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The Complexation of 2,3-Anthracenedicarboxylate by β- and γ-Cyclodextrins in Mixed Solvent Systems as Studied by Induced Circular Dichroism. The Role of Cosolvent Hydrophobicity

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Abstract. The complexation of 2,3-anthracenedicarboxylate (ADC) by β- and γ-cyclodextrins in water containing an organic solvent has been studied by induced circular dichroism. It has been shown that an increase of organic solvent ratio causes the degradation of the 1:1 ADC : β-CD complex and the liberation of one guest molecule from the 2:1 ADC : γ-CD complex in water. The higher the hydrophobicity of the cosolvent, the weaker the complexation of ADC by β-CD.

Key words. Inclusion, stoichiometry, induced circular dichroism, β-cyclodextrin, γ-cyclodextrin, 2,3-anthracenedicarboxylate.

1. Introduction

Cyclodextrins, CDs, are torus-shaped cyclic oligosaccharides which serve as small hydrophobic environments in aqueous solution [1]. These compounds are able to act as hosts for one or two aromatic molecule(s) [2] since they have a hydrophobic cavity, in contrast to their hydrophilic exterior which is produced by the large number of hydroxyl groups on the cavity edges.

An organic solvent, when added to an aqueous solution containing CD and aromatic molecules may interact with both hosts and guests. Since the aromatic molecules are believed to be preferentially solvated by the organic component in a water-rich mixed solvent system [3], the concurrent process to cyclodextrin complexation may occur, although the inclusion of the organic solvent into the CD cavity cannot be ruled out.

Water-rich mixed solvent systems are very often used in CD complexation studies as well as in many other applications. For example, they are used as an eluent in high-performance liquid chromatography with a CD-bound column [4] or a CD-containing mobile phase [5], this method is also used for estimation of equilibrium constants of CD complexes. The modified CDs bearing aromatic moieties for biomimetic catalysis can be used only in mixed solvents because of their poor solubility in water [6, 7]. In the course of CD complexation studies on slightly water soluble guests such as pyrene, the solution was prepared by mixing a stock

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organic solvent solution of pyrene and an aqueous solution containing CD [8, 9]. Therefore a systematic study on the influence of organic solvent addition to CD complexation in aqueous solution seems to be desirable.

The aim of the present study is to systematically examine how addition of an organic solvent to an aqueous solution containing the ADC : CD complex influences the stability and stoichiometry of the CD complex. To our knowledge, such studies are scarce in the literature. The degradation of the 2 : 1 pyrene γ-CD complex to a three-component complex after 2-methyl-1-propanol addition has been detected by fluorescence spectroscopy [10]. Recently, pyrene : CD complexes in 10% aliphatic alcohol–water mixtures have been studied in terms of fluorescence lifetime [11] as well by structure-sensitive monomer emission [12]. On the other hand, a few studies [6, 7, 13] have presented the influence of medium composition on fluorescence or induced circular dichroism (icd) spectra of chromophore-attached CDs.

We chose for our study 2,3-anthracenedicarboxylate (ADC) as a model guest compound. This molecule has an aromatic moiety and water soluble neighbouring functional groups. Therefore it seems ideal for CD complexation studies. When a compound like anthracene sulphonate [14] or ADC forms a 1 : 1 complex with β-CD, they produce a positive icd around 260–270 nm. Its maximum matches approximately the wavelength of the absorption maximum which is ascribed to the longitudinally polarized ^1B_b transition [15, 16]. In contrast, when a 2 : 1 stoichiometry of the ADC : γ-CD complex is confirmed, they produce a clear exciton coupling type icd with an inflection point matching the wavelength maximum of the icd spectrum of the 1 : 1 complex [8]. If no complexation occurs, then, of course no icd signal is produced. Therefore, from the magnitude and the shape of the icd signal we can judge the degree of complexation of the ADC molecule as well as the stoichiometry of the inclusion complex. This method seems to be more useful for CD complexation studies in water-rich mixed solvent systems than the fluorescence based methods. The fluorescence spectrum is very sensitive for probe environment [17] but the fluorescence signal may be influenced by both CD complexation and preferential solvation, which may lead to an ambiguous interpretation. The icd signal does not appear unless a CD makes an inclusion complex with the chromophore molecules.

