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Macrotricyclic quaternary ammonium ions: membrane current caused by feeble forces between hydrophobic groups and bilayer membranes†

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The ^{1}H NMR study on the encapsulation of iodide ion by controlled derivatives of macrotricyclic quaternary ammonium ions ($RMQA^{4+}$; R=H, CH_3, $CH_3(CH_2)_3$, $C_6H_5CH_2$ and $C_{10}H_7CH_2$) shows the higher stability of their complexes, having the larger hydrophobic space around their intramolecular cavity, with iodide ion. Here the stability constants were estimated from the ^{1}H NMR spectra for endocyclic protons. Both benzyl and 2-naphthalenemethyl derivatives can act as ion carriers across phospholipid bilayer membranes. Feeble forces between the increasingly hydrophobic space around their own cavity and the bilayer membrane play an important role in their permeation through the phospholipid bilayer as well as the stability of their encapsulated complex with iodide ion. The selectivity of the benzyl derivative for iodide ion has been confirmed, in the presence of chloride ion, by the drastic and decreasing change in membrane current: its decrease may originate from the change of the apparent valency of 4 to 3 due to the inclusion of I^-.

INTRODUCTION

The encapsulation of anionic guest into the hydrophobic pocket of artificial receptors has become the subject of growing interest in recent days[1] from the view of their indispensable role in many chemical and biochemical processes.[2] Methylated macrotricyclic quaternary ammonium ions have been identified as host for the inclusion of halide anions in both solid and liquid states[3,4]. Since the binding sites consist of four positively charged nitrogens arranged tetrahedrally around the cavity, the charged host is soluble in hydrophobic solvents and cannot itself translocate through the phospholipid bilayer membrane. The question that has yet to be explored is the influence of outercycle hydrophobic groups of the derivatives of macrotricyclic quaternary ammonium ions ($RMQA^{4+}$) on the stability of the encapsulated host-guest complex as well as the permeation through the membrane. Since $RMQA^{4+}$ is characteristic of an anion receptor, it is certainly different from the lipophilic anions and cations, such as tetraphenylboron and tetraphenylphosphonium, which typically have permeability across the bilayer membrane. It is much more important that the electric current caused by translocation of $RMQA^{4+}$ through the membrane can be controlled by the inclusion of iodide ion into its own intramolecular cavity.

This work describes the studies on iodide ion complexation of controlled derivatives of macrotricyclic quaternary ammonium ions with the aid of ^{1}H-NMR measurements in both aqueous and methanol solvents. We report that feeble forces between outercycle hydrophobic groups of $RMQA^{4+}$ and the bilayer membrane cause permeation across the membrane. We also show that the current across the bilayer can be controlled by the inclusion of iodide ion into the intramolecular cavity of $RMQA^{4+}$

EXPERIMENTAL

Syntheses

Macrotricyclic amine **1** (scheme): The synthesis of the parent macrotricyclic amine **1** was accomplished by three successive cyclizations under literature condition:[5]

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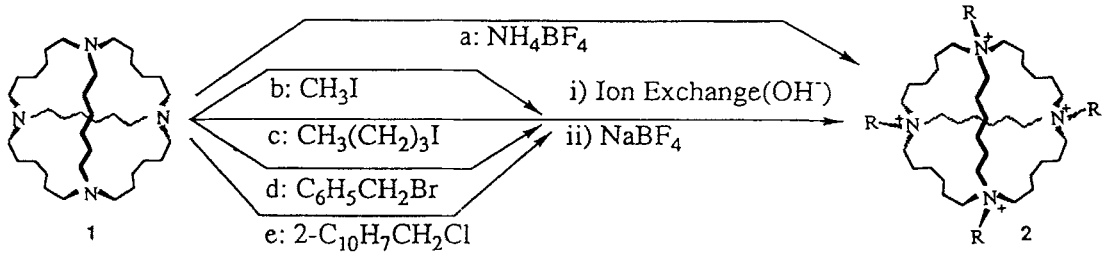
† This work has been appeared in 8th ISMRI Conference (July 31-August 5, 1994).



