

Spectroscopic Studies on the Reaction of Superoxide Ion with Non-Redox Metalloporphyrins

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The reactions of superoxide ion, O_2^- , with some non-redox metalloporphyrins were investigated at room temperature by spectroscopic methods (UV/vis, ESR) in dimethyl sulfoxide (DMSO).

Addition of the O_2^- solutions prepared from KO_2 to DMSO solutions of the meso-tetraphenylporphyrin which is complexed with group II metals such as Zn(II), Mg(II) and Cd(II), caused the visible spectra to shift to the red and made the g_1 component of the ESR spectrum due to O_2^- at 77 K shift to the higher field. Addition of methanol decomposed the superoxo complexes to the corresponding metalloporphyrins. These results suggest that the reaction products between these metalloporphyrins and O_2^- are the O_2^- adducts. Other non-redox metalloporphyrins containing group VIII metals such as Ni(II) and Pd(II) do not produce the O_2^- adducts under the experimental conditions.

The reasons for the red shifts caused by the formation of the O_2^- adducts and for the difference in the reactivities of metalloporphyrins towards O_2^- are discussed.

Introduction

Metalloporphyrins, especially iron porphyrins (heme), are biologically important, because hemoglobin, myoglobin and cytochrome P_{450} contain the heme and function in the oxygen-binding or the oxygenation of substrates.

The dioxygen complexes of metalloporphyrins are good models for understanding the oxygenated hemoproteins. Several dioxygen complexes of metalloporphyrins have hitherto been synthesized. In some dioxygen complexes of metalloporphyrins, the oxygen has been shown to be in O_2^- state. For example, in $Cr(TPP)(py)(O_2)$ [1], $Fe(TpivPP)(base)(O_2)$ [2] and $Co(TPP)(base)(O_2)$ [3], O_2^- is shown to be the axial ligand. These dioxygen complexes are formed by oxidative addition of molecular oxygen to the metalloporphyrins in a lower oxidation state.

However, the superoxo complexes cannot be produced directly by the reaction of the non-redox metalloporphyrins with dioxygen.

Recently, Valentine *et al.* showed that Zn(TPP) associates with O_2^- to form a superoxo complex [4]. From their report, it is expected that the metalloporphyrins containing the group II metals in the periodic table may directly associate with O_2^- to form the superoxo complexes.

In this paper, we report that the metalloporphyrins containing the group II metals such as Zn(II), Mg(II) and Cd(II) associate easily with O_2^- to form the superoxo complexes, but those of group VIII do not associate with O_2^- at room temperature and that formation of the O_2^- adducts causes the visible spectra of metalloporphyrins to shift to the red.

Experimental

Materials

ZnTPP [5, 6], MgTPP [7], CdTPP [5], NiTPP [7] and PdTPP [7] were prepared by literature methods. Potassium superoxide (KO_2) was purchased from Alfa Products, Inc. Dicyclohexyl-18-crown-6 (Nippon Soda Co. Ltd.) was used without further purification. Ferricytochrome *c* (cyt. *c*) from horse heart was purchased from Sigma Chemical Co. Other reagents were commercially available. Dimethyl sulfoxide (DMSO) was distilled at reduced pressure from CaH_2 and stored over freshly activated 4A molecular sieves under dry argon. Dichloromethane (CH_2Cl_2), which had been distilled, was passed through an alumina column to remove stabilizers and impurities. Other solvents were distilled immediately prior to use.

Preparation of Solutions of Superoxide Ion

Potassium superoxide was ground to a fine powder with a mortar and pestle in a dry atmosphere. Powdered KO_2 and dicyclohexyl-18-crown-6 were

TABLE I. Visible Absorption Spectra after Mixing of the Metalloporphyrins Containing Group II Metals with DMSO in Noncoordinating Solvent, CH_2Cl_2 .

