Selective Na^+/K^+ Effects on the Formation of α-Cyclodextrin Complexes with Aromatic Carboxylic Acids: Competition for the Guest

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We investigated the effects of K^+ and Na^+ ions on the formation of α-cyclodextrin complexes with ionized aromatic carboxylic acids. Using solution calorimetry and ^1H NMR, we performed the thermodynamic and structural investigation of α-cyclodextrin complex formation with benzoic and nicotinic acids in different aqueous solutions containing K^+ and Na^+ ions as well as in pure water. The experiments show that the addition of sodium ions to solution leads to a decrease in the binding constants of the carboxylic acids with α-cyclodextrin as compared to pure water and solutions containing potassium ions. From another side, the effect of potassium ions on the binding constants is insignificant as compared to pure water solution. We suggest that the selectivity of cation pairing with carboxylates is the origin of the difference between the effects of sodium and potassium ions on complex formation. The strong counterion pairing between the sodium cation and the carboxylate group shifts the equilibrium toward dissociation of the binding complexes. In turn, the weak counterion pairing between the potassium cation and the carboxylate group has no effect on the complex formation. We complemented the experiments with molecular modeling, which shows the molecular scale details of the formation of cation pairs with the carboxylate groups of the carboxylic acids. The fully atomistic molecular simulations show that sodium ions mainly form direct contact pairs with the carboxylate group. At the same time, potassium ions practically do not form direct contact pairs with the carboxylate groups and usually stay in the second solvation shell of carboxylate groups. That confirms our hypotheses that the selective formation of ion pairs is the main cause of the difference in the observed effects of sodium and potassium salts on the guest−host complex formation of α-cyclodextrin with aromatic carboxylic acids. We propose a molecular mechanism explaining the effects of salts, based on competition between the cations and α-cyclodextrin for binding with the ionized carboxylic acids.

Introduction

Cyclodextrins (CDs) attract considerable interest nowadays due to their widespread applications as encapsulating materials for medicines and food ingredients.[1−5] In this connection, CD complexes with biologically active molecules are extensively studied.[6,7] However, much less attention was paid to the effects of biological aqueous environments on the CD complex formation. The biologically relevant salts can make significant effects on biomolecules,[8−10] and, therefore, they should be also considered in the host−guest binding.

The study has been motivated by the fact that, although the selective salt effects are intensively studied in the protein[11−16] and colloidal sciences,[17,18] they are not sufficiently explored in CD chemistry. Generally, the influence of salts on CD complex formation is explained in the following ways:[19−21] (1) salting-in or salting-out effects that influence the structure of solution and shift equilibriums, changing by this way the stability of the complexes; (2) formation of ternary complexes, where the ions stabilize the inclusion complex through the additional interactions with both host and guest molecules; and (3) competition between guests and large anions for the CD cavity. However, possible competition between CD and cation for guests with ionized polar groups was not taken into account. It is necessary to note that cations can selectively interact with charged guests via ionic pairing, and these effects can play a considerable role in the mechanisms of host−guest complexation.

In this study, we investigate the comparable effects of sodium and potassium cations on the mechanism of α-CD complex formation with benzoic and nicotinic acids in water. There are several works where the selective interactions of biologically active cations with carboxylate groups of glycine,[22] formate,[8,23] acetate,[22−24] α-poly-L-glutamic acid,[25] and protein anionic side groups[26] have been studied by experimental and theoretical methods. These works demonstrated that K^+ and Na^+ cations have different binding affinities to carboxylates. As compared to potassium, much stronger attraction of sodium to carboxylates has been observed. This is consistent with the empirical Law of Matching Water Affinities by Collins,[10] according to which the ions having a similar hydration energy form more stable contact ionic pairs. Thus, as discussed in the work of Aziz et al.,[22] the hydration energies of sodium and carboxylate are very close, and, as a result, these ionic molecular species form rather strong counterion pairs in water.

In this work, we examined the selective effects of Na^+ and K^+ on the α-CD complex formation with the aromatic carboxylic acids (ACA) in aqueous solutions containing these ions

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(NaOH, KOH, NaCl, and KCl solutions). In our study, we used ^{1}H NMR and calorimetric measurements complemented by atomistic molecular modeling. Nicotinic and benzoic acids were chosen as guest molecules because they are the elementary representatives of ACAs with biological and pharmacological activity. Nicotinic acid is known as a constituent of B-family vitamins, which is involved in a number of physiological processes in living organisms. Moreover, it has been used for many years in the treatment of hyperlipemia, pellagra, and high levels of cholesterol. Benzoic acid is also widely used in food, pharmaceutical, and cosmetic products. Although these acids are considered as safe, they can cause some irritation effects (e.g., flushing, hepatotoxicity).[27,28] Inclusion complex formation with CDs can eliminate these undesired side effects and improve the physicochemical properties and biological activity of the encapsulated compounds.

