α,ω –Alkyl Dicarboxylic Acids: Characterization by Isocratic Anion-Exchange Chromatography

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Abstract

A homologous series of 7 α,ω-alkyl dicarboxylic acids is characterized using isocratic anion-exchange chromatography at different mobile phase salt concentrations to determine the characteristic charge and the equilibrium ion-exchange constant (and the corresponding Gibbs free energy of ion-exchange). The characteristic charge agrees closely with the number of carboxylic acid groups in the samples, namely 2, and the ion-exchange constant seemed to remain almost unaffected by the number of methylene groups in the sample. Succinic acid, having 2 methylene groups between the terminal carboxylic acid groups, always produces 2 chromatographic peaks despite the proven chemical purity of the sample. The characteristic charge calculated for both peaks of succinic acid is approximately 2, as is that of the other homologues. The Gibbs free energy of ion-exchange calculated for the weakly retained peak of succinic acid is similar to that of the other samples, whereas that of the strongly retained peak is lower by 2.1 kJ/mol. The 2 peaks of succinic acid are explained in terms of an equilibrium between two conformers in solution, one binding the solution counterions tightly and the other loosely.

Introduction

The search for suitable ion-exchange protein displacers (i.e., inexpensive, nontoxic, homogeneous compounds with sufficient affinity for binding to the chromatographic surface that, however, can be readily desorbed for column regeneration) (1,2) is the central role of the present authors. Such displacers can effect the simultaneous separation and concentration of mixtures of similar proteins. Before testing the displacers for separation, the steric mass action affinity parameters are determined: the characteristic charge, the equilibrium ion-exchange constant, and the steric factor (3). The characteristic charge is the effective charge of the sample upon binding or the number of ionic bonds between the sample and the surface. The equilibrium ion-exchange constant is the ratio of the partition coefficients between the stationary phase and the mobile phase of the sample to the column counterion. The steric factor is the number of inaccessible surface sites per adsorbed sample molecule under the conditions of surface saturation with sample.

Both high-molecular-weight (4,5) and low-molecular-weight polymeric displacers (6) have been employed. More recently, monomeric and oligomeric compounds were examined (7–12), some of which proved to be efficient displacers. In a recent report (13), the ion-exchange characterization of nine benzene oligocarboxylic acids that can be potentially used as anion-exchange displacers were described. These compounds bore 1 to 6 carboxylic acid groups in the benzene ring and included isomers with the same number of carboxylic acid groups placed at different positions. The flat, cyclic geometry of the benzene ring dictates a concentric placement of the carboxylic acid groups. The most important finding in that work was that the ion-exchange affinity of the compounds is not solely defined by the number of functional groups but also by their geometrical arrangement. Closely placed carboxylic acid groups in a sample seem to complexate with solution counterions, rendering their adsorption to the surface less favorable.

In this work, a series of compounds with linear rather than circular geometry are characterized. These molecules contain a fixed number of functional groups at the ends and a varying number of methylene spacer groups. In particular, the homologues investigated are α,ω-alkyl dicarboxylic acids. The names and chemical formulas of their dissociated forms are shown in Figure 1.

Experimental

Materials

Oxalic acid, malonic acid, succinic acid, glutaric acid, adipic acid, pimelic acid, suberic acid, tris(hydroxymethylene) aminomethane (Tris), tris(hydroxymethylene) aminomethane hydrochloride (Tris HCl), sodium chloride, and a 0.5M sodium hydroxide solution were all purchased from Aldrich (Gillingham, Dorset, U.K.). Distilled, de-ionized water was used for the preparation of the mobile phases and the solutions of the samples. An

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analytical (100 × 5 mm) Waters (Waterford, Herts, U.K.) Protein-Pak Q 8HR strong anion-exchange column packed with 8-µm quaternary methylamine-derivatized polymethacrylate beads of 100-nm average pore size was used for the experiments.

