# Stability Constants of Cyclic Polyether Complexes with Univalent Cations

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**Abstract:** The stability constants for the 1:1 complexes of 22 cyclic polyethers (12- to 60-membered rings of C–C–O units with various substituents including nitrogen and sulfur) with several cations ($Li^+$, $Na^+$, $K^+$, $Cs^+$, $NH_4^+$, $Ag^+$) in water and in methanol have been determined by potentiometry with cation-selective electrodes. The methanol stability constants are three to four decades higher than the aqueous ones, presumably because water competes more strongly for the cation than does methanol. Selectivity toward the different cations varies with polyether ring size, the optimum ring size being such that the cation just fits into the hole, *i.e.*, 15–18 for $Na^+$, 18 for $K^+$, and 18–21 for $Cs^+$. The effects of side rings fused onto the main polyether ring are comparatively small and difficult to predict. Substitution of N or S for O reduces affinity for alkali ions but greatly strengthens complexing of $Ag^+$. Evidence of 2:1 polyether:cation complexes was found for several systems in which the cation was larger than the hole of the ring. These results are consistent with Pedersen's conclusion that the alkali cation is held in the hole of the polyether ring by ion–dipole forces, though covalent bonding plays a part in the $Ag^+$ complexes of polyethers containing N or S.

In a number of recent papers Pedersen[1] has described his preparation of over 50 macrocyclic polyethers, his discovery that these compounds form complexes with many salts (mostly of group I and II cations), and extensive studies of the nature of the complexes both in the solid state and in solution by a number of different techniques: isolation of the solid crystalline complexes, solubilization of salts in nonpolar solvents, extraction studies, and ultraviolet spectroscopy. The complexes were found to be mostly of 1:1 stoichiometry, and Pedersen concluded that the cation is held in the hole of the polyether ring by ion–dipole forces. In some cases where the cation appears to be too large to fit into the hole, he found 2:1 and even 3:2 polyether: cation complexes,[1b] which he postulated to be "sandwich" structures.

Bright and Truter[2] have recently published the X-ray crystal structure of the 3:2 dibenzo-18-crown-6–rubidium complex, the first of the polyether complexes to be so analyzed. They found the unit cell to contain four molecules of 1:1 complex and two uncomplexed polyether molecules, and the $Rb^+$ ion to be located somewhat above the plane of the eight oxygen atoms. Thus there is no "sandwich" structure in this 3:2 complex, though such structures cannot be ruled out in other complexes, especially of the 2:1 type.

Complexing of alkali cations by neutral molecules is an uncommon and only recently observed phenomenon. The most comprehensive collection of stability constants,[3] which covers the literature through 1960, does not list a single instance of it. However, it has now been observed not only with the cyclic polyethers but also with a number of antibiotics, such as valinomycin, nonactin, monactin, etc., which are macrocyclic structures containing ester, ether, and peptide groups. Stability constants for several antibiotic–alkali cation systems have been reported recently.[4]

The stability constants, *i.e.*, equilibrium constants for complexing, of a few cyclic polyethers have been reported to date.[5–7] Izatt, *et al.*,[5] have used a calorimetric titration technique to obtain aqueous stability constants and thermochemical quantities for the two isomers of dicyclohexyl-18-crown-6 with a number of mono- and divalent cations. McLaughlin, *et al.*,[6] deduced the aqueous stability constants of di-(*tert*-butylcyclohexyl)-18-crown-6 complexes of the alkali cations from potential and conductance measurements on phospholipid bilayers. Wong, Konizer, and Smid[7] used spectroscopic methods for obtaining the stability constants of cyclic polyether complexes of fluorenyl alkali salts in tetrahydrofuran.

The present study was undertaken to provide a quantitative measure of the strength of complexing in solution as a function of polyether structure, cation size and type, and solvent. To this end the stability constants of a great variety of cyclic polyethers with several cations in aqueous and methanolic solution have been determined by potentiometry with cation-selective electrodes. Unlike most others, this method can be applied to systems with stability constants ranging over six or more orders of magnitude.

## Experimental Section

**Materials.** The "crown" nomenclature of Pedersen[1a] has been adopted for the cyclic polyethers. The examples in Figure 1 illustrate how the name is made up of the side-ring substituents, the total number of atoms in the polyether ring, the word crown, and the number of oxygen atoms in the main ring. All the dibenzo- and

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[1] (a) C. J. Pedersen, *J. Amer. Chem. Soc.*, **89**, 7017 (1967); (b) *ibid.*, **92**, 386 (1970); (c) *ibid.*, **92**, 391 (1970); (d) *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 27, 1305 (1968).

[2] D. Bright and M. R. Truter, *Nature (London)*, **225**, 176 (1970); *J. Chem. Soc. B*, 1544 (1970).

[3] L. G. Sillén and A. E. Martell, *Chem. Soc., Spec. Publ.*, **No. 17** (1964).

[4] (a) H. K. Wipf, L. A. R. Pioda, Z. Stefanac, and W. Simon, *Helv. Chim. Acta*, **51**, 377 (1968); (b) J. H. Prestegard and S. I. Chan, *Biochemistry*, **8**, 3921 (1969); *J. Amer. Chem. Soc.*, **92**, 4440 (1970).

[5] (a) R. M. Izatt, J. H. Rytting, D. P. Nelson, B. L. Haymore, and J. J. Christensen, *Science*, **164**, 443 (1969); (b) R. M. Izatt, D. P. Nelson, J. H. Rytting, and J. J. Christensen, *J. Amer. Chem. Soc.*, in press.

[6] S. G. A. McLaughlin, G. Szabo, G. Eisenman, and S. Ciani, Abstracts, 14th Annual Meeting of the Biophysical Society, Baltimore, Md., Feb 25–27, 1970, p 96a.

