

Articles

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pK_a of Zinc-Bound Water and Nucleophilicity of Hydroxo-Containing Species. Ab Initio Calculations on Models for Zinc Enzymes

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Received July 11, 1989

Ab initio Hartree–Fock calculations have been performed on a series of zinc complexes containing a coordinated water molecule in order to understand the factors governing the pK_a of the coordinated water and the nucleophilicity of the Zn–OH and Zn–OH₂ groups. The systems investigated include Zn(NH₃)_nOH₂²⁺ ($n = 3–5$), Zn(NH₃)₂(COOH)OH₂⁺, Zn(NH₃)₂(SCH₃)OH₂⁺, Zn(NH₃)(SCH₃)₂OH₂, and their deprotonated partners. The order of nucleophilicity for the various compounds is presented, as well as the order of acidity of the aqua complexes. The latter order is compared with the values found in metalloproteins.

Introduction

The hydrolytic and hydration reactions that are encountered in biochemistry are often related to the capacity of a Zn–OH₂ moiety in metalloenzymes to be transformed into a zinc–hydroxo group.² This fundamental step has been extensively investigated through model chemistry and theoretical approaches, especially in relation to CO₂ hydration, the simplest hydration of biological importance.^{3–7}

We have reinvestigated through ab initio Hartree–Fock calculations the deprotonation reaction of a zinc-coordinated water molecule in various model systems, in order to try to understand (1) the factors determining the pK_a of the coordinated water and (2) the nucleophilicity of the various species toward the substrates to be hydrolyzed. With this in mind we have calculated the energies and charge distributions of Zn(NH₃)₃OH₂²⁺, Zn(NH₃)₄OH₂²⁺, and Zn(NH₃)₅OH₂²⁺ on one side and of their hydroxo complexes on the other. The effect of the presence of negatively charged ligands has been investigated through the substitution of ammonia ligands by HCOO[–] or CH₃S[–] groups. We have examined in detail the variation of the fractional charges on the oxygen atom in the various model systems, as well as the energies of the HOMO having oxygen lone pair character.

Description of the Model Systems

The systems investigated are reported in Table I. The zinc complexes (1–3) in Table I have three to five NH₃ ligands and one H₂O or OH[–] ligand. In all of the above complexes, the Zn–N distances were 2.0 Å and the Zn–O distances were 2.0 Å for the H₂O ligand (1a–3a) and 1.95 Å for the OH[–] ligand (1b–3b).⁹ The geometries of the H₂O and NH₃ ligands were the experimental ones.¹⁰ The O–H distance and the Zn–O–H angle were optimized to 0.957 Å and 139.5°, respectively, in the calculation for the

Table I. Total Energies of the Analyzed Systems and Energy Differences between the Zn–OH₂ and Zn–OH Complexes

no.	complex formula	E_{SCF} , hartrees	ΔE , kJ mol ^{–1}
1a	{Zn(NH ₃) ₃ (OH ₂) ²⁺ }	–2019.8419 (–2019.8598) ^a	717 (716) ^a
1b	{Zn(NH ₃) ₃ (OH)} ⁺	–2019.5688 (–2019.5871) ^a	
2a	{Zn(NH ₃) ₄ (OH ₂) ²⁺ }	–2075.9373 (–2075.9638) ^b	764 (769) ^b
2b	{Zn(NH ₃) ₄ (OH)} ⁺	–2075.6462 (–2075.6708) ^b	
3a	{Zn(NH ₃) ₅ (OH ₂) ²⁺ }	–2132.0437	814
3b	{Zn(NH ₃) ₅ (OH)} ⁺	–2131.7337	
4a	{Zn(NH ₃) ₂ (COOH)(OH ₂) ⁺ }	–2151.9623	1063
4b	{Zn(NH ₃) ₂ (COOH)(OH)} ⁺	–2151.5573	
5a	{Zn(NH ₃) ₂ (SCH ₃)(OH ₂) ⁺ }	–2400.6412	1084
5b	{Zn(NH ₃) ₂ (SCH ₃)(OH)} ⁺	–2400.2284	
6a	{Zn(NH ₃)(SCH ₃) ₂ (OH ₂)}	–2781.2857	1440
6b	{Zn(NH ₃)(SCH ₃) ₂ (OH)} [–]	–2780.7371	
7a	{Zn(NH ₃) ₃ (OH ₂)(OH ₂)(H)} ³⁺	–2095.6225	422
7b	{Zn(NH ₃) ₃ (OH)(OH ₂)(H)} ²⁺	–2095.4616	
8a	{Zn(OH ₂) ²⁺ }	–1851.1841 (–1851.3288) ^c	428 (454) ^c
8b	{Zn(OH)} ⁺	–1851.0211 (–1851.1560) ^c	
9a	OH ₂	–75.8980 (–76.0568) ^c	1760 (1700) ^c
9b	OH [–]	–75.2275 (–75.4103) ^c	

