

# Stabilities and Structures of Metal Ion Complexes of Adenosine 5'-O-Thiomonophosphate (AMPS<sup>2-</sup>) in Comparison with Those of Its Parent Nucleotide (AMP<sup>2-</sup>) in Aqueous Solution

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Received August 23, 1996<sup>⊗</sup>

**Abstract:** The stability constants of the 1:1 complexes formed between Mg<sup>2+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, or Cd<sup>2+</sup> and AMPS<sup>2-</sup>, i.e., of the M(AMPS) complexes, were determined by potentiometric pH titrations (25 °C; I = 0.1 M, NaNO<sub>3</sub>). For the Mn<sup>2+</sup>/AMPS, Co<sup>2+</sup>/AMPS, Ni<sup>2+</sup>/AMPS, and Cd<sup>2+</sup>/AMPS systems also the protonated species M(H;AMPS)<sup>+</sup> were quantified, and for the Zn<sup>2+</sup>/AMPS system, the stability of the hydroxo species Zn(AMPS)(OH)<sup>-</sup>, which results from the Zn<sup>2+</sup>-thio coordination, could be determined. On the basis of previously established log K<sub>M(R-MP)</sub><sup>M</sup> versus pK<sub>H(R-MP)</sub><sup>H</sup> straight-line plots (R-MP<sup>2-</sup> = simple monophosphate ester ligands without further coordinating groups; Sigel, H.; et al. *Helv. Chim. Acta* **1992**, 75, 2634), it is concluded that the alkaline earth ions in the M(AMPS) complexes are coordinated to the thiophosphate group with the same intensity as to a normal phosphate group. For the M(AMPS) complexes of Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, and Cd<sup>2+</sup>, it is shown by comparison with the corresponding M(AMP) complexes and by employing the mentioned straight-line plots that the stability increase is larger than may be expected due to macrochelate formation, which means that the metal ions also bind to the sulfur atom of the thiophosphate group. The stability increases amount for Mn(AMPS), Zn(AMPS), and Cd(AMPS) to about 0.2, 0.7, and 2.4 log units, respectively, and the estimated approximate percentages of the sulfur-coordinated species are about 30, 80, and 100%, respectively. Furthermore, comparisons between these stability increases and the solubility products for the corresponding metal ion sulfides, M<sup>II</sup>S, as well as with the stability increases due to the M<sup>2+</sup>-thioether interaction observed for the complexes of tetrahydrothiophene-2-carboxylate, which also result in straight-line plots, further support the conclusions about metal ion-sulfur binding in the mentioned M(AMPS) complexes. The indicated correlations allow also an estimate for the extent of the M<sup>2+</sup>-sulfur interaction in Pb(AMPS) and Cu(AMPS). The various isomers of the M(H;AMPS)<sup>+</sup> species are analyzed in a microconstant scheme, and estimations about their formation degrees are presented; for example, for the Cd<sup>2+</sup> system, (H•AMPS•Cd)<sup>+</sup> is the dominating isomer, which has the proton at N1 and Cd<sup>2+</sup> at the thiophosphate group. It is evident that for metal ions like (Mn<sup>2+</sup>), Zn<sup>2+</sup>, or Cd<sup>2+</sup> the metal ion binding properties of the parent compound AMP<sup>2-</sup> and its thio analogue AMPS<sup>2-</sup> differ considerably, and therefore, great care should be exercised in enzymatic studies where AMPS<sup>2-</sup> is employed as a probe for AMP<sup>2-</sup> in the presence of metal ions. Regarding studies of ribozymes, it is of interest that plots are presented (pseudo-first-order rate constants versus complex stabilities) which suggest that on top of a sulfur-metal ion interaction during the transition state of the rate-determining step of the hydrolytic cleavage of an oligonucleotide containing a bridged internucleotide 5'-phosphorothioate RNA linkage also an oxygen-metal ion interaction occurs and that the two effects are "additive".

## 1. Introduction

Nucleotides play a central role in the metabolism of living cells.<sup>2</sup> They serve as substrates for the enzyme-catalyzed transfers of nucleotidyl or phosphoryl groups—reactions which depend on the presence of divalent metal ions.<sup>2,3</sup> Nucleotides are also involved in the information storage via RNA and DNA or in energy transfer processes.<sup>2,4</sup> These examples illustrate the

importance of nucleotides and explain the efforts undertaken to elucidate their metal ion binding properties<sup>3,5</sup> as well as their detailed roles<sup>2-4</sup> in enzymatic reactions.

The use of structurally altered nucleotides as probes provides one way to study reactions of enzymes which involve nucleotides as substrates. Indeed, all three parts of nucleotides have been systematically modified: for example, (i) the nucleobase was enlarged as in *lin*-benzoadenine nucleotides<sup>6a-c</sup> or in 1,*N*<sup>6</sup>-ethenoadenosine or in 3,*N*<sup>4</sup>-ethenocytidine derivatives;<sup>6d-g</sup> (ii) the ribose residue has been replaced by glucose<sup>7</sup> or by an aliphatic chain;<sup>8</sup> and (iii) in the phosphate moiety the bridging oxygen(s) was (were) substituted by a methylene,<sup>9,10</sup> a peroxy,<sup>11</sup> an imido,<sup>9a,10b,12</sup> a sulfur,<sup>9a,13</sup> or a disulfide bridge.<sup>14a</sup> Moreover, replacement of other oxygen atoms in a phosphate residue by sulfur atoms has also been achieved.<sup>9a,14,15</sup>

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<sup>⊗</sup> Abstract published in *Advance ACS Abstracts*, January 1, 1997.

(1) Work was carried out during an optional and ungraded *Advanced Laboratory Course in Inorganic Chemistry* within the diploma curriculum at the University of Basel (R.K.O.S.) and during a study leave from the Zhongshan (Sun Yatsen) University in Guangzhou, People's Republic of China (B.S.).

