ChemComm

Degeneracy in Cryptophane-Xenon Complex Formation in Aqueous Solution

Journal:	*ChemComm*
Manuscript ID:	CC-COM-10-2014-008601.R1
Article Type:	Communication
Date Submitted by the Author:	04-Dec-2014
Complete List of Authors:	Korchak, Sergey; Physikalisch-Technische Bundesanstalt, Medical Physics and Metrological Information Technology Kilian, Wolfgang; Physikalisch-Technische Bundesanstalt, Medical Physics and Metrological Information Technology Mitschang, Lorenz; Physikalisch-Technische Bundesanstalt, Medical Physics and Metrological Information Technology

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Degeneracy in Cryptophane-Xenon Complex Formation in Aqueous Solution

Sergey Korchak, Wolfgang Kilian, and Lorenz Mitschang*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

The reversible binding of xenon to cryptophane molecules is currently heavily explored for application as a reporter system in NMR. Herein, for aqueous solution, first evidence of degenerate exchange in this host-guest system is presented based on a novel approach using hyperpolarized ^{129}Xe.

The large signal enhancement of several orders due to hyperpolarization (hp) and its large chemical shift dispersion make ^{129}Xe a highly sensitive probe for investigations of molecular environments by nuclear magnetic resonance (NMR).[1,2] The non-toxic gas is dissolvable in blood and other biological fluids and can be delivered through inhaling for in-vivo imaging of lungs and tissue.[2,3] In combination with cryptophane molecules applicability and sensitivity of the approach are further expanded. Cryptophanes are a diversified family of cage molecules composed of two semi-spherical cyclotriveratrylenes connected by linkers of various lengths which bind small, apolar molecules non-covalently and reversibly.[4] For xenon binding crytophane-A molecules with three ethylene linkers are very attractive because just a single atom is enclosed with high binding constant and xenon residence times which are slow on the NMR timescale.[5,6] Complex formation with hp ^{129}Xe can thus be explored for additional signal amplification by chemical exchange saturation transfer (hyperCEST)[7,8] while modifications in the host-guest interaction, e.g. induced by deformations of the cage, can translate directly in a change of transfer rate.[9,10] The exceptional xenon binding properties make cryptophane-A which can be functionalized to bind desired targets currently the candidate of choice for xenon biosensing with the aim to image biomolecules and their interaction in tissue and organisms.[11]

The complex formation of xenon with cryptophane-A and derivatives has been previously quite extensively studied as a system governed by van der Waals forces.[5,12] The analyses focused on organic solutions assuming a simple dissociation process where xenon enters or leaves the otherwise xenon-free host. Interestingly, Bartik et al. found in tetrachloroethane solvent indications for a further exchange mechanism, the replacement of host-bound xenon directly by freely dissolved xenon.[5] To our knowledge, no other study on the evidence of such a degenerate exchange process has been published to date. Apparently, the understanding of the processes of reversible xenon binding to cryptophane is still far from complete, especially in aqueous solution. This situation is pressing, also with respect to applications because the prevailing exchange mechanisms in any particular situation can affect sensitivity of the method of choice, e.g. hyperCEST, or the interpretation of data.[7,13] This work thus attempts to determine the exchange kinetics in the cryptophane-xenon host-guest system in aqueous solution with particular emphasis on the detection of the degenerate exchange mechanism. NMR exchange experiments on hyperpolarized ^{129}Xe and the water-soluble derivative monoacetic acid cryptophane-A (CrAma) are employed for qualitative and quantitative analysis.

A xenon high field NMR spectrum of the cryptophane-xenon host-guest system in solution shows two well separated signals, which are indicative of the slow exchange between the pools of free and cryptophane-bound xenon, respectively (see Figure S1 in the Supporting Information). The signal intensities reflect the partition of xenon in between both pools. At dynamic equilibrium the magnetization of free and cryptophane-bound xenon, M_{Xe} and M_{CXe}, respectively, are related by $M_{Xe}k_{on}=M_{CXe}k_{off}$ with k_{on} and k_{off} as the transition rates for transfer of free xenon to the cryptophane-bound pool and vice versa. However, a degenerate exchange mechanism (Fig. 1)

