The Association of Inclusion Complexes of Cyclodextrins with Azo Dyes

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Abstract. Orange II (**4**) and γ cyclodextrin (CD) form 2 : 1 and 2 : 2 complexes. The complexes self-associate and microscopy indicates the formation of a fibroid aggregate. In the induced c.d. spectrum, the $\pi \rightarrow \pi^*$ band of this complex appears at $\sim$500 nm in solution, but in the aggregate it changes to a *J*-band due to the head-to-tail stacking of **4** and a *H*-band due to its parallel stacking; this indicates that the aggregation expands not only in the direction of the symmetry axis of the CD, but also in the other two dimensions. ^{2}H-NMR spectroscopy from deuteron exchange and solvation between the aggregate and deuterium oxide exhibits quadrupole splitting in the region of $0-0.2$ KHz. The orientation behavior obtained from this splitting suggests the formation of a liquid-crystaloid substance. ^{13}C-T_1 NMR indicates that molecules **4** and γ CD show the same T_1 values even at 333 K; this complex behaves like a single molecule. The behaviors of other azo dye-CD complexes are also discussed.

Key words: Association, orientation, ^{2}H-NMR, ^{13}C-NMR, circular dichroism, cyclodextrin-azo dye complex.

1. Introduction

In previous publications the following aspects of cyclodextrin (CD)-azo dye complexes have been studied: (1) the formation of the complexes and their various stacking modes have been detected by circular dichroism (c.d.) spectra [1]. The c.d. spectra in the inclusion complexes of CDs with azobenzene derivatives generally show a single peak and a positive sign in the 1st $\pi \rightarrow \pi^*$ region of the N $=$ N group, indicating that the long and slim azobenzene derivatives are included into the cavity from the long axis side [2]. The c.d. spectra sometimes form splitting patterns which may be caused by exciton interaction of two molecules of the chromophoric dye each in the form of 1 : 1 complexes with γ CD. The splitting patterns change signs and magnitudes according to the substitution on the compo-

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** Dedicated to Professor József Szejtli.

TABLE I. Azo dyes used.

1 Tropaeolin

NaO_3S—〈 〉—N=N—〈 〉—NH—〈 〉

2 Metanil Yellow

NaO_3S—〈 〉—N=N—〈 〉—NH—〈 〉

3 Methyl Orange

NaO_3S—〈 〉—N=N—〈 〉—$N(CH_3)_2$

4 Orange II

NaO_3S—〈 〉—N=N—〈HO naphthalene〉
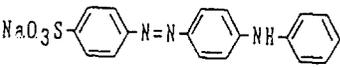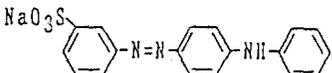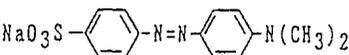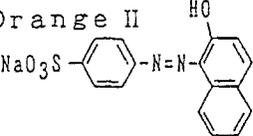
nent molecules and prove the formation of the various stacking modes. When the complex self-associates, a linear dichroism band becomes dominant; these signals may not be due to the absorption of light by the individual molecule, but due to the molecular arrangement [3]. (2) ^{2}H-nuclear magnetic resonance (NMR) spectra have been used to quantitatively estimate the orientation of the aggregate as it shows splitting of the signal by the aligned structure. A change of the aggregate induced by magnetic field, dilution, temperature, and solvent has been followed quantitatively by this splitting [4]. (3) From the relaxation time (T_1) of ^{13}C-NMR [5], the correlation time for molecular re-orientation (τ) of the azo dye included into CD and an average T_1 value of the individual aromatic ring which constitutes the azo dye have been measured and the portion of the guest molecule which interacts with the host molecule has been estimated. This paper presents an overview of the association phenomena found with azo dye-complexes.

