Fluorescence detection of the electrostatic interactions in the molecular recognition between protonated amino-cyclodextrins and some anilinonaphthalenesulfonate anions

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Strong fluorescence enhancements have been observed when some anilinonaphthalenesulfonate anions formed inclusion complexes with protonated amino-cyclodextrins in aqueous solution. The role of electrostatic interactions between anilinonaphthalenesulfonate anions and chemically modified β-cyclodextrin such as mono(6-aminoethylamino-6-deoxy)-β-cyclodextrin and mono(6-*N*,*N*-dibutylaminoethylamino-6-deoxy)-β-cyclodextrin was clarified by fluorescence spectroscopy. Modified β-cyclodextrins could become positively charged by the protonation of their amino group in some pH regions. Stability constants of the formed inclusion complexes were found to be increased by electrostatic interactions and in some cases the extension of the hydrophobic environment at the periphery of the inclusion site. The possible structure of the inclusion complexes was also discussed in detail.

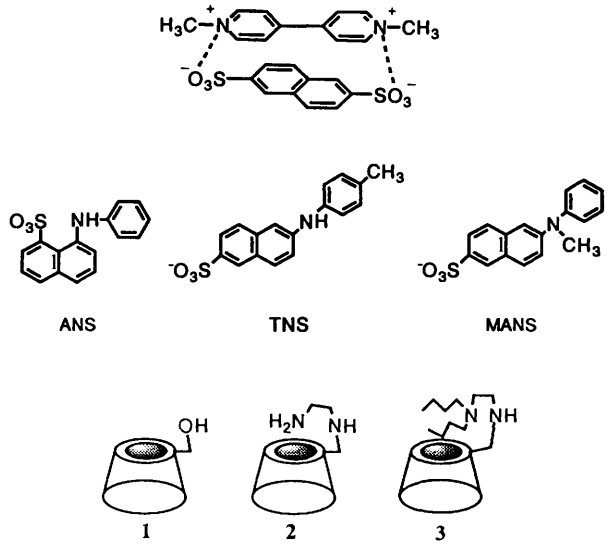
Introduction

The molecular recognition phenomena by modified cyclodextrins have been a subject of special attention in the past two decades as suitable models[1] for enzyme–substrate interactions in which various weak interactions play an important role. Several weak interactions such as van der Waals forces, hydrogen bonding, hydrophobic interactions, charge-transfer and electrostatic interactions are operative in the biological recognition processes. For example, the electrostatic interactions (salt bridges) between α- and β-subunits of haemoglobin are indispensable for cooperativity and allosteric regulation of its various molecular functions such as oxygen binding.[2] The importance of quantification of electrostatic contributions have been recognized in other biological processes such as the enzyme–substrate binding mechanism by site-directed mutagenesis[3] and polyamine binding to nucleic acids.[4]

Complementary orientation of positive and negative charge in host and guest molecules turns out to be a valid principle not only for the design of supramolecular ion-paired complexes,[5] but also for quantitative prediction of corresponding binding strengths.[6,7] For example, the Coulomb attractive force between methyl viologen† and naphthalene-2,6-disulfonate was estimated to be 16.0 kJ mol^{-1} in H_2O at 25 °C.[6]

Cyclodextrins (CD_x) can form inclusion complexes with a variety of organic and inorganic molecules and ions. Some anilinonaphthalenesulfonate anions as the anionic fluorescent probe for hydrophobic environments have been well known to bind with the native CD_x.[8] The fluorescence characteristics of these *N*-arylaminonaphthalenesulfonate anions make these dyes useful probes of the polarity at protein binding sites.[9] Generally, fluorescence intensities of the anilinonaphthalenesulfonate anions increase significantly with inclusion within the inner hydrophobic CD_x cavity.

Attempts to detect the electrostatic interaction between the protonated amino-CD_x and some azo guest molecules by UV–VIS and NMR spectroscopies resulted in failure.[10] In the present study, direct detection of electrostatic interactions between anilinonaphthalenesulfonate anions and the positively charged species of mono(6-aminoethylamino-6-deoxy)-β-cyclodextrin (β-CD_{xen}; **2**) and mono(6-*N*,*N*-dibutylaminoethylamino-6-deoxy)-β-cyclodextrin (β-CD_{xdben}; **3**) was carried out by fluorescence spectroscopy.



Experimental

Materials and synthesis

The potassium salt of 2-(4-toluidino)naphthalene-6-sulfonate (TNS) and the sodium salt of *N*-methyl-2-anilinonaphthalene-6-sulfonate (MANS) were purchased from Nacalai Tesque Inc. The free acid of 1-anilinonaphthalene-8-sulfonate (ANS) was supplied from Molecular Probe Ltd. Guaranteed-grade β-cyclodextrin as received from Wako Pure Chemical Ind. Ltd. was used without further purification for the synthesis of the various β-CD_x derivatives. Tosylated β-cyclodextrin (β-CD_{xots}) was formed by reaction of β-CD_x with toluene-*p*-sulfonyl chloride in pyridine at room temperature for 1.5 h. Matsui and co-workers also used the same method[11] except for reaction temperature and reaction time. The β-CD_{xots} obtained was dissolved in excess anhydrous ethylenediamine (en; Nacalai

† Recommended IUPAC name is 1,1′-dimethyl-4,4′-bipyridinium.