^a Basis set 2. ^b $R(\text{Zn–N}) = 2.1 \text{ \AA}$. ^c Basis set 3.

Zn(NH₃)₃OH⁺ system (1b) and then kept constant along the hydroxo series, unless otherwise specified. The coordination geometry for the water molecule was such as to maintain the zinc ion in the H–O–H plane on the bisectant of the H–O–H angle. This choice is consistent with previous energy minimizations on a Zn–OH₂ system,³ although nonplanar geometries have been also reported.¹¹ However, it can be safely assumed that a small displacement of the Zn–OH₂ moiety from planarity will not affect our main conclusions. The coordination geometries were tetrahedral for complexes 1, square pyramidal for complexes 2, and octahedral for complexes 3. In the square-pyramidal geometries, the oxygen ligands were placed in the axial positions and the angles with the in-plane ligands were taken equal to 95°. In a second series of zinc complexes, also listed in Table I (4–6), the effect of the presence of a negatively charged ligand was investigated. In the formate complexes (4), the Zn–O distance was 2.0 Å⁹ and

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Table II. Charge Distributions Derived from the Mulliken Population Analysis for the Systems Reported in Table I (Energies (eV) of the HOMO with Oxygen Lone-Pair Character Given in Parentheses)

complex	charge distribn								
	Zn	H ₂ O/OH ⁻	O	H	NH ₃	COOH ⁻	SCH ₃ ⁻	H ₂ O	H ⁺
1a	+1.30	+0.13	-0.73 (-24.6)	+0.43	+0.19				
1b	+1.21	-0.66	-0.96 (-13.4)	+0.30	+0.15				
2a	+1.33	+0.07	-0.73 (-24.0)	+0.40	+0.15				
2b	+1.29	-0.69	-0.96 (-12.7)	+0.27	+0.10				
3a	+1.31	+0.09	-0.71 (-23.4)	+0.40	+0.12				
3b	+1.32	-0.67	-0.94 (-12.1)	+0.27	+0.07				
4a	+1.28	+0.10	-0.72 (-20.9)	+0.41	+0.16	-0.70			
4b	+1.23	-0.68	-0.97 (-9.5)	+0.29	+0.11	-0.77			
5a	+1.02	+0.10	-0.72 (-20.5)	+0.41	+0.16		-0.44		
5b	+1.04	-0.69	-0.97 (-9.2)	+0.28	+0.12		-0.59		
6a	+0.85	+0.09	-0.69 (-16.7)	+0.39	+0.12		-0.53		
6b	+0.92	-0.70	-0.93 (-5.8)	+0.23	+0.08		-0.65		
7a	+1.29	+0.08	-0.72 (-27.5)	+0.44	+0.20			+0.08	+0.95
				+0.36 ^a					
7b	+1.23	-0.69	-0.92 (-16.5)	+0.23	+0.15			+0.10	+0.91
8a	+1.81	+0.19	-0.77	+0.48					
8a ^b	+1.82	+0.18	-0.76	+0.47					
8b	+1.44	-0.44	-0.82	+0.38					
8b ^b	+1.48	-0.44	-0.87	+0.39					
9a		0.0	-0.64	+0.32					
9a ^b		0.0	-0.64 (-13.6)	+0.32					
9b		-1.0	-1.09	+0.09					
9b ^b		-1.0	-1.17 (-1.0)	+0.17					

