Supramolecular Chemistry

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Activation and Stabilization of Drugs by Supramolecular pK_a Shifts: Drug-Delivery Applications Tailored for Cucurbiturils**

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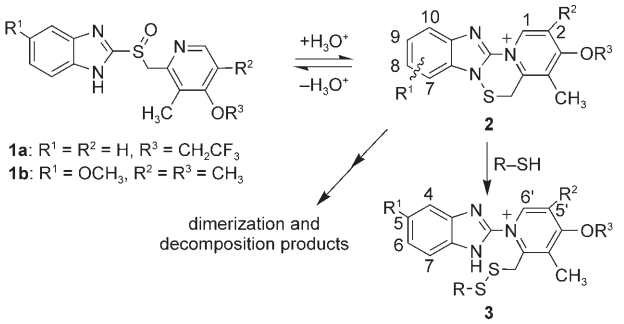
There is rapidly developing interest in the potential of macrocyclic host molecules to modify the effective pK_a values of included guests. Following early reports of small pK_a shifts of included guests in cyclodextrins ($\Delta pK_a \approx 1$)[1,2] and cucurbit[6]uril[3] ($\Delta pK_a \approx 1$), we have used cucurbit[7]uril (CB7, $\Delta pK_a \approx 2$-3)[4,5] and sulfonatocalixarenes ($\Delta pK_a \approx 2$) as additional hosts and proposed structure–reactivity relationships.[6] Complexation by CB7 has also been reported to cause shifts of the excited-state protonation equilibria.[7] Most recently,[8] Pluth et al. have provided impressive examples of pK_a shifts in a metal-organic supramolecular host, with values up to 4.5 found, thus approaching biological examples of pK_a shifts, which range up to 5 pK_a units.[9] Herein, we demonstrate that similarly large pK_a shifts can be achieved with simple macrocycles such as CB7 when, for example, benzimidazoles are selected as guests. With respect to applications, we have previously exploited pK_a shifts in protolytic displacement assays, where the protonation of indicator dyes in the complex enhances the fluorescence response.[5,10] In an impressive enzyme-mimetic example,[11] Pluth et al. exploited pK_a shifts in acid-catalyzed reactions. Herein, we demonstrate how supramolecular pK_a shifts can be used to activate and stabilize proton-pump inhibitors in aqueous solution. The inhibitors used are orally administered commodity drugs such as lansoprazole (**1a**, Scheme 1) and omeprazole (**1b**) which are being prescribed worldwide to cure diseases related to the secretion of gastric acid such as gastroesophageal reflux as well as gastric and duodenal ulcers.[12–16]

Chromophoric guests are advantageous for studies of complexation-induced pK_a shifts, because they allow a direct determination of their pK_a values in both their complexed and free forms.[4–6] Our exploratory studies with drugs concerned benzimidazoles, for example, thiabendazole (TBZ), a widely used agricultural fungicide.[17,18] In the course of these studies we observed that CB7, besides enhancing the solubility of the guests in the desired manner,



Scheme 1. Decomposition and reaction pathways.

preferentially binds the benzimidazole residue and shifts its pK_a value by up to 4 units, the largest directly determined value so far reported for an organic macrocyclic host. The mode of inclusion of TBZ ($K = (1.8 \pm 0.4) \times 10^6\ \text{M}^{-1}$), for example, was established by the characteristic shifts in the NMR spectrum (see the Supporting Information), while the preferential protonation of the benzimidazole ring in the complex was quantified by UV titrations (Figure 1). The

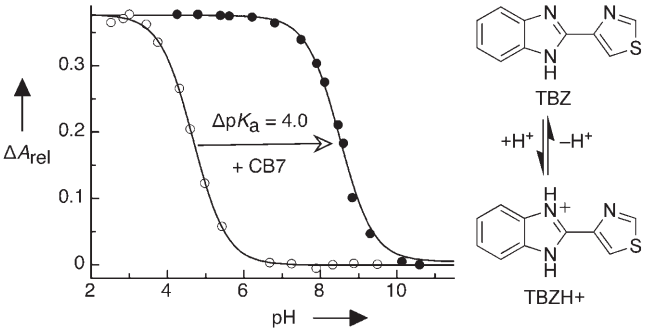


Figure 1. pH titration by monitoring the UV absorption band of TBZ ($\lambda_{mon} = 318$ nm, 15 μM, in water) in the absence (open circles) and presence of 2.5 mM CB7 (filled circles). The pertinent protonation equilibrium is shown on the right.

higher pK_a value of TBZ in its CB7 complex is a consequence of the cation receptor properties of this class of macrocyclic hosts,[19–22] which cause a selective stabilization of the protonated form of the guest.