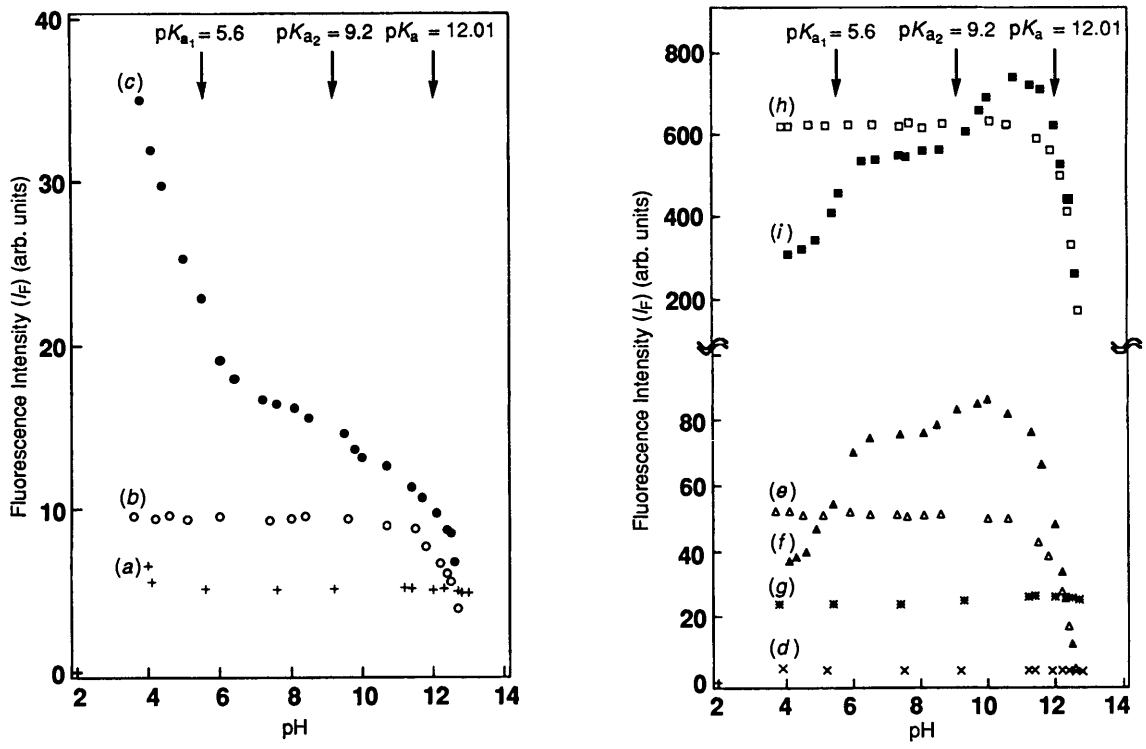


Fig. 1 pH Dependence of the fluorescence intensity (I_F) of ANS, TNS and MANS and their inclusion complexes with **1**, **2** and **3** at 25 °C. [**1**] = [**2**] = [**3**] = 5×10^{-4} mol dm^{-3} and [ANS] = [TNS] = [MANS] = 1.2×10^{-5} mol dm^{-3} (*a*) ANS (λ_{ex} = 350.0 nm, λ_{em} = 492.0 nm); (*b*) **1**–ANS; (*c*) **2**–ANS; (*d*) TNS (λ_{ex} = 366.0 nm, λ_{em} = 454.4 nm); (*e*) **1**–TNS; (*f*) **2**–TNS; (*g*) MANS (λ_{ex} = 320.0 nm, λ_{em} = 469.6 nm); (*h*) **1**–MANS; (*i*) **2**–MANS. The excitation and emission bandwidth were both set at 5.0 nm.

Tesque Inc.) or *N*,*N*-dibutylethylenediamine (dben; Tokyo Kasei) and the solutions were heated at 70 °C for 1.5 h with stirring. The reaction mixture was poured into a large excess of acetone and the white precipitate was collected. Purification of **2** and **3** was achieved by ion-exchange column chromatography through cation-exchange resin (Toyopearl 650M; 0.05 mol dm^{-3} NH_4HCO_3 aqueous solution as eluent). A thin layer chromatogram [Silicagel 70 plate-Wako; AcOH–$CHCl_3$–H_2O, 8:1:3 (v/v/v)] of **2** and **3** thus purified exhibited a clear single spot of R_f 0.45 and 0.50 [diphenylamine (4 g) in aniline–acetone–phosphoric acid (4:400:40 cm^3)]. The elemental analysis of **2** and **3** was satisfactory, although solvent water molecules were usually bound [Found for **2**: C, 41.95; H, 6.5; N, 2.2. Calc. for $C_{44}H_{76}O_{34}N_2 \cdot (H_2O)_{4.5}$: C, 42.00; H, 6.80; N, 2.22%. Found for **3**: C, 44.9; H, 7.3; N, 2.0. Calc. for $C_{52}H_{92}O_{34}N_2 \cdot (H_2O)_{5.5}$: C, 44.98; H, 7.47; N, 2.01%]. The ^{1}H NMR spectrum (internal reference DSS; JEOL α 500 FT NMR spectrometer) of **2** shows characteristic peaks of the α-protons to amino nitrogen at δ 2.6–2.7 in D_2O: **2**, δ 2.65 (4 H, N–CH_2), 3.4–3.55 (14 H, 2-H and 4-H), 3.6–3.9 (28 H, 3-H, 5-H and 6a,6b-H). New minor peaks which would be assigned to 6-H at the modified glucose ring were observed also at δ 2.8–3.4, 4.93 (7 H, 1-H). The ^{1}H NMR spectrum of **3** could not be obtained owing to poor solubility in D_2O. Mass spectral data also coincide with the structural formula [MS (FAB) *m*/*z* 1177 for **2** (M^+) and 1289 for **3** (M^+)].

Measurements

Stability constants (K_f) of the inclusion complexes were determined fluorometrically using a Shimadzu RF-5000 recording fluorescence spectrometer. The pH values in solution were obtained using a Horiba pH meter B-112. The temperature was maintained at 25 ± 0.1 °C by means of an external circulating water bath (Thomas Kagaku Co. Ltd., TRL-108H). The pH-fluorescence data were obtained by use of buffer solutions made for each pH value. Below pH 5.5, acetate buffer was used and above this pH, phosphate buffer, NH_4Cl–NH_3 and/or NaOH were used. Relative fluorescence intensities were measured at a constant wavelength around the emission maximum. The values of the excitation wavelength (λ_{ex}) for ANS, TNS and MANS were chosen to be 350.0, 366.0 and 320.0 nm, respectively. The wavelengths for emission measurements of ANS, TNS and MANS for the inclusion systems were 492.0, 454.4 and 469.6 nm, respectively. The NMR spectra of the inclusion complexes were obtained at room temperature with a JEOL EX-400 FTNMR spectrometer (with TMS as an external reference, pD = 3.5).

Results and discussion

pH Profiles of the fluorescence intensity

Fig. 1 shows the pH dependence of the fluorescence intensity (I_F) of ANS, TNS and MANS and their inclusion complexes with **1** and **2**.‡ The probes alone exhibit only a very weak fluorescence in water and no pH dependence of I_F. In both the probes alone and in the fluorescence β-CD_x complexes of ANS, TNS and MANS, the pH dependence of I_F was found to be flat in the wider pH regions from 3.5 to *ca.* 11.0. In these pH regions, no protolytic equilibria occur in both β-CD_x and the probes. The order of their relative fluorescence intensity in these pH regions was **1**–ANS < **1**–TNS ≪ **1**–MANS, which is roughly parallel to the hydrophobicity of the guest molecules and/or the stability of the complexes. The drastic decrease in I_F at pH > *ca.* 11.0 for **1**–ANS and **1**–TNS could be ascribed to the deprotonation of the anilino NH proton of the guest[8g] and/or the dissociation of the secondary hydroxy group of β-CD_x (pK_a = 12.01).[12] Since this abrupt decrease is also observed in the β-CD_x complex of MANS having no dissociable NH proton

‡ There will be some error as I_F also changes a little due to the spectral shift upon complexation as seen in Fig. 2(*a*). Ideally, spectral areas or quantum yields should be used instead of I_F in order to obtain the physical constants.