[7] K. H. Wong, G. Konizer, and J. Smid, *J. Amer. Chem. Soc.*, **92**, 666 (1970).

dicyclohexyl-crowns used in this work had the hydrocarbon rings in symmetric positions at opposite sides of the polyether ring.

The polyethers were prepared by the methods described by Pedersen[1a] and purified by recrystallization or fractionation. They contained at most a few per cent impurities, as judged by tlc, glpc, and melting point.

Since the cyclohexyl derivatives were made by hydrogenation of the corresponding benzo compounds, cis–trans isomerism at the bridge bonds is a possibility.[1a] However, nmr spectra suggest that all the bridge bonds of dicyclohexyl-18-crown-6 are trans.[8] This accords with the fact that only one isomer was found in cyclohexyl-15-crown-5 and cyclohexyl-18-crown-6, while dicyclohexyl-18-crown-6 contained only two, presumably a meso and a *dl* form. Dicyclohexyl-18-crown-6 was separated into its two component isomers (A, mp 61–62°; B, mp 69–70°) by elution from a neutral alumina column with ether–hexane mixtures.[8] At this time it is not known which of the two isomers has the meso and which one the *dl* structure. No attempt was made to separate the isomers of the other dicyclohexyl derivatives; their stability constants refer to the isomeric mixtures.

The salts, chlorides except for $AgNO_3$, were ACS reagent grade and were not further purified except for drying. The solvents were deaerated deionized water and absolute methanol (reagent ACS, less than 0.05% water).

**Apparatus.** The activity of the uncomplexed cation was measured by potentiometry with ion-selective electrodes. The following measuring electrodes were used: Corning NAS 11-18 (Cat. No. 476210) sodium-ion electrode for $Na^+$; Corning monovalent-cation electrode (Cat. No. 476220) for $Li^+$, $K^+$, $Cs^+$, and $NH_4^+$; silver|silver chloride electrode for $Ag^+$. The monovalent-cation electrode was conditioned to each new cation by soaking it overnight in a 0.01–0.1 *M* solution of its chloride. One such electrode cracked on immersion in methanol. Other monovalent-cation electrodes were successfully used in methanol by stepwise conditioning in aqueous solutions of increasing methanol content up to pure methanol, in which they could then be kept indefinitely.

Initially 3 *M* calomel electrodes were used as reference, but they proved troublesome, especially in methanol, owing to pluggage of the salt bridge, fluctuating junction potential, and leakage of measurable quantities of potassium into the solution. These difficulties were avoided by using silver|silver chloride electrodes as reference for cations other than $Ag^+$, which was made feasible by using chloride salts and keeping the chloride concentration substantially constant during each run. They were prepared by flash coating silver-ingot electrodes for about 15 sec at 50 mA in 0.1 *M* HCl.

The emf was measured with a Corning Model 12 pH meter which permits readings to within 0.1 mV. Each electrode system was calibrated for every cation–solvent system. Mean activity coefficients (for measurements with Ag|AgCl reference) were taken from the literature[9] where available or estimated by the Debye–Hückel theory,[9] as were single-ion activity coefficients (for measurements with calcomel reference). The calibration plots (emf *vs.* log *a*) were linear from 0.0003 to 0.01 *M*, and their slope differed by at most 3% from theory. This difference is more likely due to uncertainties in the activity coefficients than to nonideal electrode behavior.

A closed glass cell equipped with inlets for the two electrodes, two serum caps, and a magnetic stirrer was used for the measurements. Its capacity was 20–35 ml. Titrant was added from a 2.5-ml micrometer buret through a syringe needle. All measurements were conducted in a constant-temperature room kept at 25 ± 0.5°.

**Experimental Procedure.** Each emf measurement was obtained by taking readings at 5-min intervals until three successive readings differed by 0.2 mV or less. The solution was stirred except for 1 min before and during each reading. The difference between the emf of the stirred and of the quiescent solution was generally negligible with the Ag|AgCl reference. With the calomel reference it was often appreciable, increased with dilution, and was largest for methanol solutions, sometimes amounting to several millivolts. However, 1 min without stirring was usually long enough to reach a steady reading.

Each run was started by measuring the emf of the salt solution. The polyether was added by one of two different procedures: (1) titration and (2) addition by weight. Method 1, used when polyether solubility and supply were adequate, *i.e.*, for most of the methanol measurements, consisted of adding a four- to tenfold excess of a 0.05 or 0.1 *M* solution of the polyether in eight to ten increments and measuring the emf after each addition; then the cell was refilled with the original salt solution and the emf remeasured; the average of this value and the initial emf was used in the calculations. In method 2, which was used for some methanolic and all aqueous solutions, one to four increments of polyether were added by weight.

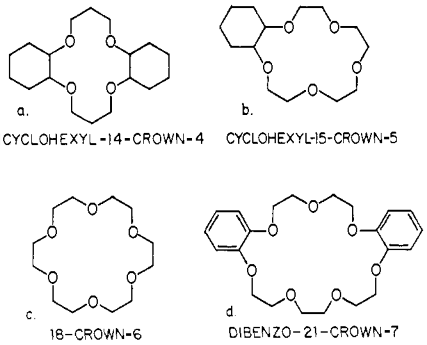

Figure 1. Structural formulas and "crown" names. The *Chemical Abstracts* names are as follows: (a) hexadecahydro-6*H*,15*H*-dibenzo[*b*,*i*][1,4,8,11]tetraoxacyclotetradecin; (b) tetradecahydro-1,4,7,10,13-benzopentaoxacyclopentadecin; (c) 1,4,7,10,13,16-hexaoxacyclooctadecane; (d) 6,7,9,10,12,13,20,21,23,24-decahydrodibenzo[*b*,*k*][1,4,7,10,13,16,19]heptaoxacycloheneicosin.