The proton-pump inhibitors **1** were subsequently selected as medicinally relevant benzimidalzoles[14–16] with the idea of utilizing supramolecular pK_a shifts for the activation and stabilization of these drugs. The reaction pathways of **1** have been the subject of intensive mechanistic investigations (Scheme 1). One of the two major limitations of the medicinal use of these drugs is their slow conversion into the cyclic

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sulfenamide **2**, because it is this active form of the drug which reacts with cysteine residues of gastric (H^+-K^+)-ATPase[14] and reduces the production of gastric acid. The second limitation is the rapid dimerization and decomposition of the active form (within minutes) at strongly acidic pH values in water, which is the physiologically condition in the stomach.[14]

For both **1a** and **1b**, the two most widely used drugs, the addition of CB7 results in pronounced beneficial effects on both the activation and stabilization. First, CB7 catalyzes the rapid and highly efficient formation of the active form of the drug (**2**). Second, CB7 stabilizes the cyclic sulfenamide **2**, such that decomposition pathways are effectively suppressed. The kinetics of the activation and decomposition of the drug could be directly followed by UV spectroscopy in aqueous solution at pH 2.9 (Figure 2),[23] since the UV absorption band at

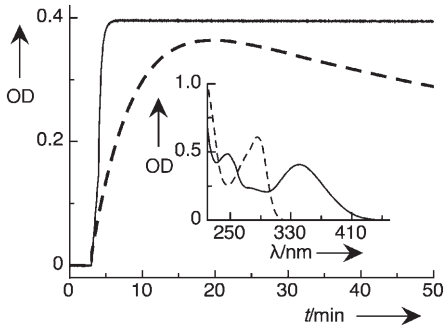


Figure 2. Evolution of the active form upon dissolution of **1a** (50 μM) in aqueous solution at pH 2.9 followed by UV spectroscopy (λ_{mon} = 340 nm) in the absence (dashed line) and presence (solid) of 0.2 mM CB7. The inset shows the UV spectra of **1a** (dashed line, immediately after dissolution without CB7, λ_{max} = 287 nm) and of the complex **2a**·CB7 (solid line, with 1 mM CB7 after 3 min, λ_{max} = 340 nm).

340 nm is characteristic of the active form. The estimated half-life for its formation was decreased from about 5 minutes in the absence of CB7 to about 20 seconds in its presence (0.05–5 mM),[24] which corresponds to a rate enhancement from 0.2 min^{-1} to 3 min^{-1}. Accordingly, CB7 increases the kinetics of the acid-promoted formation of the active form by at least 15 fold.[25] Moreover, without CB7, the active form degraded rapidly at pH 2.9, with a half-life of about 60 minutes. In contrast, in the presence of the macrocyclic stabilizer (5 mM), the cyclic sulfenamide **2** was extremely stable, and degraded with a half-live of more than 3 weeks (see the Supporting Information). This value corresponds to a stabilization factor of 500 or more. The addition of CB7 even allowed us to record a clean ^{1}H NMR spectrum of the reactive intermediate **2** in D_2O (Figure 3a); previously the ^{1}H NMR spectrum had only been obtained in organic solvents.[15,16] The close structural relationship between different proton-pump inhibitors means that the stabilizing potential of CB7 is not limited to lansoprazole (**1a**). Experiments carried out with omeprazole (**1b**) revealed similarly favorable effects, namely the faster formation of the active form and its long-term stabilization (see the Supporting Information).

