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# Heat-Capacity Changes in Host–Guest Complexation by Coulomb Interactions in Aqueous Solution

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**Abstract:** Heat-capacity changes ($\Delta C_p^0$) were determined for the complexation of 1-alkanecarboxylates with protonated hexakis(6-amino-6-deoxy)-α-cyclodextrin (per-$NH_3^+$-α-CD) and heptakis(6-amino-6-deoxy)-β-cyclodextrin (per-$NH_3^+$-β-CD). $\Delta C_p^0$ decreased with an increase in the binding constant ($K$) and plateaued at $K = 4000\,M^{-1}$. The complexes of 1-pentanoate, 1-hexanoate, and 1-heptanoate with per-$NH_3^+$-α-CD are classified as the host–guest system in which the size of the guest fits the CD cavity well. In such a system, van der Waals interaction is the major force for complexation, leading to a negative $\Delta H^0$ value. Simultaneously, the water molecules around the hydrophobic alkyl chain of the guest and inside the CD cavity are released to the aqueous bulk phase, leading to a positive $\Delta S^0$ value. The negative $\Delta C_p^0$ value in such complexation is ascribed to dehydration of the hydrophobic alkyl chain of the guest and extrusion of the water molecules inside the CD cavity. Meanwhile, the complexes that show positive $\Delta C_p^0$ values are characterized by complexation in which the guest molecules are significantly smaller than the CD cavities. In such a case, the complexation is endothermic and driven by the entropy gain. When the guest is much smaller than the CD cavity, the main binding force should be Coulomb interaction. To form an ionic bond, dehydration of the charged groups must occur. This process is endothermic and leads to positive $\Delta H^0$ and $\Delta S^0$ values. As the top of the CD cavity is capped by a small but hydrophobic alkyl chain, the water molecules inside the CD cavity may form the iceberg structure. This process must be accompanied by a positive $\Delta C_p^0$ value. Hence, the complexation of a small guest with the CD with a large cavity through Coulomb interactions shows positive and large $\Delta C_p^0$ values. These conclusions were applied to the electrostatic binding of proteins with an anionic ligand.

**Keywords:** calorimetry · cyclodextrins · electrostatic interactions · hydrophobic effect · proteins · thermodynamics

## Introduction

Heat-capacity changes ($\Delta C_p^0$) have been widely utilized to study the unfolding of proteins as well as protein–protein, protein–ligand,[1,2] protein–DNA,[3] and protein–membrane interactions.[4] It is assumed that protein unfolding causes exposure of the hydrophobic part of the protein to the aqueous bulk phase to yield ordered water structures around the exposed part of the protein, thus resulting in a positive $\Delta C_p^0$ value.[5] Conversely, binding of a hydrophobic ligand to a protein gives a negative $\Delta C_p^0$ value due to dehydration from both protein and ligand. Such an effect is called "hydrophobic interaction".[6] The solvent-accessible surface area (ASA) is presumed to correlate the exposed hydrophobic area with $\Delta C_p^0$.[5b] Meanwhile, hydration of a polar or charged solute and dehydration of polar or ionic groups are considered to lead to negative and positive $\Delta C_p^0$ values, respectively.[3,5–7] Numerous examples showing negative or positive $\Delta C_p^0$ values in biological systems have been mainly interpreted in terms of solvent reorientation. There is no doubt, however, that factors other than solvent reorientation participate in $\Delta C_p^0$; the question is how much each factor contributes to the total $\Delta C_p$ value. Sturtevant predicted positive $\Delta C_p^0$ values in events such as 1) exposure of nonpolar groups to the aqueous phase, 2) hydrogen-bond formation, and 3) generation of internal vibrational modes.[5a] Indeed, the explanation of $\Delta C_p^0$ in terms of ASA has been in doubt.[8,9] On the basis of their study of the combination of a random-network model of water and Monte Carlo calculations, Sharp and co-workers demonstrated that the change

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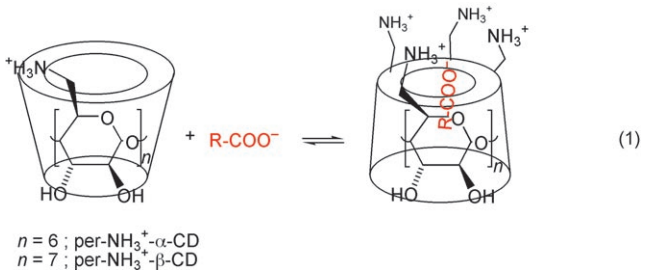
in heat capacity upon solubilizing a solute in water depends on the number of hydrogen bonds participating in the first hydration shell of a hydrophobic solute or in the first and second hydration shells of a polar solute.[9] The difference in the structures of hydrated water around nonpolar and polar solutes was considered to cause the difference in the signs of $\Delta C_p^0$.[9b] Although there is controversy over a detailed theory of $\Delta C_p^0$ in aqueous solution, it is evident that $\Delta C_p^0$ depends on the formation or decomposition of the water structures around hydrophobic solutes. The problem is an understanding of $\Delta C_p^0$ in the cases of polar and charged solutes. There are individual examples of positive $\Delta C_p^0$ values in the complexation of charged host and guest pairs. For example, Privalov and co-workers reported positive $\Delta C_p^0$ values in electrostatic interactions between a DNA-binding protein (HMG-D100) with a positively charged (15+) peptide chain at an end of *Drosophila melanogaster* (HMG-D) and a DNA duplex, whereas complexation with HMG-D75 with a 5+ charge showed a negative $\Delta C_p^0$ value.[3b] They simply concluded that dehydration from polar groups results in positive $\Delta C_p^0$ values.[3b] Similarly, electrostatic binding of a cell-penetrating peptide, CPP R9, with an anionic heparan sulfate proteoglycan in lipid membranes was reported to show positive $\Delta C_p^0$ values at $\geq 37\,^\circ$C.[4a] Meanwhile, De La Cruz and co-workers interpreted the positive $\Delta C_p^0$ value in the complexation of actomyosin VI with ADP in terms of a temperature-dependent enthalpy change due to endothermic isomerization of actomyosin VI that has to occur prior to binding with ADP.[10] Endothermic distortion of DNA was also assumed to be an origin of the positive $\Delta C_p^0$ value in the complexation of DNA with Sac7d mutants.[3a] The interpretation of $\Delta C_p^0$ in the complexation of polar or charged solutes is chaotic.