Scheme The synthesis of derivatives of macrotricyclic quaternary ammonium salts. 2a for R=H; 2b for R=CH_3, 2c for R=$CH_3(CH_2)_3$; 2d for R=$C_6H_5CH_2$ and 2e for $C_{10}H_7CH_2$.

^{1}H NMR (CD_3Cl, TMS) δ 1.32 (m, 48H, CH_2), 2.33 (t, 24H, NCH_2), $C_{36}H_{72}N_4$(560.9), field desorption mass spectrum, m/z=560(43%, M^+).

2a: Both **1** and NH_4BF_4 were added into chloroform/methanol (3:1 v/v, scheme). The mixture was warmed with occasional shaking. The clear solution was then kept in a desiccator containing diethyl ether for two days. The tiny crystals were separated by decantation of the liquid phase and recrystallized from methanol at room temperature. Yield 70%; ^{1}H NMR (CD_3OD, TSP) δ 1.16 (t, 4H, NH), 1.51 (b, s, 24H, γ-CH_2), 1.77 (b, s, 24H, β-CH_2), 3.20 (t, 24H, α-CH_2); Anal. calcd for $C_{36}H_{76}N_4B_4F_{16}$(912.16); C, 47.40; H, 8.39; N, 6.14; Found; C, 47.36; H, 8.41; N, 6.09.

2b–2e: 1 was stirred in acetonitrile under dry conditions. To this suspension were added the corresponding alkyl halide (Scheme) and sodium carbonate, and the mixture was refluxed for 3 days. The solution was separated by filtration and the solvent was evaporated under reduced pressure. Each of the products was used in the next step without purification. The residue was then dissolved into 3mL of methanol and chromatographed using rasine (OH^-, prepared by ion exchange of Cl^- by 1M NaOH solution in a short column) for anion exchange. Thus the eluent obtained from methanol was concentrated by evaporation with reduced pressure and diluted by addition of water/methanol (1:1 v/v). To this solution $NaBF_4$ dissolved in water was added and the mixture was kept at 0°C for two days to precipitate the expected products.

2b: Yield 70%; ^{1}H NMR (CD_3OD, TSP) δ 1.52 (b, s, 24H, γ-CH_2), 1.84 (b, s, 24H, β-CH_2), 2.29 (s, 12H, CH_3), 3.38 (t, 24H, α-CH_2); Anal. calcd for $C_{40}H_{84}N_4B_4F_{16}$ (968.35); C, 49.61; H, 8.74; N, 5.75; Found; C, 40.04; H, 8.81; N, 5.70.

2c: Yield 62%; ^{1}H NMR (CD_3OD, TSP) δ 1.02 (t, 12H, CH_3), 1.41 (q, 8H, γ'-CH_2), 1.51 (b, s, 24H, γ-CH_2), 1.71 (q, 8H, β'-CH_2), 1.82 (b, s, 24H, β-CH_2); 3.22 (b, 8H, α'-CH_2), 3.37 (t, 24H, α-CH_2); $C_{52}H_{108}N_4B_4F_{16}$ (1136.56); C, 54.95; H, 9.57; N, 4.93; Found; C, 54.81, H, 9.41, N, 4.88.

2d: Yield 60%; ^{1}H NMR (CD_3OD, TSP) δ 1.48 (b, s, 24H, γ-CH_2), 1.89 (b, s, 24H, β-CH_2), 3.31 (t, 24H, α-CH_2), 4.49 (s, 8H, α'-CH_2), 7.52 (m, 20H, Ar-H); $C_{64}H_{100}N_4B_4F_{16}$ (1272.64); C, 60.40; H, 7.91; N, 4.40; Found; C, 60.23; H, 7.98; 4.27.

2e: Yield 26%; ^{1}H NMR (CD_3OD, TSP) δ 1.32 (b, s, 24H, γ-CH_2), 1.81 (b, s, 24H, β-CH_2), 3.36 (t, 24H, α-CH_2), 4.58 (s, 8H, α'-CH_2), 7.61–8.29 (m, 28H, Ar-H); $C_{80}H_{108}N_4B_4F_{16}$ (1472.87); C, 65.24; H, 7.38; N, 3.80; Found; C, 65.06; H, 7.29; 3.84

^{1}H NMR spectra and titration curve

The complexation behavior of **2** towards iodide ion in both D_2O and CD_3OD was studied by ^{1}H NMR (JEOL, 400) using TSP as an internal standard. Since the solubility of **2b–2d** is lower in pure water, methanol was added at 10% (v/v); **2e** is insoluble into water. The addition of guest substrate ($[I^-]_0$ = 0–10mM) to each host ($[RMQA^{4+}]_0$ = 5mM) led to the appreciable change of the endocyclic proton signals in both solvents in a similar fashion, whereas the exocyclic protons were minimally perturbed.