We adopt in our studies nine solvents which differ considerably in their properties such as polarity, acidity–basicity and hydrophobicity. This is because only by the use of a wide range of solvents, is it possible to deduce which property of the added solvent influences most the CD complexation process in water-rich mixed solvent systems.

2. Experimental

2.1. MATERIALS

ADC was prepared according to the literature [17, 18] and recrystallized from ethanol. Analysis: calc. for $C_{16}H_{10}O_4$: C, 72.18; H, 3.79, found: C, 72.33; H, 3.70. β-CD (Nakarai Chemicals, guaranteed reagent) and γ-CD (gift from Dr. N. Nakamura (Japan Maize Products Ltd.)) were used without further purification.

The solvents: propylene carbonate, PC (Tokyo Kasei), dioxane, DX (Koso Chemicals), tetrahydrofuran, THF, acetonitrile, ACN (Wako Chemicals), dimethylsulphoxide, DMSO, ethylene glycol, EG (Kanto Chemicals), butanol, BuOH, 2-methyl-1-propanol, 2MP and 2-butoxyethanol, 2BE (Nakarai Chemicals) were guaranteed reagents and were used without further purification.

2.2. MEASUREMENTS

Icd spectra were measured on a JASCO J-400X spectropolarimeter and absorption spectra on a Shimadzu UV-250 spectrophotometer.

2.3. PROCEDURES

In all cases, first the spectra of 0.1 mM ADC + 1 mM β- or γ-CD in phosphate buffer (pH = 9) were recorded using a 1 mm cell. It was confirmed by means of a Job continuous variation plot that 1 : 1 and 2 : 1 complexes with β-CD and γ-CD, respectively, mainly exist in this solution (Figure 1). The known amount of organic solvent was added directly to the cell using a microsyringe, mixed well and then spectra were recorded. No effect of aging of the solution has been observed. The change of chromophore concentration after volume expansion was taken into account in the calculation of molecular ellipticity, θ. The error of quantities evaluated from the icd spectra was estimated to be 0.4×10^5 deg cm^2 dmol^{-1} for θ and *ca.* 2 nm for band peak wavelengths. When the recorded icd signal was smaller than 2×10^{-4} deg/cm, it was taken as zero.

In the case of the β-CD complex, the maximum volume concentration of organic solvent was 30%. Where phase separation occurred as was the case for 15% PC or

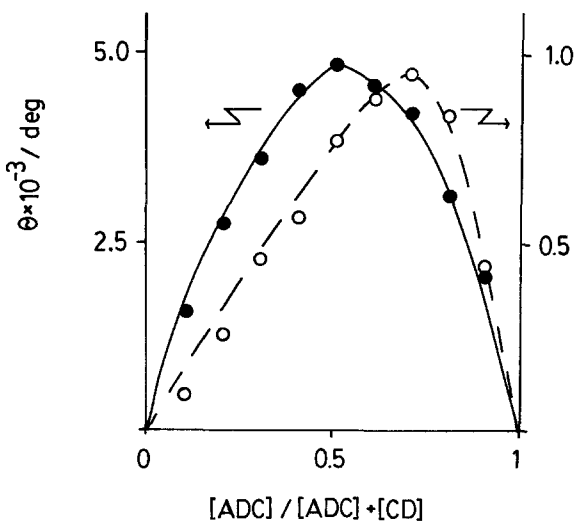

Fig. 1. Job's continuous variation plots for the determination of the complex stoichiometry; [ADC] + [β-CD] = [ADC] + [γ-CD] = 1×10^{-3} M (constant), monitored at either 384 (β-CD system, solid circle) or at 388 nm (γ-CD system, open circle) using 5 or 20 mm cell, respectively.

10% BuOH solution, the spectrum was not recorded. The maximum volume concentration of organic solvent in the experiments with the ADC : γ-CD complex was 7.8%. The further addition of organic solvent did not affect the magnitude of the icd signal, although the signal-to-noise ratio gradually increased.

3. Results

The addition of organic solvents to an aqueous solution containing the ADC : CD complexes scarcely affects the absorption spectra but dramatically alters the icd spectra for all systems studied.