Herein, we report a well-designed host–guest system in which $\Delta C_p^0$ switches regularly between positive and negative values in the complexation of positively charged cyclodextrins with negatively charged 1-alkanecarboxylates ($C_nCO_2^-$) of increasing hydrophobicity. Hexakis(6-amino-6-deoxy)-α-cyclodextrin (per-$NH_2$-α-CD) and heptakis(6-amino-6-deoxy)-β-cyclodextrin (per-$NH_2$-β-CD; $pK_a = 6.9$–$8.5$[11]) were chosen as the hosts. At pH 6.0, all the amine groups of these cyclodextrins were protonated to yield the polycationic hosts (per-$NH_3^+$-α-CD and per-$NH_3^+$-β-CD), which included alkanecarboxylates ($pK_a = 4.7$–$4.9$) through both electrostatic and van der Waals and/or hydrophobic interactions [Eq. (1)].[12] The participation of van der Waals and/or hydrophobic interactions in the complexation with per-$NH_3^+$-α-CD, which has a narrower cavity, may be much larger than that for per-$NH_3^+$-β-CD with the larger cavity. The present system enables us to measure $\Delta C_p^0$ values for host–guest complexation as a function of the balance between intermolecular van der Waals and/or hydrophobic and electrostatic interactions.




(1)

## Results and Discussion

### Complexes of $C_nCO_2^-$ with Per-$NH_3^+$-α- and -β-CD

Thermodynamic studies of alkanecarboxylates and benzoate with native α- and β-cyclodextrins (α- and β-CDs) are reported elsewhere.[13] In most cases, the complexation of the carboxylate anions with native CDs showed positive entropy changes ($\Delta S^0$). A detailed study of the complexation of *p*-methylbenzoate with per-$NH_3^+$-α- and -β-CD was previously carried out by means of UV/Vis and $^1$H NMR spectroscopy.[12] In the present study, the complexation was followed by means of isothermal titration calorimetry (ITC), which provides the binding constant for complexation ($K$) and the enthalpy change ($\Delta H^0$) directly. A typical result of the calorimetric titration is shown in Figure 1. Complexation of 1-butanoate ($C_3CO_2^-$) with per-$NH_3^+$-α-CD is an endothermic process, and analysis of the titration curve indicated the formation of the 1:1 host–guest complex. Meanwhile, the titration curves obtained for other $C_nCO_2^-$ ions ($n = 4$–$6$) could not be fitted to the equation for 1:1 complexation and were instead analyzed by assuming simultaneous formation of the 2:1 $C_nCO_2^-$/per-$NH_3^+$-α-CD complexes. The thermodynamic

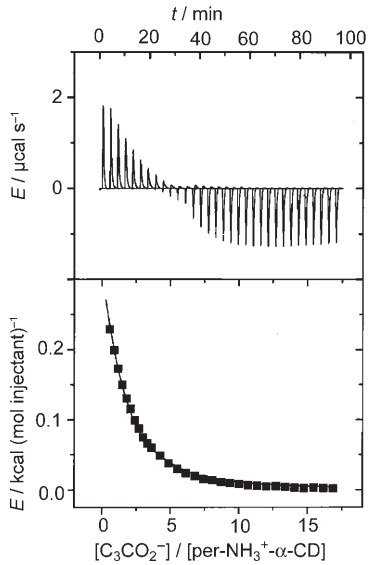



Figure 1. Calorimetric titration of per-$NH_3^+$-α-CD ($9\times10^{-4}$ M per cell) with successive injections of $C_3CO_2^-$ ($7.5\times10^{-2}$ M in a syringe, 10×5-μL and 20×10-μL aliquots) in 0.02 M aqueous NaCl at pH 6.0 and 298.15 K. Top: Raw data showing the amount of heat generated after each injection of $C_3CO_2^-$. Bottom: Plot of the amount of heat generated per injection as a function of the $C_3CO_2^-$/per-$NH_3^+$-α-CD molar ratio. The solid line represents the best fit of the data to a model of 1:1 stoichiometry.

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Table 1. Thermodynamic parameters for the complexation of anionic guests with per-$NH_3^+$-α-CD in 0.02 M aqueous NaCl at pH 6.0 and 298.15 K.

| Guest | $K_1$ [M$^{-1}$] | $\Delta H^0$ [kJ mol$^{-1}$] | $T\Delta S^0$ [kJ mol$^{-1}$] | $K_2$ [M$^{-1}$] | $\Delta H^0$ [kJ mol$^{-1}$] | $T\Delta S^0$ [kJ mol$^{-1}$] |
|---|---|---|---|---|---|---|
| $C_3CO_2^-$ | 664 ± 18 | 3.24 ± 0.04 | 19.3 ± 0.1 | – | – | – |
| $C_4CO_2^-$ | 4168 ± 299 | −4.10 ± 0.07 | 16.5 ± 0.1 | 23 ± 17 | −1.5 ± 1.2 | 5.3 ± 3.5 |
| $C_5CO_2^-$ | 12200 ± 1400 | −7.29 ± 0.14 | 16.1 ± 0.1 | 58 ± 49 | −1.3 ± 1.0 | 7.2 ± 4.1 |
| $C_6CO_2^-$ | 22060 ± 1111 | −10.1 ± 0.1 | 14.8 ± 0.1 | 530 ± 80 | 3.3 ± 0.3 | 19 ± 1 |

parameters for complexation of $C_nCO_2^-$ with per-$NH_3^+$-α-CD obtained by ITC are summarized in Table 1.

$K_1$ increased and $\Delta H^0$ decreased with an increase in the alkyl-chain length of $C_nCO_2^-$. These results are reasonable because van der Waals interactions between the host and the guest should be strengthened as the alkyl-chain length increases. Nonetheless, the complexation of $C_nCO_2^-$ with per-$NH_3^+$-α-CD is promoted by positive and large entropy changes. Dehydration from both host and guest seems to be the essential factor for promoting the complexation. The contribution of the entropy term is more remarkable in the complexation of $C_nCO_2^-$ ions with shorter alkyl chains. This result is apparently explained by the enthalpy–entropy compensation effect.

Although the analysis of the ITC data suggests the 2:1 complexes, the binding constant for the second step ($K_2$) for each system is so small compared with $K_1$ for the first step that the Job plots for the changes in the chemical shifts of the signals of per-$NH_3^+$-α-CD upon addition of $C_nCO_2^-$ apparently showed a 1:1 stoichiometry of the complexes (see Supporting Information). The structures of the $C_nCO_2^-$/per-$NH_3^+$-α-CD complexes were confirmed by 2D NMR spectroscopy. Figure 2 shows the ROESY spectrum for the $C_6CO_2^-$/per-$NH_3^+$-α-CD system as a representative example. All the cross-peaks indicate the formation of a complex whereby the carboxylate group is anchored by the ammonium groups and the hydrophobic alkyl chain of the guest is placed inside the CD cavity.

