

## Observation of Entropic Effect on Conformation Changes of Complex Systems under Well-Controlled Temperature Conditions

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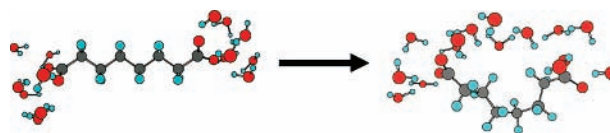
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We report direct observation of an entropic effect in determining the folding of a linear dicarboxylate dianion with a flexible aliphatic chain [ $^-O_2C-(CH_2)_6-CO_2^-$ ] by photoelectron spectroscopy as a function of temperature (18–300 K) and degree of solvation from 1 to 18 water molecules. A folding transition is observed to occur at 16 solvent water molecules at room temperature and at 14 solvent molecules below 120 K due to the entropic effect. The  $^-O_2C-(CH_2)_6-CO_2^-(H_2O)_{14}$  hydrated cluster exhibits interesting temperature-dependent behaviors, and its ratio of folded over linear conformations can be precisely controlled as a function of temperature, yielding the enthalpy and entropy differences between the two conformations. A folding barrier is observed at very low temperatures, resulting in kinetic trapping of the linear conformation. The current work provides a simple model system to study the dynamics and entropic effect in complex systems and may be important for understanding the hydration and conformation changes of biological molecules.

Temperature plays a critical role in determining the structure and reactivity of complex molecules in both gaseous and condensed phases.<sup>1–3</sup> Molecular beam techniques are used routinely to cool small molecules in the gas phase. Helium droplets and rare-gas tagging have recently been applied to produce cold molecules and clusters.<sup>4–6</sup> However, precise temperature control and tuning have been challenging for gaseous molecules. Recent advances in ion trap technology<sup>7</sup> have made it possible to create cold ions down to very low temperatures<sup>8–10</sup> and to allow temperature-tuning. Here we report the observation of temperature-induced structural changes of a complex doubly charged anion in a cold ion-trap over a wide temperature range. We produced hydrated suberate dianions,  $^-O_2C-(CH_2)_6-CO_2^-(H_2O)_n$ , and probed their structural changes as a function of temperature and hydration using photoelectron spectroscopy (PES). The bare suberate possesses a linear conformation, and a folding transition occurs at  $n = 16$  at room temperature.<sup>11,12</sup> We find that the folding transition depends on temperature and occurs at  $n = 14$  below 120 K due to entropic effect (Scheme 1). Interestingly, a potential barrier is observed to inhibit folding at very low temperatures, resulting in kinetic trapping of the metastable linear conformation. The folding of the hydrated suberate is analogous to protein folding and may be used as simple models to study the dynamics of protein folding.<sup>13</sup> The current experiment suggests temperature-dependent PES can be used to study the structure and dynamics and solvent and electrostatic effects of complex molecules and clusters.