2. Materials and Method

All methods were the same as described previously [1, 3–6]. Continuous variation plots of the visible spectra show that the host : guest ratio in the complexes was 1 : 1, except for the γ CD complexes where the ratio was 1 : 2 [7]. In this paper, azo dyes in which one or two sides of the N = N bridge bear benzene or naphthalene nuclei (1–4) are used (Table I) and inclusion compounds with α-, β-, γ-CDs, 2, 6-dimethyl- and 2,3,6-trimethyl-β-CDs (DM-β CD and TM-β CD), low molecular mass fractions of water soluble α-, β-, and γ-CDs polymer products – epichlorohydrin (Ep) and ethyleneglycol-bis(epoxypropyl)ether (DiEp) – [6] are investigated.
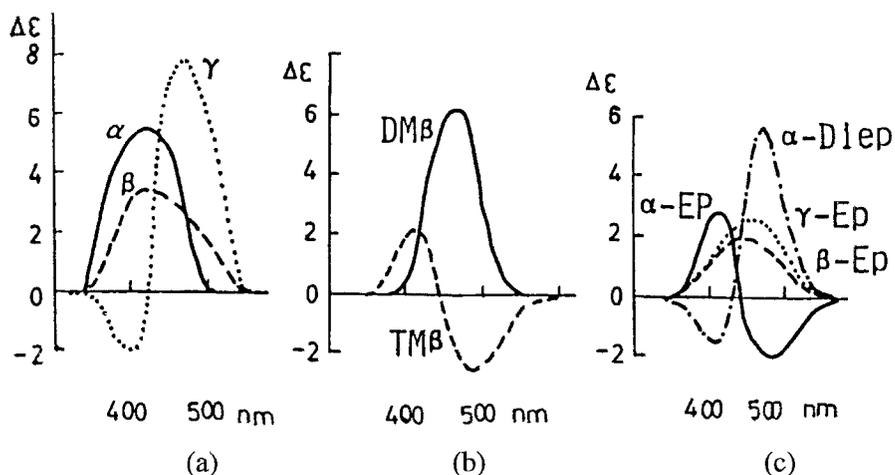
Fig. 1. c.d. Spectra of tropaeolin (**1**) complexes with (a): α CD (——), β CD (- - - -), γ CD ($\cdots$); (b): 2,6-Dimethyl-β CD (——), 2,3,6-Trimethyl-β CD (- - - -); (c): Products with the linking agents: α CD-epichlorohydrin (——), β CD-epichlorohydrin (- - - -), γ CD-epichlorohydrin ($\cdot\ \cdot\ \cdot$), α CD-ethyleneglycol bis(epoxypropyl) ether (-·-·-).
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3. Results and Discussion

3.1. THE C.D. SPECTRA

3.1.1. Complexes of Tropaeolin (1)

The $\Delta\varepsilon$ values of the single peak in the complexes of **1** are in the order of DM-β CD > α CD > β CD > γ CD-Ep > β CD-Ep [1] (Figure 1). The order is the same as that for the complex formation constants [7]. Substitution by an O-methyl group at O-2 and O-6 in β CD lengthens the cavity $\sim$8 to $\sim$11 Å and enhances the inclusion ability. Further substitution by the O-methyl group on the inner side of β CD (the 3-OH group) narrows the inner diameter of the cavity and causes negative split-type Cotton effects. The $\Delta\varepsilon$ values of both extrema in the splitting pattern are the same, and give information about the interaction of the two molecules of the chromophoric dye each in the form of a 1 : 1 complex.

The γ CD complex gives a small splitting pattern, indicating the presence of greater association of the complex or distortion of the guest molecules. The complex with α CD-Ep gives negative split type Cotton effects and the $\Delta\varepsilon$ of both extrema are the same. The complex with α CD-DiEp sows a positive split type Cotton effect and the $\Delta\varepsilon$ of the extrema are not the same. Judged from the model of the associated molecules calculated using the exciton splitting and molecular orbital theories [8], the negative split Cotton effects observed in the TM-β CD and α CD-Ep complex series suggest that the angle between two associated complexes is less than 90° and the positive Cotton effects observed in the α CD-DiEp complex series suggest that the above angle is greater than 90°. Thus the substituents on the CD torus may play
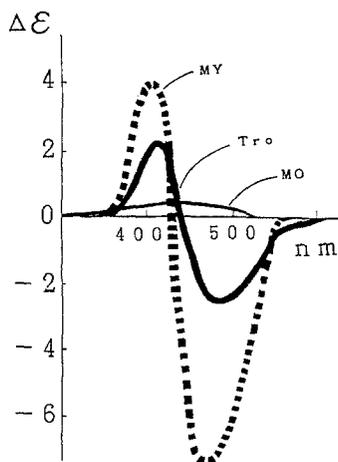
Fig. 2. c.d. Spectra of 2,3,6-trimethyl-β CD complexes: Tropaeolin (**1**) (——); Metanil yellow
(**2**) (- - - -); Methyl orange (**3**) (——).
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an important factor in deciding the angle between the longitudinal axis of the two interacting azo dye molecules and the tight fit between the host-guest interaction. On the other hand when the dyes are included with a loose fit, as in the β CD-Ep and γ CD-Ep cavities, the pattern shows a single Cotton effect.