Measurements of membrane current through the phospholipid bilayer materials

The phospholipid used was partially purified soybean phospholipid. A small amount of the derivatives of $RMQA^{4+}$ (ca. 9μM) in CH_3CN (1 mM) was added into the buffer solution (50 mM Tris-HCl, pH 7.4); the aqueous solutions of KI (200μM, 400μM and 1 mM) were also used to supply the iodide ions.

Formation of planar bilayer membrane and measurement of I–V curve[6–9]

The planar lipid bilayers were formed by the folding method: the cell used was made of Teflon and consisted of two chambers, the volume of which was 2.0 mL each. The above buffer solution in each chamber was connected through a septum with an aperture, 100–200μM in diameter, covered by the bilayer membrane; the utility of the formed membrane was confirmed by measuring the capacitive current passed under an applied voltage (triangular-shaped wave). The capacitance of membrane was 0.5–1 μF/cm^2, and the membrane resistance was more than 200 GΩ. The steady transmembrane current

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was observed by successive increases in every 20 mV voltage from 100 mV to -100 mV.

RESULTS

Complexation of 2 with iodide ion and NMR spectra of endocyclic proton in $RMQA^{4+}$

The emergence of the new signals at the downfield side along with the original ones (Figure 1) has originated from the encapsulated complex formation with much slower chemical exchange between free $RMQA^{4+}$ and its complex, on the NMR time scale. The penetration of the negatively charged guest into the cavity through coulombic interaction causes the changes of the local electronic environment of the binding sites showing the deshielding of endocyclic protons.[10] The more pronounced shift for the α-protons in comparison with β- and γ-protons is consistent with this prediction. For **2c** and **2d** the R (=$[I^-]_o/[RMQA^{4+}]_o$=0–2) dependence of the ^1H NMR spectra in both water + methanol (10% v/v) and methanol at 25°C shows the gradual increase of the peaks for the complex with the increase of R (Figure 2): it is concluded that a gradual increase in the amount of complex formed takes place in the system, whereas the total integration intensity of the free host and the host-guest complex was preserved. The molar fraction of the host-guest complex, x defined by [HG]/([H] + [HG]), was derived

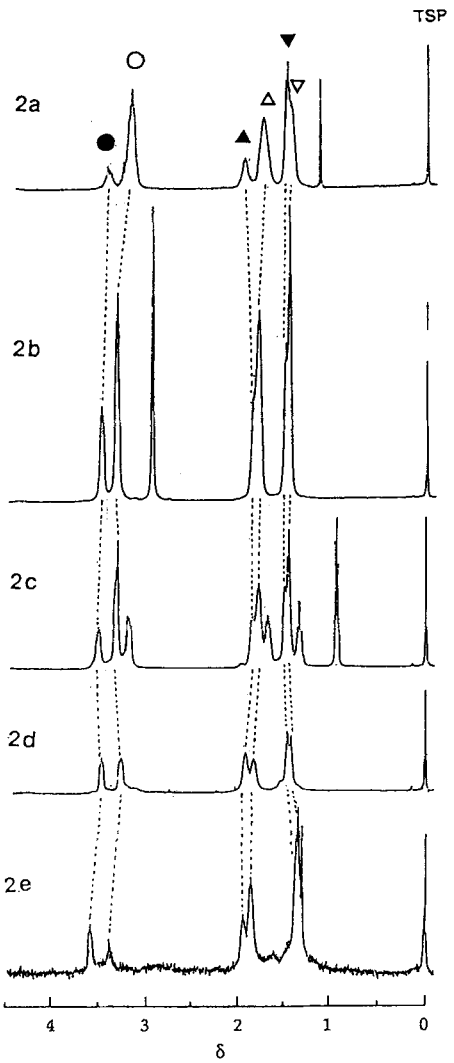

Figure 1 Partial ^1H NMR spectra of **2** in the presence of I^- for R = 1 in water + methanol (10% v/v) at 25°C: ○, ● = α-CH_2; △, ▲ = β-CH_2, ▽, ▼= γ-CH_2 for endocyclic protons, where open marks are for the free hosts and solids ones for the complexes.

