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Binding of the Cationic Fluorophore Auramine-O to Neutral and Charged Poly(vinylbenzo-18-crown-6) in Water

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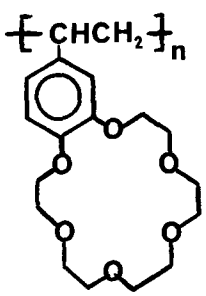
Summary

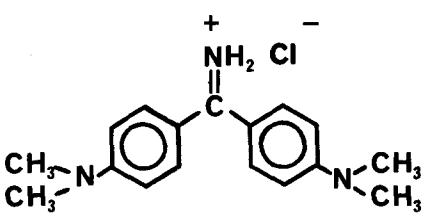
The cationic fluorophore Auramine-O strongly interacts with poly(vinylbenzo-18-crown-6), a polymer which in water behaves as a typical polysoap. The intrinsic binding constant at 25°C was found to be 2.2×10^4 M^{-1}. The binding can be modified by adding crown ether-complexable cations such as K^+ or Cs^+. Complexation converts the neutral polycrown ether into a polycation causing repulsion of the cationic fluorophore and a decrease in the observed fluorescence. There is some indication that the $>C=NH_2^+$ cation of the dye specifically interacts with a crown ligand.

Introduction

Poly(vinylbenzo-18-crown-6) (P18C6), dissolved in water, is known to interact strongly with organic solutes (WONG and SMID, 1977 and 1980; KIMURA and SMID, 1981). Due to its tightly coiled



P18C6



Auramine O

structure, the polymer behaves as a typical polysoap. For ionic organic solutes such as picrate anions, the hydrophobic interaction with P18C6 can be augmented electrostatically by converting the neutral polymer into a polycation. This occurs when cations (K^+, Cs^+) are added that are capable of forming cation-crown complexes with P18C6 in water. In previous work we exclusively used anionic organic solutes, the binding of which is strongly enhanced on addition of crown-complexable cations. Both optical (picrate, tetraphenylboride, methyl orange, hydroxybenzeneazobenzoate) and fluorescent probes (ANS, TNS) were employed in these investigations. The binding

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of cationic dyes to P18C6 is expected to be hindered by charging the polycrown ether with alkali ions. To study this effect, we used the cationic fluorophore auramine O (AuO, see structure above) which has been used in probing the structures of proteins (CHEN, 1977), polynucleotides (OSTER and NISHIJIMA, 1964) and synthetic polyacids (ERNY and MULLER, 1979; FENYO et al, 1979). The results of this study are reported in this paper.

Experimental

The synthesis of poly(vinylbenzo-18-crown-6) has been reported previously (KOPOLOW et al, 1973). The number average molecular weight of the polymer was 63,000. A sample of pure auramine O was kindly supplied to us by Drs. Fenyo and Muller of the University of Rouen. The fluorescence spectra were measured on a Perkin-Elmer 650-10S spectrofluorimeter at 25° $\pm$ 0.1°C and at an excitation wavelength of 366 nm. Conditions were chosen such that inner filter effects were negligibly small. Reagent grade inorganic salts were used without further purification.

Results and Discussion

The fluorescence of AuO in water (λ_{em}506 nm) is very weak (CHEN, 1977; ERNY, B. and MULLER, G., 1979) but addition of P18C6 strongly enhances the fluorescence intensity. At high [P18C6] ($\approx 10^{-3}$M), when all AuO is bound, the increase amounts to nearly a factor 200. The increase is in part due to the less polar environment of bound AuO, but this effect is known to be much smaller than for such fluorescent probes as TNS or ANS. Much of the increase in the fluorescence of bound AuO appears to be the result of rotational restrictions imposed upon the rather flexible structure of this fluorophore by the polymer domain to which the dye becomes bound (OSTER and NISHIJIMA, 1956; CHEN, 1977).

The binding of AuO to P18C6 was measured as a function of the AuO concentration. The observed fluorescence, F, is given by the expression $1/F = 1/F_m' + 1/KF_m'[AuO]$, where [AuO] denotes the free dye concentration, K is the intrinsic binding constant and F_m' the fluorescence intensity under conditions where P18C6 is saturated with the fluorophore. As shown in Figure 1, the plot of 1/F (in arbitrary units) vs 1/[AuO] is linear. The total AuO concentration was substituted for the free [AuO] since experimental conditions were such that the fraction of bound dye was less than 0.02. The intrinsic binding constant calculated from the slope and intercept of the plot was found to be 2.2×10^4 M^{-1}. This value is comparable with intrinsic binding constants found for the anionic solutes TNS (6.18×10^4 M^{-1}) and ANS (5.38×10^4 M^{-1}) to P18C6 (KIMURA and SMID, 1981), but higher than the reported values for AuO bound to proteins (CHEN, 1977). Preliminary studies indicate that the minimum number, N, of crown monomer units needed to bind one AuO molecule is about 70, close to the value 80 found for ANS.