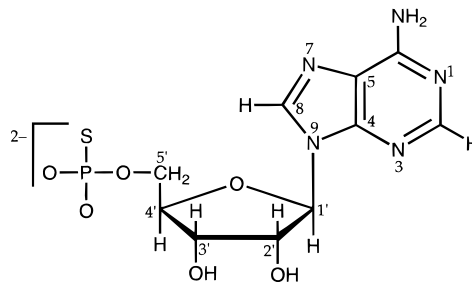
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The latter alteration leads to so-called nucleoside phosphorothioates<sup>14a</sup> or thionucleotides.<sup>16</sup> Such compounds were first synthesized 30 years ago.<sup>14a</sup> Nowadays they are heavily used as probes in enzymic studies<sup>15b,17</sup> including evaluations of isomeric metal complex species;<sup>14b,18</sup> they are employed in the antisense strategy,<sup>15a,19</sup> and they serve as biochemical tools for sequencing<sup>20</sup> and mutagenizing<sup>21</sup> DNA and for elucidating the active site(s) and the role(s) of metal ions in ribozymes.<sup>13bc,22</sup> Surprisingly, their metal ion-binding properties<sup>16</sup> as quantified by equilibrium constants have hardly been studied; apparently



**Figure 1.** Chemical structure of adenosine 5'-O-thiomonophosphate (AMPS<sup>2-</sup>)<sup>23</sup> in the *anti* conformation.<sup>24</sup>

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only a single equilibrium study<sup>16</sup> exists which deals with the affinity of Mg<sup>2+</sup> and Cd<sup>2+</sup> toward thio derivatives of adenosine 5'-diphosphate (ADP<sup>3-</sup>) and adenosine 5'-triphosphate (ATP<sup>4-</sup>).

The present study concentrates on adenosine 5'-O-thiomonophosphate (AMPS<sup>2-</sup>; see Figure 1),<sup>23,24</sup> which was first synthesized in 1968;<sup>25</sup> an improved synthesis was given in connection with its radiolabeling.<sup>26</sup> The acidity constants of H<sub>2</sub>(AMPS)<sup>±</sup> have recently been determined<sup>27</sup> by potentiometric pH titrations in aqueous solution, and the micro acidity constants regarding the protonation of N1 and the thiophosphate group were evaluated. This means the stability constants of the 1:1 complexes formed between Mg<sup>2+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, or Cd<sup>2+</sup> (=M<sup>2+</sup>) and AMPS<sup>2-</sup> could now also be measured<sup>28</sup> and compared with those of the M(AMP) complexes.<sup>5,29</sup> In the AMPS systems with Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, and Cd<sup>2+</sup> also a monoprotonated complex, M(H<sub>2</sub>AMPS)<sup>+</sup>, was discovered and quantified and its various isomers are discussed. Most surprising is the observation that Zn(AMPS)(aq) shows an increased tendency to form hydroxo complexes.

## 2. Experimental Section

**2.1. Materials.** The same three lots of dilithium salts of AMPS<sup>2-</sup> as used recently<sup>27</sup> were also employed now. During all of the studies no differences between the three samples could be detected. The aqueous stock solution of AMPS<sup>2-</sup> was freshly prepared daily, and the pH was adjusted to about 7.5. The exact concentrations of the AMPS<sup>2-</sup> stock solutions were determined each time.

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(23) Abbreviations: AMP<sup>2-</sup>, adenosine 5'-monophosphate; AMPS<sup>2-</sup>, adenosine 5'-O-thiomonophosphate, which is also known as adenosine 5'-O-monophosphorothioate or adenosine 5'-[α-thio]-monophosphate; M<sup>2+</sup>, general divalent metal ion; R-MP<sup>2-</sup>, phosphate monoester (R may be any organic residue, e.g., phenyl or nucleosidyl; in some instances also phosphonate derivatives are included in this abbreviation); THTC<sup>-</sup>, tetrahydrothiophene-2-carboxylate; Tris, tris(hydroxymethyl)aminomethane = 2-amino-2-(hydroxymethyl)-1,3-propanediol. Further abbreviations are defined in the legend of Figure 2. Species which are given in the text without a charge either do not carry one or represent the species in general (i.e., independent from their protonation degree); which of the two versions applies is always clear from the context.

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Potassium hydrogen phthalate, 1,2-diaminoethane-*N,N,N',N'*-tetraacetic acid (Na<sub>2</sub>H<sub>2</sub>EDTA), HNO<sub>3</sub>, NaOH (*Titrisol*), and the nitrate salts of Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, and Cd<sup>2+</sup> (all *pro analysi*) were from Merck AG, Darmstadt, FRG. All solutions were prepared with bidistilled CO<sub>2</sub>-free water.

The titer of the NaOH used for the titrations was established with potassium hydrogen phthalate and that of the M<sup>2+</sup> stock solutions with EDTA.

**2.2. Potentiometric pH Titrations.** These were carried out with the same equipment and in exactly the same way as described recently<sup>27</sup> (cf. also refs 29b and 30).

At the beginning our titrations were seriously hampered if combined single-junction macro glass electrodes (Metrohm: 6.0202.100, JC) were used. AMPS induced a "poisoning" of the electrode, which means that during the titration the plotted pH versus mL of NaOH curve became unsteady. This poisoning effect was somewhat less pronounced if the titration solution contained also divalent metal ions. An improvement could be achieved by rigorously excluding dioxygen and by adding the necessary HNO<sub>3</sub> only shortly before starting the titration (for details, see ref 27). Excellent titration curves were obtained by using two separated electrodes, i.e., a pH measuring electrode (Metrohm: 6.0133.100) in combination with an Ag/AgCl reference electrode (Metrohm: 6.0726.100).<sup>27</sup>

The results given in section 3 are the averages obtained from all experiments (always at least one titration for each system was carried out with separated electrodes). The indicated difficulty is the main reason why in some instances the results carry relatively large error limits (see section 3).