$$Xe^* + CXe \overset{k}{\leftrightarrow} Xe + CXe^*$$

where freely dissolved xenon is denoted as *Xe*, or *Xe**, and cryptophane-xenon complexes as *CXe*, or *CXe**, remains unrecognized in this spectrum because it does not affect the intensity or pattern of the spectral lines further. In order to find means to identify such a process, consider the situation in NMR exchange spectroscopy with the magnetization in the freely dissolved xenon pool being selectively inverted and subsequently allowed to exchange freely.[14] A depletion of the magnetization in the cryptophane-bound xenon pool occurs because, firstly, magnetization is lost at rate k_{off} due to dissociation of the complex, and, secondly, negative magnetization is gained at rate k_{on} from the freely dissolved xenon pool when the complex associates anew. A derivation of the depletion rate $R_d=k_{on}+k_{off}$ by the Bloch-McConnell equations which take into account chemical exchange can be found in the Supporting Information. Moreover, in any case of fairly diluted host molecules $M_{CXe}<<M_{Xe}$ can be achieved by a proper choice of freely dissolved xenon concentration. In consequence, $k_{on}<<k_{off}$ in dynamic equilibrium and the depletion rate then simplifies to $R_d = k_{off}$. The degenerate exchange process will contribute with rate $k[Xe]$ to the transition rate k_{off} because the frequency of collisions for replacement of cryptophane-bound xenon is proportional to the concentration of freely dissolved xenon, [*Xe*]. Similarly, the complex can also

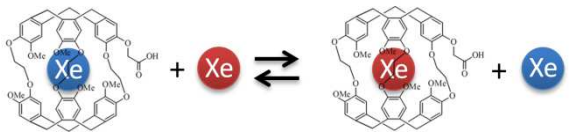

Figure 1 Scheme for degenerate exchange in xenon binding to monoacedic acid cryptophane-A (Me = CH_3).

possibly dissociate through the replacement of cryptophane-bound xenon by guest molecules other than xenon present in the solution. Such mechanisms as well as the spontaneous decay of the cryptophane-xenon complex occur at a rate independent of the free xenon concentration and may be collectively addressed as xenon-independent dissociation processes. It is known that cryptophanes can host a range of small guest molecules, including water, helium and possibly molecular oxygen, or nitrogen.[4–6] Some of the species are essentially present in most common solvents, particularly water, and may than contribute rates proportional to their concentration to k_{off}. Under condition of constant concentration these rates, as well as the rate of spontaneous decay, are constant such that k_{off} is the sum of a constant component, c, stemming from xenon-independent dissociation, and a component proportional to free xenon concentration, specific to degenerate exchange,

$$R_d = c + k[Xe].$$

Thus, by a series of exchange experiments differing in the concentration of freely dissolved xenon, the presence of degenerate exchange can be clearly proven: it is causative for a linear dependence of R_d on the dissolved xenon concentration. Any mechanisms inducing xenon-independent dissociation of the complex account for a constant offset in the depletion rate.

A solution of CrAma in pure water (~6.5 µM) was prepared (see SI) and filled in a commercial screw-cap NMR tube supplemented by a home built tube setup to enable bubbling of hp ^{129}Xe gas through the sample. A gas mixture of helium, nitrogen, and xenon (^{129}Xe in natural abundance of 26%) was provided by a home built xenon polarizer[16] and lead to a nearby 9.4 T wide bore NMR spectrometer where the experiments were conducted. The mixture had constant total and nitrogen partial pressures of 3 bar and 0.2 bar, respectively, while xenon and helium partial pressures were varied complementarily. At each setting of xenon partial pressure, p_{Xe}, the polarizer was allowed to settle for stable generation of hyperpolarized ^{129}Xe and run until xenon saturated flow through the temperature equilibrated sample (at 298 K) was achieved. Directly prior to execution of the pulse sequence bubbling was stopped and the sample was allowed to settle for 2 s to recycle any material from built up foam. Thus, for given p_{Xe} the solution was assumed to be saturated at a xenon concentration given by Henry´s law, $[Xe] = s\, p_{Xe}$, where s = 4.32 mM/bar is the xenon solubility in water at 298 K.[17]