ITC suggested that per-$NH_3^+$-β-CD forms 1:1 complexes with $C_nCO_2^-$ ($n$ = 3–6), and no 2:1 complexes were detected. In all cases, the complexation was endothermic and was promoted by positive and large $\Delta S^0$ values (see below).

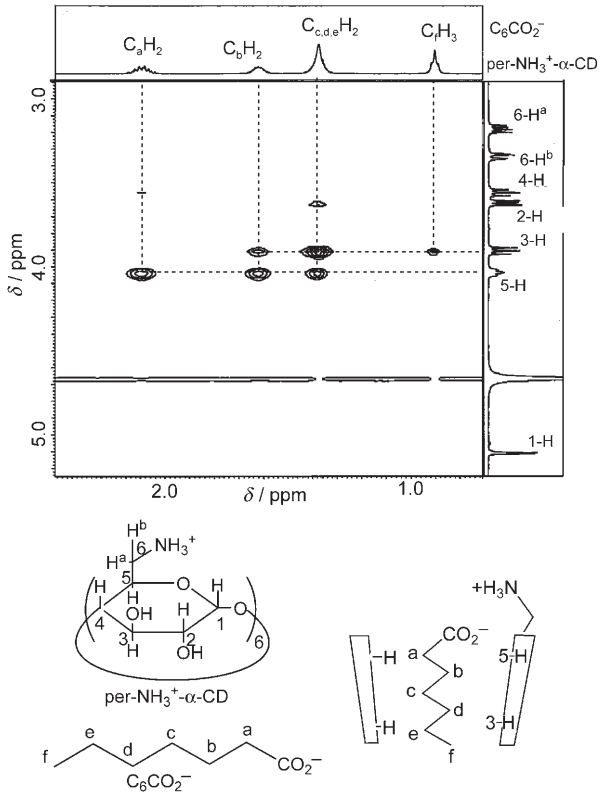


Figure 2. ROESY spectrum of the $C_6CO_2^-$/per-$NH_3^+$-α-CD complex in a solution of NaCl (0.02 M) in $D_2O$ at pD 6.0 and 298 K. [$C_6CO_2^-$] = [per-$NH_3^+$-α-CD] = $1 \times 10^{-2}$ M, mixing time = 250 ms.

## Heat-Capacity Changes

Temperature-dependent enthalpy ($\Delta H^0$) and entropy changes ($\Delta S^0$) are represented by Equations (2) and (3), respectively:

$$\Delta H^0 = \Delta C_p^0 (T - T_H) \quad (2)$$

$$\Delta S^0 = \Delta C_p^0 \ln\left(\frac{T}{T_S}\right) \quad (3)$$

$\Delta C_p^0$ is the isobaric heat-capacity change, and $T_H$ and $T_S$ are the absolute temperatures at which $\Delta H^0$ and $\Delta S^0$, respectively, are zero. The $\Delta C_p^0$ values for the complexation were obtained from the calorimetric measurements. An example of the linear relationship between $\Delta H^0$ and $T$ is shown in Figure 3. The thermodynamic parameters determined for the various host–guest pairs are summarized in Table 2. Only 1:1 complexation was considered in the cases of the $C_nCO_2^-$/per-$NH_3^+$-α-CD pairs that form 2:1 complexes with small $K_2$ values.

In all cases, the complexation was accompanied by positive and large $\Delta S^0$ values, which suggests that dehydration from both host and guest promotes formation of the inclusion complex.[12]

Figure 4 shows the relationship between $K$ and $\Delta C_p^0$. In all the complexations of 1-alkanecarboxylates with per-$NH_3^+$-β-CD, the $\Delta C_p^0$ values were positive and decreased linearly as $K$ increased ($\Delta C_p^0 = -0.22K + 800$, $R^2 = 0.96$). This is the first example of positive $\Delta C_p^0$ values in inclusion-complex formation of CDs. Meanwhile, all the host–guest

  

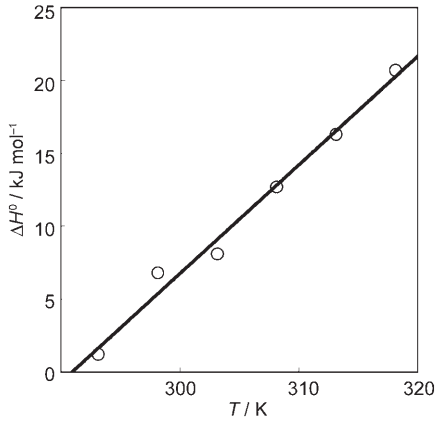


Figure 3. Plot of $\Delta H^0$ versus $T$ for the $C_3CO_2^-$/per-$NH_3^+$-β-CD pair in 0.02 M aqueous NaCl at pH 6.0.

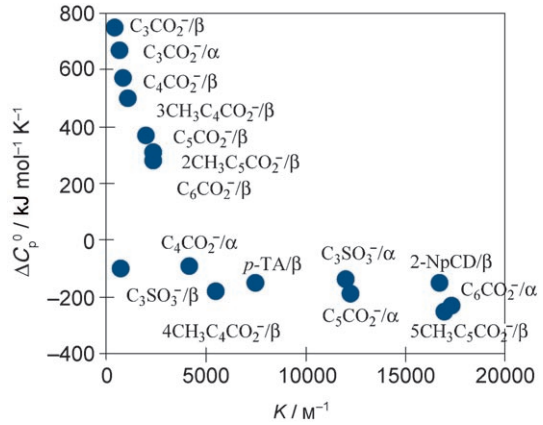


Figure 4. Plot of $\Delta C_p^0$ versus $K$. α and β stand for per-$NH_3^+$-α- and -β-CD, respectively. The abbreviations are defined in the footnote of Table 2.

pairs with the larger $K$ values due to better size fitting showed negative $\Delta C_p^0$ values and did not obey the above linear relationship, though $\Delta C_p^0$ also tended to decrease with increasing $K$. The results shown in Figure 4 indicate that $\Delta C_p^0$ is positive in the complexation of a guest whose molecular size is too small to fit the CD cavity, and that it decreases steadily with an increase in the size of the guest.