Equipment

A Polymer Laboratories (Church Stretton, Shropshire, U.K.) LC1150 Quaternary Pump was used to deliver the solvent at a flow rate of 0.5 mL/min. A Polymer Laboratories LC1200 ultraviolet/visible detector was used to monitor the column effluent. An online Polymer Laboratories PL-DCU data collection unit and a computer were used for data acquisition. The samples were introduced to the system using a 7125 Rheodyne (Rohnert Park, CA) injector.

Methods

Two mobile phase solutions were prepared, one with 1.0M NaCl and the other without any salt added. The pH of both solutions was maintained at 8.0 using Tris buffer containing an additional 28mM Cl^- (from Tris HCl). Solutions of the α,ω-alkyl dicarboxylic acids at concentrations 10 mg/L were prepared by dissolving the solid sample (in protonated form) in the salt-free mobile phase and subsequently adjusting the pH to 8.0 with the addition of 0.5M NaOH solution (when necessary). Sample volumes of 20 µL were introduced using the Rheodyne injector. The absorbance was monitored at 215 nm. Experiments were performed at various mobile phase chloride concentrations that covered the 128–328mM range by online mixing the appropriate proportions of the salt-free and 1.0M NaCl mobile phases.

Table I. Dissociation Constants of the α,ω-Alkyl Dicarboxylic Acids*

Empirical name	IUPAC name	pK^{I}	pK^{II}
Oxalic acid	ethanedioic acid	1.23	4.19
Malonic acid	propanedioic acid	2.83	5.69
Succinic acid	butanedioic acid	4.34	5.42
Glutaric acid	pentanedioic acid	4.19	5.48
Adipic acid	hexanedioic acid	4.42	5.41
Pimelic acid	heptanedioic acid	4.48	5.42
Suberic acid	octanedioic acid	4.52	5.40

* Taken from Reference 14.

Results and Discussion

All experiments for this study were performed at pH 8, where all carboxylic acid groups of all samples are fully dissociated. Table I lists the dissociation constants (p*K*s) of the α,ω-alkyl dicarboxylic acids, which range from 1.2–5.7 (14).

The retention times (t_R) of the samples determined under isocratic elution conditions were converted to capacity factors (k') using the following formula (15):

$$k' = (t_R - t_0)/t_0' \quad \text{Eq 1}$$

where t_0 is the dead time of the column (the elution time of an unretained solute) and t_0' is the column dead time corrected for noncolumn