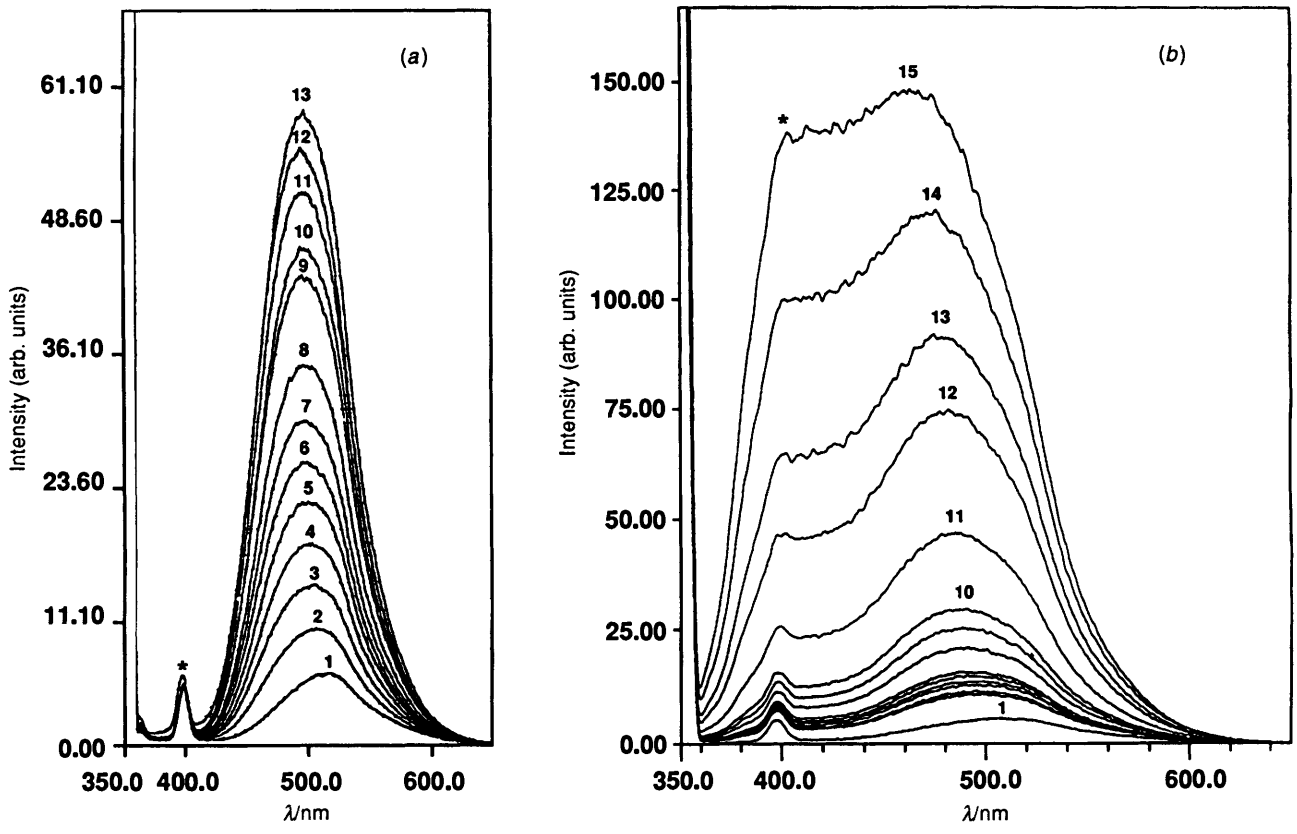


Fig. 2 Fluorescence spectra change of ANS at the various CD_x concentrations. (*a*) [β-CD_x] = 0.00 (**1**)–9.00 × 10^{-3} mol dm^{-3} (**13**) at 25 °C and pH = 7.0. [ANS] = 1.2 × 10^{-5} mol dm^{-3}, *H_2O Raman. (*b*) [β-CD_{xen}] = 0.00 (**1**) to 7.00 × 10^{-3} mol dm^{-3} (**15**) at 25 °C and pH = 3.5. [ANS] = 2 × 10^{-5} mol dm^{-3}, *H_2O Raman.

and the dependence of I_F on pH is not observed in the probes alone, the latter interpretation seems to be reasonable.

The pH dependence of I_F for **2**–ANS is quite different to that for **1**–ANS. The value of I_F for **2**–ANS increases with decreasing pH. The increase in the I_F value for **2**–ANS at lower pH appears to be closely related to the protonation of the ethylenediamine group of **2**. The pH values at the inflection points in Fig. 1 do not always correspond to the pK_a values of **2** (pK_{a_1} = 5.6 and pK_{a_2} = 9.2).[11b] An intriguing difference in the I_F–pH profiles is observed between **2**–ANS and **2**–TNS (**2**–MANS), suggesting that the association modes between ANS and TNS (MANS) with **2** are quite different. Particularly, the I_F for **2**–TNS and **2**–MANS begins to drop gradually at lower pH values in contrast to that for **2**–ANS. The pH values at the inflection point at the acidic, slightly alkaline (*ca.* pH = 9) and alkaline pH region are in agreement with the pK_{a_1} (=5.6) and pK_{a_2} (=9.2) of **2**, and the pK_a (=12.01) of the secondary hydroxy group of β-CD_x, within experimental error. The drop in I_F at lower pH values would be caused by the more external position of the anilino moiety of TNS (MANS) from the CD_x cavity which is enforced by the electrostatic interaction between the $-SO_3^-$ site of TNS (MANS) and the $-NH_2^+$-$CH_2CH_2NH_3^+$ site of **2** (*vide infra*).

Johnson *et al.* reported an interesting pH dependence of I_F for the ANS–α-chymotrypsin (CHT) system; an appreciable decrease in I_F is observed as the pH value increases from 3 to 8 and there is a red shift of λ_{max}(em) from 484 to 505 nm.[14] This pH effect on I_F of ANS–α-CHT can be interpreted in terms of a pH-dependence equilibrium between α-CHT conformers differing in the degree of mobility of polar residues and water molecules at the ANS binding site or structural changes in the ANS binding site.

Stability constants of the β-cyclodextrin complexes

A typical example of the fluorescence spectral change of ANS in the presence of β-CD_x at a constant pH is shown in Fig. 2(*a*). A gradual increase in fluorescence intensity and a slight blue shift in λ_{max} were observed. It has been already observed that ANS, TNS and MANS showed pronounced fluorescence enhancement when they bind with α-, β- and γ-cyclodextrins.[8] ANS could form a 1 : 1 inclusion complex with β-CD_x. If the observed fluorescence increments (ΔI) are proportional to the concentration of the formed inclusion complex, then the relationship shown in eqn. (1) is derived under the condition of

$$\Delta I^{-1} = \frac{K_f^{-1}}{\Delta I_{\infty 1:1}[\beta\text{-}CD_x]_{tot}} + \frac{1}{\Delta I_{\infty 1:1}} \quad (1)$$

$[\beta\text{-}CD_x]_{tot} \gg [ANS]_{tot}$, where $[\beta\text{-}CD_x]_{tot}$, K_f and $\Delta I_{\infty 1:1}$ are the total concentration of β-CD_x, the stability constant of the 1 : 1 inclusion complex and the fluorescence intensity at infinitive [β-CD_x] when all ANS are bound to β-CD_x. The binding curve between β-CD_x and ANS at pH 7.0 is shown in Fig. 3(*a*). The plot of ΔI^{-1} *vs.* $[\beta\text{-}CD_x]^{-1}$ is linear as shown in Fig. 4(*a*). The values of K_f and $\Delta I_{\infty 1:1}$ are evaluated from the slope and intercept of the straight line, respectively. The data thus obtained are finally optimized for a 1 : 1 host–guest model by a non-linear least-squares method (Damping Gauss–Newton method).

