Metal Ion Binding Properties of Dihydroxyacetone Phosphate and Glycerol 1-Phosphate

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Abstract: The acidity constants of $H(R\text{-}MP)^-$, where $R\text{-}MP^{2-}$ = dihydroxyacetone phosphate ($DHAP^{2-}$) and glycerol 1-phosphate ($G1P^{2-}$), and the stability constants of the binary M(R-MP) complexes (M^{2+} = Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}) were determined by potentiometric pH titrations in aqueous solution (I = 0.1 M, $NaNO_3$; 25 °C). The stability of the ternary Cu(Arm)(R-MP) complexes (Arm = 2,2'-bipyridyl or 1,10-phenanthroline) were also measured. On the basis of recent results for simple phosphate monoesters, $R\text{-}MP^{2-}$, where R is a strictly noncoordinating residue (Massoud, S. S.; Sigel, H. *Inorg. Chem.* **1988**, *27*, 1447-1453), it is established that the stability of all the M(DHAP) and M(G1P) complexes is governed by the basicity of the phosphate group of $DHAP^{2-}$ and $G1P^{2-}$. There are no indications in aqueous solution for the participation of the oxygen atom of the carbonyl or hydroxy groups at C-2 of these ligands in complex formation, which would on steric grounds be possible. However, measurements with Cu^{2+} and $DHAP^{2-}$ or $G1P^{2-}$ in water containing 30 or 50% (v/v) 1,4-dioxane (I = 0.1 M, $NaNO_3$; 25 °C) prove that to some extent seven-membered chelates involving the mentioned oxygen atoms may be formed. This may also be surmised for the other mentioned divalent metal ions under appropriate conditions, because it is well-known that they all can interact with the oxygen of carbonyl or hydroxy groups, especially when the solvent has poorer solvating properties than water. This condition exists in active-site cavities of enzymes; therefore, the indicated type of metal ion interaction could play a role in certain metabolic processes involving DHAP (or GAP; see below) and G1P. It is further concluded that the proton and metal ion affinities of glyceraldehyde 3-phosphate (GAP^{2-}) correspond in a first approximation to those of $G1P^{2-}$ because both ligands contain the same structural unit, i.e., $-CH(OH)CH_2OPO_3^{2-}$, which is responsible for the proton and metal ion binding properties, as shown now for $G1P^{2-}$. This conclusion regarding GAP is meaningful because this ligand may hardly be studied directly due to its conversion into DHAP.

Dihydroxyacetone phosphate ($DHAP^{2-}$) and glycerol 1-phosphate ($G1P^{2-}$)[2] are important intermediates in biological systems.[3-5] For example, the most abundant membrane lipids in many cells are constructed upon a backbone of glycerol 1-phosphate.[3] DHAP is, next to glyceraldehyde 3-phosphate (GAP^{2-}), produced in the catabolism of glucose:[4,5] once taken up by the cell, glucose is phosphorylated in position 6 with ATP by hexokinase, isomerized to fructose 6-phosphate, further phosphorylated with a second ATP by phosphofructokinase to fructose 1,6-diphosphate and then split by an aldolase into DHAP and GAP, which can be rapidly interconverted into each other by triosephosphate isomerase.[4,6]

The so-called α-glycerophosphate shuttle[3] transforming DHAP and G1P into each other is important for NADH oxidation in insect flight muscles as well as in many tissues, including brain and smooth muscle, of other organisms.[3,5] It is depictured in a simplified way in Figure 1, together with the chemical structures of the two title compounds considered in this study.

Many of the metabolic steps indicated above depend on metalloenzymes or on metal ion-depending enzymes; this is especially true for transphosphorylations[7] and, e.g., ATP^{4-} is employed as substrate only in the form of a metal ion complex, mostly as $Mg(ATP)^{2-}$.[8] The split of fructose 1,6-diphosphate into DHAP and GAP is also catalyzed by a metalloaldolase.[9] The stabilities and structures[10] of $M(ATP)^{2-}$ and of many related[11] nucleoside phosphate complexes in solution are relatively well characterized today. However, to our knowledge there are no such data available for DHAP and only a few for G1P.[12,13] Therefore, we have now measured the stabilities of the complexes formed between these two phosphate monoesters, $R\text{-}MP^{2-}$, and the alkaline earth ions, several divalent 3d metal ions, and Zn^{2+} or Cd^{2+}; the results also allow conclusions regarding the proton and metal ion affinities of GAP^{2-}. We endeavored to answer the question: is the stability of these complexes solely determined by the basicity of the phosphate group or may one of the other groups, i.e., the oxygen atom of the carbonyl or a hydroxy group (see Figure 1), also participate in complex formation?

1. Experimental Section

1.1. Materials. The disodium salt of D,L-α-glycerophosphate hexahydrate (approximately 95%) and the lithium (1.5 equiv) salt of dihydroxyacetone phosphate (purity 97%; with ca. 5 mol % inorganic phosphorus) were from Sigma Chemical Co., St. Louis, MO, and used as obtained (see also section 1.2). However, from the potentiometric pH titrations and the curve-fit procedure for the determination of $pK^{H}_{H(R\text{-}MP)}$ the exact concentrations of G1P and DHAP were measured (see below). Acetone, $HClO_4$ (70%), $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, and 1,4-dioxane (extra pure) were from Merck AG, Darmstadt, FRG. The perchlorate salts of Cd^{2+} and the other metal ions were from Johnson Matthey GmbH, Alfa Products, Karlsruhe, FRG, and Fluka AG, Buchs, Switzerland, respectively. All other reagents were the same as used previously.[14] All

(1) Work done in Basel during leaves from the Academy of Beijing Traditional Medicine (G.L.) and from the University of Nanjing (D.C.), China.

