The crystal structure of **1** shows there are three hexaaquacobalt(II) complexes per cyclic unit that are located between the cyclic units in the honeycomb layer and act as countercations, and a further six complexes that are located between the honeycomb layers. The anionic honeycomb layers and the cationic layers composed of hexaaquacobalt(II) cations lie alternately along the *c* axis and results in a canceling out of their electrical charge.

The electronic absorption spectrum of **1** in the solid state has an intense band centered at 383 nm, which is dissimilar to that of the ligand alone with two π-π* transition bands centered at 293 and 336 nm, and is therefore attributed to the metal-to-ligand charge-transfer transition in the cyclic core. The magnetic susceptibilities of **1** over the temperature range 2–300 K show a monotonous decrease in the $\chi_m T$ value (from 7.07 emu K mol^{-1} at room temperature) with decreasing temperature.

In this work we have succeeded in the design and synthesis of a novel cyclic module with a hexacarboxylate derivative. These results have important implications for the design of nano-scaled materials.

Experimental Section

1: H_6L (prepared from a literature procedure[11]) was added to an aqueous NaOH solution. An acetonitrile solution of $Co(NO_3)_2 \cdot 6H_2O$ was carefully layered on top of the aqueous solution and the mixture allowed to stand for one month. The red prismatic crystals obtained were collected by filtration (65% yield). Elemental analysis calcd for $C_{18}H_{22}Co_3N_6O_{23} \cdot 4H_2O$: C 23.02, H 3.22, N 8.95; found: C 22.70, H 2.80, N, 9.27.

X-ray structure determination of **1**: All data were measured on a Rigaku/MSC Mercury CCD diffractometer with graphite-monochromated $Mo_{K\alpha}$ radiation. The structure was solved by Patterson methods (DIRDIF92 PATTY). The water molecules of the $[Co(H_2O)_6]^{2+}$ ion encapsulated in the central cavity of the cyclic hexamer were disordered, and were modeled as oxygen atoms with 33.33% occupancy factors. Non-hydrogen atoms which were not disordered were refined anisotropically, except for the solvent water molecules. All the water molecules were modeled as oxygen atoms. Refinement was carried out with full-matrix least-squares on F^2. All calculations were performed with the TEXSAN crystallographic software package. Crystal data for $C_{18}H_{22}Co_3N_6O_{23} \cdot 12.33H_2O$: $M_r = 1089.34$, crystal size $0.20 \times 0.20 \times 0.20$ mm, trigonal, space group $R\bar{3}$ (No. 148), $a = 22.632(3)$, $c = 39.794(3)$ Å, $V = 17652(3)$ Å^3, $Z = 18$, $\rho_{calcd} = 1.844$ g cm^{-3}, $\lambda(Mo_{K\alpha}) = 0.71069$ Å, $F(000) = 10085.58$, $\mu(Mo_{K\alpha}) = 13.80$ cm^{-1}, $T = 25\,^\circ$C, $2\theta_{max} = 54.2^\circ$. Of the 32745 reflections collected, 8580 were unique ($R_{int} = 0.043$). For 4373 reflections with $I > 4.00\sigma(I)$, 518 parameters; $R(R_w) = 0.087(0.238)$. Min./max. residual electron density $-0.88/1.10$ e$^-$ A^{-3}.

Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-161830. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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Chaotropic Anions Strongly Stabilize Short, N-Capped Uncharged Peptide Helicies: A New Look at the Perchlorate Effect**

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In the course of an analysis of the pH-dependent helicity of short alanine peptides N-capped with helix-initiating templates of the Ac-Hel family,[1] we noted a remarkable helix stabilization caused by binding of anions with low hydration volumes, the so-called chaotropic anions.[2] These are well-known to bind both to pH-denatured proteins[3] and to helically disposed polycationic peptides,[4] electrostatically shielding and thereby stabilizing partially folded conformations such as helices. We now demonstrate efficient anion-induced helix stabilization for short N-capped polyalanines that lack charged groups. In addition to the helix-inducing N-cap these polyalanine peptides also contain recently reported N- or C-caps that solubilize the polyalanine se-