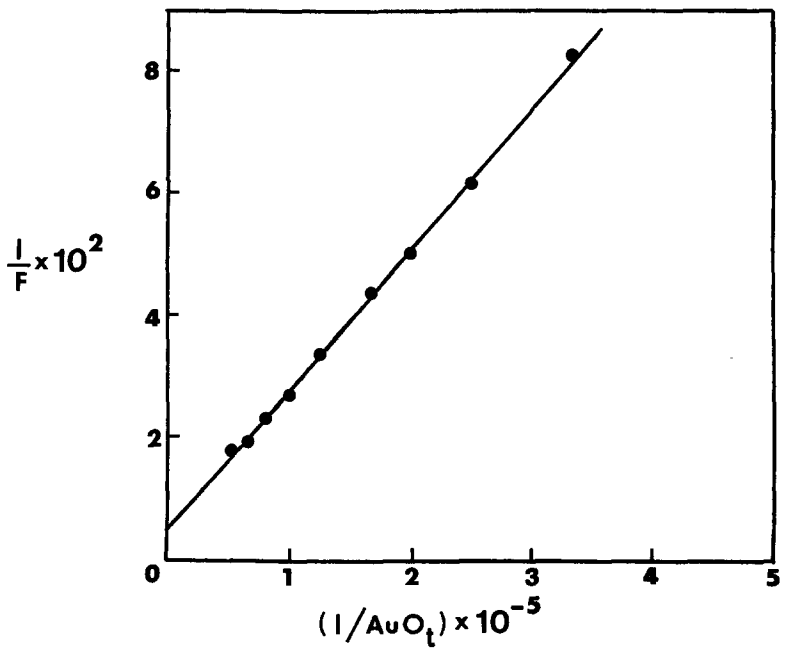

Fig. 1. Plot of 1/F vs $1/[AuO]_t$ for binding of Auramine O to P18C6 in water at 25°C. [P18C6] = 9.66×10^{-5} M

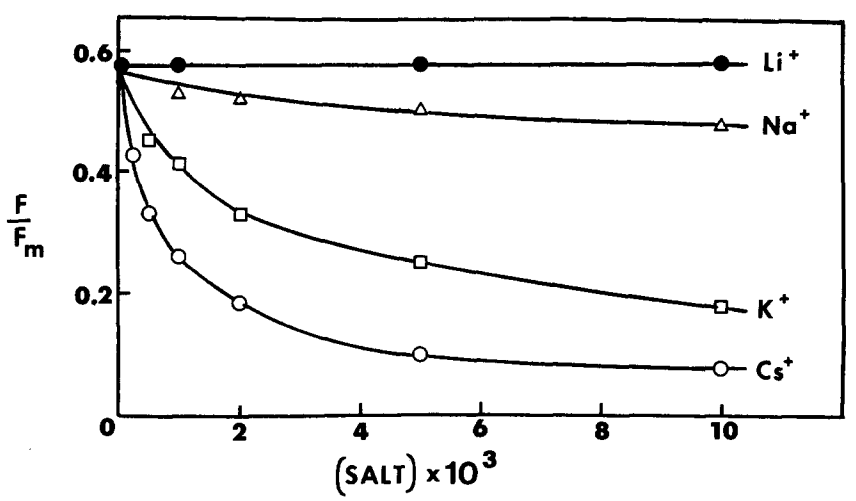

Fig. 2. Effect of salt on the fraction of P18C6-bound Auramine O

Figure 2 depicts the effect of salts on the binding of AuO to P18C6. The function F/F_m is plotted versus the concentration of added Li^+, Na^+, K^+ or Cs^+ cations, where F_m denotes the maximum observed fluorescence in the presence of excess, neutral P18C6, i.e., when all AuO is bound. Assuming that crown-bound cations do not affect the fluorescence of P18C6-bound AuO, the ratio F/F_m represents the fraction of bound AuO. This assumption is reasonable except for cations which are strong fluorescence quenchers, e.g., Tl^+ or Pb^{2+} (KIMURA and SMID, 1981).

Starting with conditions where in the absence of salt about 60% AuO is P18C6-bound ($F/F_m \approx 0.6$, see figure 2), the ratio F/F_m decreases rapidly on addition of Cs^+ and K^+, less for Na^+ and there is no change with Li^+ up to 10^{-2} M. The decrease in F/F_m results from the conversion of neutral P18C6 into a polycation when the alkali ions complex with the crown ligands of P18C6. The positively charged polymer repels the cationic fluorophore, the effect being most pronounced for the cation with the largest binding constant to P18C6. The order $Cs^+ > K^+ \gg Na^+ \gg Li^+$ is the same as found for anionic solutes (WONG and SMID, 1980; KIMURA and SMID, 1981) except of course that for the latter the fraction of bound solute increases due to electrostatic attraction. The ratio F/F_m appears to level off at higher Cs^+ concentration to a finite value. This may be due to the fact that the binding constants of cations to P18C6 decrease as more cations become bound to P18C6 (WONG and SMID, 1980). Hence, due to repulsion, the charge density on P18C6 does not change much once a certain fraction of crown ligands are complexed to cations.

The strong binding of AuO to P18C6 may in part be due to a specific interaction of the $\geq C = NH_2^+$ ion with a benzo-18-crown-6 ligand, similar to those known to exist for primary ammonium and uanidinium type cations. These complexes are usually unstable in water, but may be more stable in the less polar polymer domain. If such an interaction exists, it may also affect the rotational motions of the bound AuO and, therefore, the fluorescence quantum yield. We are presently investigating the binding of this fluorophore to poly(vinylbenzoglyme) (PVBG), a polymer with polysoap properties very similar to P18C6 (SINTA and SMID, 1980) except that the polymer-bound glyme ligands in water do not form stable complexes with alkali ions. Previous results have shown that binding of anionic organic solutes (e.g., TNS, ANS, picrate) to PVBG is slightly better than to P18C6. Preliminary data with AuO show a much smaller fluorescence enhancement in the presence of PVBG than with P18C6. Dialysis and fluorescence measurements are being carried out to determine whether the higher fluorescence in the presence of P18C6 is caused by a higher binding constant of AuO to P18C6, or to a lower fluorescence quantum yield of PVBG-bound AuO.

Acknowledgement

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