This Article is organized as follows. In the Experimental Section, we explain details of experimental techniques we used in our study. Next, in the Molecular Modeling section, we explain details of the molecular modeling methods we used in this work. In the Results section, we present the results of NMR experiments, calorimetric studies, and molecular modeling. In the Discussion section, we discuss the obtained results in the light of literature on selective ion pairing and salt effects on the guest−host CD complexes with organic molecules. We conclude our Article with a short Conclusion section.

Experimental Section

Materials. α-CD (Fluka), benzoic acid (Sigma-Aldrich), and nicotinic acid (MP Biomedicals) were of analytical reagent grade. Sodium and potassium chlorides were obtained from Sigma-Aldrich and used without further purification. The purity of NaCl and KCl was ≥99.99% and ≥99.5%, respectively. We note that analysis of the lists of impurities provided by Sigma for these chemicals shows that none of the impurities have significant affinity to α-CD. At the same time, those impurities that can bind the carboxylic acids (like Li^+ or Mg^{2+}) have negligibly small concentrations (0.05−0.5 mg/kg). Therefore, due to the low amounts of these species in the impurities, we considered their influence on ACA complexation with α-CD as negligible.

All the solutions were prepared with distilled and deionized water by weight. Experiments with nicotinic acid were done in pure water where zwitterionic species are dominant.[29] To obtain the prevalence of benzoic acid anions (benzoates) in solution, we added some amount of either KOH or NaOH to adjust pH to 9. The pH of solutions was controlled before and after the measurements, and it was constant within the error limits. The base concentration was 0.03 mol/kg.

^{1}H NMR. ^{1}H NMR experiments were carried out at 298.15 K on a Bruker-AV-500 spectrometer operating at 500 MHz. Cyclohexane was applied as external reference. ^{1}H NMR spectra were measured in D_2O of 99.9% isotopic purity. ^{1}H NMR chemical shifts of α-CD protons were measured at constant α-CD concentration (0.005 mol/kg) and variable concentrations of nicotinic acid (0−0.15 mol/kg) and benzoate (0−0.04 mol/kg) with and without salt addition (0.2 M NaCl or KCl). The chemical shift changes ($\Delta\delta$) induced by complex formation were calculated as follows:

$$\Delta\delta = \Delta\delta_{\text{complexed}} - \Delta\delta_{\text{free}} \quad (1)$$

TABLE 1: Stability Constants of α-CD Complexes with Benzoate and Nicotinic Acid in Aqueous Solutions Containing Na^+ and K^+ Ions at 298.15 K Obtained from ^{1}H NMR

complex	solution	K (kg/mol)
α-CD/benzoate	0.03 M KOH	19.1 ± 1.5
	0.03 M NaOH	17.5 ± 1.6
	0.03 M KOH + 0.2 M KCl	18.9 ± 1.6
	0.03 M KOH + 0.2 M NaCl	16.0 ± 0.6
	0.03 M NaOH + 0.2 M NaCl	17.2 ± 2.0
α-CD/nicotinic acid	H_2O	27.0 ± 0.4
	0.2 M KCl	26.1 ± 0.6
	0.2 M NaCl	23.5 ± 0.7

Stability constants of the complexes (K) were evaluated from the concentration dependences of $\Delta\delta$ by the nonlinear curve fitting procedure reported early.[30] Values of K are presented in Table 1.

Calorimetry. Thermal effects of dissolution of crystalline α-CD in pure water and in aqueous solutions of nicotinic acid were measured by means of a solution calorimeter.[31] The detailed description of the calorimeter and the method was given previously.[31] In the calorimetric measurements, the α-CD concentration was constant (0.001 mol/kg), whereas the nicotinic acid concentration was changed from 0 to 0.14 mol/kg. The same set of the experiments was performed in the presence of 0.2 M NaCl and 0.2 M KCl.