In the case of the ADC : β-CD complexes, the addition of organic solvent causes a decrease of the positive icd intensity, although the wavelength of the signal maximum does not change. Some of the representative spectra recorded after addition of EG or DX are presented in Figure 2. In order to facilitate inspection of the organic solvent effect, we plot the θ/θ_w against molar fraction of organic solvent (Figure 3), where θ and θ_w are the ellipticity at 266 nm in organic solvent-water mixtures and in pure water, respectively. It is clearly visible that decrease of θ is more pronounced in the following order: EG < ACN < DMSO < DX < BuOH < PC < 2MP < 2BE. Only in the case of about a 10% molar fraction of such solvents as EG, ACN and DMSO, does the icd signal still exist.

By the addition of an organic solvent to an aqueous solution containing the ACD : γ-CD 2 : 1 complex (Figure 1), the icd spectrum changes generally as follows

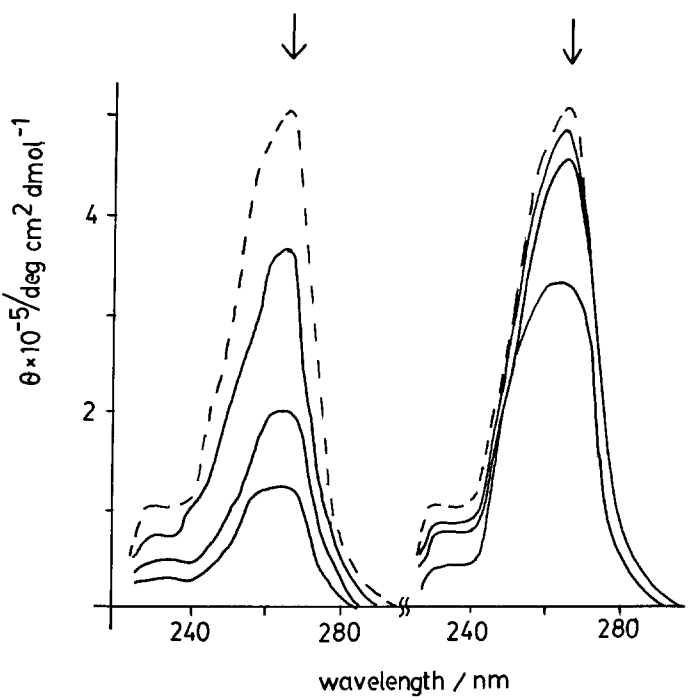

Fig. 2. The icd spectra of the ADC : β-CD system recorded in phosphate buffer (pH = 9) in water (dashed line) and after addition of 1.6, 3.2 and 7.8 volume percent of DX (left side) and EG (right side). The arrows indicate the change of spectra with increasing organic solvent concentration.

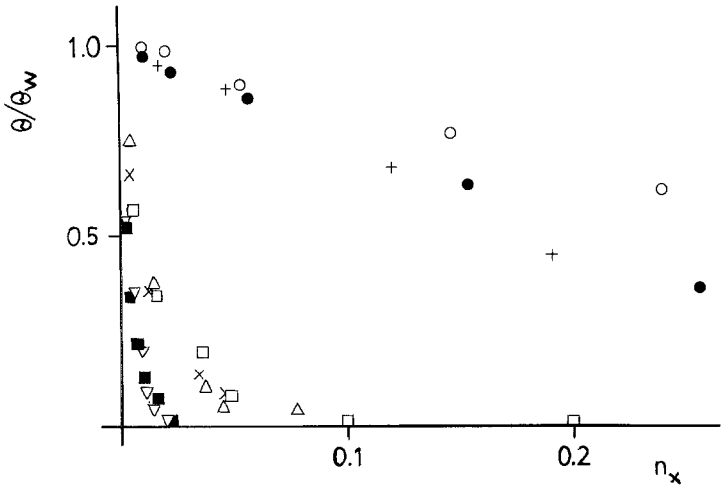

Fig. 3. The value of the ratio of θ at maximum (266 nm) recorded in water–organic solvent mixture to those recorded in water, θ/θ_w, for ADC : β-CD system vs. molar fraction of organic solvents as DMSO (+), EG (○), ACN (●), DX (□), BuOH (×), PC (△), 2BE (▽) and 2MP (■).