**2.3. Determination of the Stability Constants.** The conditions for the determination of the stability constants  $K_{M(AMPS)}^M$  of all M(AMPS) complexes, and in the cases of Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, and Cd<sup>2+</sup> also for  $K_{M(H;AMPS)}^M$  of the monoprotonated M(H;AMPS)<sup>+</sup> complexes, were the same as given in ref 27 (*I* = 0.1 M, NaNO<sub>3</sub>; 25 °C) for  $K_{H_2(AMPS)}^H$  and  $K_{H(AMPS)}^H$  of H<sub>2</sub>(AMPS)<sup>±</sup>. This means [HNO<sub>3</sub>] = 0.56 mM and [AMPS] = 0.23 mM, except that now NaNO<sub>3</sub> was partly or fully replaced by M(NO<sub>3</sub>)<sub>2</sub>. For Mg<sup>2+</sup> and Ca<sup>2+</sup>, [M(NO<sub>3</sub>)<sub>2</sub>] was 33.33 or 26.67 mM, i.e., AMPS:M<sup>2+</sup> ≈ 1:145 and 1:116, respectively. For Ba<sup>2+</sup> only 33.33 mM solutions were employed. With Mn<sup>2+</sup> and Co<sup>2+</sup>, [M(NO<sub>3</sub>)<sub>2</sub>] was 16.67 mM (AMPS:M<sup>2+</sup> ≈ 1:72). For Cd<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, and Zn<sup>2+</sup>, [M(NO<sub>3</sub>)<sub>2</sub>] was 8.333 mM (AMPS:M<sup>2+</sup> ≈ 1:36), for Ni<sup>2+</sup> and Zn<sup>2+</sup>, it was in addition 4.17 mM (1:18), and for Cd<sup>2+</sup>, also 3.33, 0.833, and 0.500 mM, i.e., the AMPS to Cd<sup>2+</sup> ratios were about 1:15, 1:3.6, and 1:2.2. For each system at least six independent pairs of titrations were made and at least two different AMPS to M<sup>2+</sup> ratios (except for Ba<sup>2+</sup>) were employed.

The stability constants  $K_{M(AMPS)}^M$  for the Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Ba<sup>2+</sup> complexes were calculated with an IBM compatible desk computer (with a 80486 processor) connected with a Hewlett-Packard 7475A plotter and a M1509 printer for each pair of titrations by taking into account the species H<sup>+</sup>, H<sub>2</sub>(AMPS)<sup>±</sup>, H(AMPS)<sup>-</sup>, AMPS<sup>2-</sup>, M<sup>2+</sup>, and M(AMPS).<sup>31</sup> Throughout, the data were collected (every 0.1 pH unit) from about 10% complex formation to a neutralization degree of about 90% or the beginning of the hydrolysis of M(aq)<sup>2+</sup>; the latter was evident from the titrations without AMPS. The values calculated individually for log  $K_{M(AMPS)}^M$  showed no dependence on pH or on the excess amount of M<sup>2+</sup>.

For the AMPS systems with Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, and Cd<sup>2+</sup>, the experimental data were analyzed with a curve fitting procedure<sup>32</sup> using the mentioned computer by taking into account in addition the species M(H;AMPS)<sup>+</sup> to obtain  $K_{M(H;AMPS)}^M$  and  $K_{M(AMPS)}^M$ . Application of this analysis to the Mg<sup>2+</sup>, Ca<sup>2+</sup>, or Ba<sup>2+</sup> systems gave the same result for  $K_{M(AMPS)}^M$  as the above procedure but did not yield a value for  $K_{M(H;AMPS)}^M$ , indicating that the concentration of the M(H;AMPS) complex is negligible. This conclusion also applies for the Zn<sup>2+</sup> system, but comparison of the titration curves for Zn<sup>2+</sup>/AMPS with those for Zn<sup>2+</sup> alone revealed that in the first case a further proton was liberated

before the onset of the hydrolysis of Zn(aq)<sup>2+</sup>. This can only mean that Zn(AMPS) forms a hydroxo complex more easily than Zn<sup>2+</sup> itself. Indeed, by taking into account the species H<sup>+</sup>, H<sub>2</sub>(AMPS)<sup>±</sup>, H(AMPS)<sup>-</sup>, AMPS<sup>2-</sup>, Zn<sup>2+</sup>, Zn(AMPS), and Zn(AMPS)(OH)<sup>-</sup>, a satisfactory curve fit and a value for  $K_{Zn(AMPS)(aq)}^H$  could be obtained.

The experimental data for the AMPS systems with Mg<sup>2+</sup>, Ca<sup>2+</sup>, or Ba<sup>2+</sup> were also evaluated with the mentioned computer by determining with a curve fitting procedure using a Newton–Gauss nonlinear least-squares program for each titration pair the apparent acidity constant  $K'_a$ . With  $K'_a$  and the acidity constant  $K_{H(AMPS)}^H$ , values for the stability constants  $K_{M(AMPS)}^M$  were obtained with eq 1:<sup>33,34</sup>

$$K_{M(AMPS)}^M = (K'_a - K_{H(AMPS)}^H) / (K_{H(AMPS)}^H [M^{2+}]) \quad (1)$$

These constants agreed excellently with those as determined above.

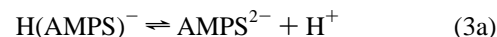
### 3. Results and Discussion

**3.1. Some Relevant Properties of Adenosine 5'-O-Thio-monophosphate.** As a purine derivative,<sup>35</sup> AMPS is expected to self-stack. Replacement of an oxygen by a sulfur in the phosphate residue should not alter this property, and the self-association constants for AMPS and AMP<sup>36</sup> should be similar or even identical. Hence, as calculated previously for AMP,<sup>29b</sup> one expects that in 0.3 mM solutions more than 99% of the AMPS species exist as monomers. Consequently, in the present study ([AMPS] = 0.23 mM), self-association of AMPS is certainly negligible.

