# Cation Radius and Deoxycholic Acid Polymer-Like Structure Stability

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**The stability of the polymer-like structure displayed by deoxycholic acid gels under certain conditions has been investigated. The influence of cation dimensions, hydration, and hydrogen bonds is shown. A peculiar behavior of deoxycholic acid quaternary ammonium salts is discussed. Some biological implications are mentioned briefly.**

DEOXYCHOLIC ACID (DCA) is a unique molecule from its physical, chemical, and biological aspects. The physicochemical properties of DCA have been reported by Giacomello (1, 2), and more recently by Rich (3, 4). It has been found that under appropriate conditions sodium deoxycholate aggregates in solution to form a gelatinous complex of macromolecular dimensions. X-Ray diffraction studies of fibers of this complex showed that the molecules had assumed an elongated helical configuration 36 Å. in diameter (3, 4). The formation of a helical complex of macromolecular dimension from sodium deoxycholate is highly specific since closely related compounds do not exhibit this effect.

In previous work the influence of counterions on the conformation of either synthetic or natural polyelectrolytes (5, 6) has been shown. Therefore, it seemed worthwhile to study DCA gels in order to acquire further information on the many factors which influence the stability of these structures. It was the objective of the present study to determine if the formation of gels of macromolecular dimensions was in some way affected when deoxycholic acid salts other than sodium deoxycholate were used. The effects of cation dimensions, hydration, and hydrogen bonds on the stability of the structure were also investigated.

The importance of DCA and related structures to the physicochemical properties of biological membranes, as well as the interactions between these polymer-like structures and drugs, must not be overlooked. The study of the variation of the ionic activity of quaternary ammonium ions in a polyelectrolyte solution and the resulting influence on the conformation of the structure is of basic importance in order to interpret, on physicochemical grounds, biological problems such as nerve transmission.

## EXPERIMENTAL

**Material**—DCA (Merck) was recrystallized from alcohol before use.

Solutions of different DCA salts were prepared by neutralizing DCA solution with the hydroxides of different cations. The DCA salt solutions thus obtained were then lyophilized.

Stock solutions of $Li^+$, $Na^+$, $K^+$, $Rb^+$, $Cs^+$, $NH_4^+$, and quaternary ammonium bases were freshly prepared and standardized with a solution of 0.1 *N* HCl.

Lithium hydroxide solution was prepared from $Li_2SO_4$ and $Ba(OH)_2$. A small excess of $Li_2SO_4$ was employed so as to have a minimum of $Ba^{2+}$ in the LiOH solution. The $BaSO_4$ was separated by filtration, and the LiOH solution free of sulfate and carbonate was obtained by passing the solution through an anion exchange column.[1]

Rubidium hydroxide and CsOH solutions were prepared by exchanging RbCl and CsCl solutions through the above-mentioned ion exchanger.

In a similar way quaternary ammonium bases were prepared by exchanging the corresponding iodides. The absence of halogens ($Cl^-$ or $I^-$) from these solutions was checked.

All reagents used were of analytical grade.

## PROCEDURE

The different DCA salts were prepared by dissolving DCA with an amount, slightly less than that required, of hydroxide solutions of the corresponding cations. The excess of acid was removed by filtration. The solution was evaporated and the DCA salt recovered.

DCA gels were obtained by dissolving known amounts of the DCA salts in water and then adjusting the pH to a value of 6.8–6.9 by adding very small amounts of HCl under continuous and constant stirring. All gels obtained were stored at a temperature of about 5°.

Conductivity measurements were carried out in a constant-temperature bath regulated within 0.01° for temperatures from 20° to 30° and within 0.3° for higher or lower temperatures. The complete filling and closing of the cell prevented evaporation of the solution. A WTW bridge (model $LF_3$) was employed for such measurements.

## RESULTS AND DISCUSSION

In Fig. 1 specific conductivity data recorded in a solution of NaDCA are plotted against temperatures. The break in the trend, corresponding to a particular value of temperature, indicates a transition which is a function of the concentration of DCA in solution and of the radius of the cation which neutralizes DCA. As shown in Fig. 1, graphs of different monovalent cations (Li, Na, K, Rb, Cs, and $NH_4$) have the same general shape but are shifted toward higher or lower temperatures.