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quence and isolate it from potential helix-modifying effects of the K_4 solubilizing region and the UV reporter group tryptophane (Trp; W). For example, the cap *t*LInp$_2$K$_4$W contains the isolating element *t*LInp$_2$, derived from the α-amino acid *tert*-leucine (*t*L, *tert*-butylglycine) and the γ-amino acid isonipecotic acid (Inp, 4-carboxypiperidine). We have previously shown[5] that regions of a peptide that are separated by *t*LInp$_2$ contribute independently to the circular dichroism (CD) spectrum and that under the conditions of this study, the reporting, solubilizing, spacing region *t*LInp$_2$K$_4$W contributes insignificantly to the molar ellipticity per residue at 222 nm ($[\theta]_{222}$), used in this study as a helicity monitor.

Direct 1:1 binding of a chaotropic anion with the helix molecule must be invoked to explain the observed effects, such as the dependence of $[\theta]_{222}$ on the perchloric acid concentration for WK$_4$Inp$_2$*t*LGHel-A$_8$-NH$_2$ (**3**) displayed in Figure 1. Acid- and aggregation-induced helix stabilization

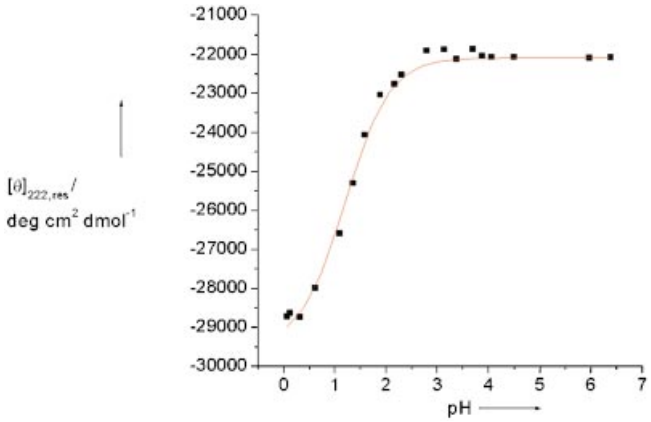

Figure 1. CD spectroscopic investigation of the titration of peptide **3** with perchloric acid at 2 °C. The squares represent experimental values and the red line the best fit obtained by least-square fit analysis.

were excluded by the absence of a detectable change of $[\theta]_{222}$ on the addition of HCl and by the invariance of $[\theta]_{222}$ over a 100-fold dilution (see Supporting Information).

As seen in Figure 2, replacement of perchloric acid HClO$_4$ by sodium perchlorate gives a comparable helix stabilization

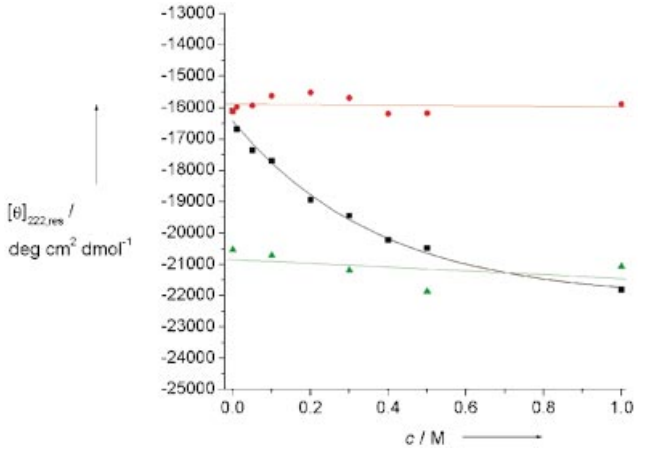

Figure 2. CD spectroscopic investigations of the salt titrations of peptides **5** and **9** at 2 °C. ■ NaClO$_4$ (entry 5a in Table 1); ● NaCl (entry 5c), ▲ NaClO$_4$ (entry 9).

but other common salts do not. An anomalous salt dependence of $[\theta]_{222}$ that is unrelated to helicity was ruled out by measuring ^{13}C NMR chemical shift changes for ^{13}C=O labeled peptides,[6] which parallel the changes in $[\theta]_{222}$, as do changes in the s-*trans*/s-*cis* ratio of the NMR helix reporter property of our N-terminal cap Ac-Hel[7] (see Supporting Information). Thus, three independent methods confirm that the ClO_4^- ion induces a strong helix-stabilizing effect in alanine-rich peptides.