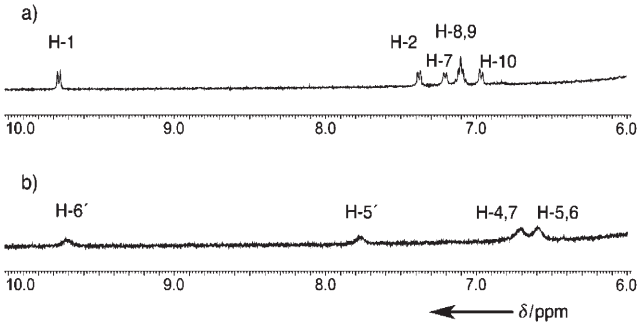


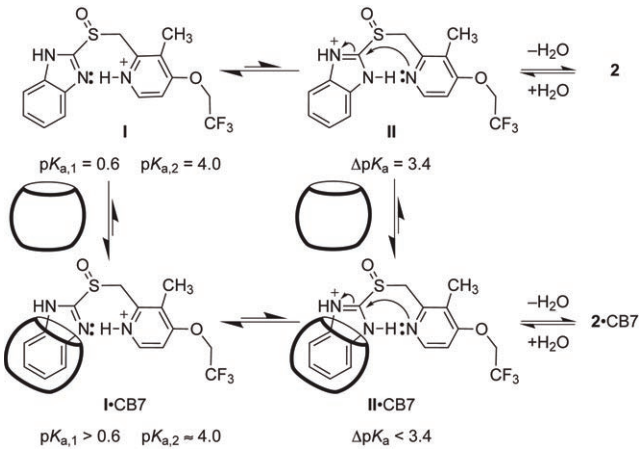
Figure 3. Aromatic region of the ^{1}H NMR spectra recorded in D_2O: a) of the **2a**·CB7 complex (2 mM **1a**, 5 mM CB7) at pD 3.3 and b) the disulfide adduct **3a** obtained in situ from the **2a**·CB7 complex (0.75 mM **1a**, 3 mM CB7) upon addition of 2.0 mM cysteine.

Since it is known from other structurally related compounds (Figure 1) that CB7 forms a strong inclusion complex with the benzimidazole residue, we propose a similar binding mode for the proton-pump inhibitors **1**. The formation of such an inclusion complex between **2a** and CB7 was independently established by the addition of pentane-1,5-diamine (cadaverine, see the Supporting Information). Cadaverine forms a strong inclusion complex with CB7[10,19–21] and displaced the active form into free solution, where it underwent a similarly fast decomposition (half-life 60 min) as in the absence of stabilizer (Figure 2).

The formation of an inclusion complex also accounts for the excellent long-term stability of the cyclic sulfenamide **2** in the presence of CB7. In contrast to the activation rate constant,[24] the degree of stabilization was dependent on the amount of stabilizer present (half-life 3 weeks for 5 mM versus 9 days for 1 mM), which is consistent with a slow decomposition through a residual equilibrium amount of the free form. By assuming that this decomposition depends linearly on the concentration of the free form, and by considering the more than 500-fold stabilization factor with 5 mM CB7 (see above), we could estimate a binding constant for the **2a**·CB7 complex of greater than $10^5\,M^{-1}$. A direct determination of the binding constants of **1** and **2** with CB7 (as well as of the absolute pK_a shift) was unfortunately prevented by the high reactivity of both free species in aqueous solution.

The factors governing the acid-promoted formation of the cyclic sulfenamide **2** from **1** have been intensively studied. The essential thermodynamic equilibrium in Scheme 2 (top) shows the two monoprotonated forms of **1a** (the abundance of the diprotonated as well as the nonprotonated forms are not included, because they are considered unreactive).[14–16] The structure with a protonated pyridine ring (**I**) is always more abundant because of its higher pK_a value (4.0 versus 0.6), while the structure with a protonated benzimidazole but unprotonated pyridine residue (**II**) attains a small equilibrium concentration of less than 0.1%.[26] The latter species is mechanistically important, because the formation of the active form **2** is presumed to occur from structure **II** by intramolecular nucleophilic attack and subsequent dehydration.[14–16]

The challenge for medicinal chemistry is to increase the basicity of the benzimidazole group while maintaining the



Scheme 2. Thermodynamic equilibrium for **1a**.

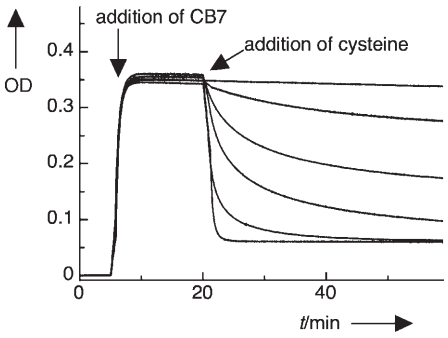


Figure 4. Formation of the active form of omeprazole (**1b**, 50 μM) catalyzed by CB7 (200 μM) and its subsequent reaction with various concentrations of cysteine (0, 20, 30, 60, 80, 120 μM, from top to bottom), monitored by UV spectroscopy at the absorption maximum of **2b** (λ_{obs} = 390 nm).