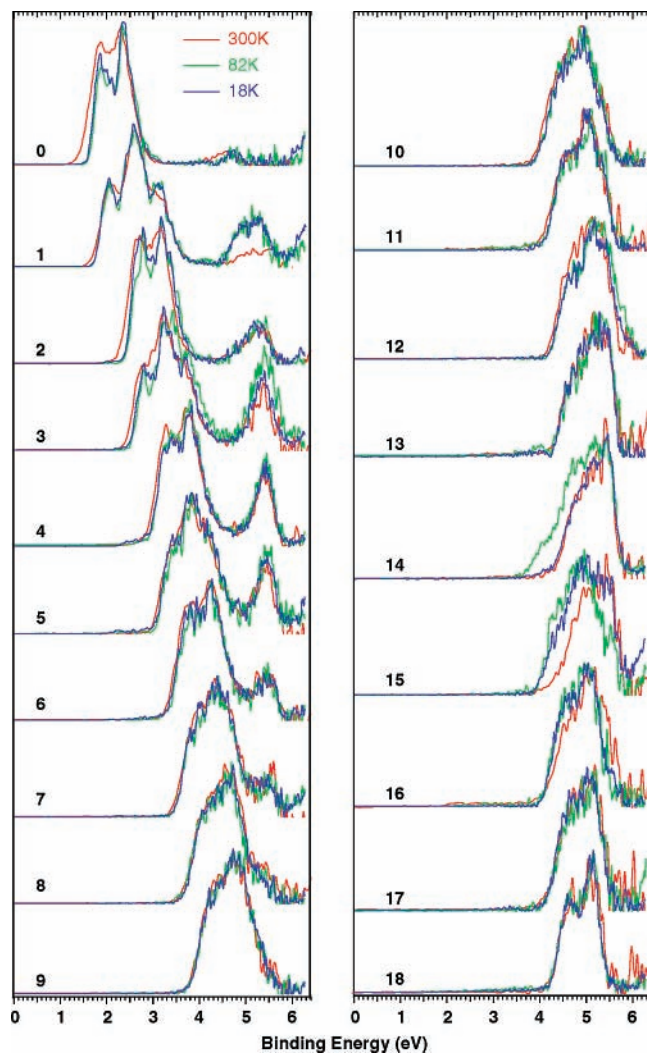
The experiment was performed with an electrospray PES apparatus coupled with a temperature-controlled ion trap.<sup>8,14</sup> The hydrated suberate clusters were produced from a solution of

SCHEME 1



suberic acid in a mixed methanol/water solvent (3/1 volume ratio) under basic conditions. Anions from the source were guided by a radio frequency octopole and quadrupole and then bent  $90^\circ$  into a Paul trap, which was attached to the cold head of a closed-cycle helium refrigerator. Temperatures of the cold head can be controlled between 10 and 350 K. The anions were trapped and collisionally cooled with a background gas ( $\sim 1$  mTorr helium containing 20%  $H_2$ ). Ions were trapped for a period of 20–100 ms and cooled via translational and rovibrational energy transfers to the background gas (the number of collisions was estimated to be  $\sim 2000$ – $10\,000$ ). The trapped ions were pulsed out into the extraction zone of a time-of-flight mass spectrometer at 10 Hz repetition rate. The ions of interest were mass-selected and decelerated before being intercepted with a 193 nm (6.424 eV) laser beam in the interaction zone of the magnetic-bottle photoelectron analyzer. The laser was operated at a 20 Hz repetition rate with the ion beam off at alternate shots for background subtraction. The photodetached electrons were collected with nearly 100% efficiency by the magnetic-bottle and analyzed in a 5-m long electron flight tube. The electron energy resolution was  $\Delta E/E \sim 2\%$ , i.e., 20 meV for 1 eV electrons. Photoelectron time-of-flight spectra were collected and then converted to kinetic energy spectra, calibrated by the known spectra of  $ClO_2^-$  and  $I^-$ . The electron binding energy spectra presented were obtained by subtracting the kinetic energy spectra from the detachment photon energies.

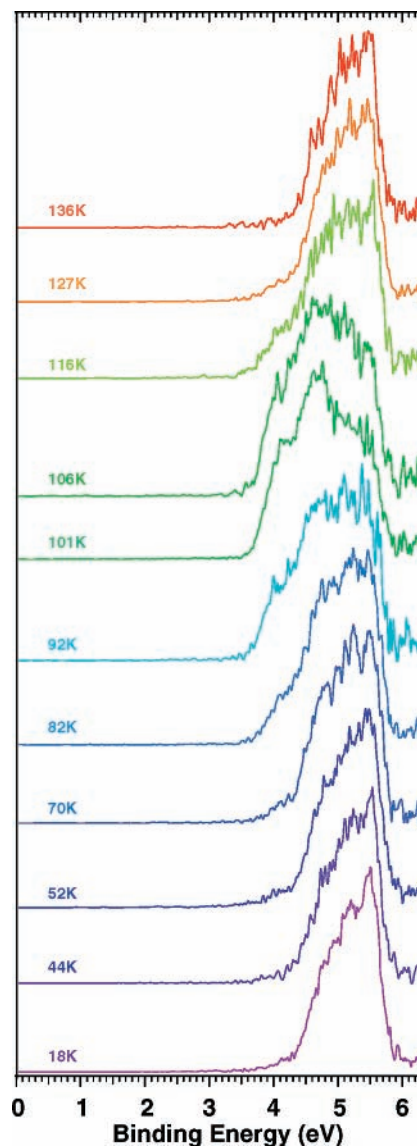
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**Figure 1.** Comparison of the photoelectron spectra of hydrated suberate,  ${}^{-}\text{O}_2\text{C}(\text{CH}_2)_6\text{CO}_2{}^{-}(\text{H}_2\text{O})_n$  ( $n = 0-18$ ) at 193 nm (6.424 eV) at 82 K (green) and 18 K (blue) with those at room temperature (red).