The acid–base reactions relevant in the pH range of this study are the two deprotonation steps of H<sub>2</sub>(AMPS)<sup>±</sup>:



$$K_{H_2(AMPS)}^H = [H(AMPS)^-][H^+] / [H_2(AMPS)^\pm] \quad (2b)$$



$$K_{H(AMPS)}^H = [AMPS^{2-}][H^+] / [H(AMPS)^-] \quad (3b)$$

The corresponding acidity constants have recently been determined:<sup>27</sup>  $pK_{H_2(AMPS)}^H = 3.72 \pm 0.03$  and  $pK_{H(AMPS)}^H = 4.83 \pm 0.02$ . These constants are separated only by  $\Delta pK_a = 1.11 \pm 0.04$ , indicating that the pH ranges in which these two deprotonation reactions occur are overlapping. This means, H(AMPS)<sup>-</sup> exists in two forms: one where the proton is at N1, as in this pH range this adenine site is protonated,<sup>24</sup> (H•AMPS)<sup>-</sup>, and one with the proton at the thiophosphate group, (AMPS•H)<sup>-</sup> (see Figure 1).<sup>27</sup> The corresponding two micro acidity constants are<sup>27</sup>  $pK_{H\cdot AMPS\cdot H}^{AMPS\cdot H} = 3.84 \pm 0.02$  and  $pK_{H\cdot AMPS\cdot H}^{H\cdot AMPS} = 4.34 \pm 0.14$ ; they allow determination<sup>27</sup> of the ratio *R* of the two mentioned monoprotonated species:

$$R = \frac{[(AMPS\cdot H)^-]}{[(H\cdot AMPS)^-]} = \frac{k_{H\cdot AMPS\cdot H}^{AMPS\cdot H}}{k_{H\cdot AMPS\cdot H}^{H\cdot AMPS}} = \frac{10^{-3.84}}{10^{-4.34}} = 10^{0.50} = 3.16 = \frac{76}{24} \approx \frac{3}{1}$$

(AMPS•H)<sup>-</sup> is dominating and occurs to about 75%, whereas (H•AMPS)<sup>-</sup> forms only to about 25%. In addition, the proton

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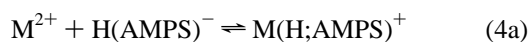
(31) Griesser, R.; Sigel, H. *Inorg. Chem.* **1970**, *9*, 1238–1243.

(32) Sigel, H.; Griesser, R.; Prijs, B. *Z. Naturforsch. B* **1972**, *27B*, 353–364.

at the thiophosphate residue in  $(\text{AMPS}\cdot\text{H})^-$  is mainly bound<sup>27</sup> to the terminal oxygens (cf. Figure 1) as was concluded from bond-order considerations<sup>37a</sup> and semiempirical calculations.<sup>27</sup>

Hence,  $\text{AMPS}^{2-}$  is formed in two ways, i.e. from  $(\text{H}\cdot\text{AMPS})^-$  and  $(\text{AMPS}\cdot\text{H})^-$ . The micro acidity constant for the latter species,  $\text{p}K_{\text{AMPS}\cdot\text{H}}^{\text{AMPS}} = 4.71 \pm 0.04$ ,<sup>27</sup> quantifying the basicity properties of the thiophosphate group will be needed below in the evaluation of the stability constants of the various metal ion complexes.

**3.2. Stabilities of Adenosine 5'-O-Thiomonophosphate Complexes.** Aside from equilibria 2 and 3, the following ones involving metal ions needed to be considered in the evaluation of the  $\text{M}^{2+}/\text{AMPS}$  systems:



$$K_{\text{M}(\text{H};\text{AMPS})}^{\text{M}} = [\text{M}(\text{H};\text{AMPS})]/[\text{M}^{2+}][\text{H}(\text{AMPS})^-] \quad (4b)$$



$$K_{\text{M}(\text{AMPS})}^{\text{M}} = [\text{M}(\text{AMPS})]/[\text{M}^{2+}][\text{AMPS}^{2-}] \quad (5b)$$



$$K_{\text{M}(\text{AMPS})(\text{aq})}^{\text{H}} = [\text{M}(\text{AMPS})(\text{OH})^-][\text{H}^+]/[\text{M}(\text{AMPS})(\text{aq})] \quad (6b)$$

The acidity constant of equilibrium 7, which connects equilibria 4 and 5, may be calculated with eq 8:



$$K_{\text{M}(\text{H};\text{AMPS})}^{\text{H}} = [\text{M}(\text{AMPS})][\text{H}^+]/[\text{M}(\text{H};\text{AMPS})^+] \quad (7b)$$

$$\text{p}K_{\text{M}(\text{H};\text{AMPS})}^{\text{H}} = \text{p}K_{\text{H}(\text{AMPS})}^{\text{H}} + \log K_{\text{M}(\text{H};\text{AMPS})}^{\text{M}} - \log K_{\text{M}(\text{AMPS})}^{\text{M}} \quad (8)$$

In all  $\text{M}^{2+}/\text{AMPS}$  evaluations equilibria 2a and 3a had to be considered. In the systems containing  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Ba}^{2+}$ , equilibrium 4a did not play any role; we were unable to observe under our experimental conditions the formation of the mono-protonated  $\text{M}(\text{H};\text{AMPS})^+$  complexes. Hence, the experimental data could completely be explained by taking into account eqs 2b, 3b, and 5b as long as the evaluation was not carried into the pH range where hydroxo complexes form (see section 2.3). In the  $\text{Zn}^{2+}/\text{AMPS}$  system, equilibrium 4a was also negligible, but equilibrium 6a became important, and we were able to quantify the formation of the hydroxo complex  $\text{Zn}(\text{AMPS})(\text{OH})^-$  (see sections 2.3 and 3.8).