Two to four runs were made for each cation–polyether–solvent system at cation concentrations from 0.2 to 10 m*M*, so that the stability constants reported for each system measured by method 1 represent 16–40 separate measurements, while those measured by method 2 represent 4–20 measurements.

**Calculations.** The thermodynamic stability constant for a 1:1 complex, $K_1$, is defined by

$$K_1 \equiv (f_C[MCr^+])/(f_M[M^+]f_{Cr}[Cr]) \qquad (1)$$

where $[MCr^+]$, $[M^+]$, and [Cr] are the molar concentrations of complex cation, uncomplexed cation, and uncomplexed polyether, respectively, while $f_C$, $f_M$, and $f_{Cr}$ are the corresponding activity coefficients. The concentration stability constant $K_1'$, which will be reported in this paper, since $f_C$ and $f_M$ are unknown, is given by

$$K_1' \equiv K_1 f_M/f_C = [MCr^+]/([M^+][Cr]) \qquad (2)$$

where $f_{Cr}$ has been taken as unity, which is reasonable for the electrically neutral polyether molecule at these low concentrations. Similarly, the stability constant for addition of a second polyether molecule to the 1:1 complex is

$$K_2' \equiv K_2 f_C/f_D = [MCr_2^+]/([MCr^+][Cr]) \qquad (3)$$

where $[MCr_2^+]$ and $f_D$ are the concentration and the activity constant of the 2:1 complex, respectively. Both $K_1'$ and $K_2'$ are expressed in liters per mole.

If *m* ml of $M_0$ molar cation solution is mixed with *c* ml of $C_0$ molar polyether solution, the two stability constants are given by

$$K_1' = (1 - u - y)/\{uD[cC_0/m - M_0(1 - u + y)]\} \qquad (4)$$

and

$$K_2' = y/\{D(1 - u - y)[cC_0/m - M_0(1 - u + y)]\} \qquad (5)$$

where the dilution factor $D \equiv m/(m + c)$, *u* is the fraction of cation left uncomplexed, and *y* is the fraction of cation in the 2:1 complex.

Simultaneous solution of eq 4 and 5 gives

$$y = 1 - uR - [(1 - uR)^2 - (1 - u)^2]^{1/2} \qquad (6)$$

where $R \equiv 1 - K_1'/2K_2'$. Thus if *u* is known and a value of *R* is assumed, $K_1'$ and $K_2'$ can be calculated.

As the ionic strength, and thus presumably the activity coefficient, was constant, emf differences within any particular run could be taken to reflect concentration ratios. Dilution by titrant must be taken into account to obtain the mole ratio *u* from the concentration

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[8] E. G. Brame and H. K. Frensdorff, manuscript in preparation.

[9] B. E. Conway, "Electrochemical Data," Elsevier, Amsterdam, 1952.

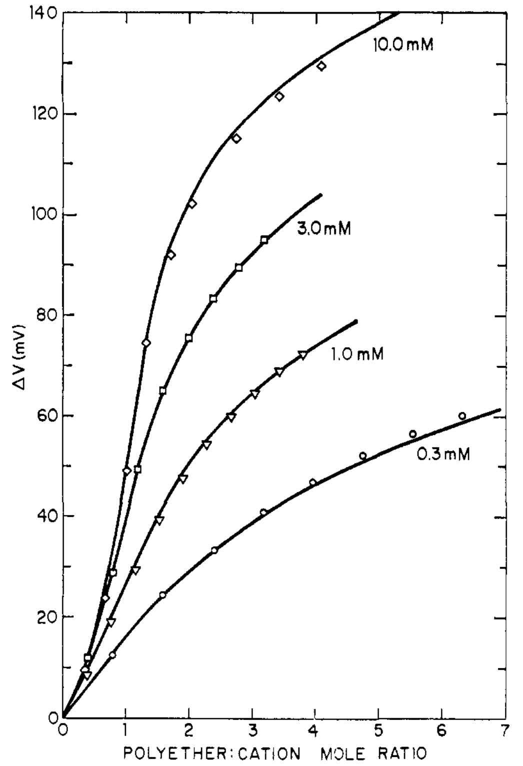

Figure 2. Titration of sodium chloride with cyclohexyl-15-crown-5 in methanol. Numbers on curves are initial NaCl concentrations, $M_0$. Points are observed, curves calculated for $K_1' = 5300$ l./mol.

ratio and to correct for its effect on the chloride activity which governs the potential of the Ag|AgCl reference electrode. Since calibration showed the electrode pairs to behave ideally (see above), the ideal Nernst slope was used to give the following relations. For the calomel reference

$$u = (10^{-\Delta V/59.16})/D$$

and for the Ag | AgCl reference

$$u = (10^{-\Delta V/59.16})/D^2 \qquad (7)$$

where $\Delta V$ is the difference between the emf of the salt solution and that of the salt-polyether solution in millivolts.

For all titration runs and weight-addition runs with enough points, $K_1'$ was obtained by nonlinear regression analysis on the assumption of 1:1 complexing; *i.e.*, the value of $K_1'$ was found which minimized the sum of the square deviations of $\Delta V$ given by eq 4 and 7 with $y = 0$. If this analysis or independent observation[1b] suggested the possibility of 2:1 complexing, regression values of $K_1'$ and $K_2'$ were computed analogously with $y$ as given by eq 6. For runs with few points no regression analysis was attempted, and $K_1'$ was calculated directly for each point and then averaged.

**Errors and Reliability.** The assumptions that the cation concentration is equal to the salt concentrations and that the chloride activity is constant imply that ionic dissociation is complete, as it certainly is in the aqueous solutions. Dissociation in the methanol solutions can be estimated from the ion-pairing equilibrium constants, which are about 10 l./mol for both NaCl and KCl.[3] This corresponds to 99% dissociation at 1 m$M$, 97% at 3 m$M$, and 92% at 10 m$M$. Ionic dissociation is expected to be even more complete for the complexed salts, where the polyether partly shields the cationic charge. It was not considered worthwhile to correct for these small effects.