Figure 1 compares the PES spectra of  ${}^{-}\text{O}_2\text{C}(\text{CH}_2)_6\text{CO}_2{}^{-}(\text{H}_2\text{O})_n$  ( $n = 0-18$ ) at three trapping temperatures. Photodetachment takes place from the carboxylate groups and should be sensitive to the solvent and electrostatic environments. The 300 K spectra (red) display an even-odd effect at smaller  $n$ , indicating hydration occurs at the charged groups alternately: those with even solvent numbers are similar to the bare dianion, whereas those with odd solvent numbers exhibit an extra band due to the nonequivalent two carboxylate ends.<sup>12</sup> The room-temperature hydrated clusters show continuous increase of electron binding energy until  $n = 16$ , where the PES spectrum exhibits a sudden shift to lower electron binding energy, signaling an increase of Coulomb repulsion. At this size, the two negative charges are screened enough, and it becomes thermodynamically less favorable to have two separate solvation centers. The merging of the two separate water droplets results in a significant energy gain due to the additional cooperative water-water H-bonding effect, at the same time pulling two negative charge closer and excluding the aliphatic chain by folding (Scheme 1).<sup>12</sup>

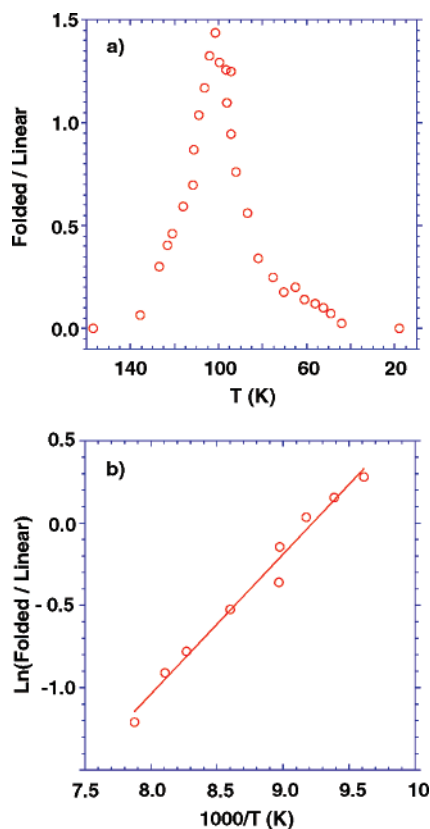
Because of the entropic effect, it is expected that the number of water molecules required to induce the folding transition should be smaller at lower temperatures. Indeed, we observed that at 82 K (green) the PES spectrum of  $n = 14$  displays a



**Figure 2.** Temperature-dependent photoelectron spectra of  ${}^{-}\text{O}_2\text{C}(\text{CH}_2)_6\text{CO}_2{}^{-}(\text{H}_2\text{O})_{14}$ .

lower binding energy feature indicating the onset of folding. The 82 K spectrum of  $n = 15$  shows a major shift to lower binding energies implying it assumes the folded conformation at low temperatures. The spectra of the smaller clusters at 82 K are similar to the 300 K spectra except that they are slightly better resolved. The threshold electron binding energy of  $n = 14$  at 82 K decreased to 3.7 eV relative to 4.3 eV at 300 K, indicating an increase of 0.6 eV in the intramolecular Coulomb repulsion between the two carboxylates in the folded conformation.