In Fig. 2 data obtained from solutions of different concentrations (0.05 and 0.10 *M*) of the above-mentioned DCA salts are reported. The transitional temperatures are plotted against the crystallographic radius of the different cations. The thermal stability of the helical complex decreases with the increasing crystallographic radius following the cation sequence.

In the case of NaDCA the influence of concentration on the transitional temperatures is shown in Fig. 3. The trend of the plot shows that the stability of the complex is temperature-dependent below a concentration of about $6 \times 10^{-2}$ *M*. Above

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[1] Ionenaustauscher III, Merck.

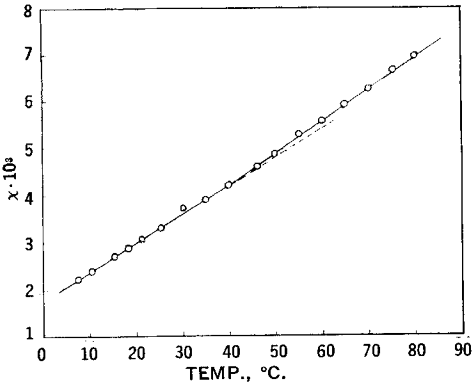

*Fig. 1—The specific conductivity $\chi$ of a NaDCA solution ($3 \times 10^{-2}$ M) as a function of temperature. The break in the trend indicates a transition. Graphs of different monovalent cations have the same general shape.*

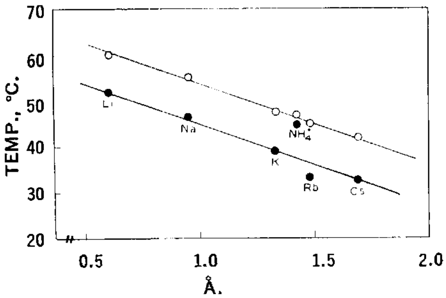

*Fig. 2—The thermal stability of the helical complexes decreases with the increasing crystallographic radius following the cation sequence. Deoxycholate concentrations: ●, 0.05* M*; ○, 0.10* M.

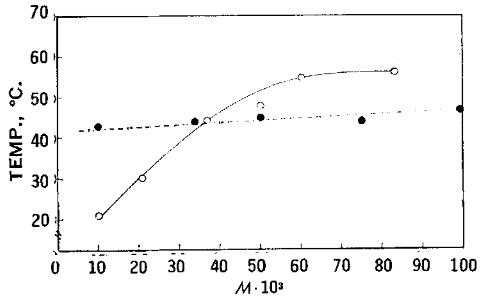

*Fig. 3—Transitional temperatures dependence upon concentration of NaDCA (○) and NH₄DCA (●) solutions.*

this value, the transitional temperatures are no longer affected by DCA concentration in solution.

In Fig. 2 the anomalous position in the plot of $NH_4^+$ which seems to have almost the same transitional temperature for the two different concentrations, 0.05 and 0.10 *M*, is evident. This is due to the fact that, in the case of $NH_4DCA$, the trend of the plot of transitional temperature *versus* concentration differs from those of the other cations. In a range of concentration between 0.01 and 0.10 *M*,

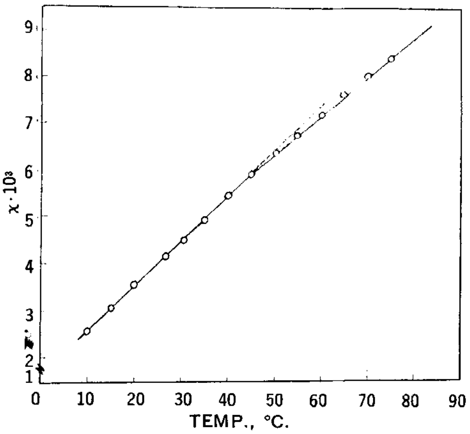

*Fig. 4—The specific conductivity $\chi$ of an $NH_4DCA$ solution ($5 \times 10^{-2}$ M) as a function of temperature. The plot differs in trend from that of Fig. 1.*