Enthalpies of transfer ($\Delta_{tr}H(w \rightarrow w + y)$) of α-CD from water to nicotinic acid solutions were calculated via the following way:

$$\Delta_{tr}H(w \rightarrow w + y) = \Delta_sH(w + y) - \Delta_sH(w) \quad (2)$$

where $\Delta_sH(w)$ and $\Delta_sH(w + y)$ are the experimentally obtained enthalpies of solution of α-CD in water (w) and in aqueous solutions of nicotinic acid (w + y). In the case when sodium and potassium chlorides were present in the solutions, the $\Delta_sH(w + \text{salt})$ and $\Delta_sH(w + y + \text{salt})$ were used in eq 2.

Calculations of the enthalpies of complex formation (Δ_cH) and stability constants of the complexes (K) were done on the basis of concentration dependences of $\Delta_{tr}H(w \rightarrow w + y)$ using nonlinear least-squares fitting as was described previously.[31] The changes of free energy (Δ_cG) and entropy (Δ_cS) of complex formation were then obtained from well-known thermodynamic equations:

$$\Delta_cG = -TR \ln K \quad (3)$$

$$\Delta_cG = \Delta_cH - T\Delta_cS \quad (4)$$

Thermodynamic parameters of complex formation of α-CD with nicotinic acid are given in Table 2.

It was not possible to perform accurate calorimetric measurements for complex formation of α-CD with benzoate due to the low solubility of benzoic acid in water and a relatively weak α-CD−benzoate binding constant. As a consequence, the binding isotherms in this case do not have a well pronounced plateau, and, therefore, one cannot make a reasonably accurate estimation of the enthalpy of complex formation for this system.

Molecular Modeling. We performed fully atomistic molecular dynamics (MD) simulations of separate salt and bulk water solutions of benzoic acid, nicotinic acid, and α-CD using the Desmond 2.2 package.[32] In the salt solutions, the concentration

TABLE 2: Thermodynamics of Complex Formation of α-CD with Nicotinic Acid in Water and in Aqueous Solutions of NaCl and KCl at 298.15 K Obtained from Calorimetry

complex	K (kg/mol)	$\Delta_c G$ (kJ/mol)	$\Delta_c H$ (kJ/mol)	$T\Delta_c S$ (kJ/mol)
α-CD/nicotinic acid (H_2O)	27 ± 4	−8.2	−25.2 ± 0.3	−17.0
α-CD/nicotinic acid (0.2 M KCl)	30 ± 5	−8.4	−24.0 ± 0.3	−15.6
α-CD/nicotinic acid (0.2 M NaCl)	19 ± 4	−7.3	−29.3 ± 0.4	−22.0

of NaCl or KCl was constant and equal to 0.3 M. In the simulations, we used the TIP4P model for water[33,34] and the OPLSAA-2005 force field[35−42] for ions and organic compounds. We used the RESPA integration algorithm with two integration time steps: the integration time step for the short-range interactions was 2 fs, and the time step for the long-range interactions was 6 fs. For the treatment of the long-range electrostatic interactions, we used the smooth particle mesh Ewald method[43] with the cutoff radius of 9 Å.

In every simulation, the solutes were placed in a periodic cubic cell of size 50 × 50 × 50 Å^3. Next, 21 ion pairs were placed randomly in the cell volume excluding the solute volume (in the case of benzoate, we placed an extra sodium cation in the cell to neutralize the system). Afterward, water molecules were added in the simulation cell; water molecules overlapping with any solute atom or any ion were removed; and the final amount of water molecule was 3791 for every system except the α-CD solutions (in this case, there were 3753 water molecules in the box). We used the molecular structures of benzoic acid, nicotinic acid, and α-CD obtained from the Cambridge Structural Database.[44] During the simulations, the positions of carbon atoms were harmonically restrained to their original positions in space. These restraints should not influence the density distribution functions discussed in this Article because the functions operate with relative positions of the atoms. The restraints were introduced for the sake of convenience of the distribution functions calculations.

Simulations were carried out in two stages for all systems. During the first stage, we equilibrated the systems in the NPT ensemble for 2 ns. We simulated the systems at the temperature of 300 K and the pressure of 1 atm, which were kept constant by using the Berendsen thermostat and barostat with the relaxation times of 1 and 2 ps, correspondingly. Before the equilibration, the potential energy of every system was minimized using the standard relaxation procedure[32] to avoid sterical clashes between the atoms.

Next, we performed productive simulations in the NVT ensemble at constant $T = 300$ K using the Berendsen thermostat with the relaxation time equal to 1 ps. To collect sufficient statistics of ion pairing, we simulated all systems for 130 ns.