Metalloporphyrin	$\lambda_{\text{max}}/\text{nm}$ ($\log \epsilon$)		
	Soret	β	α
ZnTPP	419.5(5.79)	549.0(4.38)	587.0(3.64)
ZnTPP(DMSO)	426.0(5.83)	558.5(4.35)	598.0(3.96)
MgTPP	425.0(5.72)	564.0(4.29)	603.5(4.01)
MgTPP(DMSO)	426.0(5.72)	564.0(4.27)	604.0(4.01)
CdTPP	430.5(5.18)	564.0(4.05)	604.5(3.77)
CdTPP(DMSO)	434.5(5.42)	571.5(3.97)	613.0(3.83)

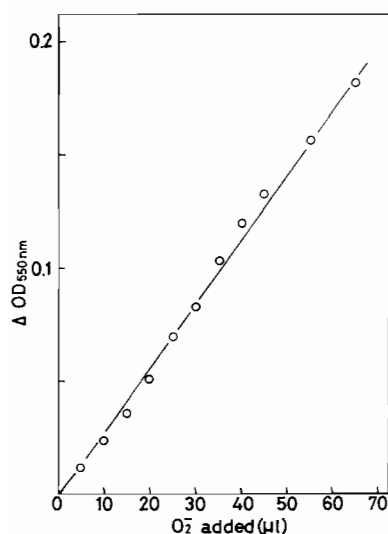


Fig. 1. Titration of ferricytochrome *c* by O_2^- . A 1-cm cuvette contained $2 \times 10^{-5} M$ ferricytochrome *c* in a volume of 2 ml phosphate-buffered aqueous solution (pH 8.0). Various amounts of O_2^- in DMSO were successively added to the cuvette. A reference cuvette contained the same additions without O_2^- . The increase in absorbance at 550 nm due to ferrocycytochrome *c* was followed.

dissolved in dry DMSO as described in the literature [8].

Preparation of Halide and Hydroxide Solution

Potassium halides and dicyclohexyl-18-crown-6 were dissolved in dry DMSO by stirring for one hour. Potassium hydroxide was ground to a fine powder with a mortar and pestle in a dry atmosphere. Powdered KOH and dicyclohexyl-18-crown-6 were dissolved in dry DMSO.

Determination of Superoxide Ion

The concentration of O_2^- was determined by the method described by Bielski *et al.* [9]. That is,

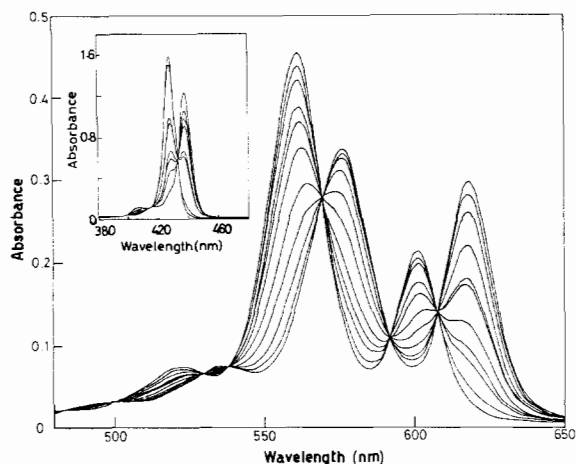


Fig. 2. Visible spectral change observed during successive additions of O_2^- to ZnTPP. The change in the Soret band which is reduced by factors of 0.1 is shown in the insert. Starting concentration of ZnTPP is $1.90 \times 10^{-5} M$. Final concentration of O_2^- is $1.60 \times 10^{-3} M$. Volume is held essentially constant (2 ml).

O_2^- was mixed with large amounts of an aqueous cyt. *c* buffered with phosphate at pH 8.0, and the absorbance change at 550 nm ($\epsilon = 2.11 \times 10^4 M^{-1} \text{cm}^{-1}$) [10] was followed thereafter. A typical titration curve is shown in Fig. 1.

Reaction of Metalloporphyrins with Superoxide Ion

Two milliliters of 10^{-5} – $10^{-6} M$ metalloporphyrins in DMSO was placed in a 1-cm capped quartz cuvette. Aliquots of 10^{-2} – $10^{-3} M$ KO_2 /dicyclohexyl-18-crown-6 were added rapidly by microsyringe. After the reactions were started by mixing the reactants, visible spectra were recorded immediately.

Reactions of Metalloporphyrins with Anions

Aliquots of $10^{-2} M$ halides or hydroxide were mixed with 2 ml of DMSO solutions of metalloporphyrins and the reaction mixtures were subjected to the spectral measurements.