In the exchange experiments on the xenon-saturated solution the magnetization of freely dissolved xenon was selectively inverted and allowed to exchange freely for a period τ_{ex}; subsequently, the magnetization of CrAma-bound xenon was selectively excited and detected (Fig. 2(a)). Upon integration of the signal in the Fourier-Transform spectrum, signal decay could be monitored in dependence of τ_{ex}. In total, six series of exchange experiments were conducted with settings for p_{Xe} of 0.02, 0.1, 0.2, 0.4, 0.8, and 1.2 bar and eight fixed exchange periods τ_{ex} between 0 and 256 ms. The data of each series were approximated by the mono-exponential function $A\exp\{-R_d\tau_{ex}\}+B$ in a non-linear least squares fit (Fig. 2(b)). As the central result, in Figure 2(c) the dependence of the depletion rate on the xenon partial pressure is presented. It is evident that the rate increases with partial pressure (or, free xenon concentration) which cannot be accounted for solely by xenon-independent dissociation processes. The presence of a degenerate exchange mechanism can be deduced by approximating the data by a linear function. A linear least squares fit with $[Xe] = s\, p_{Xe}$ reveals the kinetic rate constants for xenon-independent dissociation and degenerate exchange $c = 19 \pm 1$ s^{-1} and $k = 5600 \pm 800$ M^{-1}s^{-1}, respectively.

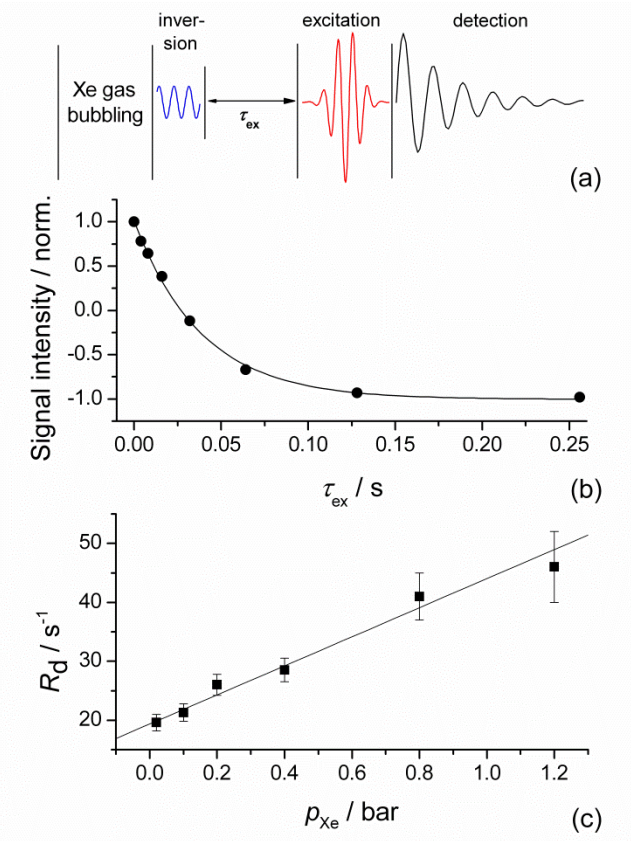

Figure 2 (a) Scheme of the NMR exchange experiment. (b) Normalized intensities (dots) of CrAma-bound xenon magnetization at p_{Xe} = 0.2 bar in dependence of exchange delay τ_{ex}. Mono-exponential curve fitting (solid line) reveals a depletion rate $R_d = 26.0 \pm 1.8$ s^{-1}. (c) Depletion rate in dependence of xenon partial pressure with linear fit.

In the analysis any contributions to the depletion rate by longitudinal relaxation of free or cryptophane-bound xenon were ignored (see SI). As is known for water soluble derivatives of crytophane-A, longitudinal relaxation of free or crytophane-bound xenon occurs with time constants of the order of minutes or tens of seconds, respectively, in aqueous solution.[11(g),12(d)] This is much longer than the exchange periods of maximally 256 ms used in the experiments such that the observed depletion is dominated by chemical exchange. Also the variation of helium concentration in the exchange experiments (helium partial pressure was between 1.6 and 2.78 bar while xenon partial pressure varied between 1.2 and 0.02 bar) was assumed to have no effect on the results. Because of the very low polarizability of the helium electron shell the binding affinity to CrAma may be much smaller in comparison to xenon. Furthermore, due to the small size of helium, the ability of a single atom to expel xenon from CrAma is likely to be very limited as xenon only occupies less than 50% of the cage volume of 89 Å^3.[6]

