Effect of Na\(^+\) and K\(^+\) on the cucurbituril-mediated hydrolysis of a phenyl acetate

Nazar Rad\(^*\) and Volodymyr Sashuk\(^*\)

Sodium and potassium are the most abundant cations in living cells. Due to the ion-specific proteins embedded into the membrane, the concentration of potassium is much higher in the cell than in seawater (140 mM vs. 5 mM), while the sodium concentration is lower (30 mM vs. 140 mM). The cell utilizes this imbalance to regulate cell transport, volume maintenance and signal transduction. Sodium and potassium also play a specific role in the activation of some enzymes. These cations can either prevent the substrate encapsulation\(^1\) or lead to the formation of a ternary complex\(^2\) altering the position of the encapsulated guest inside the macrocyclic cavity (Na\(^+\) and Ag\(^+\)).\(^{18,19}\) Transition metal cations coordinated to cucurbiturils promote desilylation (Ag\(^+\)),\(^{15}\) and increase the chemoselectivity of deazotation (Ag\(^+\))\(^{16}\) and the enantioselectivity of Diels–Alder reaction (Cu\(^{2+}\)).\(^{17}\)

The catalytic activity of neat cucurbiturils can be rationalized by the excess electronic density on the two portals that accumulate hydroxonium ions around the reaction centre.\(^{18}\) These macrocycles promote the hydrolytic cleavage of amides, benzoyl chlorides,\(^{20}\) esters,\(^{21}\) ethers,\(^{18}\) triazenes,\(^{22}\) oximes,\(^{19}\) and Schiff bases,\(^{23}\) as well as the formation of hydrazones.\(^{24}\)

As a model reaction we choose the acid hydrolysis of esters (Scheme 1). This reaction is slow enough for observation at low pH. The rate of hydrolysis depends on the concentration of the tetrahedral intermediate TI, which increases with increasing concentration of ester conjugated acid PhAc-\(^H\).\(^{25,26}\) We expected CB7 to promote hydrolysis by enhancing the basicity of the encapsulated substrate.\(^{27,28}\) Although ester hydrolysis is a reversible process, the excess of water molecules almost

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Completely shifts the equilibrium towards product formation. The employed acetate derivative (PhAc) is reminiscent of the neurotransmitter acetylcholine, the concentration of which at the synapse is controlled by the hydrolytic enzyme acetylcholinesterase. As in the case of acetylcholine, a positively charged ammonium group was expected to facilitate complexation.

Initial experiments were performed in an aqueous solution of acid in the absence of any metal cations to avoid the competition of cations with the substrate. The binding constant of PhAc with CB7 determined by $^1$H NMR titration is equal to $(1.5 \pm 0.42) \times 10^6$ M$^{-1}$. The addition of CB7 to PhAc shifts all proton signals upfield [ESI† Fig. S2]. The most shifted signal belongs to aromatic proton 3c in the meta-position to the acetate group. Significantly shifted are also the proton resonances of ammonium group 5c. The less shifted signal corresponds to the proton of acetate group 1c. Based on this, we can conclude that the phenyl ring and ammonium group are buried deep inside the cavity, and the acetate group is localised close to one of the CB7 rims [Fig. 1a].

In the salt-free solution, the hydrolysis rate of PhAc $k_I$ depends linearly on the concentration of H$_3$O$^+$ ions in the range of pH between 0.75 and 2.5 [Fig. 1b]. Since both PhAc and H$_3$O$^+$ involved in the formation of the transition state are positively charged species, $k_I$ depends also on the ionic strength of the solution [eqn (1)]. A similar positive salt effect has already been noted in the hydrolysis of acetylcholine in acid solution. $^{29}$

$$k_I = k^0_I + [D^+] \cdot 10^{-2 \left( \frac{0.509}{7.06I} + 0.14f \right)}$$ (1)

where $k^0_I$ stands for the hydrolysis rate constant of free PhAc, [D$^+$] denotes the concentration of deuterium ions, and $I$ is the ionic strength.

Adding 1.1 eq of CB7 to PhAc accelerates the reaction by more than two orders of magnitude. In this case, the rate of hydrolysis $k_0$ increases linearly [Fig. 1b] as pH decreases following eqn (2).

$$k_0 = k^0_0 + [D^+]$$ (2)

where $k^0_0$ is the rate constant of CB7-promoted hydrolysis.