The major source of uncertainty in this work derives from errors in the emf measurements: an error of 1 mV in $\Delta V$ causes an error of 4% in the calculated value of $u$. The effects of slow drifts were minimized by using only emf differences, rather than absolute values, in the calculations; this was equivalent to restandardizing the electrodes for each run. The emf of a given salt solution at the end of a titration run usually differed by less than 1 mV from the initial emf, though day-to-day variations sometimes amounted to as much as 3 mV.

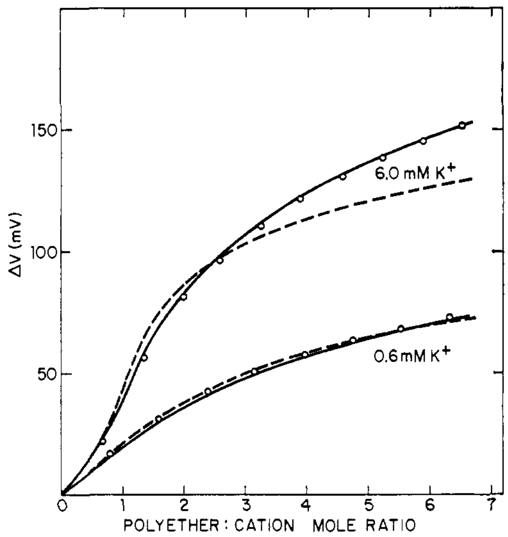

Figure 3. Titration of potassium chloride with cyclohexyl-15-crown-5 in methanol. Numbers on curves are initial KCl concentrations, $M_0$. Points are observed; full curves calculated for $K_1' = 3900$, $K_2'/K_1' = 0.02$; dashed curves calculated for $K_1' = 4500$, $K_2'/K_1' = 0$.

The relative error of $K_1'$ can be much larger than that of $u$, especially if complexing is very weak, or if it is very strong and the polyether is not present in excess of the cation. In the first case [MCr$^+$], in the second [Cr], is the small difference between two large numbers. The second condition was avoided by extending titration runs to large excesses of polyether.

The reliability of the stability constants is best illustrated by some typical results. Table I and Figure 2 show examples of runs by the weight-addition and by the titration method, respectively. Even though the measurements in Table I involve very small emf differences, quite consistent values of $K_1'$ are obtained over a tenfold range of cation and a sevenfold range of polyether concentration. Similarly, Figure 2 illustrates how well the observed points of methanol runs, which give larger emf differences, agree with the calculated curves. Thus 35 points covering 30-fold cation and even larger polyether concentration ranges are fitted to four curves with a single adjustable parameter. This good agreement between observation and theory demonstrates convincingly that the 1:1 stoichiometry is valid and that the activity coefficient ratio $f_M/f_C$ is essentially constant up to 0.01 $M$ ionic strength.

**Table I.** Complexing of 18-Crown-6 with KCl in Water

| Total concn, m$M$ | | $\Delta V$, | | $K_1'$, |
|---|---|---|---|---|
| Salt | Polyether | mV | $u$ | l./mol |
| 0.300 | 0.80 | 2.0 | 0.925 | 105 |
| 0.300 | 1.55 | 3.9 | 0.859 | 109 |
| 1.000 | 0.50 | 1.4 | 0.947 | 125 |
| 1.000 | 1.65 | 4.1 | 0.852 | 116 |
| 1.000 | 1.80 | 4.2 | 0.849 | 109 |
| 1.000 | 3.33 | 7.5 | 0.747 | 110 |
| 3.000 | 1.89 | 3.9 | 0.859 | 112 |
| 3.000 | 3.81 | 7.5 | 0.747 | 111 |
| | | | Av | 112 |

Figure 3 shows an example where the observed points just cannot be fitted to theoretical curves based on 1:1 stoichiometry at higher cation and polyether concentrations. Yet assumption of 2:1 complexing with $R = 0.02$ reproduces the data very well. Unequivocal detection of 2:1 complexing for such low values of $R$ is only possible if $K_1'$ is large enough and if measurements are carried to sufficiently high cation concentration and polyether excess.

Table I and Figure 2 are illustrative of the precision of most of the measurements. The 95% confidence limits of the $K_1'$ values obtained by regression analysis were generally ±4% or less. Since this excludes most systematic errors, an uncertainty of ±10%

(±0.04 log unit) is assigned to these stability constants. Some of the other results were of lesser reliability because of low degree of complexing, low polyether solubility, or lack of material. Estimated uncertainties for such stability constants are indicated in the tables. Estimates of maximum possible stability constants are given for systems in which no detectable complexing was observed.

## Results and Discussion

**Complexing in Methanol.** The methanol stability constants in Table II illustrate the effects of polyether ring size, cation size, and substituents on the main ring.

**Table II.** Stability Constants in Methanol at 25°

| Polyether | Log $K_1'$ [a] Na$^+$ | K$^+$ | Cs$^+$ |
|---|---|---|---|
| Tetramethyl-12-crown-4[e] | 1.41 | | |
| Dicyclohexyl-14-crown-4 | 2.18[b] | 1.30[b] | |
| Cyclohexyl-15-crown-5 | 3.71 | 3.58[d] | 2.78[b,d] |
| 18-Crown-6 | 4.32 | 6.10 | 4.62 |
| Cyclohexyl-18-crown-6 | 4.09 | 5.89 | 4.30[d] |
| Dicyclohexyl-18-crown-6 (isomer A) | 4.08 | 6.01 | 4.61 |
| Dicyclohexyl-18-crown-6 (isomer B) | 3.68 | 5.38 | 3.49 |
| Dibenzo-18-crown-6 | 4.36 | 5.00 | 3.55[d] |
| 21-Crown-7 | | 4.41 | 5.02 |
| Dibenzo-21-crown-7 | 2.40[b] | 4.30[b] | 4.20 |
| 24-Crown-8 | | 3.48 | 4.15 |
| Dibenzo-24-crown-8 | | 3.49 | 3.78[b] |
| Dibenzo-30-crown-10 | 2.0[c] | 4.60[b] | |
| Dibenzo-60-crown-20 | | 3.90[b] | |
| Pentaglyme[f] | 1.52[b] | 2.20[b] | |