No linear relationships were observed between $\Delta H^0$ and $\Delta C_p^0$, and between $T\Delta S^0$ and $\Delta C_p^0$, when all the data in Table 2 were applied (Figure 5; $\Delta C_p^0$ (kJ mol$^{-1}$K$^{-1}$) = $0.037\Delta H^0 + 0.080$, $R^2 = 0.58$; $\Delta C_p^0$ (kJ mol$^{-1}$K$^{-1}$) = $0.035T\Delta S^0 - 0.620$, $R^2 = 0.29$). However, there are fairly good linear relationships between $\Delta H^0$ and $\Delta C_p^0$ and between $T\Delta S^0$ and $\Delta C_p^0$ if the data for $C_3CO_2^-$/per-$NH_3^+$-α-CD as well as for $C_3CO_2^-$/, 4-$CH_3C_4CO_2^-$/, and 5-$CH_3C_5CO_2^-$/per-$NH_3^+$-β-CD are neglected (Figure 5).

Equations (4) and (5) show the relationships between $\Delta C_p^0$ and $\Delta H^0$ and between $\Delta C_p^0$ and $\Delta S^0$ in the complexation of the organic anions with per-$NH_3^+$-α- and -β-CD, except for the systems shown in red in Figure 5. These two equations should not be applied generally.

$$\Delta C_p^0 \text{ in kJ mol}^{-1}\text{ K}^{-1} = 0.034\Delta H^0 + 0.072,\ R^2 = 0.90 \quad (4)$$

$$\Delta C_p^0 \text{ in kJ mol}^{-1}\text{ K}^{-1} = 0.046T\Delta S^0 - 0.88,\ R^2 = 0.89 \quad (5)$$

When the analysis was limited to the 1-alkanecarboxylates ($C_nCO_2^-$) with $n = 4$–6, a better linear relationship was observed between $\Delta C_p^0$ and $T\Delta S^0$ ($\Delta C_p^0 = 0.050T\Delta S^0 - 0.99$, $R^2 = 0.95$), although the $\Delta C_p^0$ values for per-$NH_3^+$-α-CD were negative and those for per-$NH_3^+$-β-CD were positive.

Generally, a guest whose molecular size fits a CD cavity well shows a large $K$ value. This is the case for the $C_nCO_2^-$/per-$NH_3^+$-α-CD pairs. A host–guest pair with a well-fitting size forms a stable inclusion complex of which the main binding force is van der Waals interaction leading to a negative and large $\Delta H^0$ value. The enthalpy–entropy compensation rule predicts a negative $\Delta S^0$ value in such a case. However, the $\Delta S^0$ values for every $C_nCO_2^-$/per-$NH_3^+$-α-CD pair examined were positive, which suggests that dehydration from both host and guest is still an important factor even in the complexation of long-alkyl-chain 1-alkanecarboxylates with per-$NH_3^+$-α-CD. $\Delta C_p^0$ decreased

Table 2. Binding constants ($K$) and enthalpy ($\Delta H^0$), entropy ($T\Delta S^0$), and heat-capacity changes ($\Delta C_p^0$) for the complexation of various anions with per-$NH_3^+$-α- and -β-CD in 0.02 M aqueous NaCl at pH 6.0 and 298.15 K.

| CD | Guest[a] | $K$ [M$^{-1}$] | $\Delta H^0$ [kJ mol$^{-1}$] | $T\Delta S^0$ [kJ mol$^{-1}$] | $\Delta C_p^0$ [J mol$^{-1}$K$^{-1}$] |
|---|---|---|---|---|---|
| per-$NH_3^+$-α-CD | $C_3CO_2^-$ | 664 ± 18 | 3.3 ± 0.1 | 19.4 ± 0.1 | 670 ± 42 |
| | $C_4CO_2^-$ | 4168 ± 299 | −4.1 ± 0.1 | 16.5 ± 0.3 | −90 ± 15 |
| | $C_5CO_2^-$ | 12200 ± 1400 | −7.3 ± 0.1 | 16.0 ± 0.4 | −190 ± 9 |
| | $C_6CO_2^-$ | 17300 ± 400 | −10.9 ± 0.1 | 13.3 ± 0.2 | −230 ± 2 |
| per-$NH_3^+$-β-CD | $C_3CO_2^-$ | 439 ± 21 | 6.8 ± 0.1 | 21.9 ± 0.2 | 750 ± 45 |
| | $C_4CO_2^-$ | 854 ± 25 | 11.7 ± 0.1 | 28.5 ± 0.1 | 570 ± 60 |
| | $C_5CO_2^-$ | 2003 ± 68 | 6.2 ± 0.1 | 25.1 ± 0.1 | 370 ± 12 |
| | $C_6CO_2^-$ | 2347 ± 43 | 7.4 ± 0.1 | 26.7 ± 0.1 | 280 ± 24 |
| | $C_3SO_3^-$ | 11990 ± 852 | −10.2 ± 0.2 | 13.1 ± 0.1 | −140 ± 9 |
| | $C_3SO_3^-$ | 723 ± 90 | 0.32 ± 0.12 | 16.6 ± 0.3 | −100 ± 5 |
| | 3-$CH_3C_3CO_2^-$ | 1114 ± 31 | 10.8 ± 0.1 | 28.2 ± 0.2 | 500 ± 30 |
| | 4-$CH_3C_4CO_2^-$ | 5490 ± 270 | 3.6 ± 0.1 | 25.0 ± 0.2 | −180 ± 5 |
| | 2-$CH_3C_5CO_2^-$ | 2362 ± 57 | 9.1 ± 0.1 | 28.4 ± 0.2 | 310 ± 16 |
| | 5-$CH_3C_5CO_2^-$ | 16900 ± 1672 | 1.4 ± 0.1 | 25.6 ± 0.3 | −250 ± 3 |
| | p-TA | 7464 ± 281 | −3.2 ± 0.1 | 18.9 ± 0.2 | −150 ± 3 |
| | 2-NpCA | 16710 ± 662 | −6.3 ± 0.1 | 17.8 ± 0.2 | −150 ± 13 |

[a] $C_nCO_2^-$ stands for 1-alkanecarboxylate. For example, $C_3CO_2^-$ is the 1-butanoate ion. $C_3SO_3^-$ = 1-propanesulfonate, 3-$CH_3C_3CO_2^-$ = 3-methyl-1-butanoate, 4-$CH_3C_4CO_2^-$ = 4-methyl-1-pentanoate, 2-$CH_3C_5CO_2^-$ = 2-methyl-1-hexanoate, 5-$CH_3C_5CO_2^-$ = 5-methyl-1-hexanoate, p-TA$^-$ = p-methylbenzoate, 2-NpCA$^-$ = 2-naphthalenecarboxylate.