Protonated  $\text{M}(\text{H};\text{AMPS})^+$  complexes are formed with  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ , or  $\text{Cd}^{2+}$ ; hence, in these cases, equilibria 2a, 3a, 4a, and 5a had to be considered in the pH range where no hydrolysis of  $\text{M}(\text{aq})^{2+}$  occurs; the latter was evident from titrations carried out with  $\text{M}^{2+}$  only (see also section 2.3). The results obtained for the various equilibria involving metal ions are listed in Table 1.

The  $\text{Cu}^{2+}/\text{AMPS}$  system could not be studied because as soon as the reactants were mixed the solution turned slightly milky, indicating the formation of a precipitate possibly involving a

**Table 1.** Logarithms of the Stability Constants of  $\text{M}(\text{H};\text{AMPS})^+$  (Eq 4) and  $\text{M}(\text{AMPS})$  (Eq 5) Complexes as Determined by Potentiometric pH Titrations in Aqueous Solutions, Together with the Negative Logarithms for the Acidity Constants of  $\text{M}(\text{H};\text{AMPS})^+$  (Eqs 7 and 8) and for the Formation of Hydroxo Complexes (Eq 6) at 25 °C and  $I = 0.1 \text{ M}$  ( $\text{NaNO}_3$ )<sup>a,b</sup>

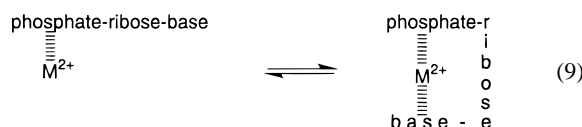
$\text{M}^{2+}$	$\log K_{\text{M}(\text{H};\text{AMPS})}^{\text{M}}$	$\log K_{\text{M}(\text{AMPS})}^{\text{M}}$	$\text{p}K_{\text{M}(\text{H};\text{AMPS})}^{\text{H}}$	$\text{p}K_{\text{M}(\text{H};\text{AMPS})(\text{aq})}^{\text{H}}$
$\text{Mg}^{2+}$		$1.28 \pm 0.04$		
$\text{Ca}^{2+}$		$1.27 \pm 0.06$		
$\text{Ba}^{2+}$		$0.99 \pm 0.03$		
$\text{Mn}^{2+}$	$1.25 \pm 0.2$	$2.03 \pm 0.10$	$4.05 \pm 0.23$	
$\text{Co}^{2+}$	$1.0 \pm 0.25$	$2.03 \pm 0.10$	$3.8 \pm 0.3$	
$\text{Ni}^{2+}$	$1.1 \pm 0.3$	$2.35 \pm 0.07$	$3.6 \pm 0.3$	
$\text{Zn}^{2+}$		$2.52 \pm 0.18$		$6.8 \pm 0.4$
$\text{Cd}^{2+}$	$3.25 \pm 0.16$	$4.62 \pm 0.12$	$3.46 \pm 0.20$	

<sup>a</sup> 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. <sup>b</sup> The acidity constants of  $\text{H}_2(\text{AMPS})^\pm$  are given in section 3.1; those of the parent nucleotide,  $\text{H}_2(\text{AMP})^\pm$  are  $\text{p}K_{\text{H}_2(\text{AMP})}^{\text{H}} = 3.84 \pm 0.02$  and  $\text{p}K_{\text{H}(\text{AMP})}^{\text{H}} = 6.21 \pm 0.01$ .<sup>29a</sup>

redox reaction. Indeed, the reducing properties of phosphorothioates are well-known<sup>38</sup> as is the formation of P–S–S–P units.<sup>38</sup>

A crude evaluation of the results reveals that despite the fact that  $\text{H}_2(\text{AMPS})^\pm$  is more acidic than  $\text{H}_2(\text{AMP})^\pm$  (see footnote b of Table 1) one observes for the  $\text{M}(\text{AMPS})$  complexes with  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  (Table 1) larger stability constants than for the corresponding<sup>5,29</sup>  $\text{M}(\text{AMP})$  complexes (Table 2, vide infra). For the  $\text{M}(\text{AMPS})$  complexes with  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ , or  $\text{Ni}^{2+}$ , the absolute values for the stability constant  $K_{\text{M}(\text{AMPS})}^{\text{M}}$  are on average about 0.2 log unit smaller than the analogous constants of the  $\text{M}(\text{AMP})$  complexes; indeed, due to the lower  $\text{p}K_{\text{a}}$  value of  $\text{H}(\text{AMPS})^-$ , this is expected. However, a detailed discussion of these observations needs some further considerations.

**3.3. Comparison of the Stabilities of the  $\text{M}(\text{AMP})$  and  $\text{M}(\text{AMPS})$  Complexes.** Already in an earlier study<sup>39a</sup> a linear relationship was established between the negative logarithms of the acidity constants of  $\text{H}(\text{R-MP})^-$  species,  $\text{p}K_{\text{H}(\text{R-MP})}^{\text{H}}$ , and the logarithms of the corresponding  $\text{M}(\text{R-MP})$  complexes,  $\log K_{\text{M}(\text{R-MP})}^{\text{M}}$ , for several simple phosphate monoester ligands ( $=\text{R-MP}^{2-}$ ), including methyl phosphate,<sup>39b</sup> and it had been shown later<sup>39c</sup> that also the points for complexes with phosphonates, like methyl phosphonate ( $\text{MeP}^{2-}$ ) or ethyl phosphonate ( $\text{EtP}^{2-}$ ), fall on the same straight baseline for a given metal ion (see also Figures 2 and 3, vide infra).<sup>29b,39c</sup> Any further interaction between a phosphate-coordinated metal ion and another group of the ligand in a given complex has to result<sup>40</sup> in an increase in complex stability, and the point due to this complex will therefore lie above the corresponding baseline. On this basis, for example, the macrochelate formation to N7 in  $\text{M}(\text{AMP})$  complexes has been investigated and quantified.<sup>29</sup> Of course, such a macrochelate will hardly form to 100%, i.e., equilibrium 9 must be considered:



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(38) (a) Fehér, F.; Vial, F. Z. *Anorg. Allg. Chem.* **1965**, 335, 113–125. (b) Neumann, H.; Steinberg, I. Z.; Katchalski, E. *J. Am. Chem. Soc.* **1965**, 87, 3841–3848.