Electrostatic effect upon stability constants

The host molecules **2** and **3** in the alkaline pH region exist in the neutral form owing to the deprotonation of amino groups, but in the acidic pH region as positively charged species. For example, in the weakly acidic pH region, the host **2** could form a positively charged species as shown in eqn. (2).

The predominant species at pH 3.5, 7.0 and 11.0 are β-$CD_{xen}H_2^{2+}$, β-$CD_{xen}H^+$ and β-CD_{xen}^0, respectively, where H denotes the amino proton. The host **3** precipitated in neutral and alkaline pH regions owing to the presence of a lipophilic group such as the *N*,*N*-dibutyl group. Fig. 2(*b*) shows the fluorescence spectral change at pH 3.5 which occur when

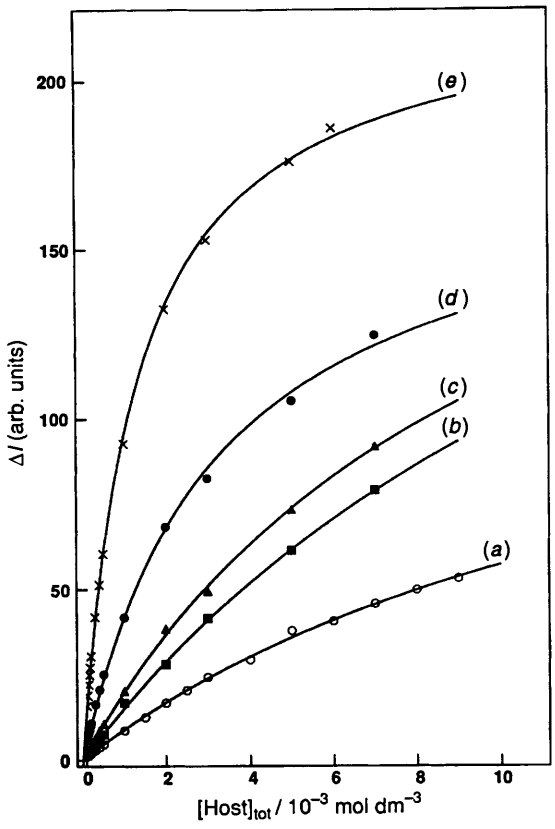


Fig. 3 Binding curves for the ANS–β-CD_x (**1**) and ANS–modified β-CD_x (**2** and **3**) systems. [ANS] = 1.2 × 10^{-5} mol dm^{-3}. (*a*) **1** (pH 7.0); (*b*) **2** (pH 11.0); (*c*) **2** (pH 7.5); (*d*) **2** (pH 3.5); (*e*) **3** (pH 3.5). The curves (*a*)–(*e*) indicate the optimized theoretical curves to the observed data points.

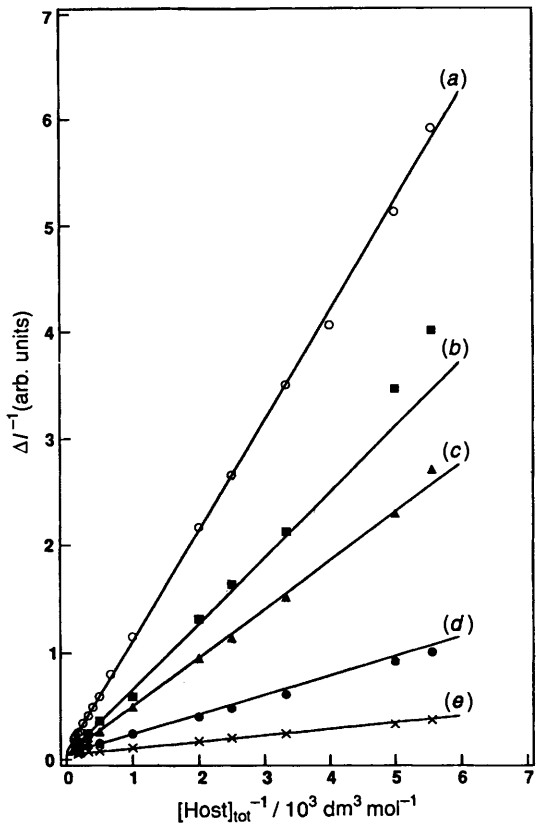
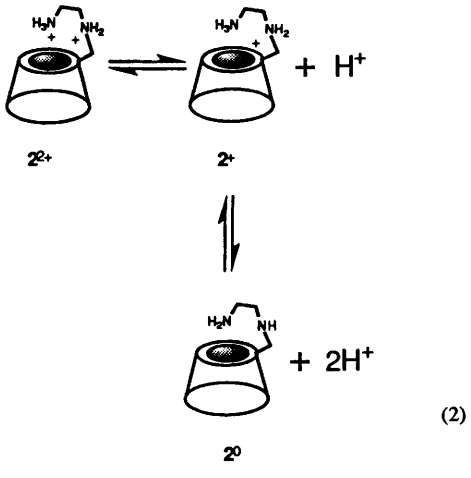


Fig. 4 Double-reciprocal plots of fluorescence intensity increment (ΔI) *vs.* host total concentration ($[Host]_{tot}$) in the ANS anion binding with **1**, **2** and **3** at 25 °C. (*a*) **1** (pH 7.0); (*b*) **2** (pH 11.0); (*c*) **2** (pH 7.5); (*d*) **2** (pH 3.5); (*e*) **3** (pH 3.5). The straight lines indicate the theoretical behaviour.



(2)

β-$CD_{xen}H_2^{2+}$ $\mathbf{2}^{2+}$ ($-NH_2^+CH_2CH_2NH_3^+$) binds to the ANS anion ($-SO_3^-$). Stronger enhancement in fluorescence intensity and a larger blue shift (*ca.* 60 nm) as compared with the β-CD_x system are observed. Fig. 3(*a*)–(*e*) shows that the order in the increment ΔI *vs.* $[Host]_{tot}$ is $\mathbf{3}^{2+}$ (pH 3.5) > $\mathbf{2}^{2+}$ (pH 3.5) > $\mathbf{2}^{+}$ (pH 7.5) > $\mathbf{2}^{0}$ (pH 11.0) > $\mathbf{1}^{0}$ (pH 7.0). It is simply suggested that the electrostatic interaction between the positively charged moiety ($-NH_2^+CH_2CH_2NH_3^+$) of $\mathbf{2}^{2+}$ and negatively charged site ($-SO_3^-$) of the ANS anion operates additionally in stabilizing the formed inclusion complex.