  

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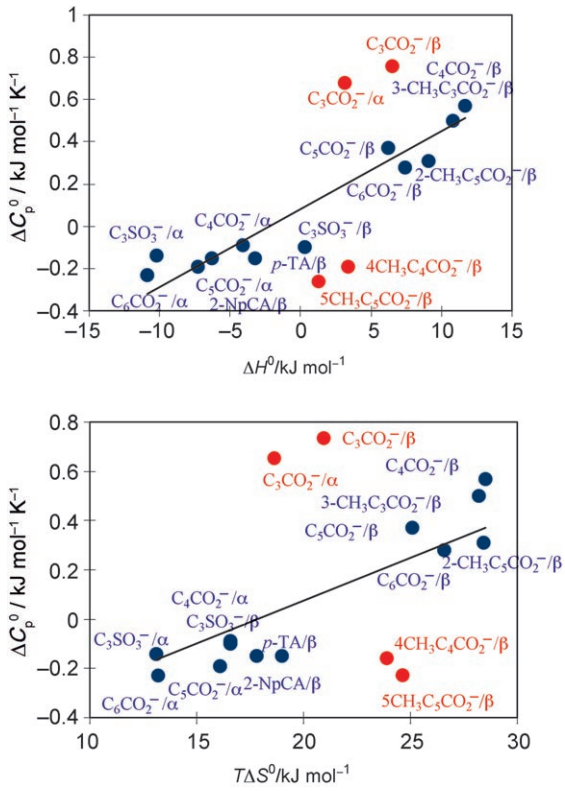
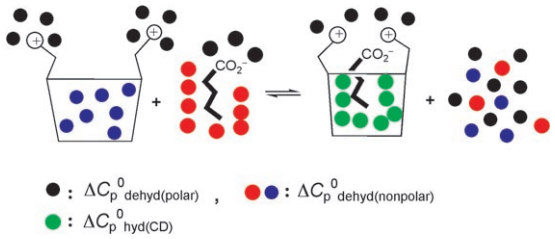



Figure 5. Plots of $\Delta C_p^0$ versus $\Delta H^0$ (top) and $T\Delta S^0$ (bottom) for the complexation of organic anions with per-$NH_3^+$-α- and -β-CD.

steadily with an increase in the alkyl-chain length of the guest. An increase in the alkyl-chain length of the guest means an increase in the contribution of hydrophobic as well as van der Waals interaction. It might be reasonable to assume that the steady decrease in $\Delta C_p^0$ in the $C_nCO_2^-$ ($n$= 4–6)/per-$NH_3^+$-α-CD systems can be ascribed to the destruction of the iceberg structures around the hydrophobic alkyl chain upon inclusion. It is well-known that complexation accompanied by breaking of the iceberg structures (hydrophobic interaction) results in a negative and large $\Delta C_p^0$ value.[2] As the experimentally obtained $\Delta S^0$ for the $C_nCO_2^-$/per-$NH_3^+$-α-CD system decreased with an increase in the alkyl-chain length, $\Delta S^0$ is not adequate for discussing hydrophobic interaction. As is widely recognized, $\Delta C_p^0$ is the good parameter for studying hydrophobic interaction. It can be concluded that the steadily decreasing $\Delta C_p^0$ in the complexation of per-$NH_3^+$-α-CD with $C_nCO_2^-$ of increasing alkyl-chain length reflects the expanding destruction of the ordered structure of water (iceberg) around the hydrophobic alkyl chain.

Each $C_nCO_2^-$/per-$NH_3^+$-β-CD pair also showed a positive $\Delta C_p^0$ value that steadily decreased with an increase in the alkyl-chain length of $C_nCO_2^-$. The pairs with positive $\Delta C_p^0$ values are characterized by their complexation of guest molecules that are significantly smaller than the CD cavity. In these cases, the complexation is endothermic ($\Delta H^0 >$ 0 kJ mol$^{-1}$) and is driven by the entropy gain. When the size of the guest is much smaller than that of the CD cavity, the main binding force should be Coulomb interaction. To form an ionic bond between hydrophilic cation and anion, dehydration from the charged groups must occur. The dehydration process is endothermic and leads to a positive $\Delta H^0$ value if the dehydration energy is larger than the ionic bond energy. It is well-known that the sign of $\Delta C_p^0$ is positive and negative when the ordered water structure (iceberg) around the hydrophobic solute(s) is formed and destroyed, respectively.[2] Conversely, $\Delta C_p^0$ has been demonstrated to be negative and positive when the water structure around the polar and/or charged solute(s) is formed and destroyed, respectively.[3–7] When limited to the $n$-alkanecarboxylates, each $\Delta C_p^0$ value can apparently be interpreted in terms of a combination of dehydration from the hydrophobic and hydrophilic (charged) parts of the guest. However, an additional factor must be considered for the present system. This factor is the contribution of hydration of and/or dehydration from the inside of the CD cavities.

Scheme 1 shows the hydration and dehydration patterns in the present system. The total heat-capacity change,




Scheme 1. Hydration and dehydration patterns in the complexation of a cationic CD with an anionic alkanecarboxylate.

$\Delta C_p^0{}_{total}$, in the complexation of an alkanecarboxylate with per-$NH_3^+$-CD is composed of three factors [Eq. (6)]:

$$\Delta C_p^0{}_{total} = \Delta C_p^0{}_{dehyd(polar)} + \Delta C_p^0{}_{dehyd(nonpolar)} + \Delta C_p^0{}_{hyd(CD)} \quad (6)$$