[a] $K_1'$ in l./mol; uncertainty ±0.04 unless noted otherwise. [b] Uncertainty ±0.08. [c] Uncertainty ±0.2. [d] Evidence of 2:1 complexing; see Table IV. [e] Cyclic tetramer of propylene oxide: J. L. Down, J. Lewis, B. Moore, and G. W. Wilkinson, *J. Chem. Soc.*, 3767 (1959). [f] $CH_3(OCH_2CH_2)_5OCH_3$.

The interaction between ring size and cation size is manifested by the selectivity order, which changes from Na$^+$ > K$^+$ for dicyclohexyl-14-crown-4, to Na$^+$ ≈ K$^+$ > Cs$^+$ for cyclohexyl-15-crown-5, to K$^+$ > Cs$^+$ > Na$^+$ for cyclohexyl-18-crown-6, to K$^+$ ≈ Cs$^+$ ≫ Na$^+$ for dibenzo-21-crown-7, and to Cs$^+$ > K$^+$ for dibenzo-24-crown-8. Moreover, the optimum ring size is 15–18 for Na$^+$, 18 for K$^+$, and 18–21 for Cs$^+$, in accord with the relative size of cations and polyether rings (Table III), from which one might have predicted that Na$^+$ just barely fits into the 15-membered ring, K$^+$ into the 18-membered ring, and Cs$^+$ into the 21-membered ring. As Pedersen[1] has observed, complexing is expected to be weak when the polyether is too small for the cation because there are fewer negatively charged oxygens and also because the cation cannot get into the plane of the oxygen atoms where the charge density is highest, or when the ring is too large, because the cation cannot be simultaneously close to all the oxygens.

**Table III.** Ionic Diameters and Hole Sizes (Å)

| Cation | Ionic diameter[a] | Polyether ring | Hole size[b] |
|---|---|---|---|
| Li$^+$ | 1.36 | 14-Crown-4 | 1.2–1.5 |
| Na$^+$ | 1.94 | 15-Crown-5 | 1.7–2.2 |
| K$^+$ | 2.66 | 18-Crown-6 | 2.6–3.2 |
| Rb$^+$ | 2.94 | 21-Crown-7 | 3.4–4.3 |
| Cs$^+$ | 3.34 | | |
| NH$_4^+$ | 2.86 | | |
| Ag$^+$ | 2.52 | | |

[a] Crystal diameter. [b] As measured on models by Pedersen;[1b] smaller size from Corey–Pauling–Koltun, larger from Fisher–Hirschfelder–Taylor atomic models.

The structure of the cyclic polyether complexes, as envisioned by Pedersen[1] and found by Bright and Truter,[2] with the cation in the center of the polyether ring, does not require stripping of the entire solvation shell of the ion, since solvent contacts are still possible in the direction perpendicular to the plane of the ring. In contrast, the macrocyclic antibiotics complex cations within their very much larger rings by wrapping themselves completely around the cation, which requires complete stripping of its solvation shell, as found in the nmr studies of Prestegard and Chan[4b] on nonactin. The sinuous shape in which these large rings wrap themselves around the cations has been directly observed by X-ray diffraction for K$^+$–nonactin[10] (32-membered ring described as "resembling the seam of a tennis ball") and K$^+$–valinomycin[11] (36-membered ring described as "three complete sine waves"). The increase of the K$^+$ stability constant between 24-crown-8 and 30-crown-10 (Table II) suggests that such "wrap-around" complexing might also occur with polyethers, provided their rings are of a suitable size.

Comparison of the stability constants of the three cations each with the crown of optimum ring size reveals that K$^+$ is the strongest by at least one order of magnitude. The macrocyclic antibiotics similarly show higher stability constants for K$^+$ than for Na$^+$ and Cs$^+$.[4] This can be considered to reflect the competition between complex formation and solvation. A small cation with its strong electric field will greatly attract both complexer and dipolar solvent molecules. Hence the largest cations have low stability constants because they do not attract the polyether very much, while the smallest cations are too strongly solvated for the polyether to compete successfully for them.

How much ring formation adds to strength of complexing is shown by the stability constants of pentaglyme (Table II), which are three to four decades lower than those of its cyclic analog, 18-crown-6. Apparently the linear polyether does not envelop the cation as completely as the cyclic one because of electrostatic repulsion between its terminal oxygens and the unfavorable entropy change involved in forcing the linear into an almost cyclic structure.

The effects of side groups, illustrated by the five 18-crown-6 derivatives in Table II, are more complex, though smaller, than those of main ring size. While the stability constants of 18-crown-6, cyclohexyl-18-crown-6, and isomer A of dicyclohexyl-18-crown-6 are within a factor of 2 for all three cations, isomer B and dibenzo-18-crown-6 behave in a more complicated manner. Isomer B is a weaker complexer than isomer A, increasingly so for the larger cations ($K_1'$ ratio isomer A:isomer B is 2.5 for Na$^+$, 4 for K$^+$, and 13 for Cs$^+$). This has the interesting effect of changing the selectivity order from K$^+$ ≫ Cs$^+$ > Na$^+$ for the A isomer to K$^+$ ≫ Na$^+$ > Cs$^+$ for the B isomer. On the other hand, compared to isomer A of the dicyclohexyl analog, dibenzo-

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[10] B. T. Kilbourn, J. D. Dunitz, L. A. R. Pioda, and W. Simon, *J. Mol. Biol.*, 30, 559 (1967).