The most remarkable enhancement in $\mathbf{3}^{2+}$ (β-$CD_{xdben}H_2^{2+}$) at pH 3.5 is ascribed to the extended hydrophobic environment due to the *N*,*N*-dibutyl group. Analysis of the plots of ΔI^{-1} *vs.* $[\beta\text{-}CD_{xen}]^{-1}$ and $[\beta\text{-}CD_{xdben}]^{-1}$ shown in Fig. 4(*a*)–(*e*) yields the stability constants (Table 1). In most cases, the inclusion complex of the charged β-CD_x is more stable than that of the native β-CD_x.

The order in stability is closely related to the increase in the positive charge, 0 < +1 < +2. Particularly, in the double positively charged **3** system, the ratio $K_f(\mathbf{3}^{2+})/K_f(\mathbf{1})$ goes up to *ca.* 10.6. As already pointed out above, this stabilization effect is due to the formation of the electrostatic interaction between the positively charged site (+2 and +1) of **2** and **3** and the negatively charged site (−1) of the ANS anion in the formed inclusion complex. However, in non-charged β-CD_{xen} (pH 11.0) system, the stability is reduced to the original value in β-CD_x system [$K_f(\mathbf{2}^0)/K_f(\mathbf{1})$ = *ca.* 0.9]. Fig. 5 shows the effect of ionic strength on the fluorescence intensity at a fixed concentration of **2**. The presence of such an effect particularly at pH 3.5 is consistent with the view that a strong electrostatic interaction is involved in the formation of the ANS–$\mathbf{2}^{2+}$ inclusion complex.

Fluorescence probe such as 1-anilinonaphthalene (1-AN) with no charge ($-SO_3^-$) could interact with the charged β-CD_x ($\mathbf{2}^{2+}$), but the stabilizing effect was not observed because of the absence of the electrostatic interaction. The stability constant for the 1-AN–$\mathbf{2}^{2+}$ system (K_f = 380 dm^3 mol^{-1}) is reduced compared with that for the 1-AN–**1** system (K_f = 710 dm^3 mol^{-1}). This reduction in stability is mainly due to the decrease in the hydrophobicity at the periphery of the positively charged site, $-NH_2^+CH_2CH_2NH_3^+$, of $\mathbf{2}^{2+}$. On the other hand, this type of decrease in the hydrophobicity at the periphery of $-NMe_2^+CH_2CH_2NMe_2H^+$ in the host $\mathbf{4}^{2+}$ (β-CD_{xtmen}) was not observed; both the stability constants of the 1-AN–β-CD_x and 1-AN–$\mathbf{4}^{2+}$ systems are almost the same (K_f = 680 dm^3 mol^{-1} for the 1-AN–$\mathbf{4}^{2+}$ system).

The role of the metal ion, Zn^{2+}, in the stabilizing effect is quite interesting. β-CD_{xen} forms a 1:1 Zn^{2+} complex in a large excess of Zn^{2+} at pH 7.0. The formed β-$CD_{xen}Zn^{2+}$ host molecule could also bind with the ANS anion. Its stability (K_f = 150 dm^3 mol^{-1}) is *ca.* 2.2 times larger than that of the β-CD_x inclusion complex (K_f = 70 dm^3 mol^{-1} in Table 1).

Table 1 Stability constants (K_f/dm^3 mol^{-1}) of the inclusion complexes of ANS with **1**, **2** and **3** at 25 °C

Host	pH	Charge	K_{f1}/dm^3 mol^{-1}	K_f(**2** or **3**)/K_f(**1**)
β-CD$_x$ **1**	7.0	0	69	1.0
			64 (± 7)[a]	
			110 (± 4)[b]	
			71 (± 5%)[c]	
β-CD$_{xen}$ ^{0} **2**^{0}	11.0	0	63	0.9
β-CD$_{xen}$H$^+$ **2**$^+$	7.5	+1	98	1.4
β-CD$_{xen}$H$_2^{2+}$ **2**$^{2+}$	3.5	+2	300	4.4
β-CD$_{xdben}$H$_2^{2+}$ **3**$^{2+}$	3.5	+2	729	10.6

[a] Ref. 8*b*. [b] Ref. 8*c*. [c] Ref. 8*d*. Error limits in K_f values are estimated to be less than ± 5%.

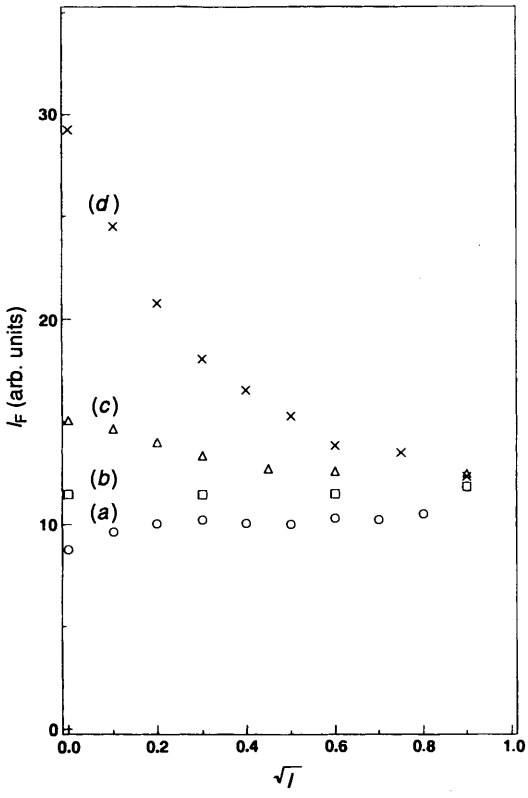


Fig. 5 Dependence of the fluorescence intensity (I_F) of ANS–**1** and ANS–**2** complexes on ionic strength (I) at 25 °C. [**1**] = [**2**] = 5 × 10^{-4} mol dm^{-3} and [ANS] = 1.2 × 10^{-5} mol dm^{-3}. Sodium chloride was added to adjust the ionic strength. (*a*) **1** (pH 7.0); (*b*) **2** (pH 11.0); (*c*) **2** (pH 7.5); (*d*) **2** (pH 3.5).