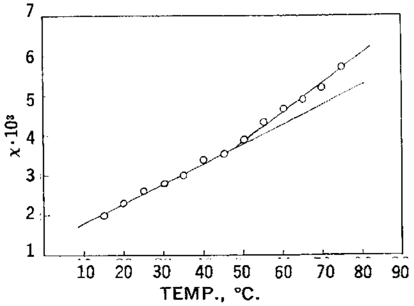

*Fig. 5—The specific conductivity $\chi$ of an $NH_4DCA$ solution ($7.5 \times 10^{-2}$ M) added of urea 6 M as a function of temperature. The plot shows the same trend of all the other deoxycholates.*

the transitional temperatures of $NH_4DCA$ go from 43° to 47°, while in the same range of concentration of NaDCA, as well as the other monovalent cations, there is a shift of about 35° (see Fig. 3).

Furthermore, the plot of specific conductivity against temperature of an $NH_4DCA$ solution shows a different trend. Below and above the transitional temperature the plot differs in slope (see Fig. 4). In addition, viscosity measurements in an $NH_4DCA$ solution show a deviation toward higher values than those obtained from solutions of other salts. This suggests that the presence of ammonium ions, through hydrogen bond formation, affects the complexity of these structures, which will grow in all directions forming a matted matrix of high viscosity. In fact, addition of urea to a solution of $NH_4DCA$ brings the plot back to the shape common to the other cations (see Fig. 5).

To further investigate the role played by ionic dimensions in these polymer-like structures, solutions of some organic quaternary nitrogen salts have been studied. They are monomethylammonium (MMA), dimethylammonium (DMA), tetramethylammonium (TMA), tetraethylammonium (TEA), and tetrabutylammonium (TBA) deoxycholate.

In Fig. 6 the transitional temperatures of the above-mentioned DCA salts are plotted against the

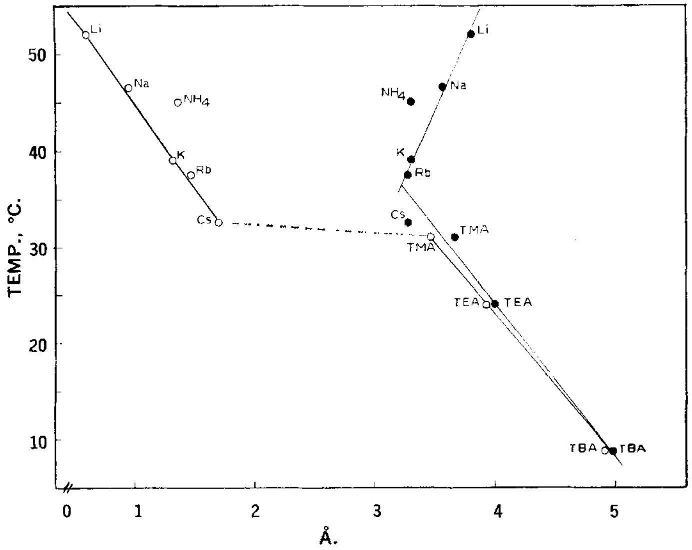

*Fig. 6—Transitional temperatures of different DCA salts as a function of the crystallographic radius* (○) *and the hydrated radius* (●).

crystallographic radius and against the hydrated radius (7). If the increment of crystallographic radius were the only reason for the increase of transitional temperatures, we should find much higher values in that part of the plot where Li, Na, K, Rb, and Cs are placed. In other words, the plot of transitional temperature as a function of crystallographic radius should be represented by the same straight line. When the hydrated radii are taken into consideration, the resulting plot shows a completely different trend. In fact, whenever there is a slight difference between crystallographic and ionic radii, that is, whenever the amount of water bound to cations is negligible, as in the case of organic cations, the resulting plots are almost identical. Instead, when the amount of structuralized or iceberg-like water bound to cations begins to be relevant, that part of the plot referring to inorganic cations exhibits a marked change in the slope and shows a discontinuity.

The experimental data summarized in Fig. 6 prove that structuralized or iceberg-like water bound to cations increases the thermal stability of the helical complex.

Water, therefore, proves to be of fundamental importance to the structural stability of such polymer-like compounds.

In conclusion, three factors were found to influence the stability of the structure: cation dimension, hydration, and hydrogen bonds.

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