(2) Abbreviations and definitions: ATP^{4-}, adenosine 5'-triphosphate; Arm, heteroaromatic N base, e.g., bpy or phen; bpy, 2,2'-bipyridyl; BuP^{2-}, *n*-butyl phosphate; $DHAP^{2-}$, dihydroxyacetone phosphate; FAD, flavin adenine dinucleotide; $FADH_2$, reduced form of FAD; GAP^{2-}, glyceraldehyde 3-phosphate; $G1P^{2-}$, glycerol 1-phosphate (= α-glycerophosphate; in many biochemistry texts also designated as glycerol 3-phosphate; in this study D,L-G1P is used); L, general ligand; M^{2+}, divalent metal ion; NAD^+, nicotinamide adenine dinucleotide; NADH, reduced form of NAD^+; phen, 1,10-phenanthroline; PO_4, if nothing else is specified, the formula PO_4 represents all related species which might be present in solution, i.e., H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}; $RibMP^{2-}$, D-ribose 5'-monophosphate; $R\text{-}MP^{2-}$, general phosphate monoester, e.g. $DHAP^{2-}$ or $G1P^{2-}$, i.e., R may be any organic residue (e.g., *n*-butyl or nucleosidyl); UMP^{2-}, uridine 5'-monophosphate.

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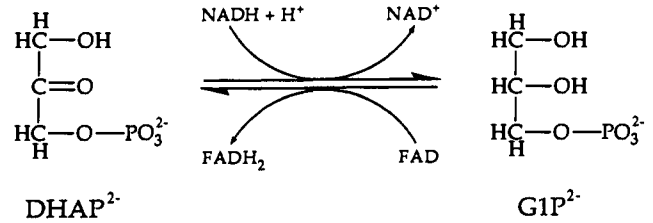

Figure 1. Simplified representation of the so-called[3] α-glycerophosphate shuttle of insect flight muscles, in which dihydroxyacetone phosphate ($DHAP^{2-}$) and glycerol 1-phosphate ($G1P^{2-}$) are interconverted into each other.[3,5]

solutions were prepared with distilled CO_2-free water.

The titer of the NaOH used for the titrations was determined with potassium hydrogen phthalate; the exact concentrations of the G1P and DHAP solutions used in the titration experiments with the metal ions (titrated in the presence of an excess of HNO_3; see section 1.4) were measured with NaOH. The concentrations of all stock solutions of the divalent metal ions were established with EDTA.

1.2. Stability of DHAP and G1P toward Dephosphorylation. The available DHAP contained some inorganic phosphate as impurity (section 1.1.); therefore, the content of free PO_4 was determined as previously[7] with carefully standardized molybdate reagent in DHAP and also in the G1P. The measurements were made with a Perkin Elmer Lambda 1 photometer.

The DHAP contained initially 3.8 ± 0.7% (3σ) of inorganic phosphate. For 0.3 mM aqueous solutions (see section 1.4) of DHAP (I = 0.1 M, $NaClO_4$; 25 °C), we observed that the liberation rate of phosphate increased from pH 4–8. At pH 4 after 5 h only an additional 0.3% of PO_4 was liberated from DHAP, while at pH 8 after the same time then in total about 10% of free PO_4 were present. This was the most unfavorable case for a titration experiment because in the daily freshly prepared stock solutions of DHAP a pH of about 8 had to be adjusted. However, the effect of this free phosphate on the stability constants of the M(DHAP) complexes was minimized by evaluating in these cases only about the first half of the titration curves (see section 1.4); this is possible because the pK_a values of $H_2PO_4^-$ and $H(DHAP)^-$ differ by 0.8 log unit (Table I; vide infra) and therefore complex formation between M^{2+} and HPO_4^{2-} occurs also at a higher pH than with $DHAP^{2-}$. Finally, it should be emphasized that dephosphorylation experiments in the presence of Ni^{2+}, Cu^{2+}, or Cd^{2+} under the conditions of the titrations (I = 0.1 M, adjusted now with $NaClO_4$; 25 °C) and in the corresponding pH range revealed that these metal ions have no remarkable effect on the liberation of phosphate during the 30 min needed for a titration experiment.

Regarding G1P the liberation of PO_4 did not constitute any problem. The purchased compound contained not more than 0.2% of inorganic phosphate. Measurements at pH 5.5 in the absence and presence of Ni^{2+}, Cu^{2+}, or Cd^{2+}, with the concentrations used in the titration experiments, showed that over 10 h only about 3% of PO_4 is liberated from G1P. At pH 8 with [G1P] = 0.3 mM (I = 0.1 M, $NaClO_4$; 25 °C) after 8 h about 0.3% of PO_4 was released.

1.3. Potentiometric pH Titrations. These were carried out as described.[14] The direct pH meter readings were used in the calculations of the acidity constants for $H(R\text{-}MP)^-$, i.e., these constants are so-called practical constants, also known as mixed or Brønsted constants.[15] The negative logarithms of these acidity constants given for aqueous solutions at I = 0.1 M ($NaNO_3$) and 25 °C may be converted into the corresponding concentration constants by subtracting 0.02 log unit.[15]

It should be emphasized that the ionic product of water (K_w) and the mentioned conversion term do *not* enter into our calculation procedures because we evaluate the *differences* in NaOH consumption between solutions with and without ligand (see section 1.4).[14,15]

1.4. Determination of Equilibrium Constants. The acidity constants $K^{H}_{H(R\text{-}MP)}$ for $H(DHAP)^-$ and $H(G1P)^-$ were determined by titrating 50 mL of aqueous 0.54 mM HNO_3 and $NaNO_3$ (I = 0.1 M; 25 °C) in the presence and absence of 0.3 mM $R\text{-}MP^{2-}$ with 1 mL of 0.03 M NaOH as described.[14] For DHAP and G1P 18 and 26 pairs of independent titrations, respectively, were evaluated between about 3% and 97% of neutralization for the equilibrium $H(R\text{-}MP)^-/R\text{-}MP^{2-}$.