(Figure 4). That is, the negative ellipticity observed at *ca.* 255 nm in water changes to positive and the intensity of the positive icd signal at *ca.* 277 nm decreases, concominant with a simultaneous blue-shift of its position. In order to facilitate inspection of the organic solvent effects, the values of θ at 256 nm and at positive peaks and the wavelength position of positive peaks are plotted against molar

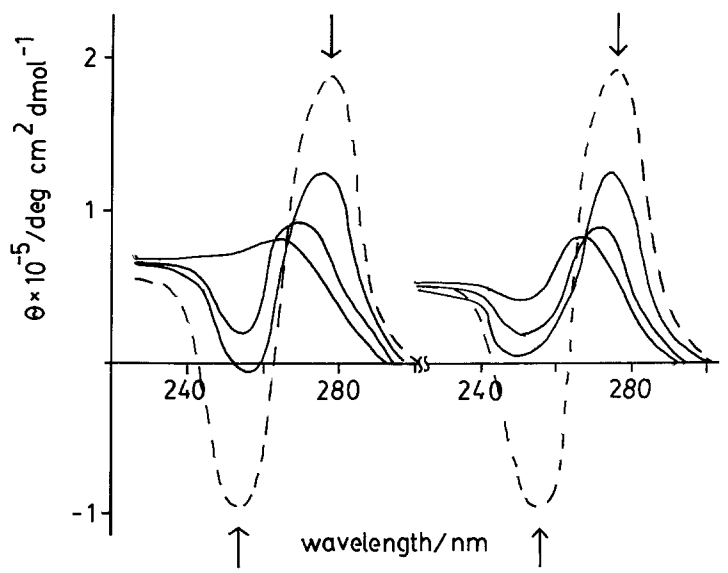

Fig. 4. The icd spectra of the ADC : γ-CD system recorded in phosphate buffer (pH = 9) in water (dashed line) and after addition of 1.6, 3.2 and 7.8 volume percent of organic solventsd as DX (left side) and EG (right side). The arrows indicate the change of spectra with increasing organic solvent concentration.

fraction of the organic solvent (Figure 5). The changes in θ or wavelength by the addition of the organic solvents are large when the fraction of organic solvent is relatively small, and at higher organic solvent concentration they saturate. The observed differences in θ or wavelength-shift among organic solvents are only slightly larger than the error estimated for these parameters. The shape of the icd spectra recorded in the ADC : γ-CD solutions containing the highest amount of organic solvent also does not differ from each other very much (Figure 6).

4. Discussion

As shown in Figure 2, ADC produced a positive icd peak corresponding to the ^1B_b transition, indicating the ADC : β-CD 1 : 1 complex formation in water (see also