^a Relative to the atom hydrogen-bonded with the other water molecule. ^b Basis set 3.

the ligand geometry was taken equal to the experimental one for formic acid after abstraction of the proton;¹⁰ all other distances and angles were the same as those of complexes **1**. In the CH₃S⁻ complexes (**5** and **6**), the Zn-S distances were 2.3 Å⁹ and the ligand geometry was taken from the experimental one for CH₃-SH,¹⁰ again after abstraction of a proton. The Zn-N and Zn-O distances, as well as coordination geometries, were the same as those described above. Complexes **7** are the same as **1**, with the addition of a water molecule bridging the coordinated water to a proton through hydrogen bonds. One of the protons of the coordinated water is hydrogen-bonded to the oxygen of the second water, whose geometry is the experimental one,¹⁰ at a 2.5-Å distance. The latter oxygen is in turn hydrogen-bonded to a proton at a 2.5-Å distance in a linear arrangement bisecting the H-O-H angle, so that the total coordinated oxygen-proton distance is 5 Å. Finally, systems **8** and **9** serve as references: in **8a** and **9a** the water geometry is the experimental one,¹⁰ and the Zn-O distance in **8a** is 2.0 Å.⁹ The geometry of **8b**, as well as the O-H distance in **9b**, is the same as that of the Zn-OH⁺ moiety in **1b**. Independently optimized geometries for **8** and **9** were also obtained (see later).

Computational Methods

"Ab initio" spin restricted Hartree-Fock gradient calculations were performed on the analyzed systems by using the GAMESS program package¹² implemented on a CRAY XMP/48 computer.

The basis set employed for zinc (basis set 1) was derived from Huzinaga's MIDI-4,¹³ with the addition of a p-type polarization function, whose exponent is 0.123.¹⁴ Huzinaga's MIDI-4 basis set was also used for carbon,¹⁵ oxygen,¹⁵ nitrogen,¹⁵ and sulfur¹⁶ atoms, while a (2s) contraction¹⁵ of Van Duijneveldt's (4s) primitive set¹⁷ was adopted for the hydrogen atoms.

Preliminary calculations have been performed on systems **1** with a second basis set (basis set 2), where the 4p orbital of the zinc atom is described by two additional basis functions, whose exponents are 0.5226 and 0.2049.¹⁸ The results obtained gave results similar to those achieved

with the smaller basis set (see later), suggesting the use of the less expensive one for the more extensive calculations.

A more expanded basis set with the inclusion of diffuse functions on the oxygen atom (basis set 3) was used in preliminary calculations performed on systems **8** and **9**. This basis set, of triple- ζ valence quality for oxygen and hydrogen,¹⁹ has been augmented with a polarization d-type function on oxygen²⁰ and a p-type function on hydrogen²⁰ and diffuse s and p functions on oxygen with exponents 0.09 and 0.06, respectively.²¹ The diffuse functions play, as expected, a crucial role in the description of **9b**, while **8b** can be acceptably described also with the smaller basis set defined above.

Results and Discussion

Properties of Coordinated Water. The energies of the various species under examination are reported in Table I, together with the energy differences between the Zn-OH₂ and Zn-OH species. The calculations on the Zn(NH₃)₃OH₂²⁺/Zn(NH₃)₃OH⁺ systems (**1**) have been also performed by using a more expanded basis set (basis set 2) as described in the previous section. The effect of the improved basis set is to lower the total energies by about 50 kJ mol⁻¹, while their difference is virtually unaffected (Table I). Therefore, all the subsequent calculations were performed by using the smaller basis set. For H₂O/OH⁻ (**9**) and Zn-OH₂²⁺/Zn-OH⁺ (**8**), a more expanded basis set (basis set 3) with the additional inclusion of diffuse functions on oxygen was also tested. The energies are reported in parentheses in Table I. The dissociation energy of H₂O into H⁺ and OH⁻, which has an experimental value of 1720 kJ mol⁻¹,²² is here reproduced with values ranging between 1700 and 1760 kJ mol⁻¹. The difference between the two basis sets is thus rather unimportant for water and even less important for Zn-OH₂. This supports our choice to use a simplified basis set to compare the results along the series.