$\Delta C_p^0{}_{dehyd(polar)}$ is the contribution from the dehydration of the charged groups of the solutes. As hydration of an ion gives a negative $\Delta C_p^0$ value,[5b,9] $\Delta C_p^0{}_{dehyd(polar)}$ should be positive. $\Delta C_p^0{}_{dehyd(nonpolar)}$ ($<0$) is the term for the collapse of the water aggregates around the hydrophobic alkyl chain of $C_nCO_2^-$ and the dehydration of the CD cavity. $\Delta C_p^0{}_{hyd(CD)}$ ($>0$) is the heat-capacity change during the formation of the water aggregates in the vicinity of the alkyl chain of $C_nCO_2^-$ inside the CD cavity. The last term becomes significant in the complexation of a smaller alkanecarboxylate with per-$NH_3^+$-β-CD with the larger cavity because, in this case, there is an enough space in the CD cavity where the water molecules are located. When $(\Delta C_p^0{}_{dehyd(polar)} + \Delta C_p^0{}_{hyd(CD)}) > |\Delta C_p^0{}_{dehyd(nonpolar)}|$, $\Delta C_p^0{}_{total}$ becomes positive. This is the case of the positive $\Delta C_p^0$ values in the complexation of the $C_nCO_2^-$/per-$NH_3^+$-β-CD pairs. In the complexation of 1-alkanecarboxylates ($n$=4–6) with per-$NH_3^+$-α- and -β-CD,

the positive value of $\Delta C_p^{0}{}_{hyd(CD)}$ must decrease steadily with increasing length and/or bulkiness of the alkyl chain of the guest. This could be the reason for the positive but steadily decreasing $\Delta C_p^{0}$ values in the complexation of per-$NH_3^+$-β-CD and the negative and steadily decreasing $\Delta C_p^{0}$ values in the complexation of per-$NH_3^+$-α-CD as the alkyl-chain length of the guest is increased.

The problem is the pairs ($C_3CO_2^-$/per-$NH_3^+$-α-CD and $C_3CO_2^-$/, 4-$CH_3C_4CO_2^-$/, and 5-$CH_3C_5CO_2^-$/per-$NH_3^+$-β-CD) whose plots of $\Delta C_p^{0}$ versus $\Delta H^0$ and $\Delta S^0$ deviate from the linear relationships. The anions in this category are roughly classified into two groups: very hydrophilic and small anions and amphiphilic anions with a methyl branch at the ends of the alkyl chains. The water structures in the vicinity of hydrophilic $C_3CO_2^-$ may be different from those of more hydrophobic $C_nCO_2^-$, as deduced from the results of Sharp and co-workers, who found that the water structure around argon is icelike, whereas a more random hydration shell is formed around $K^+$.[9] If this difference in hydration can be applied to the present system, we would be able to interpret the deviation of the plots for $C_3CO_2^-$. However, the discontinuity in $\Delta C_p^{0}{}_{dehyd(nonpolar)}$ of $C_3CO_2^-$ seems to be implausible. We then noticed $\Delta C_p^{0}{}_{hyd(CD)}$. The $\Delta C_p^{0}{}_{hyd(CD)}$ value of the $C_3CO_2^-$/per-$NH_3^+$-CD system should be positive and large because of the large space inside the CD cavity, whose top is covered by a hydrophobic butyl group. The unexpectedly large and positive $\Delta C_p^{0}{}_{total}$ value for the $C_3CO_2^-$ system seems to be ascribed to the abnormally large and positive $\Delta C_p^{0}{}_{hyd(CD)}$ value. The negative $\Delta C_p^{0}$ value for the $C_3SO_3^-$/per-$NH_3^+$-β-CD pair is suggestive of the important contribution of $\Delta C_p^{0}{}_{hyd(CD)}$ to $\Delta C_p^{0}{}_{total}$. As shown in Table 1, the $\Delta C_p^{0}$ values for the $C_3SO_3^-$/per-$NH_3^+$-α- and -β-CD systems were $(-140\pm9)$ and $(-100\pm5)$ J mol$^{-1}$ K$^{-1}$, respectively, whereas those for the $C_3CO_2^-$ complexes of per-$NH_3^+$-α- and -β-CD were $(670\pm42)$ and $(750\pm45)$ J mol$^{-1}$ K$^{-1}$, respectively. To explain such a remarkable difference in $\Delta C_p^{0}$ between $C_3SO_3^-$ and $C_3CO_2^-$, the structures of the inclusion complexes were determined from the ROESY spectra (Figure 6). No cross-peak was observed between the 3-H protons of per-$NH_3^+$-α-CD and $C_aH_2$ of $C_3CO_2^-$, whereas cross-peaks were clearly seen between 3-H and $C_cH_3$ and $C_bH_2$. This result strongly suggests that in the structure of this inclusion complex, the guest molecule projects upwards (Figure 6). In the ROESY spectrum of the $C_3SO_3^-$/per-$NH_3^+$-α-CD complex, however, the cross-peak between 3-H and $C_aH_2$ was clearly observed, thus indicating that the $C_3SO_3^-$ molecule was included more deeply into the cyclodextrin cavity (Figure 6). The penetration of $C_3SO_3^-$ causes extended extrusion of the water molecules inside the CD cavity, which weakens the contribution of $\Delta C_p^{0}{}_{hyd(CD)}$. MM2 calculations (BioMedCAChe 6.0; Figure 7) suggest that the $CO_2^-$ group of $C_3CO_2^-$ interacts with two neighboring $NH_3^+$ groups of per-$NH_3^+$-β-CD, whereas the $SO_3^-$ group of $C_3SO_3^-$ is anchored by three $NH_3^+$ groups, thus leading to a significant difference in the structures of the inclusion complexes. Consequently, the $C_3SO_3^-$ ion causes a narrowing of the rim of the side with the $NH_3^+$ groups and