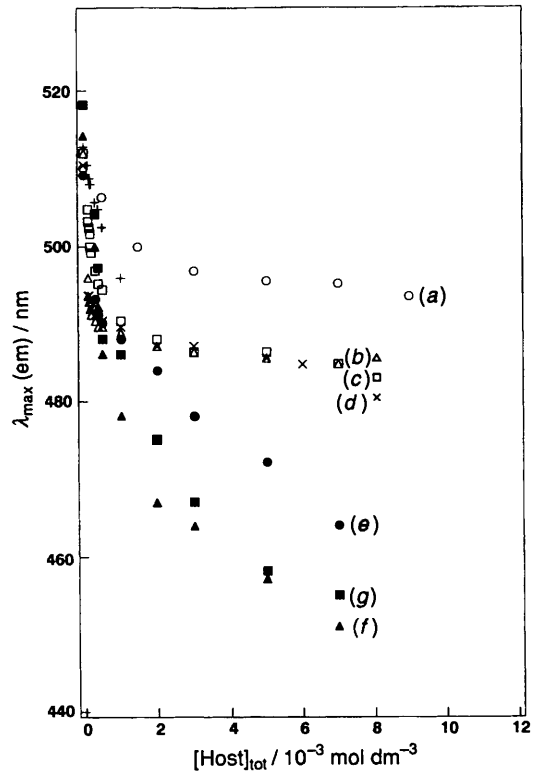
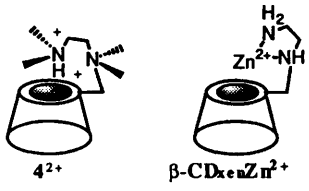


Fig. 6 Dependence of λ_{max}(em) of the ANS anion upon the various host concentrations from 0.00–7.00 (9.00) × 10^{-3} mol dm^{-3} at 25 °C. [ANS] = 1.2 × 10^{-5} mol dm^{-3}. (*a*) **1** (pH 7.0); (*b*) **3**$^{2+}$ (pH 3.5); (*c*) **4**$^{2+}$ (pH 3.5); (*d*) **4**$^+$ (pH 11.0); (*e*) **2**$^{2+}$ (pH 3.5); (*f*) **2**$^+$ (pH 7.5); (*g*) **2**^{0} (pH 11.0).



Perhaps, the negative –SO_3^- site of ANS anion interacts with the positive Zn^{2+} site of β-CD$_{xen}$$Zn^{2+}$.

Blue shifts in λ_{max}(em) of the charged β-CD$_x$ systems

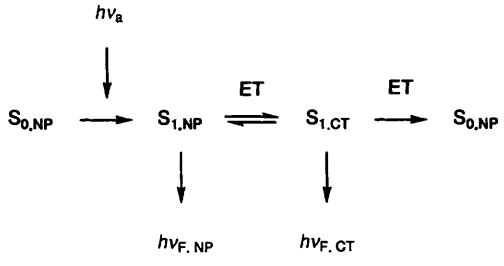
ANS has been used extensively as an anionic fluorescent probe of biological macromolecules and membranes.[14] In contrast to the weak green fluorescence of ANS in aqueous solutions, an intense blue fluorescence is exhibited when ANS binds with proteins and membranes. ANS can be used to give an indication of the degree of polarity of specific binding sites. Therefore, the emisson wavelength maximum [λ_{max}(em)] is very sensitive to the microenvironment of the anionic ANS probe. Significant blue shifts in λ_{max}(em) have been reported in some biological macromolecule systems such as α-chymotrypsin [$\Delta\lambda_{max}$(em) = 21 nm from 505 to 484 nm][13] and apomyoglobin [$\Delta\lambda_{max}$(em) = 60 nm].[15] Fig. 6 shows the host concentration dependence of λ_{max}(em) in our host–guest systems.

The tendencies of these blue shifts in λ_{max}(em) are mainly divided into three groups; (*a*) a non-charged host such as β-CD$_x$ **1**, (*b*) a charged host β-CD$_{xtmen}$ **4** (**4**$^+$) having no hydrogen-bonding ability with –SO_3^- of ANS anion and (*c*) a charged host β-CD$_{xen}$ **2** (**2**$^{2+}$ and **2**$^+$) having hydrogen bonding ability. The blue shift in λ_{max}(em) of the ANS–**1** system [$\Delta\lambda_{max}$(em) = *ca.* 17 nm] is attributable to the inclusion of the ANS anion into the hydrophobic cavity of β-CD$_x$. The presence of the electrostatic interaction as found in the ANS–**3**$^{2+}$ system causes a larger blue shift [$\Delta\lambda_{max}$(em) = *ca.* 27 nm]. Note that the host **4**$^+$ (pH 11) having no hydrogen-bonding site and **4**$^{2+}$ which has one hydrogen-bonding site also have the same value of blue shift as the host **3**$^{2+}$, which has three hydrogen-bonding sites. The presence of the –NH_3^+ or –NH_2 group would be indispensable to the large blue shift in λ_{max} as observed in **2**$^{2+}$,

2^+ and 2^0. In the presence of both the electrostatic interaction and the hydrogen bonding ability such as $-SO_3^- \cdots H_3N^+-CH_2CH_2NH_2^+-$ of the ANS–2^{2+} and ANS–2^+ complexes, the emission peak is shifted from green to blue by *ca.* 60 nm. Interestingly, even in the 2^0 (pH = 11.0) having no charge, the significant blue shifts were also observed.

In general, the dependence of λ_{max}(em) on solvent polarity can be interpreted in terms of a dipole orientation mechanism.[15,16] Chromophores with higher dipole moments in the excited state than in the ground state will exhibit this particular dependence on solvent. The dipolar excited state of the chromophore interacts with a polar solvent so as to orient the solvent dipole, whereas the solvent shell in a non-polar solvent is less perturbed. Thus, the emission is shifted to the blue in a non-polar solvent. Probably the enhancement of the fluorescence of ANS anion included in the β-CD_x cavity having an apolar character would resemble the strong fluorescence enhancement of the ANS anion surrounded by non-polar organic solvent molecule. This rule is not valid in the charged system.

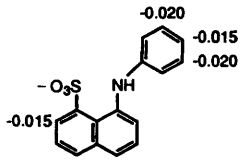
ANS exhibits variations in fluorescence maximum [λ_{max}(em)] and quantum yield (Ψ_F) with solvent polarity and substituent change. Kosower and Hanety pointed out that the fluorescences of ANS arise from two different states, a naphthalene-excited state ($S_{1.NP}$) and a charge-transfer state ($S_{1.CT}$) from the anilino moiety (δ+) to the naphthalene moiety (δ−).[17] The initially formed excited state ($S_{1.NP}$), for which λ_{max}(em) and Ψ_F vary little with solvent and substituent change, undergoes an intramolecular electron-transfer reaction (ET) to form the charge-transfer state ($S_{1.CT}$) for which λ_{max}(em) and Ψ_F vary greatly with solvent and substituent change. The latter returns to the ground state ($S_{0.NP}$) *via* another electron-transfer process. In the $S_{1.CT}$ state, a positively charged phenyl ring is non-coplanar with a negatively charged naphthalene ring.[17]



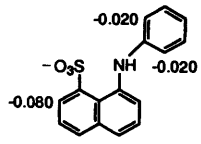
The λ_{max}(em) value in non-polar solvents arises from the $S_{1.NP}$ state and the great sensitivity of λ_{max}(em) can be explained only by the $S_{1.CT}$ state.[17] In 99.9% (v/v) dioxane–water solvent, λ_{max}(em) is 471 nm which is somewhat larger than the λ_{max}(em) found in the **2**–ANS system (451.10–464.10 nm). The electrostatic interaction and the hydrogen bonding particularly observed in the **2**–ANS system would prevent the formation of $S_{1.CT}$ and/or the radiative and non-radiative process from $S_{1.CT}$ to $S_{0.NP}$. Measurement of the fluorescence lifetime of ANS in the presence of **2** is needed to clarify the two effects on λ_{max}(em).