basicity and nucleophilicity of the pyridine ring. A classical way to achieve this goal is through the incorporation of aryl substituents and, in fact, several structurally related proton-pump inhibitors have been designed (and are being marketed, for example, **1a**, **1b**, rabeprazole, and pantoprazole) in an effort to ensure a rapid activation of the drug by fine-tuning the protonation equilibria. Our present strategy is unconventional, because it employs a selective host–guest complexation and the accompanying supramolecular pK_a shift to increase the basicity of the benzimidazole group, while affecting the basicity and nucleophilicity of the pyridine moiety (which presumably remains positioned outside the macrocyclic host) to a lesser extent. Already a pK_a shift of 1–2 units would increase the equilibrium concentration of the prototropic form **II** and therefore the activation rate 10- to 100-fold, which nicely rationalizes our kinetic findings (Figure 2).[26]

The actual medicinal activity of proton-pump inhibitors is due to the reaction of their active form with cysteine residues of gastric (H^+-K^+)-ATPase,[14] the efficiency of which under physiological conditions is limited, for example, by the yield and rate of formation of the cyclic sulfenamide **2** from the administered drug.[27] The selective and immediate formation of the **2**·CB7 complex in aqueous acidic solution offered the unique possibility to directly monitor its reaction with cysteine by UV spectroscopy. In fact, the addition of cysteine led to a rapid depletion of **2**, even in the presence of CB7 (up to 5 mM), as was demonstrated for both lansoprazole and omeprazole (Figure 4). In line with a bimolecular scavenging process, the observed reaction rate increased with the cysteine concentration, and when less than equimolar amounts of cysteine were offered, only partial conversion was achieved. The formation of the expected addition product,[16] the disulfide **3**, was further established by NMR (Figure 3b) and UV spectroscopy (see the Supporting Information). These preliminary results show that the required reactivity with sulfides is principally retained while the undesirable decomposition is efficiently suppressed.

In summary, the addition of CB7 to proton-pump inhibitors results in an impressive stabilization and rapid quantitative formation of their active sulfenamide form. This activation by CB7 can be mechanistically understood in terms of its ability to shift the effective pK_a values of included guest molecules and thereby promote acid-catalyzed reactions. Macrocyclic hosts, particularly cyclodextrins, have been frequently employed to stabilize and solubilize drugs.[28,29] Moreover, the use of cucurbiturils in the complexation of platinum- and peptide-based drugs has recently been suggested.[30–33] However, the joint beneficial effects on activation and stabilization with retention of the relevant chemical reactivity of the drug are unique.

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[1] F. Cramer, W. Saenger, H.-C. Spatz, *J. Am. Chem. Soc.* **1967**, *89*, 14–20.
[2] X. Zhang, G. Gramlich, X. Wang, W. M. Nau, *J. Am. Chem. Soc.* **2002**, *124*, 254–263.
[3] C. Marquez, W. M. Nau, *Angew. Chem.* **2001**, *113*, 3248–3254; *Angew. Chem. Int. Ed.* **2001**, *40*, 3155–3160.
[4] J. Mohanty, A. C. Bhasikuttan, W. M. Nau, H. Pal, *J. Phys. Chem. B* **2006**, *110*, 5132–5138.
[5] A. L. Koner, W. M. Nau, *Supramol. Chem.* **2007**, *19*, 55–66.
[6] H. Bakirci, A. L. Koner, T. Schwarzlose, W. M. Nau, *Chem. Eur. J.* **2006**, *12*, 4799–4807.
[7] R. Wang, L. Yuan, D. H. Macartney, *Chem. Commun.* **2005**, 5867–5869.
[8] M. D. Pluth, R. G. Bergman, K. N. Raymond, *J. Am. Chem. Soc.* **2007**, *129*, 11459–11467.
[9] F. H. Westheimer, *Tetrahedron* **1995**, *51*, 3–20.
[10] A. Hennig, H. Bakirci, W. M. Nau, *Nat. Methods* **2007**, *4*, 629–632.
[11] M. D. Pluth, R. G. Bergman, K. N. Raymond, *Science* **2007**, *316*, 85–88.
[12] J. Horn, *Clin. Ther.* **2000**, *22*, 266–280.
[13] J. M. Shin, Y. M. Cho, G. Sachs, *J. Am. Chem. Soc.* **2004**, *126*, 7800–7811.
[14] P. Lindberg, A. Brändström, B. Wallmark, H. Mattsson, L. Rikner, K.-J. Hoffmann, *Med. Res. Rev.* **1990**, *10*, 1–54.