The conditions for the determination of the stability constants $K^{M}_{M(R\text{-}MP)}$ of the binary M(R-MP) complexes and of $K^{Cu(Arm)}_{Cu(Arm)(R\text{-}MP)}$ of the ternary Cu(Arm)(R-MP) complexes were the same as given previously.[14]

Table I. Negative Logarithms of the Acidity Constants (Eqs 1 and 2) in Aqueous Solution for $H_2(DHAP)$ and $H_2(G1P)$ As Well As for Some Related Species, at 25 °C and I = 0.1 M ($NaNO_3$) as Determined by Potentiometric pH Titrations[a]

$R\text{-}MP^{2-}$	$pK^{H}_{H_2(R\text{-}MP)}$	$pK^{H}_{H(R\text{-}MP)}$
HPO_4^{2-}	1.86[b]	6.70 ± 0.02[c]
$CH_3OPO_3^{2-}$		6.2[d]
BuP^{2-}		6.72 ± 0.02[e]
$RibMP^{2-}$		6.24 ± 0.01[e]
$G1P^{2-}$	~0.7[f]	6.23 ± 0.01
$DHAP^{2-}$	~0.7[f]	5.90 ± 0.01

[a] So-called practical constants[15] are listed; see section 1.3. The errors given are *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] This value for H_3PO_4, i.e., $pK^{H}_{H_3PO_4}$, is from ref 21 (I = 0.5 M, KCl; 22 °C); in the same study is also given $pK^{H}_{H_2PO_4}$ = 6.69 in good agreement with the other value listed above. [c] This value for $H_2PO_4^-$ i.e., $pK^{H}_{H_2PO_4}$, is from ref 22; I = 0.1 M, $NaClO_4$; 25 °C. [d] From ref 23; I = 0.1 M, NaCl; 25 °C. [e] From ref 19; I = 0.1 M, $NaNO_3$; 25 °C. [f] Estimate; see text in section 2.1.

This means part or all of $NaNO_3$ was replaced in the solutions described in the preceding paragraph ($[R\text{-}MP^{2-}]$ = 0.3 mM) by $M(NO_3)_2$ (I = 0.1 M; 25 °C); the $R\text{-}MP^{2-}$:M^{2+} ratios were 1:111 (Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}), 1:89 (Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+}), 1:56 (Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}), 1:44 (Co^{2+}), 1:28 (Mn^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}), and 1:11 and 1:5.6 (Cu^{2+}, $Cu(bpy)^{2+}$, $Cu(phen)^{2+}$, see ref 14). For each DHAP system at least 6, and for each G1P system at least 8 independent pairs of titrations were recorded, evaluated, and the individual results (see below) averaged for the final results.

The stability constants $K^{M}_{M(R\text{-}MP)}$ (and correspondingly for $K^{Cu(Arm)}_{Cu(Arm)(R\text{-}MP)}$) were computed for each pair of titrations by taking into account the species H^+, $H(R\text{-}MP)^-$, $R\text{-}MP^{2-}$, M^{2+}, and $M(R\text{-}MP)$.[14] Throughout the data were collected (every 0.1 pH unit) from about 5% complex formation to a neutralization degree of about 50% in the case of DHAP (see section 1.2) and about 85% with G1P or to the beginning of the hydrolysis of $M(aq)^{2+}$; the latter was evident from the titrations without R-MP. The values calculated individually for $\log K^{M}_{M(R\text{-}MP)}$ (or $\log K^{Cu(Arm)}_{Cu(Arm)(R\text{-}MP)}$) showed no dependence on pH or on the excess amount of M^{2+}.

The acidity constants $K^{H}_{H(R\text{-}MP)}$ for $H(DHAP)^-$ and $H(G1P)^-$ and the stability constants $K^{Cu}_{Cu(R\text{-}MP)}$ for the binary Cu(DHAP) and Cu(G1P) complexes were also determined in water containing 30 or 50% (v/v) 1,4-dioxane. The same concentrations of the reagents as indicated above were used; for further details see refs 16 and 17. The results given in section 2.4 are the averages of at least four, usually eight independent pairs of titration curves.

2. Results and Discussion

2.1. Acidity Constants of $H_2(DHAP)$ and $H_2(G1P)$. Phosphate monoesters ($R\text{-}MP^{2-}$), such as $DHAP^{2-}$ and $G1P^{2-}$, are dibasic species; hence, the following equilibria have to be considered:

$$H_2(R\text{-}MP) \rightleftharpoons H(R\text{-}MP)^- + H^+ \quad (1a)$$

$$K^{H}_{H_2(R\text{-}MP)} = [H(R\text{-}MP)^-][H^+]/[H_2(R\text{-}MP)] \quad (1b)$$

$$H(R\text{-}MP)^- \rightleftharpoons R\text{-}MP^{2-} + H^+ \quad (2a)$$

$$K^{H}_{H(R\text{-}MP)} = [R\text{-}MP^{2-}][H^+]/[H(R\text{-}MP)^-] \quad (2b)$$

The first proton from monoesterified derivatives of phosphoric acid, i.e., from $H_2(R\text{-}MP)$, is released in water with $pK_a \approx 1$ (eq 1).[18] This was recently confirmed for diprotonated uridine 5′-monophosphate, $H_2(UMP)$: $pK^{H}_{H_2(UMP)} = 0.7 \pm 0.3$ (I = 0.1 M, $NaNO_3$; 25 °C).[19] As the phosphate group in UMP is bound to a ribosyl residue, the solvation by water around the phosphate group is expected to be similar to that in DHAP and G1P (see below) and therefore also the release of the first proton from the $-P(O)(OH)_2$ site should occur with a very similar pK_a in all three molecules; on this reasoning the estimate given in Table I is based.

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Table II. Logarithms of the Stability Constants of Binary M(DHAP) and M(G1P) Complexes (Eq 3) and of Ternary Cu(Arm)(DHAP) and Cu(Arm)(G1P) Complexes (Eq 4) as Determined by Potentiometric pH Titrations (Exptl)[a] in Water at 25 °C and I = 0.1 M ($NaNO_3$)[f]