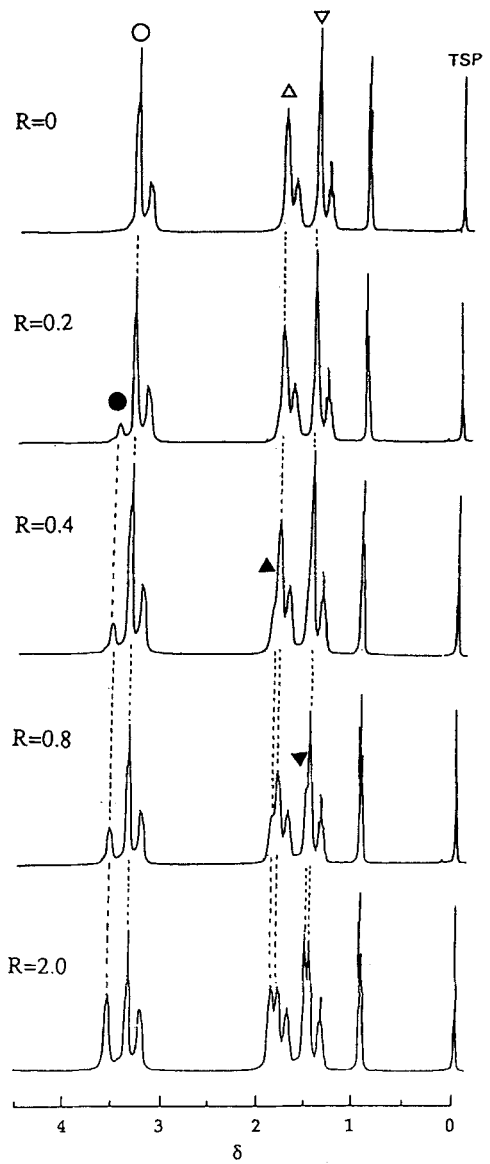

Figure 2 ^1H NMR spectra of **2c** in the presence of increasing amounts of I^- in water + methanol (10% v/v) at 25°C: ○, ● = α-CH_2, △, ▲= β-CH_2, ▽, ▼ = γ-CH_2 for the endocyclic protons, where open marks are for the free host and solids ones for the complex. The other peaks are for the exocyclic protons (3.21, α'-CH_2,; 1.69, β'-CH_2; 1.36, γ'-CH_2; 0.97, CH_3.)

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by the respective intensities of the α-protons in the NMR spectra (see Figure 1), where H and HG stand for $RMQA^{4+}$ and the inclusion complex of $RMQA^{4+}(I^-)$, respectively. Figure 3 shows the R dependence of x.

Effects of exocyclic hydrophobic space around intramolecular cavity and the halide anions encapsulated by $RMQA^{4+}$ on membrane current

The ion flux across the bilayer membrane is generally proportional to the concentration of $RMQA^{4+}$ ($R=C_6H_5CH_2$, **2d**) in the buffer solution of each chamber, as shown in Figure 4; the absolute values of the negative current observed were equal to the positive current within an experimental uncertainty. This results clearly indicates that **2d** acts as a mobile carrier in the phospholipid bilayer. Figure 5 shows the effect of the exocyclic hydrophobic groups of $RMQA^{4+}$ on the membrane current. The bulkier groups benzyl, $C_6H_5CH_2$, and 2-naphthalene-methyl, $C_{10}H_7CH_2$, in $RMQA^{4+}$ give rise to an increase in the flux of $RMQA^{4+}$ itself through the planar

