

# Chloride complexation by heptapeptides: influence of C- and N-terminal sidechains and counterion

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The channel-forming diglycolylated heptapeptides containing the amino acid sequence Gly-Gly-Gly-Pro-Gly-Gly-Gly have been found to complex chloride in  $\text{CDCl}_3$ . The strength of the interaction depends on the terminal alkyl groups and on chloride's counteraction.

Biologically important cations, such as  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$ , are exquisitely regulated in natural systems. The cation channel proteins that provide this control have been studied for more than a century and remain the object of intense scrutiny.<sup>1</sup> The first solid state structures of these remarkable molecules<sup>2</sup> appeared only within the last lustrum but have already merited the Nobel Prize. The protein channels that transport chloride ions have also been extensively studied<sup>3</sup> but structural details have become available only very recently.<sup>4,5</sup> The complexity of the ClC protein channel, evident from the solid state structure, is remarkable and details of the mechanism remain speculative.<sup>6</sup> The need for a simple system to model anion transport led us to develop a membrane-anchored heptapeptide that exhibits both selective transport and complex gating behavior.<sup>7</sup> Although transport and complexation are different phenomena,<sup>8</sup> it seems reasonable that a chloride selective channel must exhibit molecular recognition for the ion and the formation of at least a weak or transient complex.

Numerous host-guest complexes have been reported that involve various anions and a wide range of receptor molecules.<sup>9</sup> Synthetic anion receptors have been reported<sup>10</sup> by Crabtree, Gale, Mendoza, Schmidtchen, Sessler, and others. Most of these receptors are fairly

rigid macrocycles. In contrast, natural chloride transporters are peptides or proteins. Halide complexing agents derived from amino acids have been reported only recently. Ishida *et al.* bound phosphomonoesters with neutral peptides.<sup>11</sup> Anslyn *et al.* synthesized a peptide-containing receptor for ATP.<sup>12</sup> Yang *et al.* used cyclic pseudopeptides derived from D,L- $\alpha$ -aminoxy acids to bind chloride ions.<sup>13</sup> Kubik and coworkers have reported remarkably strong halide binding in aqueous/organic solutions by using cyclic polyamides individually<sup>14</sup> or covalently linked.<sup>15</sup> A cysteine containing cyclopseudopeptide was reported by Huang *et al.* as an "amphi-receptor" capable of simultaneously complexing cations and anions.<sup>16</sup> We now report that the amphiphilic heptapeptides we have developed to function as chloride transporters<sup>7,17</sup> also complex chloride anions in homogeneous solution. To our knowledge, this is the first example of chloride binding by non-cyclic receptors containing only natural amino acids.

We have previously described the preparation of **2** and **3**.<sup>18</sup> Compounds **1–6** were prepared similarly and were isolated as white solids that had the expected spectral and analytical properties.<sup>19</sup> <sup>1</sup>H-NMR was used to monitor chemical shift changes for the amide protons initially observed at 7.35 and 7.63 ppm ( $\delta$ ) when **3** (**[3]** ~ 4 mM in  $\text{CDCl}_3$ ) was titrated with  $\text{Bu}_4\text{NCl}$  (~ 80 mM in  $\text{CDCl}_3$ ) (see Fig. 1, top panel). Complexation constants (1 : 1) were calculated from these data (min. 3 replicates, stoichiometry determined by Job plot, not shown). Independent analysis for each proton gave the same values, *i.e.*  $K = 1770 \pm 20$  and  $1740 \pm 45$ .

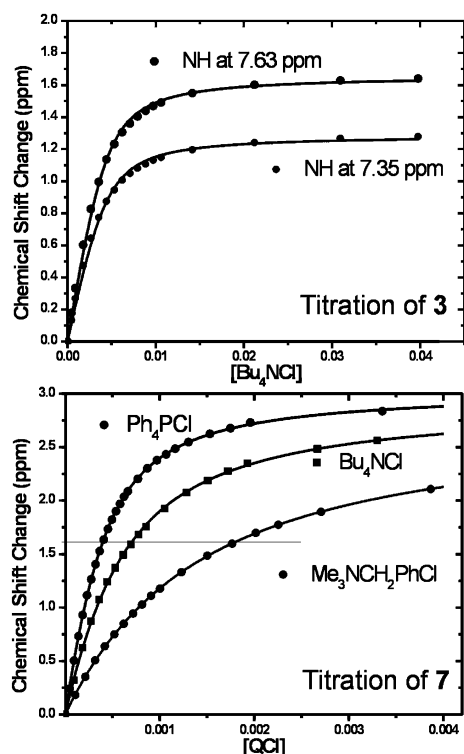
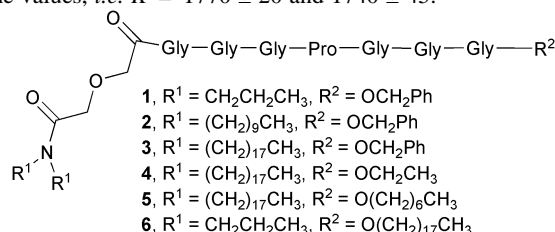