3.1.2. The TM-β CD Complexes

The TM-β CD complexes with **1** and **2** give a negative exciton splitting (Figure 2). The $\Delta\varepsilon$ value in the complex with **2** is abnormally large in the first Cotton effect and depends on the ratio of TM-β CD : **2**. At higher concentrations of the guest (1.6×10^{-4} mol) compared with that of the host molecule (0.4×10^{-4} mol), the splitting pattern shows the same magnitudes in the peak and trough. With increasing amount of TM-β CD, the relative magnitude of the minus sign increases to twice or more when the ratio of TM-β CD : **2** is ten. Continuous variation plots of the c.d. and visible spectra show the formation of the 1 : 1 complex. Argumentation of the first Cotton effect amplitude suggests the association of more than two complexes [9]. The shorter azo dye **3** is sparingly included by TM-β CD.

3.1.3. The γ CD Complexes

The γ CD complexes give a small positive splitting in the azo benzene series as shown in the complex with **1**. But in the γ CD complex with **4**, the $\pi \rightarrow \pi^*$ band shows a negative splitting chirality and the $\Delta\varepsilon$ values of both extrema are unequal (Figure 3); the two molecules of **4** may be incorporated from the long
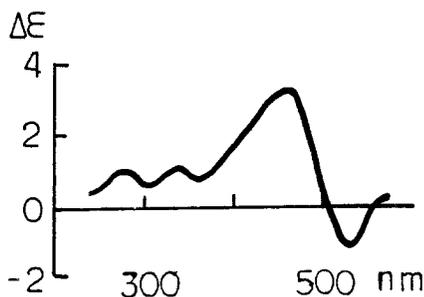
Fig. 3. c.d. Spectrum of the γ CD complex with orange II (4) in the solution state: (orange II 2×10^{-4} M and γ CD 10^{-3} M in 0.1 M phosphate buffer at pH 6.0). (Reproduced by kind permission of Elsevier Science Publishers.)
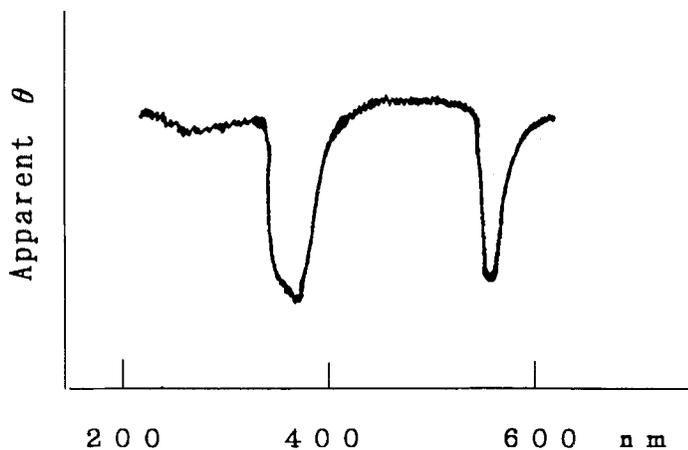
Fig. 4. c.d. Spectrum of an old aggregate in a 100 μm cell: (Orange II (4) 4.6×10^{-2} M and γ CD 10^{-1} M; 11 h after sampling). (Reproduced by kind permission of Gordon & Breach Science Publishers.)