The slope of log $k_0$ versus log[D$^+$] is equal to unity [Fig. 5b, ESI†]. Thus, the rate of CB7-mediated hydrolysis is insensitive to the ionic strength of the solution. The lack of the primary salt effect for encapsulated PhAc is explained by the presence of the hydrophobic cavity that screens the reaction centre (acetate group) from the charged anchor (ammonium group).

The efficiency of the macrocycle as a catalyst can be assessed by the acceleration factor $\alpha$. The acceleration factor, calculated as the ratio of the hydrolysis rate constants of the encapsulated and free PhAc [eqn (3)], decreases as the ionic strength of the solution increases. That is, if the substrate is charged, the ionic-strength-independent acceleration factor $\alpha^0$ should be given instead. For CB7-promoted PhAc acid hydrolysis, $\alpha^0$ is equal to 263 ± 11.

$$\alpha = \frac{k_b}{k_f}$$ (3)

Assuming that the acceleration effect of CB7 is due to an increase in substrate basicity, the concentration of PhAc@CB7-H should be 263 times higher than the concentration of PhAc-H at the same pH. Since almost all substrate (96%) is encapsulated by CB7 and the concentration of protonated ester is very low ($pK_a$ is equal about −7), the concentrations of free (PhAc) and encapsulated substrate (PhAc@CB7) should be practically the same under reaction conditions. Thus, the found $\alpha^0$ should correspond to the ratio between the basicities of the free and encapsulated substrate [eqn (4)].

$$\frac{K_{a,f}}{K_{a,b}} = \frac{[\text{H}^+] \cdot [\text{PhAc}]}{[\text{PhAc} \cdot \text{H}]} \cdot \frac{[\text{PhAc@CB7} \cdot \text{H}]}{[\text{PhAc@CB7}]} \approx \frac{[\text{PhAc@CB7} \cdot \text{H}]}{[\text{PhAc} \cdot \text{H}]} \approx \alpha^0$$ (4)

The logarithm of $\alpha^0$ is equal to 2.42 [eqn (5)] which is in the range of typical CB7-induced $pK_a$ shifts (2–3).$^{27,28}$ This confirms our assumption that the stabilization of the ester conjugated acid by CB7 is the main reason for accelerated hydrolysis.

In the next step, we studied the effect of alkali metal ions. The addition of sodium chloride sped up the hydrolysis of free PhAc due to the increase of ionic strength and reduced the activity of CB7. The obtained kinetic data do not agree with competitive inhibition, where the sodium cation competes with the substrate for the macrocycle [Fig. 2b].

Moreover, the competitive inhibition scenario does not explain the shifts in the $^1$H NMR spectrum. Upon adding salt, the signal of the 2c proton moves upfield, which can only be accounted for by the translocation of the phenyl ring deeper into the CB7 cavity [Fig. 3b].

To elucidate the source of inhibition, we recorded NMR spectra in the presence of alkali metal halides. The analysis has shown that the shielding of 2c proton signals depends strongly on the nature of the cation [ESI† Fig. S7] and does not change when varying the anion (ESI† Fig. S8). This observation is
accompanying the C7 portal next to the reactive centre to afford a PhAc@C7-Na ternary complex (Fig. 2a). The positively charged alkali cation decreases the concentration of conjugated acid of ester PhAc@C7-H to slow down the acid hydrolysis. The formation of the ternary complex is supported by the mass analysis of the isolated PhAc@C7 complex revealing a monosodium doubly charged molecular ion (ESI† Fig. S21).

The kinetic data of PhAc@C7 hydrolysis in the presence of different amounts of sodium chloride (Fig. 2b) were employed to estimate the PhAc@C7 affinity for the sodium cation according to the model of uncompetitive inhibition. The obtained binding constant is almost identical to the constant determined by NMR titration within the statistical error (Table 2). Thus, the deactivation of C7 correlates with the cation-induced dislocation of the macrocycle toward the ester group.

The binding constants calculated for other cations also yield very close values for both methods. The cation affinity for the PhAc@C7 complex (Table 2) decreases in the same order as the inhibition efficiency of the cations: Na+ > K+ > Cs+ > Li+ (Fig. 4, ESI† Table S3).

The PhAc@C7 complex binds sodium two times stronger than pure C7 (K = 21 ± 2 M⁻¹). This indicates that the organic guest reinforces the binding. The replacement of substrate PhAc by the product PhOH changes the affinity of the C7-guest complex for cations. As with the substrate, the NMR shifts of the product PhOH@C7 depend on the concentration of cations (ESI† Fig S11, S14, S16 and S18). The determined binding constants of PhOH@C7 with cations decrease in the order K+ > Cs+ > Na+ > Li+ (Fig. 4). The similarity between the sodium affinity to free C7 and PhOH@C7 shows that, in contrast to the acetyl group, the hydroxyl group is not involved in the coordination of sodium cations. Thus, both cucurbit[7]uril and the substrate are involved in cation coordination.