Possible structure of 2^{2+}–ANS inclusion complex

The fluorescence data are strongly suggestive of the presence of the electrostatic interaction in the 2^{2+}–ANS system, but its structure in solution is not clear. Complexation induced ^{1}H NMR shifts (CIS, in ppm) at 100% complexation for the ANS–β-CD_x system are shown below. Shielding effects were too weak, but discernible upfield shifts, were observed particularly in the phenyl ring groups of the ANS anion. These upfield shifts of the phenyl ring protons might be caused by C–C bond anisotropy of β-CD_x.[18] Schneider *et al.* stated that the geometry information obtained from the ROESY peaks, as well

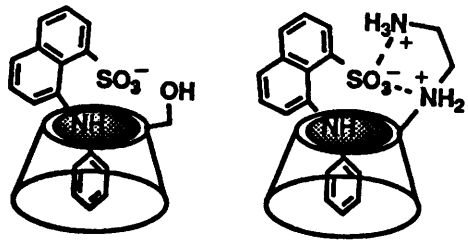


ANS–β-CDx **1**



ANS–β-CDxenH_2^{2+} 2^{2+}

as from the CIS values of the ANS–β-CD_x system, are as much consistent as surprising:[8e] only the phenyl ring of the ANS anion as the smaller component shows cross peaks and upfield CIS,[8e] although the larger naphthalene moiety is generally assumed to fit better.[19] The NMR data mentioned above clearly supports the possible structure of the ANS–β-CD_x inclusion complex as shown below.



Nishijo's ^{1}H NMR[8d] data also supports this structure of the ANS–β-CD_x inclusion complex. It is suggested from the CPK molecular model's consideration that in this conformation of the ANS anion within the β-CD_x cavity, the phenyl ring is tightly bound, but the naphthalene moiety is situated partially outside of the β-CD_x cavity due to the steric hindrance between the $-SO_3^-$ and the primary hydroxy group side of β-CD_x. A small negative enthalpy (ΔH^0 = −1.9 kcal mol^{-1} at 298 K) and a small positive entropy (ΔS^0 = 2.1 cal mol^{-1} K^{-1} at 298 K)[8d] could be interpreted by this relatively weak binding mode. In the ANS–2^{2+} inclusion complex, a significant upfield shift (−0.080 ppm) of a proton to the $-SO_3^-$ group of the ANS anion was observed. This might be due to the electrostatic interaction and/or the intermolecular hydrogen bonding between $-SO_3^-$ of the ANS anion and $-NH_2^+CH_2CH_2NH_3^+$ of 2^{2+}. This electrostatic interaction is impossible for complexation from the secondary -OH side of CD_x. The intermolecular hydrogen bonding would be related to the blue shifts in λ_{max}(em) (*vide supra*) and the electrostatic interaction found in the ANS–2^{2+} system in which the motional freedom of the ANS anion is more restricted than in aqueous solution might cause the stronger binding and stronger fluorescence enhancement.

Other guest systems (TNS and MANS)

Our observation of the formation of 1:1 and 1:2 (guest:host) inclusion complexes in both TNS–β-CD_x systems is in good agreement with earlier publications.[8c,8g,20] The results of the formation of 1:1 and 1:2 inclusion complexation in the MANS–β-CD_x system does not agree with the earlier literature report which states that only 1:1 complexation occurs,[21] as pointed out first by Catena and Bright.[8c] The plots of ΔI *vs.* $[Host]_{tot}$ and ΔI^{-1} *vs.* $[Host]_{tot}^{-1}$ are shown in Figs. 7 and 8, respectively.[8g]

The double reciprocal plots in Fig. 8 exhibit two discrete linear regions, indicating the formation of a 1:1 complex in the lower $[Host]_{tot}$ region and 1:2 complex in higher $[Host]_{tot}$ region. These fluorescence titration data are analysed using the following relationship.

Table 2 Stability constants (K_f/dm^3 mol^{-1}) of the inclusion complexes of TNS and MANS anion with **1** and **2** at 25 °C

Guest–Host	pH	Charge	K_{f1}/dm^3 mol^{-1}	K_{f2}/dm^3 mol^{-1}	$K_{f1}(\mathbf{2})/K_{f1}(\mathbf{1})$
TNS–**1**	7.0	0	2 700	15	1.0
			1 538[a]	14[a]	
			2 500 (± 100)[b]	340 (± 23)[b]	
TNS–$\mathbf{2}^0$	11.0	0	2 800	*c*	1.0
TNS–$\mathbf{2}^+$	7.5	+1	5 400	6.7	2.0
TNS–$\mathbf{2}^{2+}$	3.5	+2	11 500	15	4.3
MANS–**1**	7.0	0	11 700	*d*	1.0
	6.0	0	7 700 (± 105)[b]	320 (± 16)[b]	
MANS–$\mathbf{2}^{2+}$	3.5	+2	87 000	37	7.4

[a] Ref. 8*g*. [b] Ref. 8*c*. Error limits in K_{f1} values are estimated to be less than ± 5%. [c] 18.5 (± 36). [d] 1 (± 1).

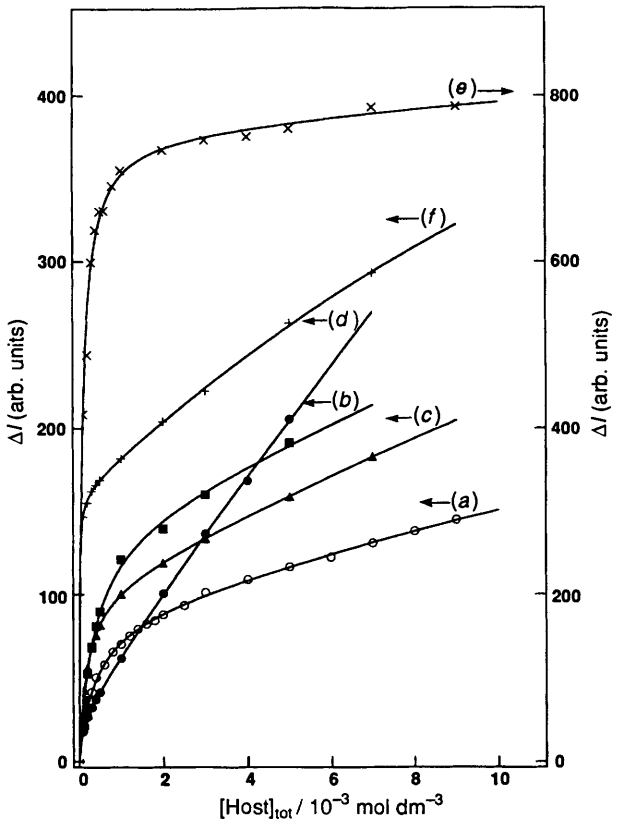


Fig. 7 Binding curves for the TNS(MANS)–β-CD$_x$ (**1**) and TNS(MANS)–modified β-CD$_x$ (**2**) systems. [TNS] = [MANS] = 1.2 × 10^{-5} mol dm^{-3}. (*a*) **1**–TNS (pH 7.0); (*b*) **2**–TNS (pH 11.0); (*c*) **2**–TNS (pH 7.5); (*d*) **2**–TNS (pH 3.5); (*e*) **1**–MANS (pH 7.0); (*f*) **2**–MANS (pH 3.5). The curves (*a*)–(*f*) indicate the optimized theoretical behaviour using the calculated parameters.