axis side in an antiparallel arrangement [10]. Compound 4 itself is easily soluble in H$_2$O, but when γ CD is added, the solution causes enhancement of the viscosity in an hour, and microscopic viewing indicates the formation of a pine needle-like self-assemblage [3]. When it is put into a 100 μm cell, the $\pi \rightarrow \pi^*$ band at $\sim$500 nm observed in the solution state is replaced by bands at $\sim$547 nm and $\sim$353 nm, respectively (Figure 4). The former is the J-band due to the head-to-tail [11, 12] stacking of 4 and the latter is the H-band due to parallel stacking [13–15]. The same pattern appears in liquid crystal polymers [16]. The aggregate is apt to move due to perturbation when filled in the 100 μm cell. To make a well-oriented longer aggregate in a fixed condition, the chiral aggregate was fastened between quartz plates. Putting enough old millet jelly into a sandwich cell ($<$ 50 nm thickness),
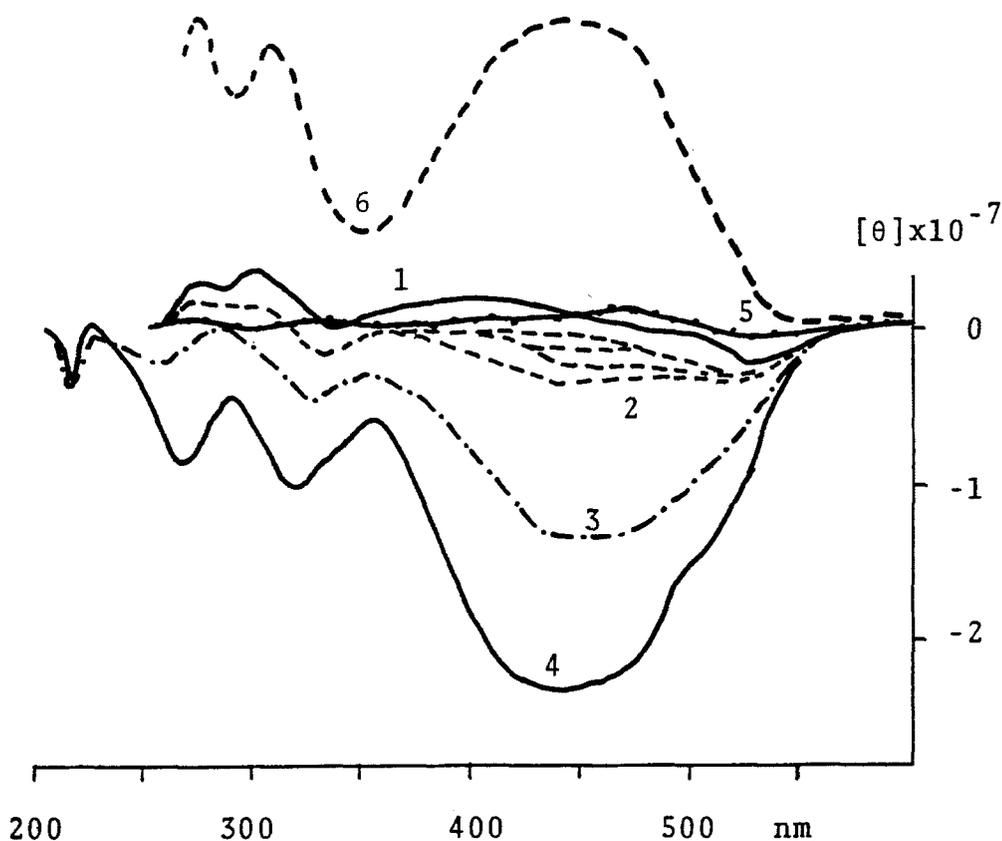
Fig. 5. c.d. Spectra of the aggregate in the sandwich cell (orange II (4) 2×10^{-2} M and γ CD 4.8×10^{-2} M): (1) 30 min. after sampling at the cell (———); (2) 1 day after sampling (- - - -); (3) 2 days after sampling (-·-·-); (4) 5 days after sampling (———); (5) solution of orange II 2×10^{-4} M and γ CD 2×10^{-3} M (0.5 cm thickness) (••••); (6) turning the cell in the plane perpendicular to an incident light beam (- - - -).
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and rubbing the upper glass plate in the transverse direction, the change of the c.d. spectrum with elapsed time was measured (Figure 5). The ϑ value of the jelly measured 11 h after sampling is nearly the same as that in the solution and the J-band cannot be observed (Figure 5, 1 and 2). No appreciable change could be detected when the cell was rotated around the light beam (Figure 5, 2). With the elapse of time, the ϑ values increase and finally reach a value forty times larger than that of the c.d. spectrum in the solution (Figure 5, 4). A spectral change could be produced by turning the cell carefully in the plane perpendicular to the incident light beam (Figure 5, 6). The aggregate contains an anisotropic part; this c.d. spectrum contains the linear dichroism related to an increase in ϑ as molecule 4 in the aggregate is oriented by rubbing [16, 17].
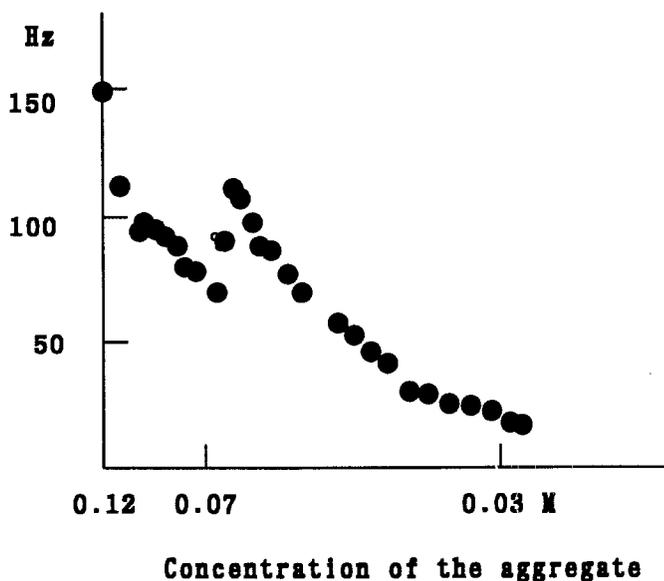
Fig. 6. Concentration dependency of the quadrupole splitting values. (Reproduced by kind permission of Elsevier Science Publishers.)