In principle, measurements of the line width of crytophane-bound xenon in dependence of p_{Xe} can be used to determine the presence of the degenerate exchange mechanism, as was anticipated by Bartik and coworkers.[5] A quantitative analysis, however, is compromised by contributions to the line width from transversal relaxation, which may well be of the order of the exchange broadening and difficult to separate from, and from inhomogeneities of the static field. These problems are alleviated in the exchange experiment approach presented here. The wide separation on the chemical shift scale of the free xenon signal

from the cryptophane-bound xenon signal (~130 ppm for CrAma) allows for very clean selective excitation and intensity determination (integration) of the latter one. The proposed approach, however, requires revision under more extreme conditions than discussed here. When the exchange and relaxation rates become of similar order depletion during τ_{ex} is governed by both processes. The simple linear dependence of the depletion rate on dissolved xenon concentration must then be replaced by a more complex expression in the matrix eigenvalues of the Bloch-McConnell equation including the relaxation rates (see SI).

It is an interesting question, whether other processes than xenon-independent dissociation or degenerate exchange can substantially contribute to the kinetics. The direct hopping of xenon from one host to the next was found to play no, or at most a very minor role in organic solvent.[12(a)] That mechanism, however, would be degenerate within the cryptophane-bound pool and not affect the freely dissolved xenon pool. An exchange of xenon in between the pools could stem from higher order dissociation mechanisms, from collisions of two or more host molecules which would result in the transfer of at least one xenon atom from its host into the solution (and, possibly, the reverse transfer). In aqueous solutions and biomedical applications the host molecules, possibly functionalized, will be highly diluted, such that higher order events too rarely occur to have a considerable impact on the kinetics. However, it remains at this point open to which extent various guest molecules different to xenon but present in the solution (nitrogen, water) affect the overall transition rates k_{on} and k_{off} individually as only their cumulative effect can be determined by the rate c. Application of the approach presented here can further progress in cases where k_{off} can be determined in dependence of the concentration of the compound in question.

In summary, for aqueous solution, a degenerate exchange mechanism for xenon bound to cryptophane host molecules was found. This exchange mechanism can be unequivocally identified by the linear dependence of the magnetization depletion rate of cryptophane-bound xenon on the free xenon concentration in a series NMR exchange experiments. In addition, spontaneous decay of the complex and further xenon-independent dissociation processes where host-bound xenon is replaced by other guest molecules manifest as a constant offset to the depletion rate. For monoacetic acid crytophane-A in pure water the rates were quantified in this way to 19 s^{-1} and 5600 M^{-1} s^{-1} for dissociation and degenerate exchange, respectively. The approach can be readily applied to unravel the kinetic network of complex formation of xenon with other members of the cryptophane family or their derivatives, including bare or functionalized host-molecules in non-pure watery solutions, like blood or cell suspensions. The understanding of the kinetics in these important host-guest systems could be further deepened and optimization strategies put forward in the applications.

This work was supported by funding from the Bundesministerium für Bildung und Forschung (01EZ1010A) and the European Community´s Seventh Framework Programme, ERA-NET Plus (grant agreement No. 217257). The authors thank L. Schröder and C. Witte at FMP for support in the experimentation.

Notes and references

[a] *Physikalisch-Technische Bundesanstalt, Division of Medical Physics and Metrological Information Technology, Abbestr. 2 – 12, 10587 Berlin, Germany, Fax: +49 30 34817553; Tel: +49 30 34817303; E-mail: Lorenz.Mitschang@ptb.de*

† Electronic Supplementary Information (ESI) available: Sample preparation, ^{129}Xe NMR experiments, data evaluation, depletion rate by Bloch-McConnell equation. See DOI: 10.1039/b000000x/