A comparison between tetrahedral, square-pyramidal, and octahedral species indicates that the differences in energy between the hydroxo and water moieties steadily increase on going from the former to the latter; i.e., the proton dissociation energy increases. This may be related to the pK_a of the corresponding water

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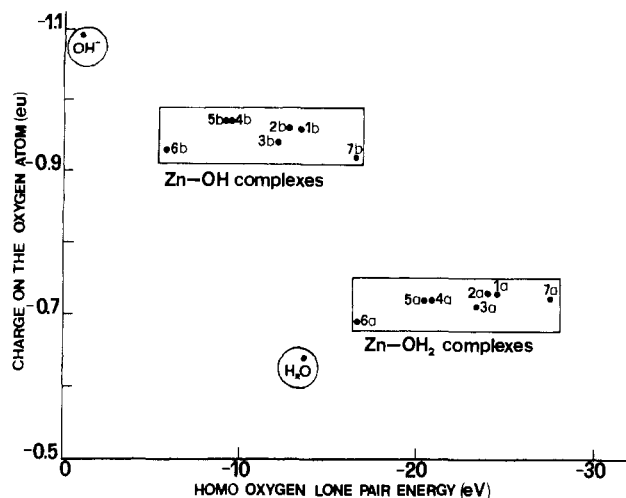


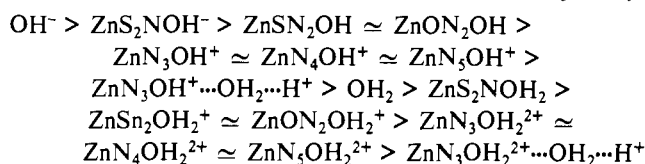
Figure 1. Mulliken charges on the oxygen atom (from Table II) against the HOMO oxygen lone pair energy for the investigated compounds. The nucleophilicity of the oxygen is expected to increase from right to left (increasing HOMO energy) as well as from bottom to top (increasing negative charge).

complexes (see later). These calculations were performed by keeping the Zn-NH₃ distance constant in all cases; however, sample calculations with a longer distance (2.1 Å) in the five-coordinated species **2** with respect to the tetrahedral species **1** show minimal changes in the energy differences (values in parentheses in Table I). Therefore, the main effect seems to be due to the change in coordination number.

Table II shows the charge distribution on all the species listed in Table I, derived from the respective Mulliken population analysis, and the energy of the HOMO with oxygen lone pair character. The charge on the zinc atom shows no significant variations on passing from the water to the corresponding hydroxo complex. It is also relatively constant in the 1-3 series (+1.21 to +1.33), as it is between **1** and **4** which, as in the H₂O/hydroxo cases, differ by one unit charge. On the other hand, the presence of an anionic sulfur ligand induces a significant decrease from +1.28 (**4a**) to +1.02 (**5a**). The substitution of a second ammonia ligand with a CH₃S⁻ moiety causes a further decrease to +0.85 (**6a**). A much higher charge of +1.81 is of course predicted for **8a**, which has no other donor atom on zinc but the water molecule. The analysis of the charge on the water or hydroxo ligands is even more instructive. The charge on the oxygen atom is systematically higher on the hydroxo complexes by a fairly constant amount (-0.20 to -0.25) for all the complexes **1-7**. The difference drops to -0.05 in **8** and rises to -0.45 in **9**. In the latter case, the hydroxide ion shows the highest negative charge (-1.09), whereas neutral water shows a value of -0.64, which is the lowest in Table II. The calculations performed with the more extended basis set leave virtually unaltered the charge on the water-containing species and increase by less than 0.1 that on the hydroxo species.