3.2. ^{2}H-NMR SPECTRA

^{2}H-NMR spectroscopy from deuteron exchange and solvation between the aggregate and deuterium oxide exhibits quadrupole splitting ($\Delta\nu$) in the region of $0 = 0.2$ KHz [4]. The orientation behavior obtained from this splitting suggests the formation of a liquid-crystaloid substance.

(1) The aggregate aligned by means of super conductive and permanent magnetic fields shows a different NMR pattern and turbidity. (2) Dilution of the 0.12 M aggregate with deuterium oxide proceeds discontinuously via the nonoriented pattern to the oriented pattern (Figure 6). (3) With an increase in temperature, the ^{2}H splitting values decrease continuously. A phase diagram that plots melting points in variable concentrations reveals separate solution and aggregate layers. (4) When the 0.06 M aggregate is diluted by deuterated solvents (water, methanol, ethanol, n-propanol, dimethyl sulfoxide, and acetone), increases in the amount added and the length of solvent molecules cause a regular reduction of $\Delta\nu$; suggesting the fragile structure of the aggregate. The deuterium signal of the methyl group in dimethylsulfoxide-d_6 exhibits a small $\Delta\nu$ value, suggesting that this solvent is included in the orientation system.

TABLE II. Rotational correlation times (τ) for complexes with tropaeolin (**1**) and cyclodextrins, and their ratios for **1** to cyclodextrin at 333 K.

	Rotational correlation times of tropaeolin included (10^{-11} s)			CD	$\tau_1{}^a/\tau_{CD}$
1[b]	NaO$_3$S $-$⟨⟩$-$ N = N $-$⟨⟩$-$ N H $-$⟨⟩				
	Ring A	Ring B	Ring C		
$+ \alpha$ CD	8	8	2	15	0.5
$+ \beta$ CD	7	10	4	16	0.6
$+$ DM-β CD	12	16	6	18	0.9
	18	18	18	18	1.0[c]
$+$ TM-β CD	16	19	50	18	1.1
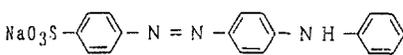
[a] The τ of tropaeolin (**1**) was calculated by using the mean value of T_1 for the carbons of ring B.
[b] Tropaeolin (**1**) itself could not be measured due to sparing solubility to H_2O.
[c] 307 K.