[15] U. Krüger, J. Senn-Bilfinger, E. Sturm, V. Figala, K. Klemm, B. Kohl, G. Rainer, H. Schaefer, T. J. Blake, D. W. Darkin, R. J. Ife, C. A. Leach, R. C. Mitchell, E. S. Pepper, C. J. Salter, N. J. Viney, *J. Org. Chem.* **1990**, *55*, 4163 – 4168.
[16] J. Senn-Bilfinger, U. Krüger, E. Sturm, V. Figala, K. Klemm, B. Kohl, G. Rainer, H. Schaefer, T. J. Blake, D. W. Darkin, R. J. Ife, C. A. Leach, R. C. Mitchell, E. S. Pepper, C. J. Salter, N. J. Viney, G. Huttner, L. Zsolnai, *J. Org. Chem.* **1987**, *52*, 4582 – 4592.
[17] M. Danaher, H. De Ruyck, S. R. H. Crooks, G. Dowling, M. O'Keeffe, *J. Chromatogr. B* **2007**, *845*, 1 – 37.
[18] B. Tang, X. Wang, H. Liang, B. Jia, Z. Chen, *J. Agric. Food Chem.* **2005**, *53*, 8452 – 8459.
[19] W. L. Mock, *Top. Curr. Chem.* **1995**, *175*, 1 – 24.
[20] J. W. Lee, S. Samal, N. Selvapalam, H.-J. Kim, K. Kim, *Acc. Chem. Res.* **2003**, *36*, 621 – 630.
[21] J. Lagona, P. Mukhopadhyay, S. Chakrabarti, L. Isaacs, *Angew. Chem.* **2005**, *117*, 4922 – 4949; *Angew. Chem. Int. Ed.* **2005**, *44*, 4844 – 4870.
[22] M. V. Rekharsky, T. Mori, C. Yang, Y. H. Ko, N. Selvapalam, H. Kim, D. Sobransingh, A. E. Kaifer, S. Liu, L. Isaacs, W. Chen, S. Moghaddam, M. K. Gilson, K. Kim, Y. Inoue, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20737 – 20742.
[23] All kinetic measurements were performed at pH 2.9, at which decomposition in the absence CB7 is sufficiently slow to be accurately followed; at higher pH values, the active form is inefficiently formed, while at lower, more acidic, pH values, decomposition is too fast (seconds) to be accurately measured by conventional methods; see Ref. [13].
[24] The activation rate (determined by UV spectroscopy) was independent, within error, of the CB7 concentration (50 μM **1a**, 0.1–4 mM CB7), which suggests that the complexation by the macrocycle is not the rate-determining step in the formation of the active form, but is instead the reaction of **1** to **2** inside the complex.
[25] The actual catalytic effect on the initial nucleophilic substitution step (Scheme 1) may be larger, because the observed rate for the formation of the active species **2** in the presence of CB7 includes a dehydration step as a follow-up reaction.
[26] At any given pH value, the ratio of the monoprotonated prototropic forms **II**/**I** is given as $10^{pK_{a,2}-pK_{a,1}}$.
[27] C. A. M. Stedman, M. L. Barclay, *Aliment. Pharmacol. Ther.* **2000**, *14*, 963 – 978.
[28] K. Uekama, F. Hirayama, T. Irie, *Chem. Rev.* **1998**, *98*, 2045 – 2076.
[29] M. E. Brewster, T. Loftsson, *Adv. Drug Delivery Rev.* **2007**, *59*, 645 – 666.
[30] Y. Jin Jeon, S.-Y. Kim, Y. H. Ko, S. Sakamoto, K. Yamaguchi, K. Kim, *Org. Biomol. Chem.* **2005**, *3*, 2122 – 2125.
[31] N. J. Wheate, D. P. Buck, A. I. Day, J. G. Collins, *Dalton Trans.* **2006**, 451 – 458.
[32] S. Kemp, N. J. Wheate, S. Wang, J. G. Collins, S. F. Ralph, A. I. Day, V. J. Higgins, J. R. Aldrich-Wright, *J. Biol. Inorg. Chem.* **2007**, *12*, 969 – 979.
[33] A. Hennig, G. Ghale, W. M. Nau, *Chem. Commun.* **2007**, 1614 – 1616.