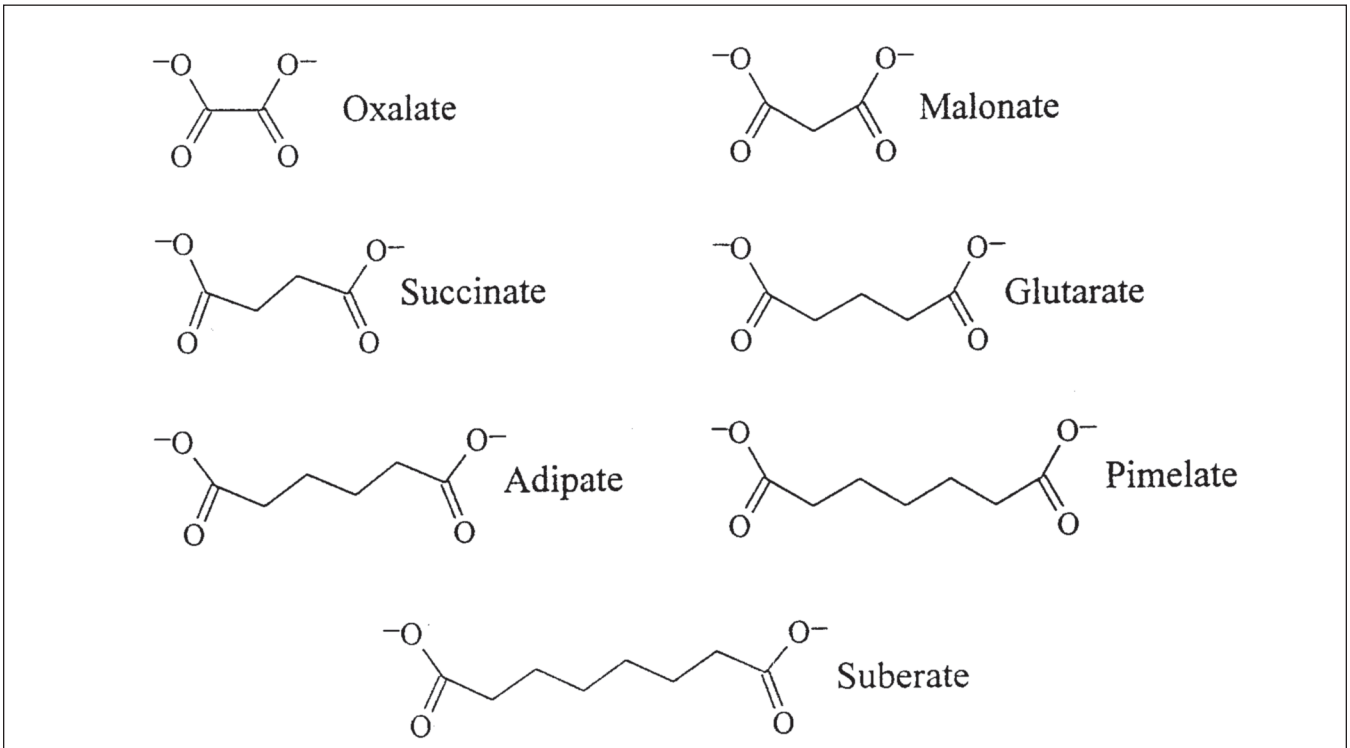

Figure 1. Chemical formulas of the 7 α,ω-alkyl dicarboxylic acids in the fully ionized state. The number of intervening methylene groups varies from 0 to 6.

volume. The capacity factors and the corresponding salt concentrations were combined to calculate the characteristic charge ν and the equilibrium ion-exchange constant K from the following equation:

$$\log k' = \log(\beta^n K \Lambda^n) - \nu \log C_{salt} \quad \text{Eq 2}$$

This equation describes Brooks and Cramer's (3) theory of steric mass action (SMA) ion-exchange under linear adsorption conditions, with the incorporation of the correction of Patrickios and Yamasaki (16,17). Knowledge of the phase ratio (β) and column capacity (Λ) is necessary for the calculation of K. The values of β and Λ were taken to be 0.5518 mL stationary phase/mL mobile phase and 487mM monovalent ions, respectively, in agreement with previous reports (4,13,18).

The ion-exchange free energy ($\Delta G^{exchange}$) was calculated from K using (3):

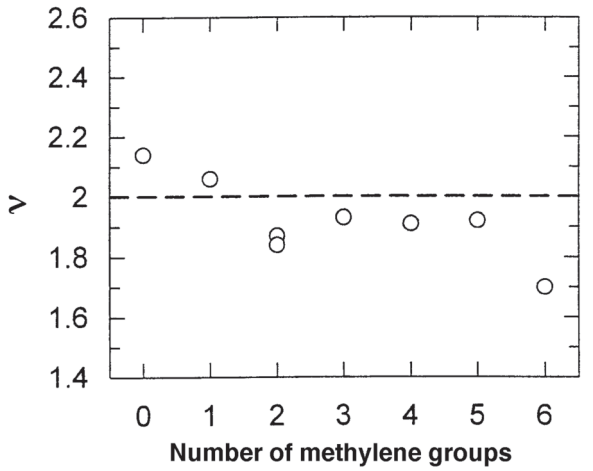

Figure 2. Dependence of the characteristic charge ν on the number of methylene groups in the α,ω-alkyl dicarboxylates.