**Table 2.** Experimentally Determined Stability Constants,  $K_{M(AMP)}^M$ , for M(AMP) Complexes<sup>a</sup> and Calculated Stability Constants,  $K_{M(AMP)_{op}}^M$ , for the Corresponding Isomers with a Sole Phosphate Coordination of  $M^{2+}$ ,<sup>b</sup> as well as the Resulting Stability Differences,  $\log \Delta_{M(AMP)}$  (Eq 13), and the Extent of the Intramolecular Chelate Formation (Eqs 9–12 and 14) in Various M(AMP) Complexes in Aqueous Solution at 25 °C and  $I = 0.1$  M (NaNO<sub>3</sub>)

$M^{2+}$	$\log K_{M(AMP)}^M$ <sup>a</sup> (eqs 5 and 11)	$\log K_{M(AMP)_{op}}^M$ <sup>b</sup> (eq 11)	$\log \Delta_{M(AMP)}$ <sup>c</sup> (eq 13)	$K_I$ (eqs 10–12)	%M(AMP) <sub>cl</sub> (eqs 9 and 14)
Mg <sup>2+</sup>	1.62 ± 0.04	1.56 ± 0.03	0.06 ± 0.05	0.15 ± 0.13	13 ± 10
Ca <sup>2+</sup>	1.48 ± 0.03	1.45 ± 0.05	0.03 ± 0.06	0.07 ± 0.14	0 (7 ± 13)
Ba <sup>2+</sup>	1.18 ± 0.04	1.16 ± 0.04	0.02 ± 0.06	0.05 ± 0.14	0 (5 ± 12)
Mn <sup>2+</sup>	2.23 ± 0.02	2.16 ± 0.05	0.07 ± 0.05	0.17 ± 0.15	15 ± 11
Co <sup>2+</sup>	2.30 ± 0.04	1.94 ± 0.06	0.36 ± 0.07	1.29 ± 0.38	56 ± 7
Ni <sup>2+</sup>	2.55 ± 0.04	1.94 ± 0.05	0.61 ± 0.06	3.07 ± 0.60	75 ± 4
Zn <sup>2+</sup>	2.38 ± 0.07	2.13 ± 0.06	0.25 ± 0.09	0.78 ± 0.38	44 ± 12
Cd <sup>2+</sup>	2.74 ± 0.05	2.44 ± 0.05	0.30 ± 0.07	1.00 ± 0.32	50 ± 8

<sup>a</sup> These values have recently been redetermined<sup>5b</sup> by taking into account (as far as possible) also the formation (in analogy to eq 4) of protonated M(H;AMP) complexes (see also the “caveat”, i.e. section 1.6 in ref 29b). However, most values are within their error limits identical with the previous ones;<sup>29a</sup> only those for the Co<sup>2+</sup>, Ni<sup>2+</sup>, and Cd<sup>2+</sup> complexes are now up to 0.07 log unit larger. 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. <sup>b</sup> Calculated with  $pK_{H(AMP)}^H = 6.21$  (Table 1) and the reference-line equations (see also Figures 2 and 3) listed in Table 5 of ref 39c; the corresponding error limits are given in Table 6 of ref 39c; these data are also summarized in Table 3 of ref 29b. <sup>c</sup> The errors given here ( $3\sigma$ ) and in the two columns at the right were calculated according to the error propagation after Gauss by using the errors listed in the two columns to the left.

The formation degree of the macrochelated or “closed” species, designated<sup>29</sup> as M(AMP)<sub>cl</sub>, is independent of the total complex concentration because it is an intramolecular equilibrium, and therefore  $K_I$ , as defined by eq 10, where M(AMP)<sub>op</sub> refers to the “open” species in equilibrium 9, is dimensionless:

$$K_I = [M(AMP)_{cl}]/[M(AMP)_{op}] \quad (10)$$

Taking this into account equilibrium 5a may be rewritten for AMP<sup>2-</sup> as below:



It has been shown<sup>29</sup> that  $K_I$  may be calculated according to eq 12:

$$K_I = (K_{M(AMP)}^M / K_{M(AMP)_{op}}^M) - 1 = 10^{\log \Delta} - 1 \quad (12)$$

where

$$\begin{aligned} \log \Delta &= \log \Delta_{M(AMP)} = \log K_{M(AMP)}^M - \log K_{M(AMP)_{op}}^M \\ &= \log K_{M(AMP)_{exp}}^M - \log K_{M(AMP)_{calc}}^M \end{aligned} \quad (13)$$

In eq 13,  $\log K_{M(AMP)_{op}}^M$  refers to the stability of the “open” species in equilibrium 9 and it equals  $\log K_{M(AMP)_{calc}}^M$ , which is calculated with the mentioned straight-line equations<sup>29b,39c</sup> and  $pK_{H(AMP)}^H$  (Table 1). The data for  $\log K_{M(AMP)_{exp}}^M$  correspond to the experimentally measured values.<sup>5b,29</sup> Finally, with  $K_I$  (eq 12) and eq 14, the percentage of the closed or macrochelated species can be calculated:<sup>29</sup>

$$\%M(AMP)_{cl} = 100[K_I/(1 + K_I)] \quad (14)$$

The earlier results for the M(AMP) complexes<sup>5b,29</sup> are summarized in Table 2.