[11] M. Pinkerton, L. K. Steinrauf, and P. Dawkins, *Biochem. Biophys. Res. Commun.*, 35, 512 (1969).

**Table IV.** 2:1 Complexing in Methanol at 25°

| Polyether | Cation | Points[a] | Log $K_1'$[b] | Log $K_2'$[b] | $K_2'/K_1'$ |
|---|---|---|---|---|---|
| Cyclohexyl-15-crown-5 | $K^+$ | 18 | 3.58 ± 0.02 | 1.88 ± 0.07 | 0.02 |
| Cyclohexyl-15-crown-5 | $Cs^+$ | 6 | 2.78 ± 0.07 | 1.91 ± 0.23 | 0.13 |
| Dibenzo-18-crown-6 | $Cs^+$ | 19 | 3.55 ± 0.02 | 2.92 ± 0.07 | 0.23 |
| 18-Crown-6 | $Cs^+$ | 16 | 4.62 ± 0.03 | 1.30 ± 0.20 | 0.0005 |
| Cyclohexyl-18-crown-6 | $Cs^+$ | 16 | 4.30 ± 0.02 | 1.52 ± 0.18 | 0.002 |
| Dicyclohexyl-18-crown-6 (isomer A) | $Cd^+$ | 22 | 4.61 ± 0.02 | 0.59 ± 0.05 | 0.0001 |
| Dicyclohexyl-18-crown-6 (isomer B) | $Cs^+$ | 16 | 3.49 ± 0.01 | *c* | 0 |
| 21-Crown-7 | $Cs^+$ | 16 | 5.02 ± 0.01 | *c* | 0 |
| Dibenzo-21-crown-7 | $Cs^+$ | 6 | 4.20 ± 0.03 | 1.9 ± 0.3 | 0.005 |

[a] Number of measurements included in regression analysis. [b] $K_1'$ and $K_2'$ in l./mol; 95% confidence limits shown. [c] $K_2' = 0$ lies within the 95% confidence limits.

**Table V.** Stability Constants in Water at 25°

| | Log $K_1'$[a] | | | | | |
|---|---|---|---|---|---|---|
| Polyether | $Li^+$ | $Na^+$ | $K^+$ | $Cs^+$ | $NH_4^+$ | $Ag^+$ |
| Cyclohexyl-15-crown-5 | <1.0 | <0.3 | 0.6 | | | |
| 18-Crown-6 | | <0.3 | 2.06[b] | 0.8 | 1.1 | 1.6 |
| Cyclohexyl-18-crown-6 | <0.7 | 0.8 | 1.90[b] | 0.8 | 1.1 | 1.7–1.9[e] |
| Dicyclohexyl-18-crown-6 | | | | | | |
| Isomer A | 0.6[c] | 1.5–1.85[d] | 2.18[b]<br>2.02[f] | 1.25<br>0.96[f] | 1.4<br>1.33[f] | 2.3 |
| Isomer B | | 1.2–1.6[d] | 1.78[b]<br>1.63[f] | 0.9 | 0.80<br>0.80[f] | 1.8<br>1.59[f] |
| Di-(*tert*-butylcyclohexyl)-18-crown-6 | <0.9[g] | 1.42[g] | 2.08[g] | 0.9[g] | 1.28[g] | |
| Dicyclohexyl-21-crown-7 | | | | 1.9 | | |
| Dicyclohexyl-24-crown-8 | | | | 1.9 | | |

[a] $K_1'$ in l./mol; uncertainty ±0.1 unless noted otherwise. [b] Uncertainty ±0.04. [c] Uncertainty ±0.3. [d] From 8 to 1 m*M* $Na^+$. [e] From 3 to 0.3 m*M* $Ag^+$. [f] Values of Izatt, *et al.*[5b] [g] Values of McLaughlin, *et al.*[6]

18-crown-6 is twice as strong for $Na^+$ complexing but ten times weaker for $K^+$ and $Cs^+$. Such differences reflect both the electrostatic and the steric effects of the side rings: the strength of the C–O dipoles is affected by the nature of the substituent (*e.g.*, less for aromatic than for alicyclic side rings); the ease of motion and the attainable conformations of the polyether ring depend on the structure near the inter-ring bridges. The detailed structures, conformations, and mobility are not well enough understood at this time to rationalize the effects of side groups on the stability constants, but these effects are relatively small, amounting to at most one decade.

**2:1 Complexing.** While the existence of 2:1 polyether–cation complexes in the solid crystalline state has been amply demonstrated,[1r] it does not follow necessarily that they also exist in solution. On the one hand, solvation alters the energetics of the complexing process; on the other hand, odd stoichiometries in the solid may be caused by the way the components pack into the lattice and other factors peculiar to the crystalline state, as exemplified by the 3:2 dibenzo-18-crown-6–$Rb^+$ complex[2] discussed in the introduction.

All the combinations of ring sizes and cations in Table IV appear to be suitable to 2:1 complex formation and, except for the last two, the cation is too large for the hole (*cf.* Table III). Yet only the first three of these show unequivocal evidence of 2:1 complex formation in methanol solution. The emf data for the remaining combinations were not inconsistent with a small amount of 2:1 complexing, though they could be fitted nearly as well without it. As indicated by the last column of Table IV, $K_2'$ is more than two decades lower than $K_1'$ in these cases.

The three cases of appreciable 2:1 complexing in solution accord closely with Pedersen's[1b] findings that benzo-15-crown-5, which he used rather than the cyclohexyl analog, formed 2:1 complexes with KCNS, CsCNS, $NH_4CNS$, and $Ba(CNS)_2$, while dibenzo-18-crown-6 formed 2:1 complexes with RbCNS and CsCNS. It was, therefore, surprising that derivatives of 18-crown-6 with zero, one, or two cyclohexyl side rings exhibit so much less, if any, 2:1 complexing with $Cs^+$ in solution. These findings, then, demonstrate that a ring size too small to accommodate the cation is not a sufficient, though it may be a necessary, condition for 2:1 complex formation.