	log $K^{M}_{M(DHAP)}$			log $K^{M}_{M(G1P)}$		
M^{2+}	exptl[a]	calcd[b,f]	log Δ_{DHAP}[c]	exptl[a]	calcd[b,f]	log Δ_{G1P}[c]
Mg^{2+}	1.57 ± 0.03	1.50 ± 0.04	0.07 ± 0.05	1.63 ± 0.03	1.57 ± 0.04	0.06 ± 0.05
Ca^{2+}	1.38 ± 0.02	1.41 ± 0.05	−0.03 ± 0.05	1.43 ± 0.02	1.46 ± 0.05	−0.03 ± 0.05
Sr^{2+}	1.23 ± 0.03	1.22 ± 0.05	0.01 ± 0.06	1.23 ± 0.03	1.25 ± 0.05	−0.02 ± 0.06
Ba^{2+}	1.14 ± 0.09	1.14 ± 0.05	0.00 ± 0.10	1.18 ± 0.03	1.16 ± 0.05	0.02 ± 0.06
Mn^{2+}	2.11 ± 0.02	2.08 ± 0.07	0.03 ± 0.07	2.21 ± 0.04	2.16 ± 0.07	0.05 ± 0.08
Co^{2+}	1.84 ± 0.02	1.87 ± 0.07	−0.03 ± 0.07	1.93 ± 0.02	1.94 ± 0.07	−0.01 ± 0.07
Ni^{2+}	1.85 ± 0.03	1.86 ± 0.06	−0.01 ± 0.07	1.90 ± 0.04	1.96 ± 0.06	−0.06 ± 0.07
Cu^{2+}	2.77 ± 0.02	2.73 ± 0.08	0.04 ± 0.08	2.83 ± 0.05	2.88 ± 0.08	−0.05 ± 0.09
Zn^{2+}	2.01 ± 0.03	2.02 ± 0.08	−0.01 ± 0.09	2.13 ± 0.04	2.12 ± 0.08	0.01 ± 0.09
Cd^{2+}	2.36 ± 0.02	2.34 ± 0.06	0.02 ± 0.06	2.43 ± 0.03	2.44 ± 0.06	−0.01 ± 0.07
$Cu(bpy)^{2+}$	2.79 ± 0.02[d]	2.78 ± 0.08[d,e]	0.01 ± 0.08	2.90 ± 0.05[d]	2.93 ± 0.08[d,e]	−0.03 ± 0.09
$Cu(phen)^{2+}$	2.77 ± 0.04[d]	2.76 ± 0.08[d,e]	0.01 ± 0.09	2.92 ± 0.05[d]	2.91 ± 0.08[d,e]	0.01 ± 0.09

[a] The error limits given are *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] The parameters of the straight-line equations are listed in Table V of ref 19; the error limits (3σ) are from Table VI of ref 19 (see also Table I in ref 32). [c] Log $\Delta_{R\text{-}MP}$ = log K_{exptl} − log K_{calcd}; note, this difference corresponds also to that defined by eq 10; the error limits (3σ) for these differences were calculated according to the error propagation method after Gauss. [d] These values refer to log $K^{Cu(Arm)}_{Cu(Arm)(R\text{-}MP)}$; cf. eq 4. [e] These values were calculated with the straight-line equations given on page 249 of ref 31. [f] The calculated stability constants for a pure metal ion–phosphate coordination (calcd) are given for comparison; these values are based on the straight-line equations[19,31] quantifying the relationships between complex stability and phosphate group basicity (see Figure 2 and Section 2.3)[b] and the $pK^{H}_{H(R\text{-}MP)}$ values of $H(DHAP)^-$ and $H(G1P)^-$ (see Table I).

In any case, the first proton from the phosphoric acid residue in H_2(R-MP) is completely ionized at pH ≥ 3 and does not overlap with equilibrium 2 and the complex formation between M^{2+} and $R\text{-}MP^{2-}$, which only occur at pH > 3.

In addition there was no indication for a release of a further proton from $DHAP^{2-}$ or $G1P^{2-}$ in the pH range covered by this study. Indeed, the deprotonation of sugar-hydroxy groups is expected to occur at pH > 12 only.[20]

The acidity constants determined for $H(DHAP)^-$ and $H(G1P)^-$ and some related data[19,21-23] are listed in Table I. Some literature values[12,13] for $pK^{H}_{H(G1P)}$, i.e., 6.08[12,24] and 6.66,[12,25] are relatively far away from our result, but this is probably due to different definitions of the constants and different experimental conditions. For example, if the concentration constant (pK_a = 6.07) given in ref 24 is transformed (+0.11 log unit)[12,13] into the "mixed" acidity constant one obtains $pK^{H}_{H(G1P)}$ = 6.18 (I = 0.1 M, KCl; 20 °C), a value closer to our result. The above conclusion is further confirmed by another published value,[26] $pK^{H}_{H(G1P)}$ = 6.231, which refers to I = 0.086 M (KCl) and 25 °C, i.e., to conditions similar to ours, and which excellently agrees with our result (Table I). For $pK^{H}_{H(DHAP)}$ a value of 5.98 (I close to 0.1 M, $NaNO_3$; 35 °C), determined by potentiometric pH titrations, was published[27] as well as an apparent[28] constant of 6.0 (I = 0.1 M; 30 °C), determined from kinetic experiments with triosephosphate isomerase. Considering the differences in the experimental conditions both constants fairly agree with our value of 5.90 (I = 0.1 M, $NaNO_3$; 25 °C).

The variations between the acidity constants in Table I may easily be rationalized: For example, a methyl group will certainly somewhat disturb solvation and therefore the release of the proton from $CH_3OPO_2(OH)^-$ should be easier than from the better screened $HOPO_2(OH)^-$ (=$H_2PO_4^-$). Similarly, the lower acidity of $H(BuP)^-$, compared to that of $H(CH_3OPO_3)^-$, may be explained by the relatively large *n*-butyl residue leading to a reduced effective dielectric constant in its vicinity,[29] thus inhibiting the formation of a further charge, i.e., the release of the proton.[30] The nearly identical acidities of $H(CH_3OPO_3)^-$, H(RibMP)$^-$, and $H(G1P)^-$ probably reflect the pronounced hydrophilicity of the ribose ring and the glycerol residue allowing a solvation of their twofold negatively charged phosphate groups similar to that in methyl phosphate. Finally, the somewhat larger acidity of $H(DHAP)^-$ compared with that of $H(G1P)^-$ may be attributed to the electron withdrawing properties of the carbonyl group at C-2, thus reducing the basicity of the phosphate residue in $DHAP^{2-}$.