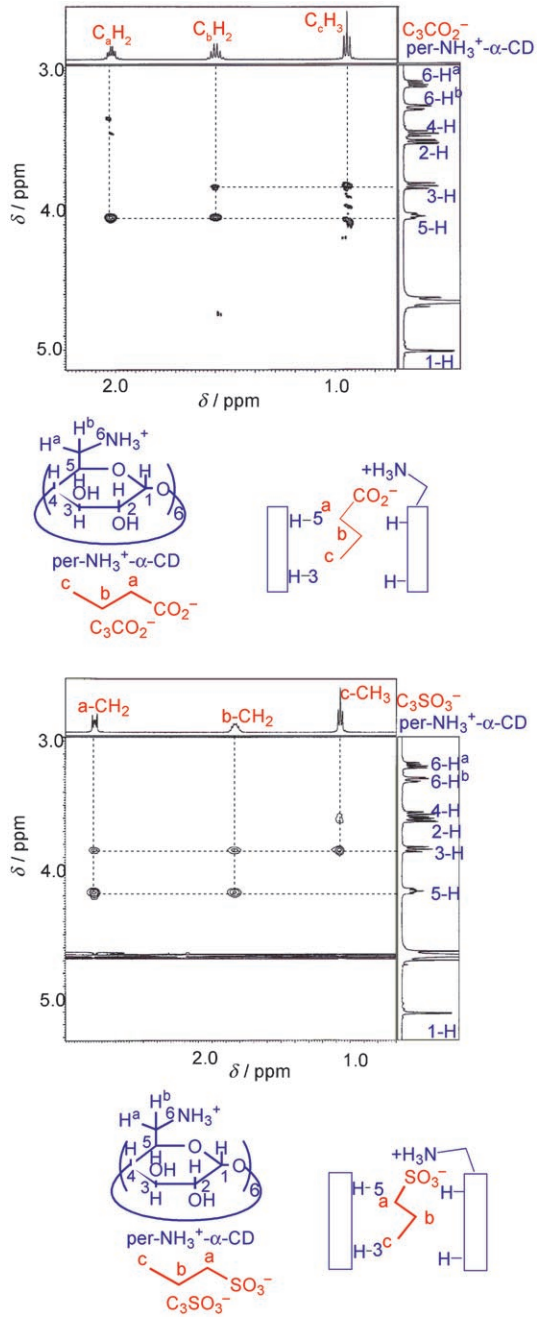



Figure 6. ROESY spectra of the $C_3CO_2^-$/perNH$_3^+$-α-CD (top) and $C_3SO_3^-$/per-$NH_3^+$-α-CD complexes (bottom) in a solution of NaCl (0.02 M) in $D_2O$ at pD 6.0 and 298 K. $[C_3CO_2^-]=[C_3SO_3^-]=$[per-$NH_3^+$-α-CD]$=0.01$ M, mixing time $=250$ ms.

penetrates more deeply into the CD cavity than in the case of $C_3CO_2^-$. 4-$CH_3C_4CO_2^-$ and 5-$CH_3C_5CO_2^-$ have the bulky methyl branches at the ends of the hydrophobic alkyl chains. Penetration of these bulky guests into the per-$NH_3^+$-β-CD cavity brings more extensive extrusion of the water molecules from the inside of the CD cavity, thus leading to very small contribution of $\Delta C_p^{0}{}_{hyd(CD)}$ to $\Delta C_p^{0}{}_{total}$. The notable difference in the $\Delta C_p^{0}$ values between 2-$CH_3C_5CO_2^-$ and 5-$CH_3C_5CO_2^-$ supports the above assumption. In conclusion,

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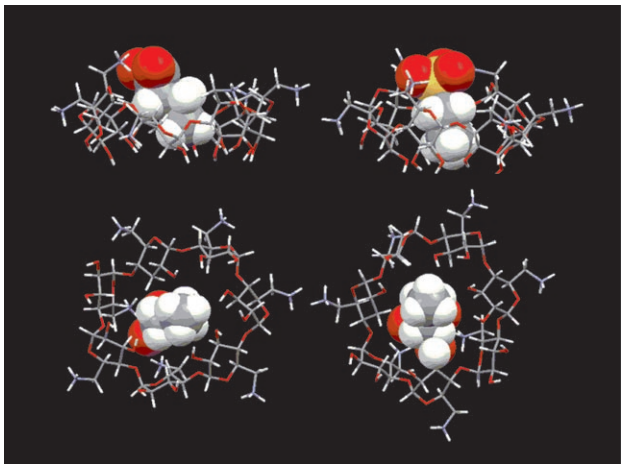

Figure 7. Energy-minimized structures of the $C_3CO_2^-$/per-$NH_3^+$-β-CD (left) and $C_3SO_3^-$/per-$NH_3^+$-β-CD complexes (right) obtained from MM2 calculations using BioMedCAChe 6.0. The effects of the solvent were not considered in the calculations.

$\Delta C_{p\ hyd(CD)}^{0}$ is the main contributor to the positive and large $\Delta C_{p\ total}^{0}$ value in the complexation of a relatively small alkanecarboxylate with a polycationic CD that has a relatively large cavity.

### Heat-Capacity Changes in the Complexation of Proteins with a Polyanionic CD

Heat-capacity changes have been widely utilized to study the unfolding of proteins and protein–ligand interactions in biological systems.[2–7] Generally, biological systems are so complex that their $\Delta C_p^0$ values warrant greater attention. As an extension of the present study, we measured temperature-dependent $\Delta H^0$ values for the complexation of cationic proteins with a polyanionic CD to determine the contribution from dehydration of the charged groups of the proteins to $\Delta C_p^0$. We used heptakis(6-carboxymethyl-2,3-di-*O*-methyl)-β-CD (CDM-β-CD) in the dissociated form as the polyanionic CD. Our previous study showed that CDM-β-CD is bound to horse heart cytochrome c (cyt c; pI 10.4) in 0.01 M aqueous NaCl at pH 7.0 through Coulomb interactions.[14] Such a simple system would be appropriate for studying $\Delta C_p^0$ in protein–ligand complexation through Coulomb interactions. In this study, the electrostatic binding of cyt c as well as metmyoglobin (metMb) with CDM-β-CD was thermodynamically examined by means of ITC. The results are listed in Table 3.

The calorimetric titration curves for the cyt c/CDM-β-CD system were well-fitted to the equation for 1:1 complex formation. As found previously,[14] the complexation is endothermic and is driven by the entropy term, which suggests the occurrence of extended dehydration from both cyt c and CDM-β-CD upon association. The plot of $\Delta H^0$ versus $T$ showed poor linearity in this system (see Supporting Information). Although the exact $\Delta C_p^0$ value for this system was hardly determined, it is likely to be positive and small ($\Delta C_p^0 = (131 \pm 136)\ J\,mol^{-1}\,K^{-1}$). At pH 7, cyt c (pI 10.4) has nine positive charges. This character of cyt c has been applied to the design of various artificial receptors that are bound to cyt c through Coulomb interactions.[15] In spite of extended dehydration, the complexation of cyt c with CDM-β-CD showed a positive and small $\Delta C_p^0$. This result corresponds to the conclusion that $\Delta C_{p\ dehyd(polar)}^{0}$ is not a major factor for the positive and large $\Delta C_p^0$ values in some complexations of $C_nCO_2^-$ with per-$NH_3^+$-CDs.