Fig. 1 (Top) Titration of **3** by  $\text{Bu}_4\text{NCl}$  in  $\text{CDCl}_3$  (see text). (Bottom) Titration of **7** by  $\text{Ph}_4\text{PCl}$  (top line),  $\text{Bu}_4\text{NCl}$  (middle line), and  $\text{Me}_3\text{NCH}_2\text{PhCl}$ , all in  $\text{CD}_2\text{Cl}_2$ .



When **3** was titrated with  $\text{Bu}_4\text{PCl}$ , rather than  $\text{Bu}_4\text{NCl}$ ,  $K$  was ~ 500, rather than ~ 1750. The general cation dependence of this system is apparent from titration experiments involving **3** with  $\text{Me}_3\text{NCH}_2\text{PhCl}$ ,  $\text{Et}_3\text{NCH}_2\text{PhCl}$ ,  $\text{Bu}_3\text{NCH}_2\text{PhCl}$ , and  $\text{Ph}_4\text{PCl}$ , the results of which are shown in Table 1. The binding constants for  $\mathbf{3} + \text{QCl} \rightleftharpoons \mathbf{3}\cdot\text{QCl}$  were ~ 1700 ( $\text{Q} = \text{Bu}_3\text{NCH}_2\text{Ph}$ ) and ~ 2400 for  $\text{Q} = \text{Et}_3\text{NCH}_2\text{Ph}$ . The equilibrium constant calculated for  $\text{Me}_3\text{NCH}_2\text{PhCl}$  was ~ 1000 or ~ 2500 depending on which amide proton was observed. The data obtained for titration of **3** with  $\text{Ph}_4\text{PCl}$  appeared reasonable but gave a value of  $K \sim 34,000 \pm 51,000$ .

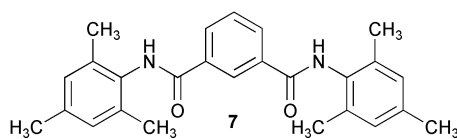
Given these large variations, we sought to confirm our technique by following the detailed and careful study involving **7** reported by Crabtree and coworkers.<sup>20</sup> We successfully reproduced the data reported therein (Table 1). We confirmed the equilibrium constant of ~ 5300 for titration of **7** with  $\text{Ph}_4\text{PCl}$ . We then extended the study of **7** to  $\text{Bu}_4\text{NCl}$ , and  $\text{Me}_3\text{NCH}_2\text{PhCl}$  (bottom panel, Fig. 1). Significantly different binding constants were observed for  $\text{Bu}_4\text{NCl}\cdot\mathbf{7}$  ( $K_a = 2480 \pm 110$ ) and  $\text{Me}_3\text{NCH}_2\text{PhCl}\cdot\mathbf{7}$  ( $K_a = 850 \pm 5$ ). Job plots confirmed 1 : 1 stoichiometry in each case.

The effect of different quaternary cations on anion activity has long been known in the context of ion-pair extraction<sup>21</sup> and phase transfer catalysis.<sup>22</sup> For example, in 1975 Herriott and Picker<sup>23</sup> surveyed 22 quaternary halides for catalytic efficiency in a two-

**Table 1** Maximum chemical shift changes and association constants for **3** and **7** with various chloride salts at 22 °C