To summarise, we scrutinised the effect of ionic strength on ester hydrolysis mediated by cucurbit[7]uril. In the absence of inorganic salts, the macrocycle screens the reaction centre from the cationic anchor making the substrate and the macrocycle insensitive to the ionic strength of the solution. However, after the addition of salts, the catalytic activity of the macrocycle is suppressed. The reason for this is the formation of a ternary complex with alkali metal cations that is less susceptible to hydronium ion attack. This type of inhibition can be described by the uncompetitive model in which the substrate remains bound

Table 1 Hydrolysis rate constants of PhAc@C7 (1.5 mM) in the presence of different salts (10 mM) at pD of 1.55

<table>
<thead>
<tr>
<th>Salts</th>
<th>k_L,M × 10⁶ M⁻¹s⁻¹</th>
<th>k_D,M × 10⁶ M⁻¹s⁻¹</th>
<th>k_L,M/ k_D,M</th>
<th>k_L,M/ k_D,0</th>
<th>k_D,0/ k_D,M</th>
<th>k_D,0/ k_D,0</th>
<th>k_D,M/ k_D,0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt-free</td>
<td>1.3</td>
<td>269.4</td>
<td>1.0</td>
<td>1.0</td>
<td>207</td>
<td></td>
<td></td>
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<tr>
<td>Cation effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LiCl</td>
<td>1.28</td>
<td>268.0</td>
<td>1.0</td>
<td>1.0</td>
<td>210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>1.25</td>
<td>180.9</td>
<td>1.0</td>
<td>0.7</td>
<td>145</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>1.27</td>
<td>183.7</td>
<td>1.0</td>
<td>0.7</td>
<td>145</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CsCl</td>
<td>1.24</td>
<td>229.9</td>
<td>1.0</td>
<td>0.9</td>
<td>185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anion effect</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>NaF⁴⁺</td>
<td>0.83</td>
<td>117.1</td>
<td>0.6</td>
<td>0.4</td>
<td>141</td>
<td></td>
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</tr>
<tr>
<td>NaCl</td>
<td>1.25</td>
<td>180.9</td>
<td>1.0</td>
<td>0.7</td>
<td>145</td>
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<tr>
<td>NaBr</td>
<td>1.33</td>
<td>179.6</td>
<td>1.0</td>
<td>0.7</td>
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<td></td>
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<tr>
<td>NaI</td>
<td>1.35</td>
<td>197.8</td>
<td>1.0</td>
<td>0.7</td>
<td>147</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

k_D,0 and k_D,M correspond to the PhAc and PhAc@C7 hydrolysis rate constants under salt-free conditions. Due to the buffering effect of NaF, the pD of the reaction mixture was higher.

Table 2 The binding constants K_M (M⁻¹) of alkali cations with PhAc@C7 and PhOH@C7

<table>
<thead>
<tr>
<th>Cation</th>
<th>PhAc@C7 kinetics data</th>
<th>PhAc@C7 NMR shifts</th>
<th>PhOH@C7 NMR shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li⁺⁷⁺</td>
<td>1.8 (0.7%)</td>
<td>1.1 (6.8%)</td>
<td>0.9 (4.5%)</td>
</tr>
<tr>
<td>Na⁺⁷⁺</td>
<td>46 (2.0%)</td>
<td>46 (4.8%)</td>
<td>19 (3.6%)</td>
</tr>
<tr>
<td>K⁺⁷⁺</td>
<td>42 (2.1%)</td>
<td>43 (6.7%)</td>
<td>30 (8.8%)</td>
</tr>
<tr>
<td>Cs⁺⁷⁺</td>
<td>12 (2.3%)</td>
<td>11 (4.8%)</td>
<td>22 (11.5%)</td>
</tr>
</tbody>
</table>

Three binding constants for the cation were determined in one experiment. The numbers in parentheses show the relative mean deviation of the calculated data from the experimental ones.
to the macrocycle instead of being displaced by the inhibitor. This work shows that Na and K cations abundant in aqueous solutions can specifically modulate the operation of artificial enzymes. Moreover, the cation selectivity observed during the formation of ternary complexes can be the basis for the development of new cation receptors.

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Conflicts of interest

There are no conflicts to declare.

Notes and references