Radial distribution functions and 3D distributions were calculated with VMD 1.8.7[45] using the following VMD plugins: Radial Distribution Function and VolMap, correspondingly.[45] The mesh size for the 3D density distributions was 0.5 Å. The 3D distributions for ions were smoothed with the Gaussian window method[45] to remove noise (the width of the window equal to the van der Waals radii of the ions).

As in refs 25, 46, the potential of mean force (in k_BT units) was calculated as the negative natural logarithm of the corresponding radial distribution function $g(r)$.

Results

NMR Experiments. In the previous publications, the possibility of nicotinic acid encapsulation by native and modified α-CD and β-CD has been investigated by several experimental techniques at different pH levels.[30,47−49] It has been shown that more stable inclusion complexes of 1:1 stoichiometric ratio are formed between α-CD and nicotinic acid zwitterions. According to literature data,[50] benzoate forms 1:1 inclusion complexes with α-CD in aqueous solution. Therefore, all thermodynamic properties considered herein and reported in Tables 1 and 2 we ascribe to the 1:1 binding process:

$$\alpha\text{-CD} + \text{R}-\text{COO}^- = \alpha\text{-CD}\cdot\text{R}-\text{COO}^-$$

$$K = \frac{[\alpha\text{-CD}\cdot\text{R}-\text{COO}^-]}{[\alpha\text{-CD}][\text{R}-\text{COO}^-]} \quad (5)$$

The ^{1}H NMR spectra of α-CD alone and in the presence of the acids under study were measured and analyzed (Figure 1). It was obtained that addition of the nicotinic acid induced the considerable upfield shifting of the signal of interior proton H3. The downfield shifting for H5 was visible, but the magnitude of $\Delta\delta$ is smaller in comparison with that for H3 proton. The same influence of benzoate on ^{1}H NMR spectrum of α-CD was observed, although the shifts of H3 and H5 are not so pronounced as in the case of nicotinic acid (Figure 1). This is due to lower binding affinity of α-CD to benzoic acid anions. The shifts of the α-CD interior protons indicate the formation of an inclusion complex where the benzoate molecule enters the cavity from the wider rim. The absence of change in the chemical shift for H6 proton additionally proves this binding mode.

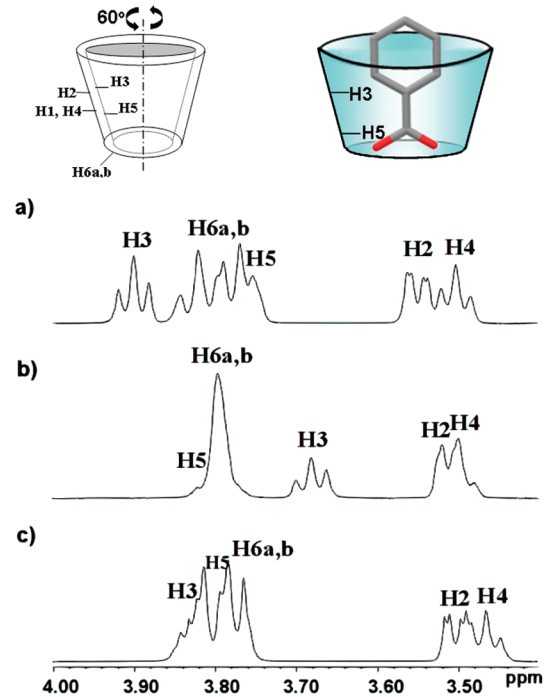

Figure 1. Partial ^{1}H NMR spectra of α-CD aqueous solution (0.005 mol/kg) at 298.15 K: (a) α-CD alone; (b) in the presence of nicotinic acid (0.04 mol/kg); (c) in the presence of benzoic acid (0.04 mol/kg). The insets on the top show the positions of the hydrogen atoms (left) and the binding mode (right) of the ACAs.