However, at the lowest temperature (18 K) achievable in our current ion trap, we observed surprisingly that the spectrum of  $n = 14$  (blue) became identical to the 300 K spectrum, whereas the spectra of all of the other clusters were similar to those at 82 K. To understand this unexpected observation, we performed a systematic temperature-dependent study on the  $n = 14$  hydrated cluster with a temperature gradient of  $\sim 5$  K from 150 down to 18 K. A selected set of these data is shown in Figure 2. We found that folding started at  $\sim 127$  K, where a weak low binding energy feature due to the folded conformation appeared. This feature increased with lowering temperature and reached a maximum at  $\sim 101$  K and then decreased with further lowering temperature. Below about 50 K, this feature completely disap-



**Figure 3.** Analysis of the temperature-dependent photoelectron spectra of  ${}^{-}\text{O}_2\text{C}(\text{CH}_2)_6\text{CO}_2{}^{-}(\text{H}_2\text{O})_{14}$ . (a) The ratio of the relative intensities of the folded versus linear conformation as a function of temperature. (b) The natural logarithmic plot of folded/linear versus  $1/T$  in the temperature range  $>101$  K (the van't Hoff plot:  $\ln(K) = -\Delta H/RT + \Delta S/R$ , where  $K$  is the equilibrium constant ( $=[\text{folded}]/[\text{linear}]$ ) and  $R$  is the gas constant), which yield a value of  $-1.68$  kcal/mol for  $\Delta H$  and  $-0.0155$  kcal/mol K for  $\Delta S$ .

peared and the PES spectrum became identical to that from 300 K. This observation suggests at very low temperatures there is no folding.

Thus, the PES spectra in the intermediate temperature range from 127 to 50 K should contain contributions from both the folded and linear conformations. If we subtract the signals due to the linear cluster, we can obtain the PES spectra due to the folded conformation (Figure S1).<sup>15</sup> Figure 3a plots the ratio of the relative intensities (taken as the integrated areas of the deconvoluted PES spectra<sup>16</sup>) between the folded and linear conformations, showing clearly a maximum at 101 K.

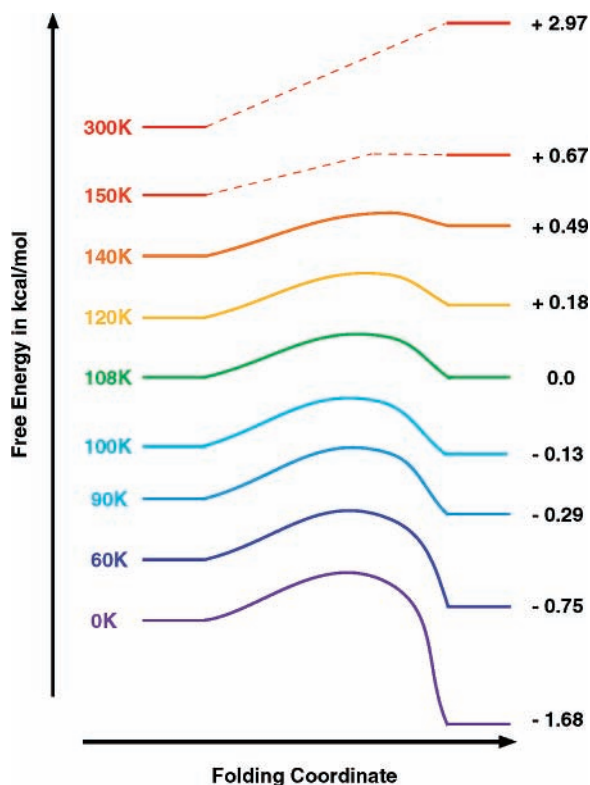
The observation of folding at a lower hydration level at low temperatures illustrates the key role that entropy ( $\Delta S$ ) plays. The linear conformation, being more floppy, possesses a higher entropy and is favored at higher temperatures. As the temperature ( $T$ ) decreases, the entropic contribution to the free energy ( $\Delta G$ ) becomes less important ( $\Delta G = \Delta H - T\Delta S$ , where  $\Delta H$  is the enthalpy). Thus, despite the increased intramolecular Coulomb repulsion, the free energy in the folded conformation is lowered relative to the linear conformation with decreasing temperature. Even for the  $n = 14$  cluster, the folded conformation can become more stable at low temperatures due to the enhanced water–water H-bonding, which overcomes the intramolecular Coulomb repulsion, giving the appearance of an “attraction” between two negative charges as they are pulled closer in the folded conformation. If the two conformations are in equilibrium, a logarithmic plot of their ratio as a function of  $1/T$  should yield a straight line (the van't Hoff plot:  $\ln(K) =$