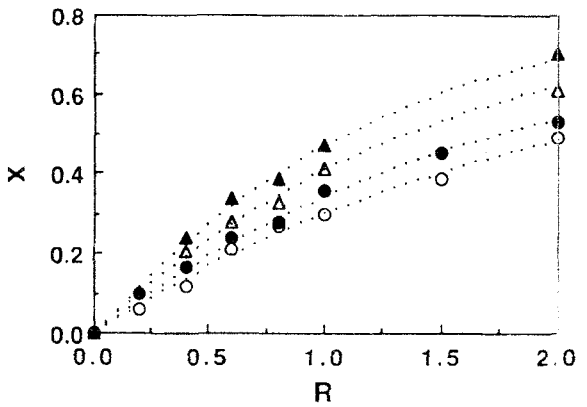

Figure 3 The R dependence of x estimated from the data on the endocyclic α-proton ^1H NMR spectra in both water + methanol (10% v/v, open marks) and methanol (solid marks) at 25°C for **2c** (○, ●) and **2d** (△, ▲), respectively. The dashed lines are obtained by computer simulation using equation $x = \{p - (p^2 - 4K_{st}^2R)^{1/2}\}/2K_{st}$, where $P = K_{st}(R + 1) + 1/[H]_0$

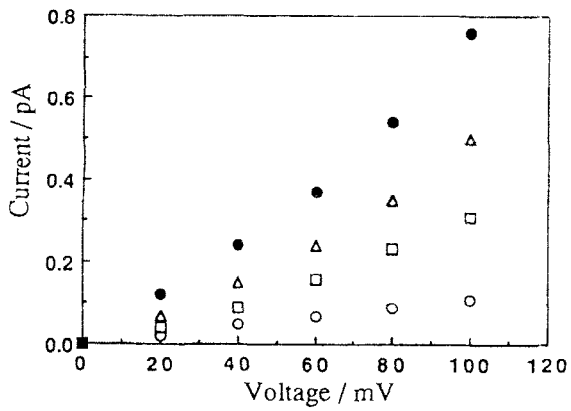

Figure 4 Concentration dependence of **2d** on membrane current: ○ = 3μM, □ = 6μM, △ = 9μM and ● = 12μM.

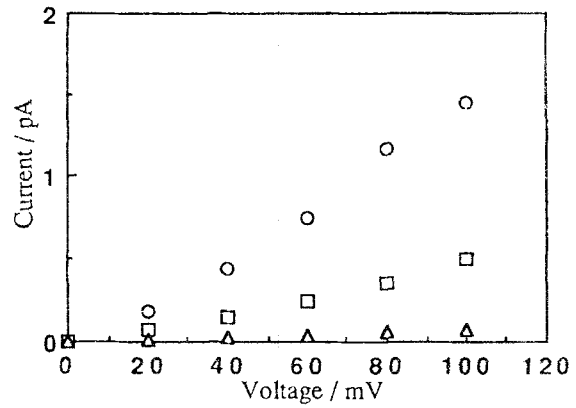

Figure 5 Effect of exocyclic hydrophobic groups around the cavity of $RMQA^{4+}$ (9μM) on membrane current: △ for **2b**, □ for **2d** and ○ for **2e**.

phospholipid bilayer. Figure 6 shows that the addition of guest I^- into the buffer solution including $RMQA^{4+}$ caused a drastic decrease in membrane current, whereas in the case of Cl^- the current was minimally perturbed.

DISCUSSION

The successful simulation of the experimental data on the R dependence of x, as shown in Figure 3, gives the stability constant, K_{st} for the reaction of H + G = HG, (G=I^-), where H is **2c** or **2d**. Table 1 shows the gradual increase in the stability of complexes due to the presence of the increasing hydrophobic space around the cavity. The anionic guest I^- encapsulated through strong electrostatic interaction due to desolvation is further stabilized by the space which consists of exocyclic hydrophobic groups around the naked cavity. The much higher stabilities for **2d** and **2e** are possibly due to the presence of the bulky group at the end of the exocyclic chain. The reason why the hydrophobic space around cavity gives rise

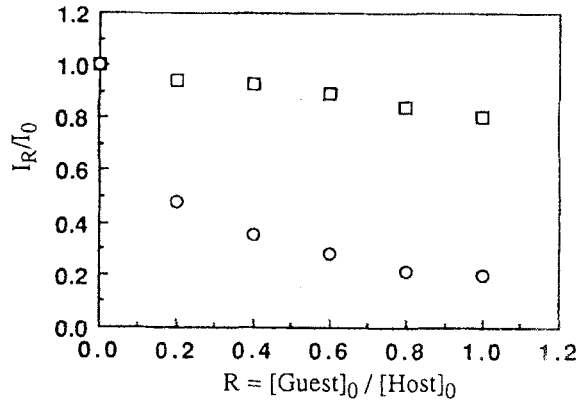

Figure 6 Influence of guests I^- (○) and Cl^- (□) on membrane current for **2d** (5μM): I_R and I_0 are the membrane current at various value of R and R = 0, respectively.