The differences between dibenzo-18-crown-6 and the other derivatives with respect to 2:1 complex formation with $Cs^+$ are most likely due to the same causes as those with respect to 1:1 complexing of $Na^+$ and $K^+$ discussed above. Again either steric factors or electrostatic ones may be responsible. If the sandwich structure proposed for the 2:1 complexes[1b] is correct, it may well be that steric factors preponderate. The exact "hole size" will determine how far the cation protrudes from the planes of the two polyethers and will thus affect the steric interference between them. Similarly the bulkiness of the side groups as well as the angles between them and the main ring will also contribute to such interference. Assignment of these causes will have to await further structural studies.

**Complexing in Water.** Stability constants of aqueous systems are collected in Table V. Since they are much lower than for the methanol solutions, the uncertainty is somewhat greater. Moreover, the number of polyethers measured is limited to those of sufficient water solubility, which excludes the benzo- and dibenzocrowns.

Unlike the methanol results, those in water could not always be explained in terms of simple stoichiometry. The data in Table VI show the most marked example of this. While $K_1'$ is essentially independent of polyether concentration at constant cation concentration (compare results at 2.6 m$M$ $Na^+$), it decreases considerably as the cation concentration rises. Such a decrease of $K_1'$ with increasing $M_0$ was found for both isomers of dicyclohexyl-18-crown-6 with $Na^+$, as well as cyclohexyl-18-crown-6 with $Ag^+$ (see Table V). In some other systems there was a similar trend, but too small to be distinguished from experimental error. This decrease of $K_1'$ is too large to be due to the effect of ionic strength on the activity coefficients, nor can it be accounted for in terms of other simple stoichiometries, such as 2:1 and 1:2. It seems, therefore, that some other, as yet unidentified, competing or coupled equilibrium is responsible.

**Table VI.** Complexing of Dicyclohexyl-18-Crown-6 (Isomer B) with $Na^+$ in Water

| Total concn, m$M$ | | | | $\Delta V$, | | $K_1'$, |
|---|---|---|---|---|---|---|
| $Na^+$ | $OH^-$ | $Cl^-$ | Polyether | mV | $u$ | l./mol |
| 1.00 | 0.20 | 0.80 | 2.12 | 1.9 | 0.929 | 37 |
| 1.00 | 1.00 | 0.00 | 2.12 | 2.2 | 0.918 | 44 |
| 2.60 | 0.20 | 2.40 | 1.74 | 1.1 | 0.958 | 27 |
| 2.60 | 0.20 | 2.40 | 2.61 | 1.9 | 0.929 | 32 |
| 2.60 | 0.20 | 2.40 | 3.26 | 2.4 | 0.911 | 32 |
| 2.60 | 0.20 | 2.40 | 6.30 | 4.0 | 0.856 | 28 |
| 2.92 | 0.04 | 2.88 | 4.06 | 2.4 | 0.911 | 26 |
| 4.00 | 0.67 | 3.33 | 3.56 | 2.0 | 0.925 | 25 |
| 7.41 | 7.41 | 0.00 | 5.98 | 2.4 | 0.911 | 18 |
| 7.88 | 0.20 | 7.68 | 3.14 | 1.0 | 0.962 | 14 |
| 8.20 | 0.20 | 8.00 | 6.50 | 2.5 | 0.907 | 18 |

Sodium complexing is anomalous in one other respect, the effect of substitution. As shown by comparison of 18-crown-6 with its cyclohexyl and dicyclohexyl analogs in Table V, side groups have as little effect on complexing in water as in methanol, except for $Na^+$ and possibly $Li^+$. It is hard to understand why $Na^+$ complexing with 18-crown-6 and cyclohexyl-18-crown-6 should be so much weaker than with the dicyclohexyl analog. This might be related to the same factors which cause the decrease of $K_1'$ with concentration.

The data collected in Table V show the stability constants to be three to four decades lower for aqueous than for methanolic solutions. This reflects the much stronger cation solvation by water, compared to methanol, with which the polyether has to compete. As with the methanol stability constants, the differences between the alkali cations also reflect their relative affinities for solvent and polyether. The $Li^+$ ion is so strongly hydrated that only one case of barely detectable complexing was found. The $Ag^+$ ion, which is quite weakly hydrated and almost the same size as $K^+$, is complexed about as strongly as $K^+$. As in methanol, $Cs^+$ is most strongly held by the 21-membered polyether; complexing of this ion by the 18-crown-6 derivatives is too weak for detection of 2:1 complexes.

The data in Table VI provide evidence that the nature of the anion does not affect complex formation. Hydroxide was added to minimize hydrogen ion interference with sodium activity measurement, and the solutions contained chloride to hydroxide ratios from 0 to 72, but there was no detectable effect on $K_1'$.

Comparison with independently determined stability constants is possible for six cation–polyether combinations which have been measured also by Izatt, *et al.*,[5] by a calorimetric method. As shown in Table V, agreement between the two methods is fair. The most precise values are those for $K^+$ with the two dicyclohexyl-18-crown-6 isomers; our values are higher by *ca.* 0.16 log unit, *i.e.*, a factor of 1.5, which is rather more than the precision limits of both methods. It is not clear at this time to what extent this discrepancy is due to systematic errors in either method, to differences in the purity of the materials, or to the concentration dependence of $K_1'$ (during each calorimetric titration the total cation concentration increased from 0 to 30 m$M$ or more[5]).