The ^{1}H NMR spectra of nicotinic acid have been early considered,[30,49] and it has been shown that the signals of the protons located near $-COO^-$ group are significantly shifted upon complex formation with α-CD, while the changes for another proton of its aromatic ring have not been observed. These results were interpreted in terms of preferential inclusion of carboxylate group into α-CD cavity in comparison with the pyridine ring carrying the positively charged NH^+ group. The revealed binding mode of nicotinic acid is consistent with that described in the literature for complex formation of α-CD with benzoic acid and benzoate in aqueous solution[51−53] as well as in the solid state.[54] In the crystalline α-CD/benzoic acid complex, the guest molecule is tightly packed in the host ring, and the carboxyphenyl group is located inside the cavity.[54] Bergeron with coauthors[51] showed by means of NMR technique that in aqueous solution both benzoic acid and benzoate are inserted into macrocyclic cavity by carboxyl group first, although the benzoate penetration is more random. Orientation of the carboxylate in the α-CD cavity has been demonstrated by Simova and Schneider[52] on the basis of ^{1}H and ^{13}C NMR measurements. The sizable complexation-induced shifts for H3 (−0.45 ppm) and H5 (−0.18 ppm) protons of HP-β-CD obtained for binding with benzoic acid are consistent with the deep insertion. In contrast to benzoic acid, values of $\Delta_c\delta$ for benzoate complexation are considerably lower, and they are equal to −0.18 and 0.04 ppm, respectively, for H3 and H5 protons. The authors discussed the possible location of $-COO^-$ inside the cavity and assumed also the probability of random inclusion. Inclusion complexes of α-CD with benzoate are substantially less stable than with benzoic acid.[51,53,55] This fact also confirms that the carboxylic group enters the cavity. Ionization of this group dramatically decreases its affinity to hydrophobic cavity because of the high polarity and strong hydration of $-COO^-$ in aqueous medium.

Stability constants of α-CD complexes with benzoate and nicotinic acid obtained from ^{1}H NMR are presented in Table 1. For complex formation with benzoate, a good agreement of our data with the literature, $K = 11.2\ M^{-1}$,[53] $12.3\ M^{-1}$,[56] $13\ M^{-1}$,[55] should be noticed here. As can be seen from Table 1, the presence of Na^+ decreases the binding affinity of α-CD to the carboxylic acids over bulk water and KCl solutions (nicotinic acid) or solutions with only K^+ cations (benzoate). Moreover, in the case of the nicotinic acid solutions where one can compare, there is no practical difference between ACA-CD binding constants in the neat water solution and the solutions containing K^+ ions.

Calorimetric Measurements. Table 2 shows the main results of calorimetric measurements of α-CD complexation with nicotinic acid. This Table presents the obtained values of stability constants as well as the enthalpy and entropy changes for α-CD/nicotinic acid complex formation in different solutions. It is evident from Table 2 that formation of inclusion complexes between α-CD and nicotinic acid is thermodynamically favorable. High negative enthalpy changes upon complex formation are mainly caused by the prevalence of attractive intermolecular interactions (van der Waals interactions and hydrogen bonding) over the penalty for desolvation of the α-CD cavity. The negative Δ_cS values are attributed to losses in rotational and translational freedom of solutes upon complexation. We also assume the possibility of hydrogen bonding between the protonated nitrogen of nicotinic acid and hydroxyls surrounding the wider rim of the α-CD cavity. This suggestion is based on the comparative analysis of enthalpy and entropy changes obtained for α-CD complexation with nicotinic acid (Table 2) and benzoate ($\Delta_cH = -16.3 \pm 1.3$ kJ/mol and $T\Delta_cS = -10.5 \pm 1.4$ kJ/mol).[50] Comparing these values, we can conclude that Δ_cH and Δ_cS are more negative for nicotinic acid complexation than for benzoate.