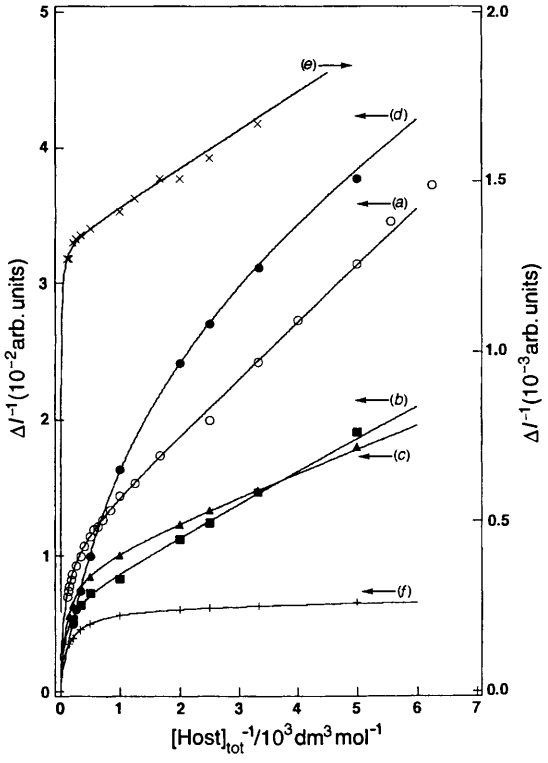


Fig. 8 Double-reciprocal plots of fluorescence intensity increment (Δ*I*) *vs.* host total concentration ([Host]$_{tot}$) in the TNS and MANS anions binding with **1** and **2** at 25 °C. (*a*) **1**–TNS (pH 7.0); (*b*) **2**–TNS (pH 11.0); (*c*) **2**–TNS (pH 7.5); (*d*) **2**–TNS (pH 3.5); (*e*) **1**–MANS (pH 7.0); (*f*) **2**–MANS (pH 3.5). The solid lines indicate the theoretical behaviour.

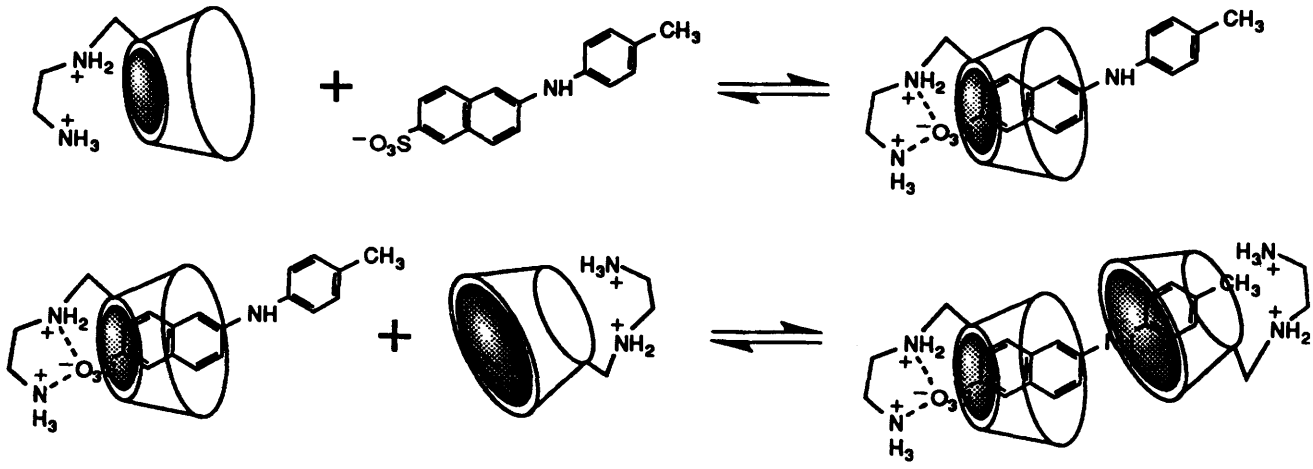
$$\text{TNS} + \beta\text{-CD}_x \overset{K_{f1}}{\rightleftharpoons} \text{TNS–}\beta\text{-CD}_x + \beta\text{-CD}_x \overset{K_{f2}}{\rightleftharpoons} \text{TNS–}(\beta\text{-CD}_x)_2 \quad (3)$$

$$\Delta I = \frac{\Delta I_{\infty 1:1}}{1 + \dfrac{K_{f1}^{-1}}{[\text{Host}]_{tot}} + \dfrac{[\text{Host}]_{tot}}{K_{f2}^{-1}}} + \frac{\Delta I_{\infty 1:2}}{1 + \dfrac{K_{f2}^{-1}}{[\text{Host}]_{tot}} + \dfrac{K_{f1}^{-1}K_{f2}^{-1}}{[\text{Host}]_{tot}^2}} \quad (4)$$

Using the parameters such as K_{f1}, K_{f2}, $\Delta I_{\infty 1:1}$ and $\Delta I_{\infty 1:2}$ obtained from the double reciprocal plots in Fig. 8, the optimized stability constants were estimated by the Damping Gauss–Newton method (Table 2).

The order of increasing K_{f1} for the 1:1 β-CD$_x$ inclusion complex is ANS (69 dm^3 mol^{-1}) ≪ TNS (2700 dm^3 mol^{-1}) < MANS (11 700 dm^3 mol^{-1}). It could be that the strong binding constant in TNS–β-CD$_x$ system is caused by the encapsulation of the naphthalene moiety of the TNS anion. Location of the *N*-substituted methyl group in the MANS anion must lead to a further enhancement in the stability of the 1:1 complex. If this postulation is correct, larger stability is expected in the TNS complex with the charged β-CD$_x$ such as β-CD$_{xen}$H$_2^{2+}$. The observed K_{f1} value for the TNS–$\mathbf{2}^{2+}$ system is 11 500 dm^3 mol^{-1} and *ca.* 4.3 times larger than that for TNS–**1** system (2700 dm^3 mol^{-1}). This enhancement factor of 4.3 is almost the same as that of 4.4 in the ANS–$\mathbf{2}^{2+}$ system. Therefore, the charged host $\mathbf{2}^{2+}$ must form the 1:1 inclusion complex with the TNS anion on the naphthalene moiety as show in Scheme 1.

The second binding step seems to be completely unaffected by the presence of the charged site of the host $\mathbf{2}^{2+}$ (K_{f2} = 15 dm^3 mol^{-1} for TNS–β-CD$_x$ and K_{f2} = 15 dm^3 mol^{-1} for TNS–$\mathbf{2}^{2+}$).



Scheme 1

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