1. T. Walker, W. Happer, *Rev. Mod. Phys.*1997, **69**, 629.
2. B. Goodson, B. M. *J. Magn. Reson.* 2002, **155**, 157.
3. S. B. Fain, F. R. Korosec, J. H. Holmes, R. O'Halloran, R. L. Sorkness, T. M. Grist, *J. Mag. Reson. Imag*. 2007, **25**, 910.
4. T. Brotin, J.-P. Dutasta, *Chem. Rev*. 2009, **109**, 88.
5. K. Bartik, M. Luhmer, J.-P. Dutasta, A. Collet, J. Reisse, *J. Am. Chem. Soc.* 1998, **120**, 784.
6. O. Taratula, P. A. Hill, N. S. Khan, P. J. Carroll, I. J. Dmochowski, *Nat. Commun.* 2010, **1:148**, doi: 10.1038/ncomms1151.
7. L. Schröder, T. J. Lowery, C. Hilty, D. E. Wemmer, A. Pines, *Science* 2006**, 314**, 446.
8. (a) Y. Bai, P. A. Hill, I. J. Dmochowski, *Anal. Chem.* 2012, **84**, 9935; (b) Y. Bai, Y. Wand, M. Goulian, A. Dirks, I. J. Dmochowski, *Chem. Sci.* 2014, **5**, 3197.
9. (a) M. Schnurr, C. Witte, L. Schröder, *Phys. Chem. Chem. Phys.* 2013, **15**, 14178; (b) M. Schnurr, C. Witte, L. Schröder, *Biophys. J.*. 2014, **106**, 1301.
10. F. Schilling, L. Schröder, K. K. Palaniappan, S. Zapf, D. E. Wemmer, A. Pines, *ChemPhysChem* 2010**, 11**, 3529.
11. (a) M. M. Spence, S. M. Rubin, I. E. Dimitrov, E. J. Ruiz, D. E. Wemmer, A. Pines, S. Q. Yao, F. Tian, P. G. Schultz, *Proc. Natl. Acad. Sci. U. S. A* 2001**, 98**, 10654; (b) Q. Wei, G. K. Seward, P. A. Hill, B. Patton, I. E. Dimitrov, N. N. Kuzma, I. J. Dmochowski, *J. Am. Chem. Soc.* 2006**, 128**, 13274; (c) A. Schlundt, W. Kilian, M. Beyermann, J. Sticht, S. Günther, S. Höpner, K. Falk, O. Rötschke, L. Mitschang, C. Freund, *Angew. Chem. Int. Ed.* 2009, **48**, 4142; (d) C. Boutin, A. Stopin, F. Lenda, T. Brotin, J.-P. Dutasta, N. Jamin, A. Sanson, Y. Boulard, F. Leteurtre, G. Huber, A. Bogeart-Buchmann, N. Tassali, H. Desvaux, M. Carriere, P. Berthault, *Bioorg. Med. Chem.* 2011, **19**, 4135; (e) G. K. Seward, Q. Wei, I. J. Dmochowski, *Bioconjugate Chem.* 2008, **19**, 2129; (f) G. K. Seward, Y. Bai, S. Khan, I. J. Dmochowski, *Chem. Sci.* 2011, **2**, 1103; (g) L. Taratula, I. J. Dmochowski, *Curr. Opin. Chem. Biol.* 2010, **14**, 97.
12. (a) T. Brotin, A. Lesage, L. Emsley, A. Collet, *J. Am. Chem. Soc.* 2000, **122**, 1171; (b) T. Brotin, T. Devic, A. Lesage, L. Emsley, A. Collet, *Chem. Eur. J.* 2001, **7**, 1561; (c) P. A. Hill, Q. Wei, R. G. Eckenhoff, I. J. Dmochowski, *J. Am. Chem. Soc.* 2007, **129**, 9262; (d) G. Huber, T. Brotin, L. Dubois, H. Desveaux, J.-P. Dutasta, P. Berthault, *J. Am. Chem. Soc.* 2006, **128**, 6239.
13. L. Schroeder, T. Meldrum, M. Smith, T. J. Lowery, D. E. Wemmer, A. Pines, *Phys. Rev. Lett.* 2008, **100**, 257603.
14. R. R. Ernst, G. Bodenhausen, A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Oxford University Press, New York, 1990.
15. D. R. Jacobson, N. S. Kahn, R. Collé, L. Laureano-Perez, Y. Bai, I. J. Dmochowski, *Proc. Natl. Acad. Sci. USA.* 2011, **108**, 10969.
16. S. E. Korchak, W. Kilian, L. Mitschang *Appl. Magn. Reson.* 2013, **44**, 65.
17. H. L. Clever, *Krypton, Xenon, and Radon: Gas Solubilities*; Pergamon Press, Oxford, 1979.