Spectral Measurements

Visible and ESR spectra were recorded on a Union Giken SM-401 spectrophotometer and a JEOL-PE-1X (X-band) spectrometer with 100 kHz field modulation, respectively. The *g*-values were obtained by comparison with a $\text{Mn}^{2+}/\text{MgO}$ marker and DPPH ($g = 2.0036$).

Results

The metalloporphyrins prepared here tend to form the aquo complexes which resist deaquaation. But,

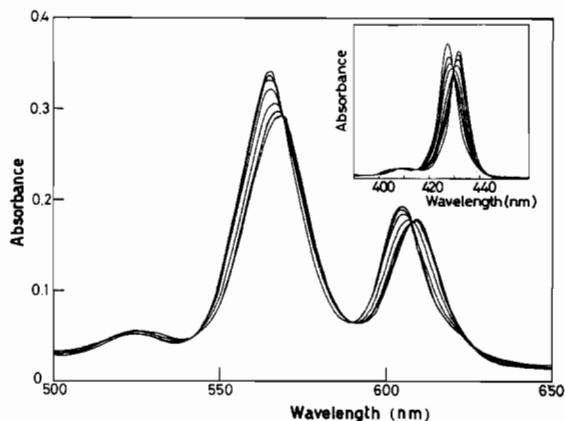


Fig. 3. Visible spectra resulting from successive additions of O_2^- to MgTPP in DMSO. The inserted figure shows the spectral change of the Soret band which is reduced by a factor of 0.1. Starting concentration of MgTPP is $5.10 \times 10^{-5} M$. Final concentration of O_2^- is $1.50 \times 10^{-3} M$. Volume is kept essentially constant (2ml).

in anhydrous DMSO solutions, an axial ligand is DMSO and not H_2O , because the visible absorption spectra of metalloporphyrins in a noncoordinating solvent such as CH_2Cl_2 showed red shifts upon dropwise addition of DMSO (Table I).

Free TPP in DMSO solution did not undergo any spectral change in the presence of O_2^- or oxygen dissolved in the medium. Dicyclohexyl-18-crown-6 did not affect the spectrum of metalloporphyrins in DMSO even though the mixtures were allowed to stand for 3 days in the dark. These observations indicate that O_2^- does not react with free TPP and that neither O_2 nor crown ether react with non-redox metalloporphyrins.

Reaction of O_2^- with ZnTPP

Valentine *et al.* [4] showed that ZnTPP binds with O_2^- to give the superoxo complex (O_2^- adduct). At first, we followed their experiments and obtained the same spectral results (Fig. 2).

The O_2^- adduct of ZnTPP appeared to be decomposed to ZnTPP upon addition of methanol, as shown by the spectral measurements. Superoxide ion in the adduct was displaced by pyridine.

Reaction of O_2^- with MgTPP

Figure 3 shows the spectral change resulting from dropwise addition of O_2^- to MgTPP in DMSO.

Addition of excess amounts of the O_2^- solutions did not cause additional spectral change. It is known that magnesium porphyrins are usually five coordinate, because the association constants for the sixth ligands are very small [11]. Therefore, these results suggest that only one O_2^- coordinates to MgTPP. The O_2^- adduct of MgTPP appeared to dissociate to

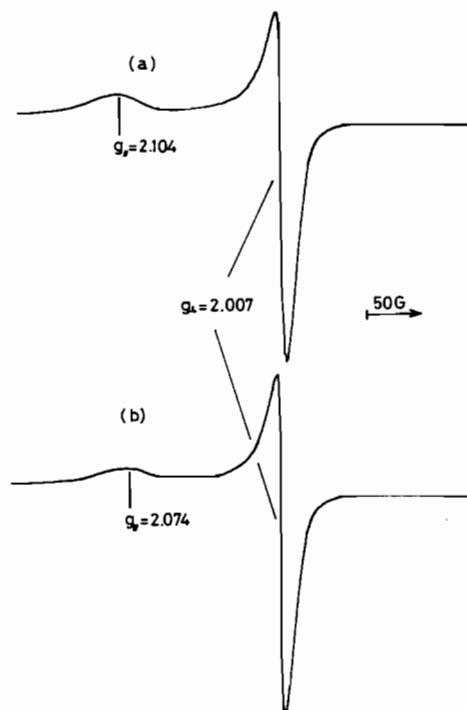


Fig. 4. ESR spectra of O_2^- at 77 K: (a) $3.90 \times 10^{-3} M O_2^-$; (b) $3.90 \times 10^{-3} M O_2^-$ plus $1.25 \times 10^{-2} M MgTPP$. Spectra were measured in DMSO.

