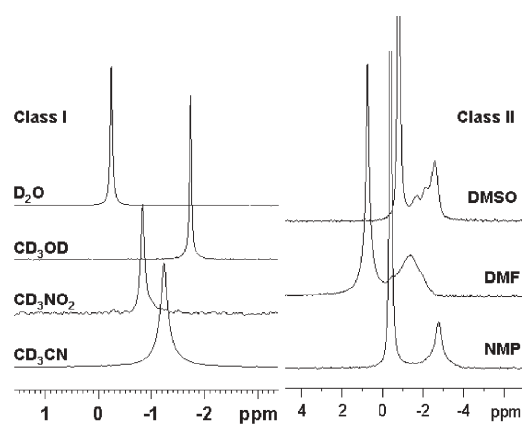

Fig. 3 ^{7}Li NMR spectra of **1** in different solvents; class I solvents, showing only one NMR peak have both relatively smaller donor numbers and smaller molecular sizes than class II solvents.

The positively charged and comparably stable complexes $[Li(solv)_n]^+$ are expected to "sit" – as in the case of several positively charged transition metal complexes[10] – preferentially above the negatively charged pores, where the release of the DMSO ligands should occur "slowly", concomitantly with the entrance of H_2O into the Li^+ coordination sphere.

From the experiment it follows that the concentration of water as active vehicle strongly influences the equilibria between the different(!) Li^+ mixed ligand, i.e. water–DMSO complexes, while the concentration of complexes with more water ligands, including $[Li(H_2O)_4]^+$, increases with increasing water content (see Fig. 2).§§ This finally also allows Li^+ to enter easily into the cavity after two or three water ligands have been released from the complex. Inside the highly charged capsule the coordination environment of entering Li^+ is governed by encapsulated water molecules in addition to the O(Mo) and O(S) functions[1,2] (see also ref. 6).

The above mentioned situation can be compared with passive cation transport in biological channels in the direction of the "electrochemical gradient", when part of the water ligands during channel trafficking is still attached to the cations so that the hydrophilic environment is similar before, during and after the uptake.¶¶ In the present case the resulting equilibrium situation (see EXSY spectrum, Fig. 2) can be especially compared with the equilibrium situation in the open K^+ leak channels which are ubiquitous in eukaryotic cells and filled with water as transport medium.[13] These channels play a critical role in normalising the membrane potential while the main net efflux of K^+ stops when the membrane potential reaches a value where the electric driving force for K^+ exactly balances the concentration gradient (corresponding to a K^+ zero electrochemical gradient). In any case, our capsule is in the sense of uptake or release an analogue to the biological counterpart where the cells are "busy places" with membrane traffic in all directions from the surface to internal compartments and back.[14] Interestingly, biological ion transports with the simple basic physical background were considered in one of the most famous textbooks of physics even before related details became known as they are responsible for the fascinating signal transfer processes.[15]‖‖

Notes and references

§ Compound **1** was synthesised and characterised as described in ref. 2a. ^{7}Li NMR spectra were recorded on a Bruker Avance 400 spectrometer at 155.51 MHz in usually non-rotating 5 mm diameter vials with the parameter settings reported in ref. 2a.

¶ Contrary to the fact that H_2O assemblies occur in all of these types of capsules,[6] the DMSO molecule and the other class II solvent molecules (Fig. 3) are too large to enter through the channels; a corresponding proof was obtained from the ^1H,^1H EXSY spectrum of the analogous system containing formate instead of sulfate inside the capsule and DMF as solvent.

‖ Though in the earlier study (ref. 2a; note the corrigendum to Fig. 4, referring to the T dependence) details of parameter influences and especially the vehicle function of water were not referred to, it could be shown that

exchange between $[Li(dmso)_n]^+$ and Li^+ sites associated with **1a** increases as the temperature increases (coalescing around 340 K). After cooling to room temperature, this initial situation is, however, not completely restored, presumably because the first Li^+ ions which are taken up occupy sites in the pore/channel region and thus in part block off the uptake of lithium ions into inner capsule sites. The low-field signal at -1.8 ppm was assigned to internal capsule sites for this reason, and the high-field components (-2.2 to -2.6 ppm) to peripheral sites; cf. also Fig. 1, B.

** There is no obvious correlation with Gutmann's acceptor numbers, employed here,[5] which vary between 13.3 {NMP} and 54.8 {H_2O} for the solvents.

†† The following solvents were excluded due to the very low solubility of **1**: acetone, ethanol, pyridine, dioxane, tetrahydrofuran, t-butylamine, i-propanol, 2-butanol, formic acid-tris(isopropylester).

‡‡ When **1** was dissolved in CD_3OD, pre-cooled to -78 °C, and immediately transferred to the pre-cooled spectrometer probe-head, only a single signal was observed.

§§ The cations $[Li(H_2O)_n]^+$ (preferably $n = 4$)[11] cannot block the pores because of the lability of the water ligands (residence time in water $\sim$1 ns corresponding to the low value of the hydration enthalpy of 519 kJ mol^{-1}).[11]

¶¶ In the case of the bacterial K^+ channel with C=O functions, however, the water ligands are released before entering.[12]

‖‖ The solvent–water mixture should reflect to some extent biological situations in the sense that the relevant medium does not have the properties of bulk water, but rather of water containing many low and high molecular mass ingredients which change the water properties drastically, e.g. with respect to the consequential density changes influencing the mobility and solubility of cations. (Note that bio-molecules are separated only by a small number of water layers.)[16]

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