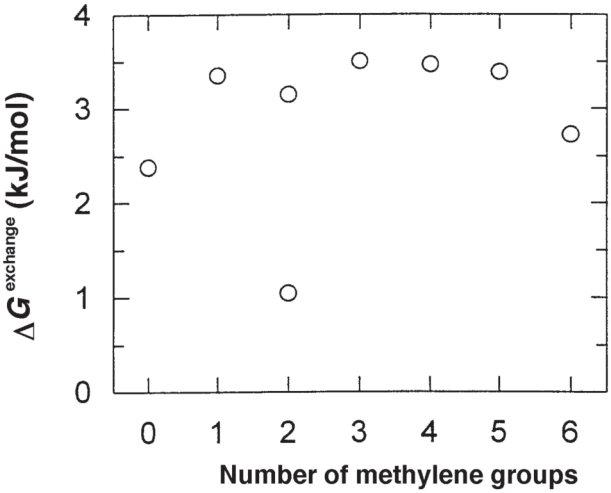

Figure 3. Dependence of the Gibbs free energy of ion-exchange $\Delta G^{exchange}$ on the number of methylene groups in the α,ω-alkyl dicarboxylates. There are 2 points for succinate (at the point corresponding to 2 methylene groups) because this sample presented 2 peaks.

$$\Delta G^{exchange} = -RT \ln K \quad \text{Eq 3}$$

where R is the gas constant and T the absolute temperature.

Figure 2 presents the characteristic charge of the 7 homologues, calculated from the isocratic experiments, as a function of the corresponding number of methylene groups in the sample. The characteristic charge of all samples is close to 2, agreeing closely with the number of dissociated carboxylic acid groups in the samples. This implies that, upon adsorption, all carboxylate groups contact the surface-forming ionic bonds to the oppositely charged quaternary methylamine groups. It is not surprising that all functional groups of the ionized α,ω-alkyl dicarboxylic acids can exchange given their linearity. The same has been observed with the benzene oligocarboxylic acids, where the flat shape of the benzene scaffold encourages the exchange of all carboxylic acid groups. In contrast, dendritic molecules characterized using similar techniques were found to contact the chromatographic surface using only a limited fraction (31–43%) of their functional groups that are distributed evenly over their spherical outer surface (8).

There are 2 points in Figure 2 for succinic acid (number of methylene groups = 2) because this sample always presented 2 peaks, with the more strongly retained peak having twice the area of the more weakly retained peak (this ratio increased slightly with the mobile phase salt concentration). Both of the calculated values of characteristic charge are close to 2, indicating that both of the peaks correspond to dicarboxylates. This excludes the possibility that one of the peaks is caused by anhydrite contamination, which is nonionic and should not be retained.

There is a slight decrease in characteristic charge (which is more significant than the error in the calculation of characteristic charge) with the number of methylene groups. The characteristic charge of the lowest homologues, oxalate and malonate, is slightly above 2 at approximately 2.1. The characteristic charge of the next 4 higher homologues, succinate, glutarate, adipate and pimelate, is slightly lower than 2 at approximately 1.9. Finally, the highest homologue, suberate, has a characteristic charge of 1.7, sensibly lower than 2. This trend might be because of a tendency of the higher homologues, suberate in particular, to form an end-on adsorption conformation that is stabilized by lateral hydrophobic interactions of the growing oligomethylene spacer. This has been exploited for the assembly of multilayered films (19). It should be noted that when the number of methylene groups in the spacer is 10 or higher, α,ω-alkyl dicarboxylates micellize in aqueous solution (20–22).