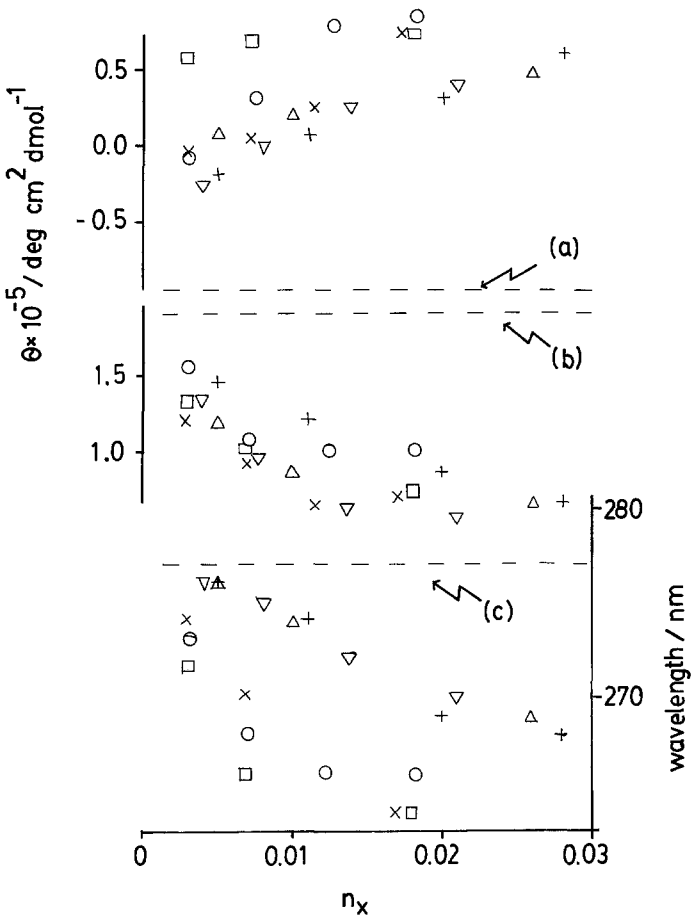

Fig. 5. The magnitude of θ at 255 nm (upper part) and at the positive peak band (middle part) and the position of the positive peak band (lower part) of the icd spectra of the ADC : γ-CD system vs. molar fraction of organic solvents: ACN (+), THF (○), EG (△), PC (□), DMSO (▽) and DX (×). The dashed lines show the magnitude of θ at 255 nm (line (a)) and at the positive peak band (line (b)) of the i.c.d. spectra recorded in water. The line (c) represents the wavelength of the positive peak band of the icd spectrum in water.

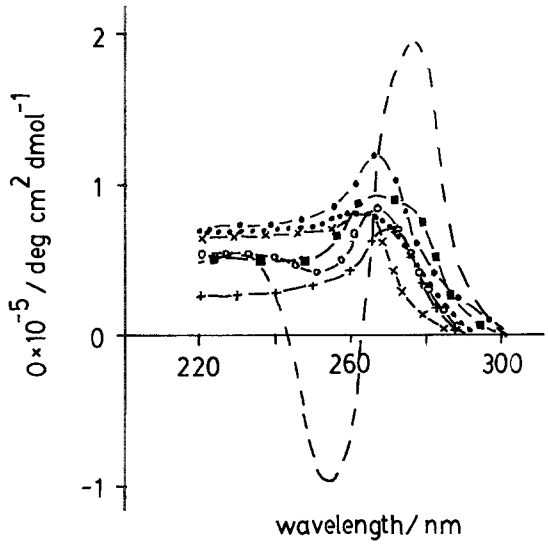

Fig. 6. The icd spectra of the ADC : γ-CD system recorded in 7.8 volume percent solution of ACN (-■-), THF (-●-), EG (-○-), PC (-×-), DMSO (-+-) and DX (· · · · ·) in phosphate buffer (pH = 9). The dashed line represents the spectrum recorded in the absence of organic solvent.

Figure 1). From the observed decay of this signal (Figures 2 and 3), one may conclude that organic solvent addition causes the decrease of the ADC : β-CD complex concentration. This is reasonable, because generally complex formation constants between aromatic molecules and CDs are smaller in organic solvents than in water [1]. The more interesting observation is that the degree of the above mentioned effect is different for different solvent addition. As can be seen from Figure 2, addition of a few volume percent of such solvent as 2MP or 2BE is enough to promote the degradation of the ADC : β-CD complex. In such systems, no inclusion complex exists when the molar fraction of 2MP or 2BE attains 0.01 as shown by the lack of the icd signal. There is no correlation between polarity or acid–base properties of the solvent and decrease of the icd intensity. The most pronounced effect by 2MP and 2BE may be correlated with hydrophobicity, since there is now evidence that these molecules in dilute aqueous solution (less than 0.03 molar fraction) can form small clusters as a result of hydrophobic interactions [20]. These small aggregates may solubilize aromatic guest molecules such as ADC and this process is concurrent to CD complexation. The decrease of solvation free energy of the preferentially solvated ADC molecule in comparison with those in aqueous solution may diminish the driving force for CD complex formation. The degradation of the complex at some organic cosolvent concentration may indicate that in this condition, this energy becomes lower than the free energy of ADC : β-CD complex formation.

It is difficult to quantify the degree of preferential solvation. Recently Haak and Engberts [21] measured the transition energy of the longest wavelength absorption band of 2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridino)phenoxide in five water-rich mixed solvent systems. This energy is known as the Dimroth–Reichardt parameter,

$E_T(30)$ [22], and it is regarded as one of the microscopic solvent polarity parameters. On the basis of ideal thermodynamic behaviour, the value of $E_T(30)$ should be linearly dependent on mixture composition. The deviation from this relationship has been proposed as a measure of the extent of preferential solvation [21]. According to this paper, the values of the preferential solvation parameter are 1.7 for ACN, 1.6 for DMSO, 2.6 for DX, 5.3 for 2MP, and 6.4 for 2BE.