Di(*tert*-butylcyclohexyl)-18-crown-6 is too insoluble in water for direct measurement, but McLaughlin, *et al.*,[6] were able to deduce the aqueous stability constants from phospholipid bilayer measurements (Table V). The complexing behavior of this compound in water is seen to be quite close to that of the other 18-crown-6 derivatives, as is also indicated by its stability constant for $K^+$ in methanol ($\log K_1' = 5.48 \pm 0.08$; *cf.* Table II).

Finding no measurable heat change on titrating the dicyclohexyl-18-crown-6 isomers with $Na^+$, Izatt, *et al.*,[5a] concluded that these polyethers complex $Na^+$ very much less than $Cs^+$. Our measurements and those of McLaughlin, *et al.*, indicate that the $Na^+$ stability constants are equal to or somewhat higher than the $Cs^+$ ones (*cf.* Table V). It appears, therefore, that thermal effects are undetectable for $Na^+$ because the enthalpy change is too small, rather than because $K_1'$ is too low.

**Nitrogen- and Sulfur-Containing Polyethers.** Substitution of N or S for O in 18-crown-6 and dibenzo-18-crown-6 greatly influences complexing, as shown by the data in Table VII. Complexing of $K^+$ is weakened appreciably by nitrogen and by sulfur substitution, the stability constants falling in the order of decreasing electronegativity, O > NR > NH > S. This is just as expected: as the negative charge on the heteroatom drops, the electrostatic attraction between it and the cation is diminished.

**Table VII.** Effect of Nitrogen and Sulfur Substitution on Stability Constants of 18-Crown-6 Derivatives at 25°

| Polyether[a] | | | Log $K_1'$ [c] | |
|---|---|---|---|---|
| Type | A | B | $K^+$ in methanol | $Ag^+$ in water |
| 1 | O | O | 6.10[d] | 1.60[d] |
| 2 | O | O | 5.00[d] | |
| 2 | NR[b] | O | 4.10 | |
| 1 | NH | O | 3.90 | 3.3[e] |
| 2 | NH | O | 3.20 | |
| 1 | NH | NH | 2.04 | 7.8[f] |
| 2 | NH | NH | 1.63 | |
| 1 | S | S | 1.15 | 4.34 |

[a] Type 1 = $A(CH_2CH_2OCH_2CH_2OCH_2CH_2)_2B$; type 2 = $A[CH_2CH_2O(o\text{-}C_6H_4)OCH_2CH_2]_2B$. [b] R = $n\text{-}C_8H_{17}$. [c] $K_1'$ in l./mol; uncertainty ±0.08 unless noted otherwise. [d] Uncertainty ±0.04. [e] Uncertainty ±0.12. [f] Uncertainty ±0.2.

The effects of N or S substitution on $Ag^+$ complexing are exactly the opposite; it is greatly increased. Evidently it is not electrostatic forces which matter here, but rather the type of covalent bonding which is involved in the many well-known complexes of $Ag^+$ with amines. Indeed the stability constants of noncyclic analogs are of comparable magnitude, *e.g.*, for $H_2NCH_2CH_2OCH_2CH_2OCH_2CH_2NH_2$ log $K_1'$ is about 7.9[12] compared to 7.8 for the cyclic diaza compound; for $CH_3OCH_2CH_2NH_2$ log $K_1'$ is 3.2[12] compared to 3.3 for the cyclic monoaza compound. That the stability constant for the dithia compound lies between those of the diaza and the unsubstituted polyethers is in agreement with the conclusion of Lotz, *et al.*,[12] that the relative stability of the silver–nonmetal bond increases in the order $O < S < N$.

The stability constants for the silver complexes of nitrogen- and sulfur-containing cyclic polyethers, therefore, provide the first evidence of complexing by other than purely electrostatic forces. However, it cannot be determined from the present evidence whether silver complexing of the straight polyethers is exclusively electrostatic or whether covalent bonding contributes to the stability. On the other hand, there is nothing to indicate that covalent bonding plays any part in the alkali metal complexes.

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# Dissociation Equilibrium and Potentiometric Titration of β-Lactoglobulin in Acidic Solutions[1]

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**Abstract:** The nonelectrostatic part of the standard free energy change accompanying dissociation of (charged) β-lactoglobulin molecules into two (charged) subunits in acidic solutions is estimated from literature values of the dissociation equilibrium constants by subtraction of electrostatic free energies obtained by numerical solution of the nonlinearized Poisson–Boltzmann equation. The nonelectrostatic portion of the standard free energy change (molality units, infinitely dilute reference state) is 10.6 ± 1 kcal/ru and is independent of pH and ionic strength. The standard free energy change in the dissociation of uncharged (*i.e.*, isoionic) β-lactoglobulin into two uncharged (isoionic) subunits is estimated from the experimental potentiometric titration curves using a method analogous to that previously applied to the helix–coil transition of poly(L-glutamic acid). The result agrees with the nonelectrostatic part of the standard free energy of dissociation of the charged molecules into charged subunits. When the dissociation is taken into account, the calculated electrostatic free energies yield theoretical potentiometric titration curves that agree rather well with experiment. The counterion radii needed to obtain a good fit are more realistic than those required when dissociation is ignored.

Aggregation of protein molecules is commonly encountered and frequently has important implications when seen in a biological context. Examination of the forces responsible is therefore of considerable interest. In some cases, dissociation of ionizable groups on the protein leads to charge repulsions that cause disaggregation of the subunits. Titration of the protein with appropriate acids or bases then enables an investigator to sweep through the entire range of states of aggregation from completely associated to completely dissociated subunits. Such a reaction is particularly susceptible to experimental study, and, since direct electrostatic interactions are the kind that are most precisely estimated, particularly susceptible to exact analysis.

The protein β-lactoglobulin displays such a dissociation equilibrium, and it has been the subject of extensive and thorough experimental investigation by Timasheff, Townend, and their coworkers.[2–8] They were able to show by various methods that the protein

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