$-\Delta H/RT + \Delta S/R$ , where  $K$  is the equilibrium constant and  $R$  is the gas constant), which can yield the enthalpy ( $\Delta H$ ) and entropy ( $\Delta S$ ) differences between the two conformations. Figure 3b shows that in the temperature range  $>101$  K, a linear curve is indeed observed, from which we obtain  $\Delta H = -1.68$  kcal/mol and  $\Delta S = -0.0155$  kcal/mol.K. Hence, the free energy difference between the folded and linear conformations as a function of temperature can be described as:  $\Delta G = -1.68 + 0.0155T$  in kcal/mol, which shows at room temperature the linear conformation is more stable than the folded one by  $\sim 3$  kcal/mol, whereas at 0 K the folded one is more stable than the linear one by 1.68 kcal/mol.

Therefore, one would expect that at low temperatures the  ${}^{-}\text{O}_2\text{C}(\text{CH}_2)_6\text{CO}_2{}^{-}(\text{H}_2\text{O})_{14}$  hydrated cluster should assume the folded structure since it has lower free energy. Why did we observe a decrease of the folded conformation below 101 K and no folding below 50 K? This was totally unexpected, suggesting that there is a potential barrier for folding. It turned out that this barrier can be understood from the intramolecular Coulomb repulsion. As the linear substrate folds, the Coulomb repulsion increases between the two negatively charged terminal carboxylate groups. However, once the two carboxylate groups fold close enough, the water molecules solvating each carboxylate can merge in the folded conformation, providing a stabilization due to the enhanced water–water H-bonding. The superposition of the long-range Coulomb repulsion between the two negative charges during folding and the short-range water–water H-bonding gives rise to the folding barrier, analogous to the repulsive Coulomb barrier in electron detachment from a multiply charged anion.<sup>11,17</sup> The  ${}^{-}\text{O}_2\text{C}(\text{CH}_2)_6\text{CO}_2{}^{-}(\text{H}_2\text{O})_{14}$  clusters are generated by electrospray under ambient environments in the linear conformation.<sup>12</sup> They are cooled in the ion trap through collisions with a helium background gas at  $\sim 1$  mTorr pressure. Apparently, below 100 K the hydrated clusters are cooled rapidly to the ion-trap temperature before folding takes place so that an equilibrium between the linear and folded conformations cannot be established at such low temperatures. The population of the folded clusters is then kinetically determined by the conversion rate over the folding barrier.<sup>18</sup> Below 50 K, the folded conformation was not measurable. Clearly, the clusters are rapidly quenched at such low temperatures that they are frozen in the thermodynamically metastable linear conformation.<sup>19</sup>

Figure 4 shows schematically the overall free energy changes between the linear and folded conformations of the  $n = 14$  hydrated cluster as a function of temperature ( $\Delta G = -1.68 + 0.0155T$  in kcal/mol). The folded conformation is stabilized due to the enhanced H-bonding interactions of the water, despite the increased Coulomb repulsion. The decrease of 0.6 eV (13.8 kcal/mol) in electron binding energy between the folded and linear conformations is a direct measure of the increased Coulomb repulsion. Thus in the folded conformation the increased H-bonding interactions amount to  $\sim 15.5$  kcal/mol, providing a net stabilization of 1.68 kcal/mol. Because of this relatively low net stabilization, the entropy effect and temperature become crucial in determining the stability of the linear vs folded conformations. Clearly, the linear conformation is more stable above 108 K and the folded conformation is more stable below 108 K. At 108 K, the two conformations have the same free energy, consistent with the observation of a 1/1 ratio in their relative intensities at this temperature (Figure 3a).