In our studies, we chose the change of ellipticity at 266 nm in a solution containing a 0.005 molar fraction of organic solvent from those recorded in water as a measure of the influence of organic solvent addition on ADC : β-CD complex formation, $d\theta/dn_x(n_x = 0.005)$, because only in this medium composition does the icd signal still exist in all the solvent systems studied. This procedure seems to be appropriate because the intensity of the icd signal is directly proportional to the concentration of the CD complex. The lack of change in absorption signal in this region means that this dependence is not affected by medium composition. From the dependence of $d\theta/dn_x(n_x = 0.005)$ on the quantified extent of preferential solvation (Figure 7), one may conclude that the higher the preferential solvation or hydrophobicity of solvent, the weaker the CD complexation. For other solvents used in this study, similar conclusions can be drawn. In the case of relatively hydrophobic solvents such as PC and BuOH, which, as mentioned above, can even cause phase separation when mixed with water, the effect is similar to those of DX. On the other hand the decrease of the positive icd signal is the smallest in the case of EG.

The icd signal recorded in the ADC : γ-CD aqueous solution (dashed lines in Figures 4 and 5) indicates that the two ADC molecules included in the γ-CD cavity have R-helicity [14]. The observed decay of the negative signal with simultaneous blue-shift of the positive band peak indicates that the addition of organic solvent causes a decrease in dimer concentration. At maximum organic solvent concentration only the 1 : 1 ADC : γ-CD complex exists, because in this condition the

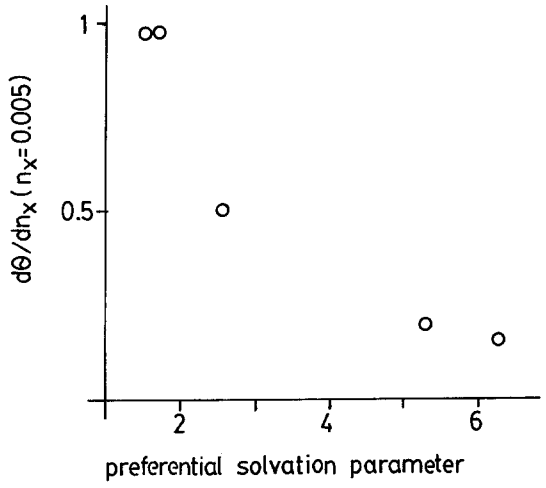

Fig. 7. The change of ellipticity at 266 nm recorded in phosphate buffer (pH = 9) solution containing the ADC : β-CD system in the presence of 0.005 molar fraction of organic solvent from that recorded in water, $d\theta/dn_x(n_x = 0.005)$ vs. extent of preferential solvation [21].

wavelength of the positive peak band is nearly the same as for the 1 : 1 ADC : β-CD complex (*ca.* 266 nm). As mentioned above, when the stoichiometry of the complex is not influenced by the presence of organic solvent additive as is the case for the β-CD complex, the position of the positive icd signal is not influenced by organic solvent addition. Therefore, one may conclude that addition of an organic solvent to the ADC : γ-CD solution causes a change of inclusion complex stoichiometry from 2 : 1 to 1 : 1. On the basis of the above discussion, the further degradation of the 1 : 1 complex is also probable, however it is difficult to extract this effect from our data because of the small magnitude of the icd signal.

It should be noted that the degradation of the 1:1 ADC : β-CD complex as well as liberation of one guest molecule from the 2 : 1 ADC : γ-CD complex may be also caused by organic solvent inclusion into the CD cavity.

5. Conclusions

It has been shown that addition of an organic solvent to aqueous solutions of the ADC : β-CD and ADC : γ-CD complexes produces the following effects: (1) The degradation of the 1 : 1 ADC : β-CD complex. (2) The liberation of one guest molecule from the 2 : 1 ADC : γ-CD complex in aqueous solution. (3) The higher the hydrophobicity of the cosolvent, the weaker the complexation of ADC by β-CD. These results show how medium composition may affect stability and stoichiometry of cyclodextrin complexes.

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