Table 1 summarizes the results of titrations of other peptide sequences. These peptides were constructed from a short core alanine-rich sequence, N-capped by Ac-Hel or X-Gly-Hel,

Table 1. Summary of salt and acid titration data derived from CD spectrscopic analysis of a series of polyalanines at 2 °C.

Entry	Sequence[a]		Additive	$\Delta[\theta]_{222,res}$[b] [%]	$c_{1/2}$ [c] [M]
1	WK$_4$Inp$_2$*t*LGHelA$_4$-NH$_2$	**1**	HClO$_4$	84	0.13 ± 0.01
2	WK$_4$Inp$_2$*t*LGHelA$_6$-NH$_2$	**2**	HClO$_4$	78	0.09 ± 0.01
3	WK$_4$Inp$_2$*t*LGHelA$_8$-NH$_2$	**3**	HClO$_4$	30	0.06 ± 0.01
4	WK$_4$Inp$_2$*t*LGHelA$_{12}$-NH$_2$	**4**	HClO$_4$	9	0.07 ± 0.01
5a	AcHelA$_8$*t*LInp$_2$K$_4$W-NH$_2$	**5**	NaClO$_4$	35	0.24 ± 0.04
5b	AcHelA$_8$*t*LInp$_2$K$_4$W-NH$_2$	**5**	NaBF$_4$	22	–[d]
5c	AcHelA$_8$*t*LInp$_2$K$_4$W-NH$_2$	**5**	NaCl	–	–
5d	AcHelA$_8$*t*LInp$_2$K$_4$W-NH$_2$	**5**	NaHSO$_4$	–	–
5e	AcHelA$_8$*t*LInp$_2$K$_4$W-NH$_2$	**5**	NaH$_2$PO$_4$	–	–
5f	AcHelA$_8$*t*LInp$_2$K$_4$W-NH$_2$	**5**	NaPF$_6$	39	–[d]
6	AcHelA$_4$KA$_3$*t*LInp$_2$K$_4$W-NH$_2$	**6**	NaClO$_4$	49	0.20 ± 0.01
7	AcHelA$_7$K*t*LInp$_2$K$_4$W-NH$_2$	**7**	NaClO$_4$	23	0.21 ± 0.02
8	AcHelA$_7\beta t$LInp$_2$K$_4$W-NH$_2$	**8**	NaClO$_4$	29	0.12 ± 0.01
9	Ac$^\beta$DHelA$_7\beta t$LInp$_2$K$_4$W-NH$_2$	**9**	NaClO$_4$	–	–

[a] *t*L: *tert*-leucine, Inp: 4-carboxypiperidine, β: β-aminoalanine, Ac$^\beta$D: *N*-acetyl-β-aspartate. [b] Difference between observed $[\theta]_{222,min}$ and $[\theta]_{222,max}$ in salt titrations. [c] Salt concentration at half saturation of the peptide binding site obtained from a least-squares analysis of experimental ellipticities. Note that $1/c_{1/2} = K$ (binding constant). [d] Data does not fit a 1:1 binding model of anion to peptide.

which have comparable helix-inducing propensities. Each peptide contains a solubilizing polylysine region, a UV reporting tryptophane (Trp) residue, and the spacing element *t*LInp$_2$ which shields the polyalanine core from helicity effects of the solubilizer and reporter.[5] Entries 3 and 5a of Table 1 show that the position of the solubilizing spacer does not influence the sensitivity of the CD ellipticity to perchlorate concentration. It should be noted that the CD spectra of peptides **1**–**9** at high salt concentrations show the typical features of a classical spectrum for a helical peptide, that is minima at 208 and 222 nm and a maximum at short wavelength (see Supporting Information).

The curve of Figure 1 was calculated by assuming stoichiometric 1:1 binding of peptide and anion; the analysis assigns $[\theta]_{222,max}$ the limiting value at high $[ClO_4^-]$ and a binding constant K. As seen in Table 1, an average value for K corresponds to half saturation of a peptide binding site at a ClO_4^- concentration of 0.1–0.2 M. The % change in $-[\theta]_{222,max}$ is largest for short peptides and for peptides that exhibit a low value for $-[\theta]_{222}$ in water alone. For the tetraalanine sequence **1**, for example, the value for $-[\theta]_{222}$ nearly doubles at high $[ClO_4^-]$, while for the dodecaalanine sequence **4**, only a 9% increase is seen.