2.2. Stability Constants of Binary M(DHAP) and M(G1P) Complexes As Well As of Some Ternary Cu(Arm)(R-MP) Complexes. The experimental data of the potentiometric pH titrations carried out with DHAP and G1P and the alkaline earth ions, several divalent 3d metal ions, as well as Zn^{2+} and Cd^{2+} (M^{2+}) may all be completely described by considering equilibria 2 and 3 (see also sections 1.4 and 2.1).

$$M^{2+} + R\text{-}MP^{2-} \rightleftharpoons M(R\text{-}MP) \quad (3a)$$

$$K^{M}_{M(R\text{-}MP)} = [M(R\text{-}MP)]/([M^{2+}][R\text{-}MP^{2-}]) \quad (3b)$$

The same is true for the ternary systems consisting of DHAP or G1P, Cu^{2+}, and an aromatic amine (Arm), i.e., 2,2′-bipyridyl (bpy) or 1,10-phenanthroline (phen); here equilibria 2 and 4 have to be considered.[14]

$$Cu(Arm)^{2+} + R\text{-}MP^{2-} \rightleftharpoons Cu(Arm)(R\text{-}MP) \quad (4a)$$

$$K^{Cu(Arm)}_{Cu(Arm)(R\text{-}MP)} = [Cu(Arm)(R\text{-}MP)]/([Cu(Arm)^{2+}][R\text{-}MP^{2-}]) \quad (4b)$$

The stability constants determined are listed in columns 2 and 5 of Table II for the M(DHAP) or Cu(Arm)(DHAP) and M(G1P) or Cu(Arm)(G1P) complexes, respectively; the data given in the other columns[19,31,32] will be discussed in section 2.3.

To our knowledge only the stability constants for the Mg^{2+} and Ca^{2+} complexes of $G1P^{2-}$ were previously determined;[12,13,24] the agreement with the present values, which are about 0.2 log unit smaller, is satisfactory. Furthermore, the stability constants listed

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in Table II are in the order previously observed for other phosphate monoester complexes.[19] In fact, a direct comparison of the data for D-ribose 5'-monophosphate ($RibMP^{2-}$)[19] and $G1P^{2-}$ is possible because their $pK^{H}_{H(R\text{-}MP)}$ values are practically identical (see Table I): the average stability difference between the log $K^{M}_{M(R\text{-}MP)}$ values for the M(RibMP) complexes[19] and the ten corresponding binary M(G1P) complexes listed in Table II is 0.057 log unit. In addition, within each series of the M(DHAP) and M(G1P) complexes the usual trends are observed:[14,19,32] Complex stability of the alkaline earth ions increases with decreasing ionic radii but is lower than that of the 3d M^{2+} ions; for these the longstanding experience[19,22,33] is confirmed that the stabilities of metal ion–phosphate complexes do not strictly correspond to the Irving–Williams series.[34]

The relative stability of ternary complexes toward their binary parent complexes is best quantified by considering the difference defined in eq 5:[35-37]

$$\Delta \log K_{Cu} = \log K^{Cu(Arm)}_{Cu(Arm)(R\text{-}MP)} - \log K^{Cu}_{Cu(R\text{-}MP)} \quad (5)$$

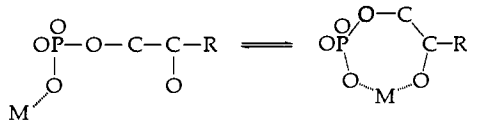
This difference of the logarithms of two stability constants is of course again a constant; it quantifies the position of the following equilibrium:

$$Cu(Arm)^{2+} + Cu(R\text{-}MP) \rightleftharpoons Cu(Arm)(R\text{-}MP) + Cu^{2+} \quad (6a)$$

$$10^{\Delta \log K_{Cu}} = \frac{[Cu(Arm)(R\text{-}MP)][Cu^{2+}]}{[Cu(Arm)^{2+}][Cu(R\text{-}MP)]} \quad (6b)$$

For the tetragonal or Jahn–Teller distorted octahedral coordination sphere of Cu^{2+} it is difficult to assess a statistical value, but an estimate[38] was made: $\Delta \log K_{Cu/statist} \simeq -0.5$. Values derived from the experimental data for the four Cu(Arm)(R-MP) complexes listed in Table II are $\Delta \log K_{Cu/bpy/DHAP} = 0.02 \pm 0.03$, $\Delta \log K_{Cu/phen/DHAP} = 0.00 \pm 0.04$, $\Delta \log K_{Cu/bpy/G1P} = 0.07 \pm 0.07$, and $\Delta \log K_{Cu/phen/G1P} = 0.09 \pm 0.07$. Evidently these mixed ligand complexes are considerably more stable than expected on a statistical basis, but this observation agrees with previous experience, e.g., for the Cu^{2+}/phen/$HCOO^-$ or CH_3COO^- systems also $\Delta \log K_{Cu}$ values close to zero had been obtained,[29,37,38] and explanations for this rather general behavior of mixed ligand complexes formed by a divalent 3d metal ion, a heteroaromatic N base, and an O donor ligand have been given.[35,36,39] Hence, one may conclude that in these mixed ligand complexes $DHAP^{2-}$ and $G1P^{2-}$ show no exceptional properties but those expected for O-donor ligands.

2.3. Structural Considerations on the M(DHAP) and M(G1P) Complexes. Space filling molecular models show that a phosphate-coordinated metal ion in M(DHAP) may also reach the carbonyl oxygen and, correspondingly, in M(G1P) the hydroxy group at C-2 (Figure 1). Hence, the question arises for the M(DHAP) and M(G1P) complexes: does an equilibrium exist in aqueous solution between a solely phosphate-coordinated species and a seven-membered chelate? This equilibrium is indicated below in a simplified form, by also neglecting the charges:



(7)

Of course a metal ion coordinated at the phosphate group of $G1P^{2-}$ may equally well reach the 2-hydroxy group in the L or the D isomer: hence, the answer to the above question remains unaffected by the use of D,L-G1P in the present study.