MgTPP after the addition of a small volume of methanol. Superoxide ion as an axial ligand was not displaced by pyridine.

The formation of the O_2^- adduct was also confirmed by ESR studies at 77 K. Superoxide ion prepared from KO_2 has a characteristically anisotropic ESR spectrum (Fig. 4a, $g_{||} = 2.104$, $g_{\perp} = 2.007$). When MgTPP was added to DMSO solution of superoxide ion, the $g_{||}$ peak shifted to higher field (Fig. 4b), but the spectral pattern was still maintained as that of O_2^- . This higher-field shift of $g_{||}$ is attributed to the complex formation of O_2^- with the metal ion [4, 12].

Reaction of O_2^- with CdTPP

Since Cd(II) has the same electronic configuration (d^{10}) as Zn(II), CdTPP will accept one axial ligand to form five-coordinate complexes [11, 13, 14]. Solutions of CdTPP in DMSO are pale-green. Addition of O_2^- caused the solutions to become deep-green. The resulting spectral changes (Fig. 5) are similar to those obtained by reacting O_2^- with ZnTPP or MgTPP. In this case, the O_2^- adduct was also decomposed to CdTPP by addition of methanol. Superoxide ion as an axial ligand was not displaced by pyridine.

TABLE II. Visible Spectral Change resulting from Additions of O_2^- to Metalloporphyrins in DMSO.

Metalloporphyrin	Original, λ_{max}/nm (log ϵ)		O_2^- adduct, λ_{max}/nm (log ϵ)			
ZnTPP	428.0 (4.92)	562.0 (3.38)	601.5 (3.05)	438.0 (4.81)	576.0 (3.25)	618.5 (3.19)
MgTPP	427.5 (5.32)	565.5 (3.84)	605.0 (3.59)	431.0 (5.29)	568.0 (3.72)	610.0 (3.40)
CdTPP	437.0 (5.44)	574.5 (4.07)	616.5 (4.00)	441.0 (5.27)	584.5 (3.99)	628.0 (4.01)

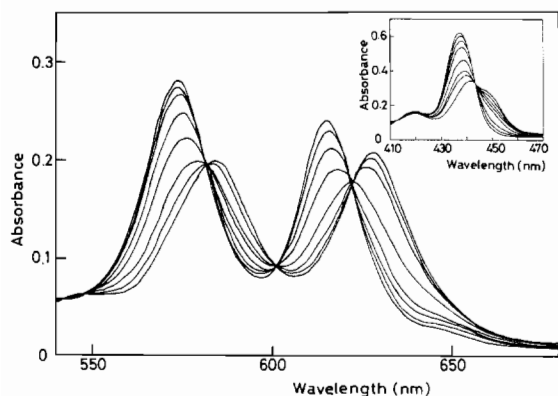


Fig. 5. Visible spectral change resulting from successive additions of O_2^- to CdTPP in DMSO. The inserted figure shows the spectral change of the Soret band which is reduced by a factor of 0.1. Starting concentration of CdTPP is $5.03 \times 10^{-5} M$. Final concentration of O_2^- is $1.94 \times 10^{-3} M$. Volume is held essentially constant (2 ml).

The ESR spectrum of the O_2^- adduct of CdTPP also showed the higher-field shift of g_{\parallel} component of the O_2^- signal, but the spectral pattern did not change.

Table II summarizes the parameters of absorption spectra (λ_{max} , log ϵ) before and after the addition of O_2^- to metalloporphyrins.

Reaction of O_2^- with Ni- and PdTPP

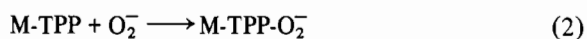
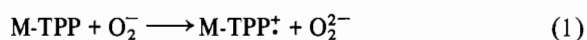
Four coordinate Ni(II)- and Pd(II)-porphyrins are shown to add one or two ligands [11]. However, NiTPP or PdTPP in the DMSO solutions did not cause any visible spectral change after addition of O_2^- . These results suggest that NiTPP and PdTPP did not associate with O_2^- .