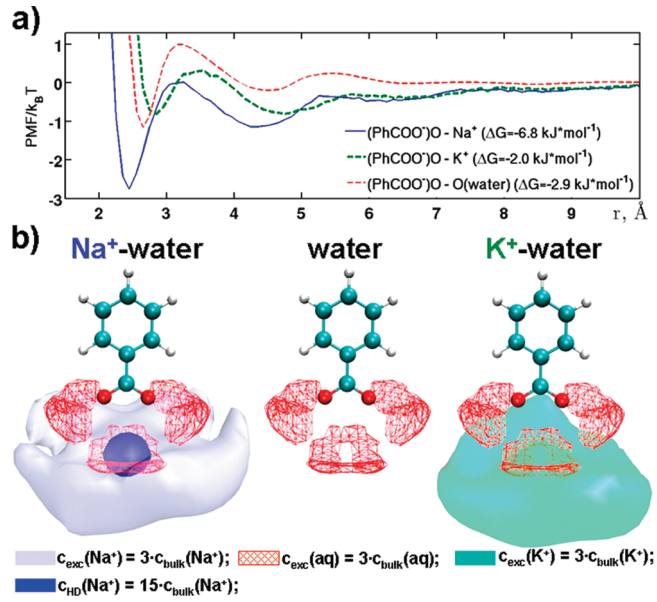

Figure 2. Water and ions distributions near the carboxylate group of benzoate acid. (a) Potentials of mean force (PMF) between the carboxyl oxygen of benzoate acid and Na^+, K^+, and water. The values of the free energies of formation direct contact pairs (ΔG) between the species are given in brackets. Solid blue line is the $(PhCOO^-)O-Na^+$ PMF; bold dashed green line is the $(PhCOO^-)O-K^+$ PMF; dashed red line is the $(PhCOO^-)O-O$ (water) PMF. (b) Water and ions isoconcentration surfaces around the carboxylate group of benzoate. The red mesh as well as the light blue and green surfaces are isosurfaces of local particle concentrations that are 3 times higher than the bulk concentrations of water (c_{bulk}(water)), Na^+ ($c_{bulk}(Na^+)$), and K^+ ($c_{bulk}(K^+)$), correspondingly. The dark blue surface is the isosurface of local Na^+ ion concentration that is 15 times higher than the Na^+ concentration in the bulk. Bulk concentrations are: $c_{bulk}(Na^+) = c_{bulk}(K^+) = 0.3$ M (1.84×10^{-4} particles per Å^{-3}); c_{bulk}(water) = 55.4 M (3.33×10^{-2} particles per Å^{-3}).

We obsereved different effects of NaCl and KCl on the enthalpy and entropy parameters. As follows from Table 2, the presence of KCl has only a slight influence on the Δ_cH and Δ_cS values. In particular, complex formation in KCl solution is less exothermic and slightly enhances disorder in the system. The difference in the binding constants for pure water and KCl solution is within the error limits. On the contrary, addition of NaCl results in a significant decrease of the apparent binding constant K (also called complex stability constant) and more negative values of Δ_cH and Δ_cS. Presumably, the Na^+ ion pairing with the anionic carboxylate group of nicotinic acid is responsible for the additional negative contribution to Δ_cH due to the favorable electrostatic interactions between the counterions. However, the formation of the sodium−carboxylate contact ion pairs also results in an increase of ordering in the system. Therefore, the favorable enthalpy gain is compensated by the negative entropy term resulting in the overall decrease of the apparent complex stability constant K.

Molecular Modeling. To investigate the molecular mechanisms of selective K^+/Na^+ binding to ACAs, we performed MD calculations for ionic pairing of Na^+ and K^+ to benzoate and nicotinic acid zwitterions. Figure 2 presents the calculated potentials of mean force and the 3D density distributions of ions and water around the carboxylate groups of the benzoate molecule. We plot the PMFs curves in k_BT units (PMF/(k_BT))

to show the scale of the effects as compared to the energy of temperature fluctuations k_BT.

The potential of mean force functions (PMF) exhibit a short distance minima corresponding to the direct ion pairing, while the less pronounced second minima correspond to the solvent-separated ion pairing. The depths of the secondary minima are around 0.3−1 k_BT, which means that the energies of the solvent-separated ions pairs are in the range of the energy of thermal fluctuations. Because of the low energies of the solvent-separated ion pairs, we assume that their effects on the α-CD−ACA complex formation are rather weak, and, therefore, in the discussion of our experimental results, we will mainly focus on the effects of direct ion pairing.

Using the PMFs, we obtained the free energies of formation of direct contact pairs (ΔG) between the molecular species as the minimum values of the corresponding PMFs. For the ΔG calculations, we used the standard definition of PMF (PMF(r) = $-k_BT \ln(g(r))$.[46]

One can see from Figure 2 that, as compared to K^+, the distribution of Na^+ around $-COO^-$ is much more dense for benzoate. This tendency is the same for both acids (the results for nicotinic acid can be found in the Support Information (Figure S1)). Thus, Na^+ ions preferably interact with the carboxylate via formation of direct contact pair, which is consistent with the literature data.[22,25,57,58] From another side, as it can be seen from Figure 2, potassium ions have much less potency to make direct contact pairs with the carboxylic group because they cannot compete with water for the first solvation shell of the group. The $-COO^-$ group remains fully hydrated as in the bulk water solution. That explains the apparent absence of K^+ effect on ACA−CD complex formation as compared to the bulk water solution.

Discussion

The review of available literature data concerning salt effects on binding of CDs with different guests showed that inorganic salts can affect the complex formation process in the following ways: (i) they can change the activity of water molecules participating in the binding; (ii) they can compete with the guest for the host cavity; and (iii) they can form a ternary complex due to additional electrostatic interactions and H-bonding. In this work, we found that Na^+ and K^+ act differently on the α-CD complex formation with nicotinic acid and benzoate presumably due to the different binding properties of these ions with regards to the ionized carboxylic group of the ACAs ($-COO^-$ group of aromatic carboxylic acids). The influence of Na^+ is more evident for all systems under study, and it is reflected in a considerable decrease of binding constants. Thus, the equilibrium described by eq 5 is shifted only to the left upon the NaCl salt addition. On the contrary, the addition of KCl shows practically no effect as compared to the bulk water solution.