We would like to use the data of Table II to understand the kinetic capability of the various species to perform a nucleophilic attack on a substrate molecule. The overall charge on the oxygen atom may give an indication of the order of nucleophilic capability. The kinetic properties are probably related to the difference in charge between the oxygen donor and the carbon acceptor. Furthermore, they are also related to the difference in energy between the oxygen orbital involved in the formation of the new bond and the energy of the accepting orbital: since the accepting orbital is always higher in energy, the higher the oxygen HOMO, the closer it will be to the accepting orbital, thereby favoring the formation of a covalent bond. If the acceptor is kept constant, then both the charge on the oxygen and the energy of the HOMO can allow us to make a scale of nucleophilicity.²³⁻²⁷ In Figure

1 the charge on the oxygen atom is plotted against the energy of the HOMO. The figure shows clearly that charge and HOMO energy, taken separately as a measure of nucleophilicity, would not give the same order. Obviously, the actual nucleophilicity must be a function of both. However, if we look at the oxygen charge, four groups of systems are easily identified: they are the free hydroxide ion, the zinc-hydroxo complexes, the zinc-water complexes, and free water. The differences in oxygen charge among the first three groups are so sizable that the latter parameter by itself can be confidently taken as a qualitative measure of the nucleophilicity. There are virtually no differences in oxygen charge within the Zn-hydroxo or the Zn-aqua series, even if the total charges of the systems within each series span over three units. On the other hand, the HOMO energy is apparently sensitive to the total charge. Therefore, within each of the two series, the nucleophilicity would seem to increase on passing from the tripositive to the bipoisitive to the monopositive to the neutral complexes. The position of free water in Figure 1 deserves a comment. In terms of oxygen charge, free H₂O would seem to be even less effective as a nucleophile than Zn-OH₂ complexes. However, the energy of its HOMO is markedly higher than that in all water complexes, thereby reversing the order given by the charge. In other words, if we consider free water as having approximately the same oxygen charge as the Zn-OH₂ complexes, then the order given by the HOMO energies correctly predicts that water is more nucleophilic than any of the zinc complexes. The latter finding makes us more confident of the order based on the HOMO energies within the Zn-OH₂ series itself and, by extension, within the Zn-OH series. As a final comment we may note that there are no appreciable differences among four-, five-, and six-coordinated complexes in terms both of charge and of HOMO energy. It can be concluded that lowering the coordination number decrease the pK_a, i.e. makes a Zn-OH complex available at lower pH (see later), without altering the nucleophilicity of the Zn-OH complex. In summary, the above analysis would suggest the following as a reasonable order of nucleophilicity:



Regarding the relationship between the present energy calculations and the pK_a of the coordinated water, the increase in proton dissociation energy is 47 kJ mol⁻¹ on passing from tetrahedral to square-pyramidal complexes and is 50 kJ mol⁻¹ on passing from square-pyramidal to octahedral complexes (Table I). This increase, if entropic and other enthalpic effects are neglected, would correspond to about 8 pK_a units, which is indeed too much for any range of experimental values. Of course solution effects, which are neglected here, may greatly alter the observed differences, although probably not the trend. Furthermore, a reaction in an enzyme-active cavity may actually be better approximated by a gas-phase model rather than by a model reaction in solution. Proton dissociation energies of the same order of magnitude were previously calculated by using less refined methods for a variety of compounds.⁷

When the charge of the complex is reduced by one unit with a carboxylate ligand, the proton dissociation energy dramatically increases. The same increase in proton dissociation energy occurs when a cysteine type of ligand is used, showing that the effect is essentially electrostatic; this is at variance with the charge on the zinc atom, which, as discussed before, is less sensitive to the total charge of the complex than to the type of donor atom. The presence of two cysteines, which brings the charge of the water complex to zero, greatly disfavors the breaking of the O-H bond.

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On the other hand, the presence of a positive charge outside the first coordination sphere in complex⁷ has a profound effect in lowering the proton dissociation energy. This effect is apparently equivalent to the effect of three ammonia ligands, as can be seen by comparing **1** with **7**.

Concluding Remarks

This research has shown that the Zn–OH moiety has a nucleophilicity close to that of the hydroxide group independent of the coordination number and of the nature of the other donor groups. This result is meaningful with respect to the many reactions catalyzed by Zn–OH₂ (which then could give rise to a Zn–OH intermediate) and Zn–OH moieties.