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Although none show the complete set of features seen with our short uncharged helices, certain peptides and proteins exhibit perchlorate-induced helicity changes.[8] Certain denatured proteins that form molten globules at low pH values can show increased helicity in the presence of chaotropic anions such as perchlorate. Goto, Takahashi, and Fink observed this effect for β-lactamase, cytochrome c, and apomyoglobin.[9] To further probe the helix-inducing effects of chaotropes Goto and Aimoto constructed a cationic peptide that folds to a double-helical conformation containing a solvent-shielded hydrophobic core.[10] All these examples exhibited titration curves and formed compact, folded, highly helical conformations in the presence of low concentrations of a range of chaotropic anions. These positively charged proteins undergo complex conformational changes in the presence of chaotropes at lower concentrations than those reported in Table 1. The exceptional sensitivity of these polycationic proteins and peptides to chaotropic anions probably reflects their greater capacity to undergo subsequent folding to hydrophobically stabilized compact structures, once salt-induced helices have formed. Our uncharged polyalanine substrates appear to be unique in undergoing a single, simple conformational change to form hydrated helices.

Our use of CD spectroscopic analysis to measure helicity is restricted to those anions that lack significant UV absorption in the 195–220 nm region and thus excludes the most efficient chaotropic anions studied by Goto et al. As seen in Table 1, salts formed from anions with normal hydration volumes, such as chloride and sulfate, induce insignificant changes in the value of $-[\theta]_{222}$. Only the chaotropic anions BF_4^-, PF_6^-, and ClO_4^- induce large changes in $-[\theta]_{222}$ that implies anion binding. Our findings underline the difficulties of conducting salt-dependent CD studies of polypeptides at low pH values in water, since no UV transparent noninteracting anion appears to be available.

How does a chaotropic anion change the helicity of our uncharged polyalanine systems? Where does perchlorate bind to the peptide? The CD spectroscopic investigation of the peptide **9**, which bears a negatively charged N-terminal cap, provides the first answers to these questions. Addition of perchlorate to **9** has no noticeable influence on the CD ellipticity $[\theta]_{222}$ (Figure 2). Helical conformations are stabilized by the presence of terminal charges, positive charges at the C-termini and negative at the N-termini, and modeling suggests a natural anion binding site for a chaotropic anion in a cleft in the Hel function, proximate to the NH group of the first amino acid of the peptide sequence. This hypothesis is supported by the fact that, among the anions we studied, only the titration behavior of perchlorate fits a simple 1:1 binding model. This suggests that not only the chaotropic nature of the anion but also its size is of vital importance for efficient binding to the peptide.

X-ray crystallographic data for binding of anions to helix N-termini of globular proteins have recently been reviewed and suggest precedents for our hypothesis.[11] We have shown that an anion bound in the vicinity of the N-terminus of a potentially helical peptide can strongly stabilize it. With our template-substituted model peptides, the binding effect was found to be specific for chaotropic anions of a certain size. The perchlorate ion was found to be strongly favored over other chaotropic anions. In addition to the strongly helix-stabilizing effect of perchlorate for short uncharged peptides, our findings may be of significant importance for folding pathways of partially helical intermediates. These intermediates may be sensitive to the presence of locally bound anions, which also may play a tuning role in complex protein-dependent signal transfer pathways.

Experimental Section

Peptides were prepared as previously described[7] and purified by repeated reverse-phase high performance liquid chromatography (RP-HPLC), and characterized by EI-MS (electrospray ionization mass spectrometry). The purity of all compounds is estimated to be higher than 95%. CD spectra were recorded on a thermostated Aviv 62DS circular dichroism spectrometer calibrated according to literature methods.[12] pH values were measured at 20 °C with a PHM240 pH-meter from Radiometer Copenhagen.

The Supporting Information contains the salt and acid titration curves for all compounds as well as ^{1}H NMR and ^{13}C NMR spectroscopical data for compounds **1** and **5**, as well as the CD spectrum of **3**.

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