Figure 3 displays the Gibbs free energy of ion-exchange against the number of methylene groups in the samples. The $\Delta G^{exchange}$ values of all the samples are approximately constant at +3 kJ/mol, with the exception of the second peak of succinate which is +1 kJ/mol. The $\Delta G^{exchange}$ values for oxalate and suberate are slightly lower than those of the other samples. In the case of suberate, this may be caused by the (partial) end-on adsorption conformation and lateral hydrophobic interactions mentioned in the previous paragraph. For the case of oxalate, this may be caused by the stiffness of this molecule or its high charge density (or both). For comparison, the $\Delta G^{exchange}$ values of benzene dicarboxylates were +0.4 kJ/mol for the phthalate (1,2-benzene dicarboxylate) and

–2.6 and –2.5 kJ/mol for the isophthalate and terephthalate (1,3- and 1,4-isomers), respectively (13). Thus, all $\Delta G^{exchange}$ values of α,ω-alkyl dicarboxylates are higher than all $\Delta G^{exchange}$ values of benzene dicarboxylates, suggesting that the adsorption of the former series of species is less favored than that of the latter. This might be due to the presence of the polar benzene ring, which may interact with the polar surface. Another possibility is that the fixed placement of carboxylates on the benzene ring enhances the adsorption affinity.

The strongly retained succinate species had a $\Delta G^{exchange}$ of approximately +1 kJ/mol, which is –2 kJ/mol more favorable than that of the weakly retained succinate peak. This difference is similar in magnitude to the value determined for the solution binding of sodium counterions to the sample (2.7 kJ/mol) in the recent work on benzene oligocarboxylic acids (13). Thus, it is suggested that the strongly retained peak is caused by the adsorption of a succinate conformer with only loosely bound sodium ions in solution. In contrast, the weakly retained peak should be caused by the adsorption of the succinate conformer with tightly bound sodium ions solution. Because all other samples display $\Delta G^{exchange}$ values equal to that of the weakly retained succinate conformer, they should also tightly bind sodium ions in solution. The appearance of the strongly retained succinate (conformer corresponding to the species binding sodium ions weakly in solution) indicates that there are difficulties in the binding of sodium ions to succinate in comparison with the other homologues. This might be related to the number of backbone atoms in succinate, which is 6: 4 carbons and 2 oxygens. Assuming that the bond angles are approximately equal to the tetrahedral angle of 109.5°, and that the length of the carbon–carbon bonds is approximately equal to the length of the carbon–oxygen single bonds, succinate can form a regular pentagon (a pentagon's internal blunt angle is 108°, very close to the value of tetrahedral angle) with the 2 charged oxygens overlapping. Molecular model construction confirms this. This conformation would be unable to form a complex with the 2 charged oxygens surrounding the sodium cation because of space limitations.

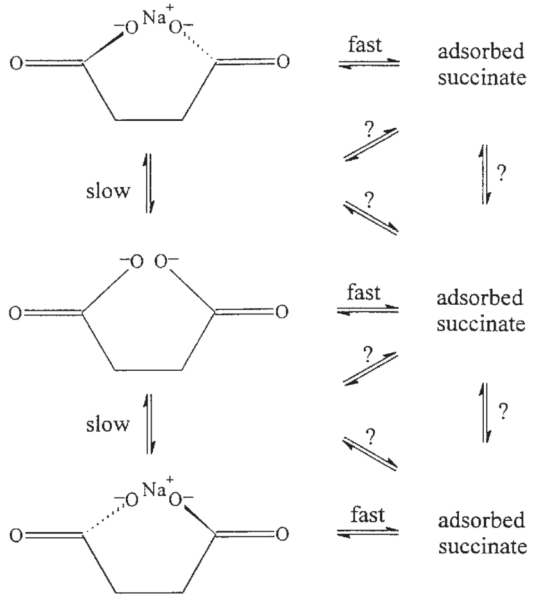

Figure 4. Schematic representation of our hypothesis for the existence of various succinic acid species in solution and on the surface.