Discussion

Superoxide ion has both oxidation and reduction abilities in aprotic solvent [15]. In the non-redox metalloporphyrins the removal (or addition) of

electrons from (or to) the π -electron core in the porphyrin ligand may occur during the reaction with the redox active compounds [16]. Therefore, π -cation or π -anion radicals of metalloporphyrins may be formed by the reaction with O_2^- . From the redox potentials of O_2^- (O_2/O_2^- , $-0.7 V$ and O_2^-/H_2O_2 , $1.3 V$) [17] and metalloporphyrins (reduction potentials: CdTPP, $-1.25 V$; MgTPP, $-1.35 V$; ZnTPP, $-1.31 V$ and oxidation potentials: CdTPP, $0.63 V$; MgTPP, $0.54 V$; ZnTPP, $0.71 V$) [16], neither π -cation nor π -anion radicals are formed in our reaction systems. The possibility of formation of the π -cation radical is also excluded by the following experimental results.

The visible spectra shown in Fig. 2 are quite different from that of the ZnTPP cation radical generated by electrochemical oxidation of ZnTPP [18]. Furthermore, since the ESR spectrum due to MgTPP and CdTPP cation radical could not be observed, the spectral changes shown in Table II probably result from O_2^- adduct formation (eqn. (2)) and not from radical formation (eqn. (1)).



The absorption spectra of the metalloporphyrins examined are shifted to the red by the addition of O_2^- as shown in Table II. These spectral properties are different from those of the OH^- complexes of the corresponding metalloporphyrins (Table III).

Therefore, the hydroxide, being a product of O_2^- decomposition by water probably does not coordinate to the metalloporphyrins. This fact is confirmed by the ESR experiments. That is, if OH^- is ligated, the higher-field shift of the g_{\parallel} component of the ESR spectrum due to O_2^- would not be observed.

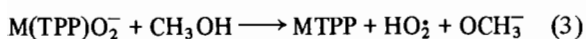
Unfortunately, we could not observe a direct interaction between the odd electron of O_2^- with ^{25}Mg ($I = 5/2$, natural abundance 10.05%) of MgTPP or ^{111}Cd ($I = 1/2$, natural abundance 12.56%) and/or ^{113}Cd ($I = 1/2$, natural abundance 12.34%) of CdTPP. These results may suggest that the bonding between

TABLE III. Visible Spectral Differences between the O_2^- Adducts and the OH^- Complexes of the Metalloporphyrins in DMSO.

Adducts	λ_{max}/nm (log ϵ)		
	Soret	β	α
ZnTPP	428.0(4.92)	562.0(3.70)	601.5(3.05)
Zn(TPP) O_2^-	438.0(4.81)	576.0(3.25)	618.5(3.19)
Zn(TPP) OH^-	438.5(4.87)	577.0(3.27)	619.0(3.23)
MgTPP	427.5(5.32)	565.5(3.84)	605.0(3.59)
Mg(TPP) O_2^-	431.0(5.29)	568.0(3.72)	610.0(3.40)
Mg(TPP) OH^-	432.0(5.32)	569.5(3.78)	610.5(3.60)
CdTPP	437.0(5.44)	574.5(4.07)	616.5(4.00)
Cd(TPP) O_2^-	441.0(5.27)	584.5(3.99)	628.0(4.01)
Cd(TPP) OH^-	445.0(5.41)	587.0(4.02)	631.0(4.11)

O_2^- and metalloporphyrin is not as tight as was thought [12, 19].

The visible spectral data indicate that methanol decomposes the superoxo complex by the following equations [4]:



These results suggest that O_2^- coordinates to the metalloporphyrins.

It is reported that, when anions such as Cl^- and F^- coordinate to metalloporphyrins, the visible spectrum is caused to shift to the red [20]. In our experiments, Cl^- and Br^- complexed with CdTPP

in a similar way to the complexation of halides to ZnTPP. However, I^- did not complex with CdTPP. The metalloporphyrins other than group II metals cannot coordinate with halides.