Quaternary chloride		Receptor			
		<b>3</b>		<b>7</b>	
		NH (7.63) <sup>a</sup>	NH (7.35) <sup>a</sup>	NH (7.52) <sup>b</sup>	CH (8.48) <sup>b</sup>
Bu <sub>4</sub> NCl	run a	1700	1790	2370	2400
	run b	1740	1750	2590	2660
	run c	1790	1760	2480	2530
	Avg.	1740 (45) <sup>c</sup>	1770 (20)	2480 (110)	2530 (130)
	$\Delta\delta_{\max}$	1.67 ppm	1.28 ppm	2.92 ppm	1.14 ppm
Log <i>K</i>		3.24	3.25	3.39	3.40
Bu <sub>3</sub> NCH <sub>2</sub> PhCl		1700 (130)	1720 (145)	—	—
Bu <sub>4</sub> PCl		510 (20)	510 (10)	—	—
Et <sub>3</sub> NCH <sub>2</sub> PhCl		2600 (220)	2300 (200)	—	—
Me <sub>3</sub> NCH <sub>2</sub> PhCl		2500 (170)	1000 (120)	850 (5)	850 (5)
Ph <sub>4</sub> PCl		35000 <sup>d</sup>	32000 <sup>d</sup>	5100 (160)	4970 (70)

<sup>a</sup> Peak position ( $\delta$ , ppm) of the initial 4.28 mM solution of **3** in CDCl<sub>3</sub> in the absence of additive. <sup>b</sup> Peak position ( $\delta$ , ppm) of the initial 0.33 mM solution of **7** in CD<sub>2</sub>Cl<sub>2</sub> in the absence of additive. <sup>c</sup> Standard deviation of three independent measurements. <sup>d</sup> Data could not be fitted to the used equation.



phase reaction and found rate variations in a standard reaction that ranged over nearly five orders of magnitude. Many of the halide complexation studies have involved rigid macrocycles that were themselves the counterion. Although recognized,<sup>24</sup> this issue has received relatively little attention. To our knowledge, there are only little data available that refer to open-chained complexing agents where the cation has been varied. Smith *et al.* showed that anion binding by synthetic hosts can be inhibited by the presence of different alkali metal cations.<sup>25</sup> Kubik and coworkers reported a variation of about 15% in strengths for binding of NaI, KI, and Me<sub>4</sub>NI by a bridged, dimeric cyclohexamide.<sup>14,15</sup> They worked in 50% D<sub>2</sub>O–CD<sub>3</sub>OD where the cation dependence is expected to be smaller than in chlorocarbon solvents.

If ion-pair, rather than chloride, complexation occurred with host molecules **1–6**, we anticipated that binding would reflect interactions with the N-terminal dialkyl groups, the C-terminal ester groups, or both. Compounds **1–6** (1.8 mM in CDCl<sub>3</sub>) were titrated with Bu<sub>4</sub>NCl. Additional studies showed that the equilibrium constants were not concentration dependent from 0.88 mM to 4.28 mM.

Compounds **1–3** all possess a C-terminal benzyl ester but differ in the dialkyl groups. The binding constants, *K*, (log<sub>10</sub> *K* in parentheses) were as follows: **1** (dipropyl) 1360 (3.13); **2** (didecyl) 1530 (3.18); and **3** (dioctadecyl) 1750 (3.24). Compounds **3–5** all possess twin octadecyl N-terminal groups but differ in the C-terminal ester. The binding constants were as follows: **3** (benzyl-oxy) 1750 (3.24); **4** (ethoxy) 1760 (3.25); and **5** (*n*-heptyloxy) 1790 (3.25). Compounds **1** and **6** possess twin propyl N-terminal groups. Changing the ester group from benzyl (**1**) to *n*-octadecyl (**6**) changed the binding constant from 1360 (3.13) to 1470 (3.17).

Heptapeptides **1–6** constitute what is, to our knowledge, the first example of open-chained peptides, comprised only of essential amino acids, that bind chloride as part of an ion pair. The cation dependence is often not considered in anion binding studies but proved to be significant in this case. Molecular mechanics calculations (not shown) suggest that the peptide coils to encompass Cl<sup>–</sup> with NH hydrogen bonds. The chain length dependence of the amide, but not ester, residues suggests that the quaternary cation interacts with the N-terminal end of the complex more than with the ester. Additional structure–activity studies are underway that are expected to clarify these interactions and their relevance to chloride channel transport.

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- Melting points: **1**, 111–112 °C; **2**, 128–129 °C; **3**, 116–118 °C;<sup>18</sup> **4**, 127–129 °C; **5**, 99–101 °C; and **6**, 135–137 °C.
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