3.3. ^{13}C-T_1 NMR SPECTRA

Previously, the molecular motions of CD complexes with **1** have been studied on the basis of ^{13}C-T_1 measurements [5]. The T_1 value of the ^{13}C with a directly attached ^{1}H is inversely proportional to the effective isotropic correlaton time for overall molecular reorientation (τ_{eff}).

$$1/NT_1 = \hbar^2 \cdot \gamma_C^2 \cdot \gamma_H^2 \cdot r_{CH}^{-6} \cdot \tau_{eff} = \text{const} \cdot \tau_{eff}$$

where N is the number of directly bonded protons. γ_H and γ_C are the gyromagnetic ratios of the ^{1}H and ^{13}C nuclei, respectively, r_{CH} is the length of the C-H bond, and τ_{eff} is the time for the whole molecule to rotate through one radian.

In the anisotropic molecule **1**, it is possible to estimate an individual τ value for each aromatic ring which constitutes the azo dye. Table II shows all τ values for the aromatic rings in **1** included in CDs (Table II). τ values for **1** are smaller than those for the CDs; the ratios for **1** to CDs were 0.5 $-$ 0.9; thus **1** rotates up to twice as fast as the CDs. In the α CD complex, the A and B rings show the same τ values, but ring C shows a quicker τ value; **1** is included by α CD at the rings A and B side. In the TM-β CD complex, the τ value of ring C is abnormally slow compared to the other rings; **1** is included by TM-β CD near the C ring and has a

TABLE III. Relaxation times $(T_1)^a$ for orange II (**4**), complexes and cyclodextrins, and their ratios for **4** to cyclodextrin.

	temp.[b] K	molar ratio	T_1 s			$T_{1,4}/T_{1,CD}$
			benzene nucleus	naphthalene nucleus	CD	
4	307		0.27	0.26		
$+\ \beta$ CD	307		0.23	0.21	0.25	0.8
	333		0.29	0.27	0.34	0.8
$+$ DM-β CD	307		0.24	0.20	0.25	0.8
$+\ \gamma$ CD	333	2 : 1	0.34	0.31	0.30	1.0
	333	1 : 1	0.29	0.29	0.28	1.0

[a] Mean values for the carbons with the directly attached ^{1}H.
[b] Temperature.

long exposed portion in this complex. In the DM-β CD complex, the ratio for τ_1 to τ_{DM_β} at 307 K indicates that the whole molecule shows the same τ value; the complex rotates like a single molecule.

In the complexes with **4**, the β and DM-β CD complexes still permit molecule **4** to rotate in the cavity, but the γ CD complex exhibits the same T_1 values in the host and guest molecules even at 333 K; the inner side of the cavity may be so crowded as to make it easy to form the aggregate (Table III).

4. Conclusion

A scheme for the formation of the aggregate is proposed. A molecule of **4** is included quickly, but loosely-fitted into the cavity of γ CD as the 1 : 1 complex [complex formation constant for the 1 : 1 complex $(K_1) = 4.18 \times 10^2$ dm^3 mol^{-1}]. The second molecule of **4** is included slowly but securely into the same cavity and forms the 2 : 1 complex [complex formation constant for the 2 : 1 complex $(K_2) = 1.68 \times 10^6$ dm^3 mol^{-1}]. The naphthalene nucleus, the exposed portion of the second molecule of **4**, is included quickly into the cavity of the second γ CD and forms the 2 : 2 complex [complex formation constant for the 2 : 2 complex $(K_3) = 1.77 \times 10^2$ dm^3 mol^{-1}]. The benzenesulfonate part, which is the exposed portion of the first molecule of **4**, is included into the cavity of the third γ CD which has already formed a 1 : 1 complex, and forms the 3 : 3 complex, and so on.

The aggregation not only expands in the direction of the symmetry axis of the CD, but also in the other two dimensions; the aggregate is a self-assemblage of γ CD linked by inclusion of **4**. The above association constants were obtained from reference [10]. Results obtained from c.d., ^{2}H- and ^{13}C-NMR spectra, and morphology are also consistent with the structure assumed above. The precise

conformation of the fragile aggregate, including the solvent interaction, is now the subject of molecular dynamics calculations.

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