Table IV shows the spectral parameters of the Cd(TPP)X and Cd(TPP)X $^-$ complexes formed. From Table IV, it is apparent that the anionic ligand, O_2^- , as well as the halides (Cl^- , Br^-) complexes with Zn-, Mg- and CdTPP. Furthermore, the red shifts observed are shown to relate to the charge, because the extent of the red shifts is larger in the complexes with anionic ligands compared with those of the neutral ligands. It was reported that the red shifts seen after complexation with the anionic ligands are caused by the increase in the transfer of charge out from the anionic ligands onto the porphyrin ring [20, 21]. We can explain therefore that the red shift is mainly caused by the charge transfer from ligand to the porphyrin ring via the central metals.

While the metalloporphyrins containing group II metals formed the O_2^- adducts, the metalloporphyrins containing group VIII metals such as Ni(II) and Pd(II) could not form the adduct with O_2^- as well as with the anionic ligands under the experimental conditions.

The most stable coordination form of Ni(II)- and Pd(II)-porphyrins is the bare species like M(P) (M, metals; P, porphyrins), which has the square planar d^8 configuration with the filled d_{z^2} -orbital allowing addition of axial ligands only to a limited extent [22]. In the metalloporphyrins containing group II metals, the complexing properties of the closed-shell d^{10} Zn(II) or Cd(II), and d^0 Mg(II) atoms derive primarily from their unoccupied $4sp^3$, $5sp^3$ and $3sp^3$ orbitals, respectively [22].

Hence, the differences between the complexations may be mainly caused by differ-

TABLE IV. Electronic Absorption Spectra of CdTPP Derivatives.

Complex	Solvent	λ_{max}/nm (log ϵ)		
		Soret	β	α
CdTPP	CH_2Cl_2	430.5(5.18)	564.0(4.05)	604.5(3.77)
Cd(TPP)THF	CH_2Cl_2	433.0(5.38)	569.0(3.97)	610.0(3.83)
Cd(TPP) CH_3CN	CH_2Cl_2	433.0(5.39)	570.0(3.95)	612.0(3.78)
Cd(TPP)DMSO	CH_2Cl_2	434.5(5.42)	571.5(3.97)	613.0(3.83)
Cd(TPP)DMF	CH_2Cl_2	433.5(5.42)	572.0(3.96)	613.0(3.81)
Cd(TPP)py	CH_2Cl_2	436.0(5.44)	575.0(3.95)	617.0(3.83)
Cd(TPP)Im	CH_2Cl_2	436.5(5.35)	576.5(4.01)	618.5(3.91)
Cd(TPP) Br^-	DMSO	439.0(5.40)	577.0(4.00)	621.0(3.95)
Cd(TPP) Cl^-	DMSO	440.5(5.47)	581.0(4.03)	624.0(4.02)
Cd(TPP) O_2^-	DMSO	441.0(5.27)	584.5(3.99)	628.0(4.01)
Cd(TPP) OH^-	DMSO	445.0(5.41)	587.0(4.02)	631.0(4.11)

ences in the electronic configurations of metalloporphyrins.

Conclusions

The metalloporphyrins containing group II metals such as Zn(II), Cd(II) and Mg(II) associate with O_2^- to form the O_2^- adducts (superoxo complexes) in DMSO. However, the metalloporphyrins containing group VIII metals such as Ni(II) and Pd(II) did not yield the O_2^- adduct. The differences between the electronic configurations of the metal ions may cause the difference in ability of the metalloporphyrins to form adducts with O_2^- .

The red shifts caused by addition of the O_2^- or the other anionic ligands were explained by charge transfer from the ligands to the porphyrin ring via the central metals.

The present work has shown that the superoxide ion is useful for the preparation of the superoxo complex.

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- 5 A. D. Adler, F. R. Longo, F. Kampas and J. Kim, *J. Inorg. Nucl. Chem.*, **32**, 2443 (1970).
- 6 Abbreviations used in this paper are as follows: TPP, *meso*-tetraphenylporphyrin; TpivotPP, *meso*-tetra($\alpha,\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)porphyrin; DMSO, dimethyl sulfide; DMF, dimethyl formamide; THF, tetrahydrofuran; py, pyridine; Im, imidazole; UV, ultra violet; ESR, electron spin resonance; cyt. *c*, ferricytochrome *c*; M, metals; P, porphyrins; DPPH, 2,2-diphenyl-1-picrylhydrazyl.
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