Any kind of chelate formation has to enhance complex stability.[10,32,40] A possibly increased stability of M(DHAP) or M(G1P), if compared with a pure phosphate coordination, could therefore be attributed to the participation of the oxygen at C-2; i.e., equilibrium 7 would then truly exist. The position of this concentration-independent equilibrium between an "open" isomer, $M(R\text{-}MP)_{op}$, and a "closed" species, $M(R\text{-}MP)_{cl}$, is defined by the dimensionless constant K_I:

$$K_I = [M(R\text{-}MP)_{cl}]/[M(R\text{-}MP)_{op}] \quad (8)$$

Values for K_I may be calculated with eq 9 from the experimentally accessible (overall) stability constant, $K^{M}_{M(R\text{-}MP)}$, provided the stability constant, $K^{M}_{M(R\text{-}MP)_{op}}$, of the open isomer is also known.

$$K_I = \frac{K^{M}_{M(R\text{-}MP)}}{K^{M}_{M(R\text{-}MP)_{op}}} - 1 = 10^{\log \Delta} - 1 \quad (9)$$

$$\log \Delta = \log \Delta_{R\text{-}MP} = \log K^{M}_{M(R\text{-}MP)} - \log K^{M}_{M(R\text{-}MP)_{op}} \quad (10)$$

Obtaining the difference defined in eq 10 is the crucial part of any evaluation, and the reliability of any calculations for K_I (eq 9) depends on its accuracy.

The needed values for $K^{M}_{M(R\text{-}MP)_{op}}$ are not accessible by a simple direct experimental determination. However, this problem was recently resolved[19] by constructing log $K^{M}_{M(R\text{-}MP)}$ versus $pK^{H}_{H(R\text{-}MP)}$ plots for M(R-MP) complexes where $R\text{-}MP^{2-}$ are simple phosphate monoesters; i.e., their group R does not participate in complex formation. Plots of this type result in straight lines for series of structurally related ligands,[40] and this is also the case[19] for simple phosphate–monoester complexes of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}; the parameters of the resulting straight reference lines (least-squares) are summarized in Table V of ref 19 (cf. also Table I in ref 32). This achievement now allows the calculation of the stability constant for a pure phosphate coordination with the known acidity constant of any monoprotonated phosphate residue.

By using the mentioned reference-line equations and the $pK^{H}_{H(R\text{-}MP)}$ values of $H(DHAP)^-$ and $H(G1P)^-$ (Table I), the logarithms of the stability constants, log $K^{M}_{M(R\text{-}MP)_{op}}$, for the open isomers $M(DHAP)_{op}$ and $M(G1P)_{op}$ were calculated (Table II, columns 3 and 6). Now the differences between the measured and the calculated stability constants can be formed according to eq 10, and these log Δ values are given in columns 4 and 7 of Table II. All these values, with the single exception of the values for Mg(DHAP) and Mg(G1P) to which we attribute at present no meaning, are zero within the error limits; this also holds for the Cu(Arm)(R-MP) complexes; hence, no significantly increased stability is observed for any of the binary M(DHAP) and M(G1P) complexes or for the ternary Cu(Arm)(DHAP) and Cu(Arm)-(G1P) complexes. This result is probably even more apparent from Figure 2 where four examples for plots of log $K^{M}_{M(R\text{-}MP)}$ versus $pK^{H}_{H(R\text{-}MP)}$ are shown: the data pairs for the Ba^{2+}, Zn^{2+}, and Cu^{2+} complexes with $DHAP^{2-}$ or $G1P^{2-}$ are clearly all falling on the reference lines within the error limits; probably this also holds for the corresponding Mg^{2+} complexes.

From the above observations follows that the intramolecular equilibrium 7 is on its left side. However, one has to be aware that the log Δ values listed in Table II carry error limits. Under the reasonable assumption that a stability increase of 0.1 log unit would have been recognized with certainty, one obtains with eq 9 $K_I < 0.26$; hence, one has to conclude that the upper limit for the occurrence of closed species according to equilibrium 7 is about 20%. In other words, it cannot be ruled out on the basis of the present results that $M(DHAP)_{cl}$ and $M(G1P)_{cl}$ occur in aqueous solution in "traces", e.g., with Mg^{2+}.

2.4. A Reduced Solvent Polarity Promotes Formation of Chelates with $DHAP^{2-}$ and $G1P^{2-}$! With the last mentioned conclusion of section 2.3 in mind, we decided to study the influence of increasing amounts of 1,4-dioxane on the stability of the Cu^{2+}

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Table III. Negative Logarithms of the Acidity Constants $H(R\text{-}MP)^-$ (Eq 2) and Logarithms of the Corresponding Binary Cu(R-MP) Complexes (Eq 3) for $R\text{-}MP^{2-}$ = $DHAP^{2-}$ and $G1P^{2-}$ as Determined by Potentiometric pH Titrations (Exptl) in Dependence on the Amount of 1,4-Dioxane Added to Water and on the Resulting Dielectric Constant (I = 0.1 M, $NaNO_3$; 25 °C)[a,e]

R-MP²⁻	% (v/v) dioxane	mol fract diox	ϵ[b]	$pK^H_{H(R\text{-}MP)}$	log $K^{Cu}_{Cu(R\text{-}MP)}$ exptl	log $K^{Cu}_{Cu(R\text{-}MP)}$ calcd[c,e]	log $\Delta_{R\text{-}MP}$[d]
$DHAP^{2-}$	0	0	78.5	5.90 ± 0.01	2.77 ± 0.02	2.73 ± 0.08	0.04 ± 0.08
	30	0.083	52.7	6.63 ± 0.01	3.70 ± 0.02	3.62 ± 0.02	0.08 ± 0.03
	50	0.175	35.2	7.09 ± 0.01	4.50 ± 0.01	4.24 ± 0.03	0.26 ± 0.03
$G1P^{2-}$	0	0	78.5	6.23 ± 0.01	2.83 ± 0.05	2.88 ± 0.08	−0.05 ± 0.09
	30	0.083	52.7	6.94 ± 0.01	3.85 ± 0.02	3.79 ± 0.02	0.06 ± 0.03
	50	0.175	35.2	7.39 ± 0.03	4.65 ± 0.02	4.41 ± 0.03	0.24 ± 0.04