The charge of the donor groups (i.e. His, Glu, or Cys) changes the energy of the Zn–OH₂ ⇌ Zn–OH + H⁺ reaction. Such energy presumably parallels the pattern of the pK_a values. If for the sake of comparison, one equates the value of 1760 kJ mol⁻¹ for the dissociation energy of H₂O into OH⁻ + H⁺ (Table I) to 14, the pK_a of water, and scales the other ΔE values in Table I in the same way, a value of 5.7 for the “pK_a” of **1a** is obtained. The pK_a increases to 6.1 and 6.5 for **2a** and **3a**, i.e., with increasing coordination number. As an example, the enzyme carbonic anhydrase with a donor set ZnN₃OH₂ has a pK_a ranging between 5 and 7 depending on the isoenzyme and the nature of the groups inside the cavity.⁸ A positive charge inside the cavity lowers the pK_a of coordinated water.²⁸

4a and **5a**, with one positive charge less than **1a–3a**, have pK_a's of 8.5 and 8.6. In the enzyme carboxypeptidase there is a Zn–

(His)₂(Glu)OH₂ moiety, but the coordinated water is hydrogen-bonded to another Glu,²⁹ which undergoes deprotonation with a pK_a of 5. The enzyme has another pK_a of 9, which has been proposed, among other possibilities, to be due to the coordinated water.³⁰

6a, with two sulfurs and no charge, has a pK_a of 11.5. Liver alcohol dehydrogenase has a zinc coordinated to two cysteines and one histidine, besides water.³¹ According to the present results, the pK_a should be sizably higher than that in carbonic anhydrase and, indeed, it is 9.2.³² The presence of a positively charged NAD⁺ coenzyme lowers the pK_a down to 7.4,³² but the presence of a neutral NADH coenzyme molecule raises it to 11.2.³²

The above comparison is of course artificial, since the energies have been scaled down arbitrarily. Nevertheless, it is also instructive in the sense that the scaled values reproduce not only the experimental trend but also the sizes of the relative variations. This is a further support to the conclusion that the pK_a's of coordinated water in metalloproteins are indeed determined by the electronic properties of the metal complex as a whole, plus the electrostatic contributions from nearby residues, in a predictable way.

Acknowledgment. Thanks are expressed to Prof. R. S. Drago for a helpful discussion.

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Stabilities of Trivalent Metal Complexes of Phenolic Ligands Related to *N,N'*-Bis(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic Acid (HBED)

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Received August 3, 1989

The stability constants and other equilibrium parameters of the trivalent metal ion complexes of three multidentate ligands have been determined: *N,N'*-bis(2-hydroxy-3,5-dimethylbenzyl)ethylenediamine-*N,N'*-diacetic acid (Me₂HBED); *N,N'*-bis(2-hydroxy-3-methyl-5-*tert*-butylbenzyl)ethylenediamine-*N,N'*-diacetic acid (*t*-BuHBED); and *N,N'*-bis(2-hydroxy-3,5-dimethylbenzyl)-*N*-(2-hydroxyethyl)ethylenediamine-*N'*-acetic acid (HBMA). Alkylation of the aromatic ring significantly increases the basicity of the ligand, as measured by protonation constants, but lowers metal ion affinity, because of steric effects. Replacement of an acetate of Me₂HBED by a hydroxyethyl donor group lowers the protonation constants of the ligand only slightly but lowers affinity for trivalent metal ions by 4–6 orders of magnitude. Stability constants involving trivalent metal ions are compared with those of five other sexadentate ligands, all of which contain two phenolate donor groups. Effectiveness of all eight ligands in binding trivalent metal ions in biological media is assessed by comparing pM values at physiological pH 7.4.

Introduction

Two phenolate-containing sexadentate ligands (Chart I) with an EDTA-type framework, ethylenebis(2-hydroxyphenyl)glycine (EHPG, **1**) and *N,N'*-bis(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED, **2**) designed to strongly bind Fe(III), have been known for many years.^{1,2} Recently a series of analogues of these parent compounds have been developed as ligands having high affinities for Ga(III), In(III), and Gd(III), as well as Fe(III), as radiopharmaceuticals, and as magnetic resonance paramagnetic contrast imaging agents.^{3–6} Also recently EHPG has been separated into its *racemic* and *meso* forms, and the stabilities of

complexes of each isomer with a wide variety of metal ions have been determined.⁷ Two additional new ligands of this general type, with two phenolate donors, a substituted ethylenediamine moiety,

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