It was found that in some cases KCl and NaCl act as salting-out agents.[59−61] Schlenk and Sand[59] experimentally observed the decrease of aqueous solubility of benzoic acid in the presence of KCl. When the salts displaying salting-out effect are dissolved in aqueous solution, they are hydrated by water molecules. This process should result in reduction of empty space for aromatic carboxylic acids and promote their inclusion into α-CD cavity. It implies the increase in binding constants, which, as an example, was demonstrated for complexation of β-CD with naphthalene,[61] 3-hydroxy-2-naphthoic acid,[62] and terfenadine.[63] It should be noted that in all these systems, complex formation was driven by hydrophobic effects. However, it has been shown previously[69] that benzoate has pronounced hydrotropic behavior, and we assume that there should be another driving mechanism of CD−ACA binding in addition to hydrophobic interactions. Indeed, the observed in our case decrease in the binding constants indicates that rearrangements in solution caused by adding of NaCl and KCl are not of primary importance, and hydrophobic interactions are not the main driving force in complexation of α-CD with nicotinic acid and benzoate.

As was mentioned above, in general, the possible influence of anions should be taken into account for explanation of salt effects. However, in the case of chlorides, the formation of ternary complexes between α-CD, carboxylic acid, and Cl^- is unlikely. To the best of our knowledge, such ternary complexes formed by Cl^- were not described in literature. Usually, anions with larger size such as ClO_4^- or CO_3^{2-} are involved in the formation of the complexes. These ions can additionally stabilize the complexes through specific interactions with the hydroxyl groups of CDs, which results in some increase of the binding constants.[20,64] If Cl^- makes a complex with α-CD, the competition between chlorides and carboxylic acids for the host cavity should decrease the binding of α-CD with ACAs. However, we note that interactions of CDs with inorganic anions have been studied in several works[65−67,70] where it was shown that Cl^- and α-CD do not form stable complexes. Additionally, we performed a molecular modeling analysis of interactions between α-CD and Cl^- using a 130 ns MD trajectory of α-CD in KCl and NaCl solutions and found no Cl^- ions in the internal α-CD cavity for any of the MD time-frames. Thus, the literature data as well as our observations for selective cation effects and CD−benzoate complexation in KOH and NaOH solutions (without the presence of any Cl^- ions) suggest that the probability of α-CD/Cl^- complex formation is very low, and, therefore, that can be neglected in our analysis.

The commonly used conceptions in the literature for interpretations of salt effects and the facts discussed above do not explain the different influence of Na^+ and K^+ on complex formation between α-CD and aromatic carboxylic acids. To explain our results, we propose a new mechanism of salt effects on α-CD complex formation with ionized aromatic carboxylic acids. According to this mechanism, Na^+ and K^+ cations compete with α-CD for the carboxylic acid. In this case, the processes that proceeded in the solution can be described via the following way (see also Figure 3):

$$\text{α-CD}\cdot R-COO^- \overset{\text{α-CD}}{\longleftrightarrow} R-COO^- \overset{Cat^+}{\longleftrightarrow} R-COO^-\cdots Cat^+ \quad (6)$$

Formation of ionic pairs ($R-COO^-\cdots Cat^+$) being in competition with complex formation (α-CD·$R-COO^-$) shifts the equilibrium (5) in the direction of complex dissociation. We illustrate this competitive mechanism by the pictorial representation in Figure 3. Complex formation of α-CD with acids under investigation is weak (lg K < 2), and, therefore, it is very sensitive to competitive processes. As a result, we observe the decrease in complex stability.