[a] The error limits are *three times* the standard errors (3σ); for details see footnotes *a*, *b*, and *c* in Table II. The entries for the aqueous solutions are from Tables I and II. [b] The dielectric constants for the dioxane–water mixtures are interpolated from the data given in ref 41. [c] The parameters of the straight-line equations are listed in Table II of ref 16; the error limits (3σ) are from Table III of ref 16 (see also Table III of ref 17). [d] log $\Delta_{R\text{-}MP}$ = log K_{exptl} − log K_{calcd}; note, this difference corresponds also to that defined by eq 10. [e] For comparison the calculated stability constants for a pure Cu^{2+}–phosphate coordination (calcd) are given, based on straight-line equations[16,17] quantifying the relationship between complex stability and phosphate group basicity (see Figure 3 and Section 2.4) and the $pK^H_{H(R\text{-}MP)}$ values of $H(DHAP)^-$ and $H(G1P)^-$ (see column 5, above).[a]

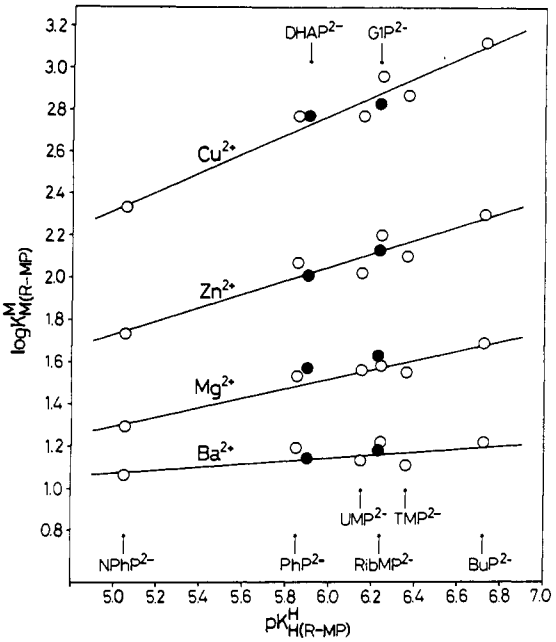

Figure 2. Relationship between log $K^M_{M(R\text{-}MP)}$ and $pK^H_{H(R\text{-}MP)}$ for M^{2+} 1:1 complexes with simple phosphate monoester ligands ($R\text{-}MP^{2-}$); i.e., 4-nitrophenyl phosphate ($NPhP^{2-}$), phenyl phosphate (PhP^{2-}), uridine 5′-monophosphate (UMP^{2-}), D-ribose 5′-monophosphate ($RibMP^{2-}$), thymidine 5′-monophosphate (TMP^{2-}), and *n*-butyl phosphate (BuP^{2-}) (○).[19] The least-squares lines are drawn through the corresponding six data sets; the equations for these reference lines are given in Table V of ref 19. The points due to the complexes formed with $DHAP^{2-}$ and $G1P^{2-}$ (●) are inserted for comparison; these constants are taken from Tables I and II. All plotted data refer to aqueous solutions at 25 °C and I = 0.1 M ($NaNO_3$).

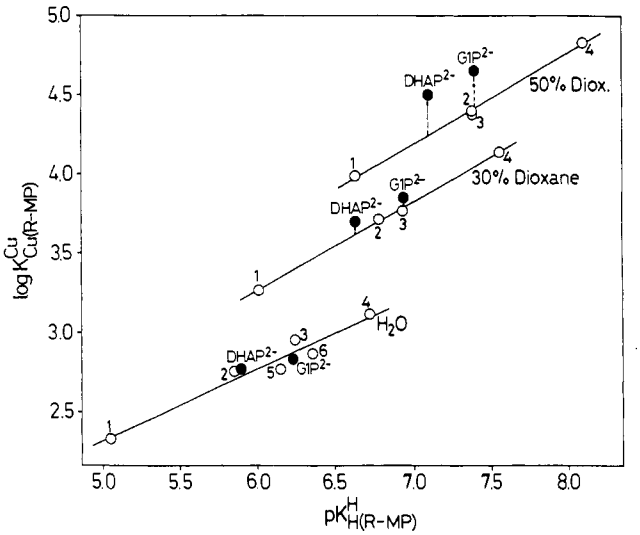

Figure 3. Evidence for an enhanced stability of Cu(DHAP) and Cu(G1P) in mixed dioxane–water solvents based on the relationship between log $K^M_{M(R\text{-}MP)}$ and $pK^H_{H(R\text{-}MP)}$ for the Cu^{2+} 1:1 complexes of 4-nitrophenyl phosphate (1), phenyl phosphate (2), D-ribose 5′-monophosphate (3), *n*-butyl phosphate (4), uridine 5′-monophosphate (5), and thymidine 5′-monophosphate (6) in water and in water containing 30 or 50% (v/v) 1,4-dioxane. The least-squares lines are drawn in each case through the data sets shown;[16,19] the equations for these reference lines are given in Table II of ref 19 (see also ref 17). All the plotted constants refer to 25 °C and I = 0.1 M ($NaNO_3$). The points due to the Cu^{2+} 1:1 complexes formed with $DHAP^{2-}$ and $G1P^{2-}$ (●) in the three mentioned solvents are inserted for comparison (see section 2.4; Table III). The vertical broken lines emphasize the stability differences to the corresponding reference lines; these differences equal log $\Delta_{R\text{-}MP}$ (see eq 10).

complexes of $DHAP^{2-}$ and $G1P^{2-}$. Increasing amounts of dioxane in an aqueous solution will render solvation of metal ions more difficult, and, hence, the participation of the oxygen at C-2 in complex formation should become facilitated. Cu^{2+} was selected for these measurements because reference lines for log $K^{Cu}_{Cu(R\text{-}MP)}$ versus $pK^H_{H(R\text{-}MP)}$ plots of simple phosphate–monoester ligands with a noncoordinating group R have previously been constructed for various water–dioxane mixtures and the straight-line equations are published.[16,17]

The acidity constants (eq 2) determined for $H(DHAP)^-$ and $H(G1P)^-$ and the stability constants (eq 3) for the corresponding Cu(R-MP) complexes using water containing 30 or 50% (v/v) 1,4-dioxane as solvent are listed in Table III.[41] The stability differences log $\Delta_{R\text{-}MP}$ (eq 10) are given in the column at the right; they increase somewhat with an increasing dioxane concentration.