The observed temperature dependence in the folding of the  ${}^{-}\text{O}_2\text{C}(\text{CH}_2)_6\text{CO}_2{}^{-}(\text{H}_2\text{O})_{14}$  hydrated substrate (Figure 3a) is quite similar to the folding of small proteins with simple two-state



**Figure 4.** Temperature-dependent folding and free energies of  ${}^{-}\text{O}_2\text{C}(\text{CH}_2)_6\text{CO}_2{}^{-}(\text{H}_2\text{O})_{14}$ . Note the appearance of the folding barrier due to the long-range intramolecular Coulomb repulsion during folding and the short-range H-bonding of the solvent. The numbers on the right are the free energy differences between the linear and folded conformations according to  $\Delta G = -1.68 + 0.0155T$  in kcal/mol.

kinetics.<sup>13,20,21</sup> Despite the complexity inherent in protein folding, the fundamental physics underlying protein folding has been modeled with simple systems that lead to major insights into folding rates and mechanisms.<sup>13,22</sup> The hydrated suberate and other dicarboxylates<sup>11</sup> with different chain lengths [ ${}^{-}\text{O}_2\text{C}(\text{CH}_2)_n\text{CO}_2{}^{-}$ ] may provide a class of model systems for protein folding. The intramolecular Coulomb repulsion gives rise to a folding barrier, which is analogous to the barriers encountered in protein folding,<sup>23</sup> but it can be accurately computed and understood. It is conceivable to probe the folding dynamics by controlling the cooling rate or the barrier height. For example, infrared heating of the metastable ions may be used to initiate folding and allow the ensuing folding kinetics to be followed. There are many negative charge carriers in biomolecules, such as the carboxylate in amino acids or phosphate in nucleotides, which are accessible to PES studies.<sup>24</sup> The temperature-dependent PES method can be a powerful technique to probe the influence of solvation and electrostatic environment on the folding and conformation of complex molecules and individual biomolecules under well controlled conditions.

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**Supporting Information Available:** Deconvolution of the temperature-dependent photoelectron spectra of  ${}^{-}\text{O}_2\text{C}(\text{CH}_2)_6\text{CO}_2{}^{-}(\text{H}_2\text{O})_{14}$ . These materials are available free of charge via the Internet at <http://pubs.acs.org>.

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- There might be different configurations in both the linear and folded conformations. But those configurations are expected to be close in energy in each conformation and should give rise to similar photoelectron spectra.
- Because photodetachment takes place from the carboxylate groups in both the linear and folded conformations, photodetachment cross sections are expected to be similar for the two conformations.
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- Because ions were continuously loaded into the ion trap, the folding time was not accurately defined in the present experiment, and the cooling rate can not be changed much due to the fixed repetition rate of detachment laser and the optimal pressure of background gas in the trap which is crucial to achieve best cooling effect. However, assuming average 50 ms reaction time and using the kinetic rate theory to fit the data in Figure 3a at low temperatures (Arrhenius plot), we obtained the barrier height of 0.67 kcal/mol, which is consistent with an electrostatic estimation (1.6 kcal/mol) by considering the Coulomb repulsion and the polarization attraction fitted with the measured enthalpy change ( $\Delta H = -1.68$  kcal/mol).
- Preliminary data of the detailed temperature-dependent studies on different size clusters indicate that folding transitions occur at  $>140$  K for  $n = 15$ , and 300 K for  $n = 16$ , both being higher than that of  $n = 14$  (127 K). This suggests folding being easier for larger clusters. The more negative  $\Delta H$  of  $n = 15$  and 16 gives rise to lower folding barriers, which can not completely block the folding transition even at 18 K as contrast to that of  $n = 14$  (Figure 1).
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