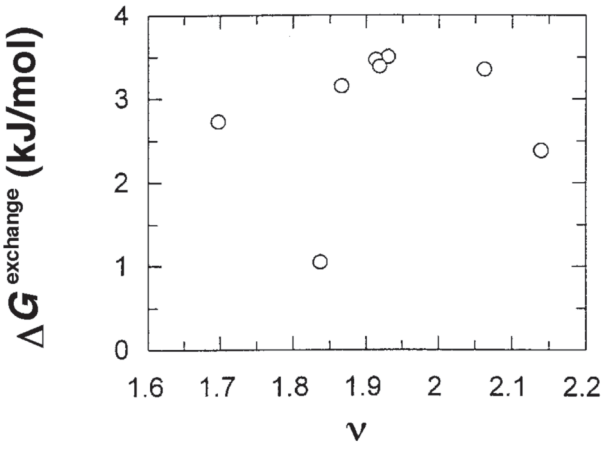

Figure 5. Dependence of the Gibbs free energy of ion-exchange $\Delta G^{exchange}$ on the characteristic charge ν of the α,ω-alkyl dicarboxylates.

Figure 4 shows a schematic representation of this explanation for the behavior of succinate. One solution conformation of succinate takes the shape of a (planar) closed ring, not allowing the binding of sodium ions between the 2 charged oxygen atoms. The strong electrostatic repulsion between the 2 oxygen atoms in this conformation may be alleviated by the weak binding of a sodium cation outside the ring. The other solution conformations shown (which are identical) are nonplanar with the 2 carbon–oxygen single bonds, opening up like a pair of scissors and accommodating a sodium cation between the charged oxygens. The existence of 2 distinct peaks suggests that the equilibrium between the planar and nonplanar conformers is slower in comparison with the time scale of the chromatography experiment (which varied from about 3 to 9 min for mobile phase sodium chloride concentrations of 0.3 and 0.1M, respectively). If there was fast exchange, the 2 succinate peaks would merge into one. The planar conformer can bind the surface with a higher affinity, because its charge is not diminished much by counterions, which is the case with the nonplanar conformer. It is likely that the adsorption conformations of the 2 conformers are different from each other and incapable of exchanging quickly between themselves. All the other possible equilibria are indicated in Figure 4 with arrows accompanied by question marks.

Figure 5 plots $\Delta G^{exchange}$ against ν. The point corresponding to the strongly retained peak of succinate appears at the lowest $\Delta G^{exchange}$ value. The other points seem to form a slight maximum that is caused by a combination of two things: the low $\Delta G^{exchange}$ values of oxalate and suberate on the one hand, and the high ν value of oxalate and low ν value of suberate on the other.

The low affinity of the α,ω-alkyl dicarboxylates, as manifested by their high $\Delta G^{exchange}$ values, may preclude their use as ion-exchange displacers of proteins. It is possible, however, that these molecules may work as efficient displacers of low-molecular-weight mixtures, such as acetic acid and glycolic acid (hydroxyacetic acid). Succinic acid, exhibiting 2 chromatographic peaks,

presents a special case. Its high-affinity peak can provide improved displacing power, but the lower-affinity peak may complicate the separation.

Conclusion

The anion-exchange chromatographic characterization of 7 homologous α,ω-alkyl dicarboxylic acids bearing 0–6 spacing methylene groups using isocratic elution at different mobile phase salt concentrations was presented. The calculated values of the characteristic charge suggest that all carboxylic acid groups of all samples bind to the surface, which is consistent with the linear shape of the molecules. The Gibbs free energy of ion-exchange of all but one sample was constant at approximately +3 kJ/mol. This is a relatively high value, suggesting that the affinity of the α,ω-alkyl dicarboxylic acids for the chromatographic surface is low. Succinic acid presented a second, high-affinity peak whose Gibbs free energy of ion-exchange was more favorable by –2 kJ/mol. This can be attributed to the existence of 2 succinic acid conformers in solution: one with tightly bound sodium counterions with low affinity for the surface, and one with loosely bound sodium and a high affinity for the surface.

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