That several of the M(AMP) complexes own an increased stability due to macrochelate formation (eq 9) is evident from

(39) (a) Massoud, S. S.; Sigel, H. *Inorg. Chem.* **1988**, *27*, 1447–1453. (b) Saha, A.; Saha, N.; Ji, L.-n.; Zhao, J.; Gregań, F.; Sajadi, S. A. A.; Song, B.; Sigel, H. *J. Biol. Inorg. Chem.* **1996**, *1*, 231–238. (c) Sigel, H.; Chen, D.; Corfú, N. A.; Gregań, F.; Holý, A.; Strašák, M. *Helv. Chim. Acta* **1992**, *75*, 2634–2656.

(40) Martin, R. B.; Sigel, H. *Comments Inorg. Chem.* **1988**, *6*, 285–314.

Figures 2 and 3: the vertical distances (dotted lines) from the M(AMP) data points (half-filled) to the reference lines correspond to the  $\log \Delta_{M(AMP)}$  values according to eq 13. If one subtracts these values (Table 2, column 4) from the measured  $\log K_{M(AMP)}^M$  constants for the M(AMP) complexes (Table 1, column 3) according to eq 15, the resulting  $\log K_{M(AMP)_{calc}}^M$  values should reflect M<sup>2+</sup> binding to the thiophosphate group (see also column 4 in Table 3; vide infra).

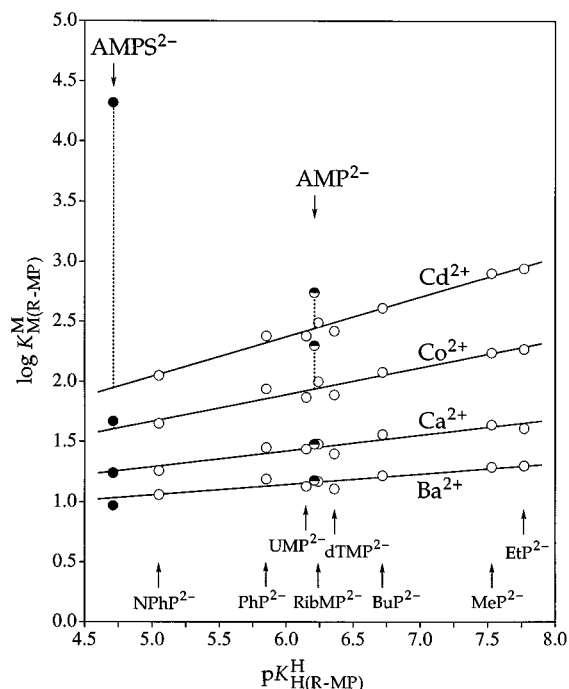
$$\log K_{M(AMP)_{calc}}^M = \log K_{M(AMP)}^M - \log \Delta_{M(AMP)} \quad (15)$$

These values are inserted in dependence on the micro acidity constant,  $pK_{AMPS-H}^{AMPS} = 4.71$  (see section 3.1), which reflects the basicity of the thiophosphate group, also into Figures 2 and 3 (full points at the left).

To conclude, the increased stability observed for the M(AMP) complexes is solely due to macrochelate formation,<sup>29</sup> whereas if macrochelate formation is accounted for (eq 15), several of the M(AMPS) complexes, especially those with Cd<sup>2+</sup> and Zn<sup>2+</sup>, still possess a stability which is beyond that expected on the basis of the basicity of the thiophosphate group; hence, this indicates that the sulfur participates in these instances in metal ion binding.

Regarding the M(AMPS) complexes of the alkaline earth ions, one may recall that it has been concluded<sup>37</sup> for the AMPS<sup>2-</sup> ion in aqueous solution that the P–S bond is a single bond with a negative charge localized on sulfur and that the P–O bond orders approach 1.5 owing to delocalization of the other negative charge unit among the two oxygens. It has also been concluded<sup>37b</sup> that metal ions will alter this charge distribution. Indeed, from the top part of Figure 3, it is evident that the data point for Mg(AMPS) falls within the error limits on its reference line. This observation allows the conclusion that Mg<sup>2+</sup> behaves equally in its binding properties toward a thiophosphate and a phosphate group.<sup>41</sup> Figure 2 shows that this is also valid for the other two alkaline earth metal ions, i.e., the Ca<sup>2+</sup> and Ba<sup>2+</sup> complexes.

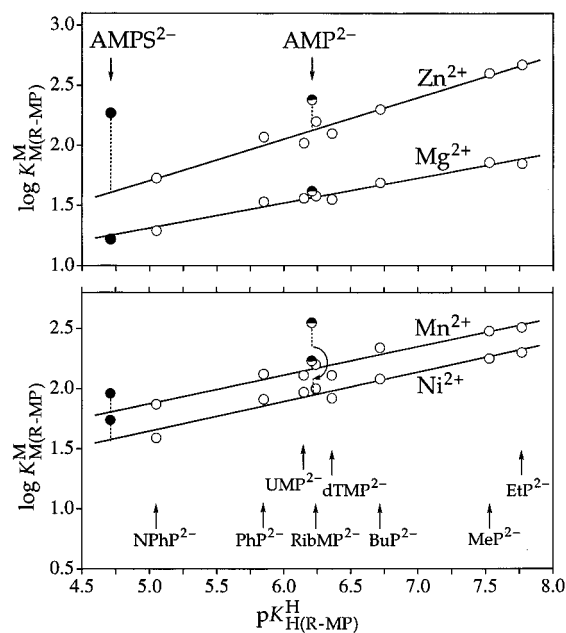
(41) A statistical consideration about the phosphate group in AMP<sup>2-</sup> and the thiophosphate group in AMPS<sup>2-</sup> gives the following result: in AMP<sup>2-</sup> the Mg<sup>2+</sup> ion can “choose” between three oxygens for binding, in AMPS<sup>2-</sup> only between two; that is, Mg<sup>2+</sup> binding will be disfavored by a factor of two-thirds, which corresponds to a stability *decrease* of 0.18 log units. However, the lower electronegativity of sulfur compared to that of oxygen should cause a higher charge density in the complexes on the two oxygens in AMPS<sup>2-</sup> than on the three oxygens in AMP<sup>2-</sup>; consequently, the oxygens of AMPS<sup>2-</sup> appear as being favored for Mg<sup>2+</sup> binding, and therefore, an *increase* in stability is expected. The result indicates that the two mentioned opposite effects evidently cancel each other.