The pI value of metMb is 7.4. Therefore, electrostatic interactions between metMb and CDM-β-CD were studied at pH 5.5. The complexation is also endothermic and is promoted by the entropy gain. Although $T\Delta S^0$ for the metMb/CDM-β-CD system was slightly smaller than for cyt c/CDM-β-CD, $\Delta C_p^0$ was much larger than for the complexation of cyt c with CDM-β-CD. Such a positive and large $\Delta C_p^0$ value cannot be interpreted in terms of dehydration of the charged groups of metMb and CDM-β-CD. One of the plausible explanations of the positive $\Delta C_p^0$ value is the increase in heat capacity by the formation of the iceberg structure of water due to exposure of the nonpolar part of metMb upon complexation with CDM-β-CD.[4,5,16,17] We then measured the circular dichroism (cd) spectral changes of metMb upon addition of CDM-β-CD (see Supporting Information). However, no spectral change was observed with metMb at 25 °C. It is evident that no significant denaturation of metMb occurs upon association with CDM-β-CD, whereas a small structural change of metMb caused by CDM-β-CD is possible. There are few examples of positive $\Delta C_p^0$ values that are ascribed to the endothermic structural changes of proteins during protein–ligand interactions.[3a,10] The small, ligand-induced structural change of a protein that does not affect the two-dimensional structure of the protein may be difficult to detect. We followed the changes in ellipticity of metMb at 222 nm ($\theta_{222}$) as a function of temperature (Figure 8), and the phase-transition temperatures ($T_M$s) in the absence and presence of CDM-β-CD were determined (Table 3). The $T_M$ of metMb was lowered by 18 °C in the presence of CDM-β-CD, which suggests that CDM-β-CD alters the intraprotein interactions and leads to some structural change in metMb. Similar results were obtained with cyt c.

Table 3. Binding constants ($K$), enthalpy ($\Delta H^0$), entropy ($T\Delta S^0$), and heat-capacity changes ($\Delta C_p^0$) for the complexation of cyt c and metMb with CDM-β-CD in 0.01 M aqueous NaCl at 298.15 K, and the phase-transition temperatures of the proteins in the absence ($T_{M0}$) and presence of CDM-β-CD ($T_M^{CD}$).[a]

| Protein | pH | $K$ [$M^{-1}$] | $\Delta H^0$ [kJ mol$^{-1}$] | $T\Delta S^0$ [kJ mol$^{-1}$] | $\Delta C_p^0$ [J mol$^{-1}$ K$^{-1}$] | $T_{M0}$ [K] | $T_M^{CD}$ [K] |
|---|---|---|---|---|---|---|---|
| cyt c | 7.7 | $(6.28\pm0.54)\times10^4$ | $11.6\pm0.4$ | $39.2\pm0.2$ | $131\pm136$ | 85 | 79 |
| metMb | 5.5 | $(7.37\pm0.58)\times10^3$ | $16.4\pm1.3$ | $38.5\pm1.5$ | $840\pm161$ | 84 | 66 |

[a] The $T_M$ values for cyt c ($1\times10^{-5}$ M) in Tris·HCl buffer (0.01 M, pH 7.0) and those for metMb ($1\times10^{-5}$ M) in succinic acid buffer ($5\times10^{-4}$ M, pH 5.5) were obtained in the absence and presence of CDM-β-CD ($5\times10^{-4}$ M) by measuring $\theta_{222}$.

  

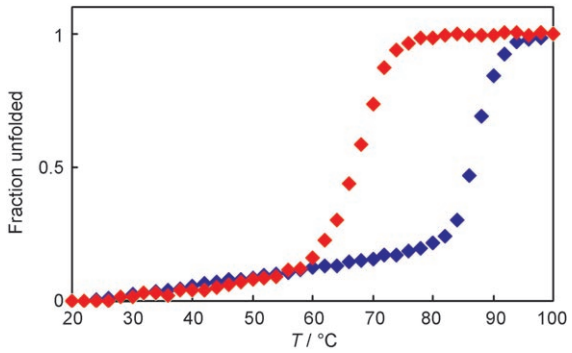


Figure 8. Thermal-denaturation profiles monitored by cd intensities at 222 nm ($\theta_{222}$) of metMb ($3\times10^{-5}$ M) in the absence (blue) and the presence (red) of CDM-β-CD ($1\times10^{-3}$ M) in $5\times10^{-4}$ M succinic acid buffer at pH 5.5.

The small structural change in the protein caused by CDM-β-CD may be the origin of the positive and large $\Delta C_p^0$ value rather than dehydration of the charged groups. Theoretical calculations predicted a positive $\Delta C_p^0$ value in noncovalent intraprotein interactions leading to structural change in the protein.[18]

## Conclusions

The complexation of amphiphilic $C_nCO_2^-$ with polycationic per-$NH_3^+$-CD through a cooperative contribution of electrostatic and inclusion interactions is an excellent subject for studying $\Delta C_p^0$ in host–guest chemistry. As is well-known, dehydration of the hydrophobic part of a guest upon inclusion results in a negative and large $\Delta C_p^0$ value. However, release of water molecules from charged groups makes a small contribution to $\Delta C_p^0$. On the other hand, the formation of the iceberg structure of water inside the cavity of a guest-loading CD results in a positive and large $\Delta C_p^0$ value. In electrostatic protein–ligand association, dehydration of the charged groups of both protein and ligand also produces a minor contribution to $\Delta C_p^0$, whereas the small structural change in the protein induced by electrostatic binding to a ligand seems to the main factor for a positive and large $\Delta C_p^0$ value.

## Experimental Section

The per-$NH_2$-α- and -β-CD used were the same as those reported previously.[12] The commercially obtained alkanecarboxylic acids ($C_nCO_2H$) were converted into their sodium salts by dissolving them in equimolar aqueous solutions of NaOH. The sodium carboxylates were isolated by evaporation and used after drying under vacuum. Horse heart cyt c (Sigma) and metMb (Sigma) were purchased and used as received. We checked the purities of these proteins by obtaining their UV/Vis spectra and observing the extinction coefficients at the Soret bands of hemin.

Microcalorimetric measurements were carried out with a Microcal VP-ITC isothermal titration calorimeter. The titration curves obtained were analyzed with the ORIGIN software program. ITC measurements for the CD system were performed in aqueous NaCl (0.02 M) to adjust the ionic strength. UV/Vis spectra were recorded on Shimadzu UV-2100 and UV-2450 spectrophotometers with thermostatic cell holders. pH values were measured with a Horiba M-12 pH meter. NMR spectra were recorded on a JEOL JNM-ECA500 spectrometer (500 MHz) in $D_2O$ (CEA, 99.9%) with sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate (TSP; Aldrich) as an external standard and NaOD for adjustment of pD.

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