Table IV. Extent of Chelate Formation (Eq 7) in the Cu(DHAP) and Cu(G1P) Complexes as Quantified by the Dimensionless Equilibrium Constant K_1 (Eqs 8 and 9) and the Percentage of $Cu(R\text{-}MP)_{cl}$ (See Text in Section 2.4) in Dependence on the Amount of 1,4-Dioxane Added to Water (I = 0.1 M, $NaNO_3$; 25 °C)[a]

R-MP²⁻	% (v/v) dioxane	log $\Delta_{R\text{-}MP}$[a]	K_1	% $Cu(R\text{-}MP)_{cl}$
$DHAP^{2-}$	0	0.04 ± 0.08	0.10 ± 0.21	9 ± 17
	30	0.08 ± 0.03	0.20 ± 0.08	17 ± 5
	50	0.26 ± 0.03	0.82 ± 0.13	45 ± 4
$G1P^{2-}$	0	−0.05 ± 0.09	0 (<0.11)	0 (<10)
	30	0.06 ± 0.03	0.15 ± 0.07	13 ± 6
	50	0.24 ± 0.04	0.74 ± 0.14	42 ± 5

[a] The values for log Δ (see eq 10) and their error limits (3σ) are from Table III; the errors for K_1 and % $Cu(R\text{-}MP)_{cl}$ were calculated according the error propagation after Gauss.

This means, Cu(DHAP) and Cu(G1P) are now more stable than expected for a sole phosphate coordination. This is even more clearly borne out from Figure 3: the data pairs for Cu(DHAP)

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and Cu(G1P) are in the mixed solvents above the reference lines. Note, the vertical distance between the points for the mentioned Cu^{2+} complexes, and the straight reference lines correspond to log $\Delta_{R\cdot MP}$ as defined by eq 10.

The values for log $\Delta_{R\cdot MP}$ of Table III can now be used to calculate K_I according to eq 9, and then the percentages of the closed isomers are also easily obtained: $\%M(R\text{-}MP)_{cl} = 100K_I/(1 + K_I)$. These results are compiled in Table IV. For both complexes, i.e., Cu(DHAP) and Cu(G1P), the formation degree of the closed species is now rather significant, at least in the 50% (v/v) water–dioxane mixture.

Conclusions

The stability of the complexes formed between divalent metal ions and dihydroxyacetone phosphate ($DHAP^{2-}$) and glycerol 1-phosphate ($G1P^{2-}$) (Figure 1) is governed by the metal ion affinity of the phosphate group. This conclusion is valid for aqueous solutions and for water containing 30 or 50% (v/v) 1,4-dioxane. However, in the last mentioned solvent mixtures the carbonyl group of $DHAP^{2-}$ and the hydroxy group of $G1P^{2-}$ can also participate in complex formation, leading to seven-membered chelates as shown for Cu(DHAP) and Cu(G1P).

On the basis of the present results it may also be surmised that glyceraldehyde 3-phosphate (GAP^{2-}: $HC(O)CH(OH)\text{-}CH_2OPO_3^{2-}$), which co-exists to about 4% in equilibrium with its ketoform[4] $DHAP^{2-}$, shows in M(GAP) complexes to a first approximation the stabilities and properties of the corresponding M(G1P) species because the structural unit, $-CH(OH)\text{-}CH_2OPO_3^{2-}$, which is responsible for the coordination properties, is identical in GAP^{2-} and $G1P^{2-}$. The above assumption is confirmed by the similarity of an apparent pK_a of 6.3 (I = 0.1 M; 30 °C) for $H(GAP)^-$, as determined by kinetic experiments with triosephosphate isomerase,[28] with the present result; i.e., $pK^H_{H(GAP)} \simeq pK^H_{H(G1P)} = 6.23$ (I = 0.1 M, $NaNO_3$; 25 °C; see Table I). It may also be recalled here (see Introduction) that the ketotriose and aldotriose, i.e., DHAP and GAP, are rapidly interconverted into each other by triosephosphate isomerase.[4,6] The preceding conclusions regarding the metal ion and also proton affinities of GAP^{2-} are important because the properties of this ligand may hardly be studied directly due to its instability[42] in, particularly alkaline, aqueous solution.

It should be emphasized that the formation of seven-membered chelates, as mentioned above for equilibrium 7 and Cu^{2+}, has also to be expected for all the other metal ions considered in this study, if the solvating properties of the solvent employed are poorer than those of water. Indeed, it is well-known[40] for all these metal ions that the oxygen of carbonyl or hydroxy groups can participate in chelate formation, if the oxygen atom is in a proper steric position.

Therefore, the oxygen atom at C-2 of $DHAP^{2-}$ (and GAP^{2-}) and $G1P^{2-}$, which can weakly interact with metal ions, may well have a special role in certain metabolic processes. The presented results manifest that chelate formation is favored under conditions of a lower polarity with poorer solvating properties than those of water, and such conditions are legion in biological systems. Since the so-called "effective" or "equivalent solution" dielectric constants in proteins[43] or in active-site cavities of enzymes[30] are reduced compared to the situation in bulk water, favored conditions for the formation of a certain amount of the described chelates (eq 7) exist in nature exactly at those places where $DHAP^{2-}$ (and GAP^{2-}) and $G1P^{2-}$ are employed as substrates.

A further point to be emphasized is that a lower polarity (dielectric constant) shifts the deprotonation reaction of phosphate groups from slightly acidic conditions into the physiological pH range (Table III). A decrease in the dielectric constant from about 80 to 35, which is well reached in an active-site cavity,[30,43] enhances the proton affinity by more than 1 log unit. Hence, a shift of such a substrate by a few Ångströms at the "surface" of a protein may drastically alter the acid–base properties of its phosphate group. Furthermore, the DHAP–GAP interconversions will affect the phosphate group basicity, and hence its proton and metal ion affinity, because as indicated above $pK^H_{H(GAP)} \simeq pK^H_{H(G1P)}$; i.e., the interconversion of DHAP into GAP will enhance the basicity of the phosphate group by about 0.3 log unit (see Table III). It is easy to imagine that nature might use this basicity difference to favor the one or other side of this and related equilibria.

Acknowledgment. The support of this research by a grant from the Swiss National Science Foundation and a fellowship to D.C. from the "Amt für Ausbildungsbeiträge des Kantons Basel-Stadt" is gratefully acknowledged.

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