Selective interactions of Na^+ and K^+ with carboxylate have been reported in several experimental and computational studies.[8,22,25,57] These works showed that Na^+ binds $-COO^-$ group much strongly than does K^+. Our MD data also show the preferential binding of Na^+ to $-COO^-$ over K^+. The revealed preferential direct contact pairing of $-COO^-$ with Na^+ determines the difference in the effects of Na^+ on thermody-

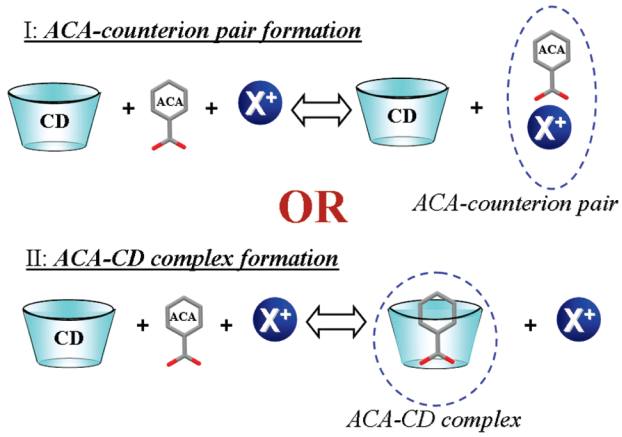

Figure 3. Schematic representation of two possible reaction mechanisms of complex formation between cyclodextrin (CD) and ionized aromatic carboxylic acid (ACA) in aqueous solutions containing counterions (X^+): (I) (top) the counterion X^+ makes a contact pair with the ACA molecule via binding the $-COO^-$ group of the molecule and, therefore, blocks formation of the ACA−CD complex; (II) (bottom) X^+ does not make a direct contact pair with the ACA molecule that permits formation of the ACA−CD complex.

namics of α-CD−ACA complex formation as compared to K^+ (Tables 1 and 2).

Because the ionic pairing coexists with complex formation, contributions of both these processes are reflected in $\Delta_c H$ and $\Delta_c S$ values reported in Table 2. The observed in NaCl solution decrease of enthalpy and entropy is attributed to increased negative contribution from electrostatic interactions between $-COO^-$ and Na^+.

We note that, in general, the formation of solvent-separated ion pairs[71−73] in the solution might have some effect on the complex formation changing the equilibrium of the complex formation reaction. However, for the systems studied in this Article, the energy of solvent-separated ion pairing is comparable with the energy of thermal fluctuations k_BT (see Figure 2 and the corresponding discussion above). The X-ray absorption results on Na−Cl and Na−OH ion pairing[72,73] also suggest that the energy of solvent-separated ions pairs is much less than the energy of contact ions pairs. Therefore, we assume that the effects of solvent-separated ion pairs are of secondary importance to the phenomena considered in this Article as compared to the effects of contact pair formation.

It is interesting to note that the opposite influence of NaCl on thermodynamic characteristics of complex formation between hydroxypropyl-β-CD (HP-β-CD) and 1-butanol in aqueous solution has been detected by Fini and co-workers.[68] The authors showed that in NaCl solution the inclusion complexes are more stable than in water and their stability is increased with rise of NaCl concentration. It was also demonstrated that enthalpy is not changed while the entropy is increased with enhancement of NaCl concentration. Thus, the variance of $\Delta_c G$ with NaCl concentration is entropy controlled. By comparing the effects of NaCl on α-CD/nicotinic acid and HP-β-CD/butanol binding, we can conclude that influence of NaCl on thermodynamics of complex formation is not the same, and it depends on the solutes nature and driving forces of interaction. In the case of butanol, complex formation is realized due to inclusion of apolar moiety of the guest molecule into hydrophobic cavity. Hydrophobic and van der Waals interactions are the main driving forces of HP-β-CD binding with butanol. Therefore, the influence of NaCl can be shown as the salting-out effect. On the contrary, the $-COO^-$ group of nicotinic acid enters the α-CD cavity upon binding, and van der Waals interactions as well as hydrogen bonding are responsible for complex formation. The decrease of binding constant observed in the presence of NaCl is determined by the partial weakening of interactions of nicotinic acid with α-CD cavity due to participation of the $-COO^-$ group in the ionic pairing with Na^+.

Conclusions

In the present work, we examined the selective effects of Na^+ and K^+ on the inclusion of nicotinic acid zwitterion and benzoate in α-CD using ^{1}H NMR, calorimetry, and MD simulations. For both aromatic carboxylic acids, we found that potassium ions make no significant effect on the formation of the CD−acid complexes in water. On the contrary, the presence of sodium ions in the solution decreases the binding constant for the α-CD−ACA complexes. To explain the obtained results, we propose a novel mechanism of salt effects based on competition between the processes of host−guest complexation and selective interaction of cations with the carboxylic groups of the guests.

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Supporting Information Available: Table S1: Force-field atom parameters used in the molecular dynamic simulations. Figure S1: Water and ions distributions near the carboxylate group of nicotinic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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