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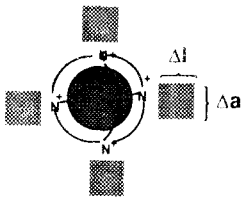
Table 1 The stability constant K_{st} calculated from the data of α-proton NMR spectra for the reaction of $RMQA^{4+} + I^- = RMQA^{4+}(I^-)$ at R = I and at 25°C

$RMQA^{4+}$	K_{st}/M^{-1}		$^{b}\Delta l \times \Delta a/Å^2$
	$D_2O + CD_3OD$ (10% v/v)	CD_3OD	
2a	95	110	~1 × 1
2b	115	155	~2 × 3
2c	122	165	~7 × 3
	124[a]	162[a]	
2d	235	334	~7 × 6
	236[a]	336[a]	
2e	—	670	~7 × 9

[a]Values are obtained by simulation of R dependence of x (Fig. 3)
[b]The approximate value estimated by CPK model designed as follows:



Schematic diagram of possible form of encapsulated complex with exocyclic hydrophobic groups.

to an increase the stability of the complex of $RMQA^{4+}$ with I^- is that the exocyclic groups with a larger value of $\Delta l \times \Delta a$, as shown in Table 1, shield the encapsulated guest from the shower of dipolar molecules of solvent. However, the presence of the analogous series of alkyl groups for **2b** and **2c** with identical thickness attached longitudinally to the binding sites can make little difference in shielding of the encapsulated guest. Consequently, the difference between stabilities is less pronounced for these two systems. It is expected that the conformational symmetry of **2a** has not changed on complexation.[11,12] This has been confirmed by the negligible perturbation of the NH peak (δ=1.16) in the ^1H NMR spectra (see **2a** in Figure 1), as well as the space filling CPK model with iodide ion. The observed higher stability of the complexes in methanol compared with that in water + methanol (10% v/v) is due to the smaller polarity of the methanol molecules compared with water molecules. Since the acceptor numbers of H_2O and CH_3OH are equal to 54.8, and 41.3,[13] (their dipole moments 1.85 and 1.7 Debye, respectively,) the encapsulated guest ion can be more easily stripped from the cavity by water molecules, compared with methanol molecules.

The substantial increase of the stability constant of encapsulated complexes should be correlated to the magnitude of membrane current caused by translocation of free $RMQA^{4+}$ as shown in Figure 5 and Table 1. It is interesting that feeble forces between the hydrophobic crowded space around the intramolecular cavity and the hydrophobic tails of the bilayer membrane give rise to the permeation of $RMQA^{4+}$ and to the membrane current.

The selectivity of **2d** for iodide ions in the presence of chloride ions[6] has been confirmed by a drastic and decreasing change in membrane current across the bilayer (Figure 6). The complex of $RMQA^{4+}$ with I^- means the decrease in its own apparent valency, compared with the free $RMQA^{4+}$. If the decrease in membrane current originates mainly from the decrease of the valency Z, the change of Z from 4 to 3 after the addition of I^- may cause roughly half of the membrane current because of the conductance proportional to valency squared;[7] the results in Figure 6 shows clearly a decrease of about half of the current after the complexation. Since the chloride ion shows no stable complex with $RMQA^{4+}$,[6] the membrane current still remains almost unchanged even after its addition.

It is concluded that the increasing hydrophobic space around the intramolecular cavity of $RMQA^{4+}$ plays an important role of the permeation of free $RMQA^{4+}$ through the phospholipid bilayer membrane as well as of the stability of encapsulated complex of $RMQA^{4+}$ with I^-.

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