**Figure 2.** Evidence for an enhanced stability of the Cd(AMPS) complex and possibly also for the Co(AMPS) complex as well as for the “expected” stability of the Ca(AMPS) and Ba(AMPS) complexes (=AMPS<sup>2-</sup>; ●), based on  $\log K_{M(\text{AMPS})}^M$  (eq 15) and on the relationship between  $\log K_{M(\text{R-MP})}^M$  and  $pK_{H(\text{R-MP})}^H$  for the 1:1 complexes of Ba<sup>2+</sup>, Ca<sup>2+</sup>, Co<sup>2+</sup>, and Cd<sup>2+</sup> with some simple phosphate monoesters or phosphonate ligands (R-MP<sup>2-</sup>): 4-nitrophenyl phosphate (NPhP<sup>2-</sup>), phenyl phosphate (PhP<sup>2-</sup>), uridine 5'-monophosphate (UMP<sup>2-</sup>), D-ribose 5-monophosphate (RibMP<sup>2-</sup>), thymidine 5'-monophosphate (dTMP<sup>2-</sup>), *n*-butyl phosphate (BuP<sup>2-</sup>), methyl phosphonate (MeP<sup>2-</sup>), and ethyl phosphonate (EtP<sup>2-</sup>) (from left to right) (○). The least-squares lines are drawn through the corresponding eight data sets, which are taken for the phosphate monoesters from ref 39a and for the phosphonates from ref 39c; the equations for these reference lines are listed in ref 29b or 39c. The data points for the M(AMP) complexes (=AMP<sup>2-</sup>; ●) are shown for comparison and are taken from ref 5b (see also column 2 of Table 2). The points for the AMPS<sup>2-</sup> systems (●) are based on the data given in Table 3 in the fourth column (vide infra) for  $\log K_{M(\text{AMPS})}^M$  (eq 15) and the micro acidity constant,  $pK_{\text{AMPS-H}}^{\text{AMPS}} = 4.71$  (section 3.1). The vertical dotted lines emphasize the stability differences to the corresponding reference lines; the differences are equal to  $\log \Delta_{M(\text{AMP})}$  for the M(AMP) complexes as defined in section 3.3 by eq 13 and to  $\Delta \log \Delta_{M(\text{AMPS})}$  as defined in section 3.4 by eq 16. All the plotted equilibrium constant values refer to aqueous solutions at 25 °C and  $I = 0.1 \text{ M}$  (NaNO<sub>3</sub>).

**3.4. Evidence That the Stability of the M(AMPS) Complexes with Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, and Cd<sup>2+</sup> Is Affected by the Sulfur Atom of the Thiophosphate Group.** The result presented in the last paragraph that the Mg(AMPS), Ca(AMPS), and Ba(AMPS) complexes behave like simple phosphate complexes despite their thiophosphate group is encouraging because it suggests that the stability data obtained for the other M(AMPS) complexes can also be judged on the basis of their  $\log K_{M(\text{R-MP})}^M$  versus  $pK_{H(\text{R-MP})}^H$  reference lines. Consequently, we calculated the stability constants,  $\log K_{M(\text{AMPS})_{\text{op}}}^M$ , simply on the basis of the basicity of the thiophosphate group ( $pK_{\text{AMPS-H}}^{\text{AMPS}} = 4.71$ ; section 3.1) and the corresponding reference lines<sup>29b,39c</sup> due to a pure phosphate binding. We define these constants as  $K_{M(\text{AMPS})_{\text{op}}}^M$ , using the asterisk as a sign that we apply here a procedure developed for phosphate ligands to a thiophosphate one. These values are listed in column 5 of Table 3.

In analogy to eq 13, now for the M(AMPS) complexes also,  $\log \Delta_{M(\text{AMPS})}^*$  may be calculated from the values listed in



**Figure 3.** Evidence for an enhanced stability of the Mn(AMPS), Ni(AMPS), and Zn(AMPS) complexes as well as for the “expected” stability of the Mg(AMPS) complex (=AMPS<sup>2-</sup>; ●), based on  $\log K_{M(\text{AMPS})}^M$  (eq 15 and column 4 of Table 3; vide infra) and on the relationship between  $\log K_{M(\text{R-MP})}^M$  and  $pK_{H(\text{R-MP})}^H$  for the 1:1 complexes of Mg<sup>2+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup>, and Zn<sup>2+</sup> with some simple phosphate monoesters or phosphonate ligands (R-MP<sup>2-</sup>) (○; see legend for Figure 2). The data points for the M(AMP) complexes (=AMP<sup>2-</sup>; ●) are shown for comparison. The vertical dotted lines emphasize the stability differences to the corresponding reference lines; the differences are equal to  $\log \Delta_{M(\text{AMP})}$  for the M(AMP) complexes as defined in section 3.3 by eq 13 and to  $\Delta \log \Delta_{M(\text{AMPS})}$  as defined in section 3.4 by eq 16. All the plotted equilibrium constant values refer to aqueous solutions at 25 °C and  $I = 0.1 \text{ M}$  (NaNO<sub>3</sub>). For further details, see the legend of Figure 2.

columns 2 and 5 of Table 3; the corresponding results are listed in column 6 of the same table. These differences are zero within their error limits for the M(AMPS) complexes of Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Ba<sup>2+</sup>, confirming the conclusions presented in the final paragraph of section 3.3.

In section 3.3 it was assumed that macrochelate formation (eq 9) in M(AMPS) and M(AMP) occurs—because of the close structural similarity of the two ligands—for a given metal ion to about the same extent. This has led to the definition given in eq 15, where  $K_{M(\text{AMPS})_{\text{calc}}}^M$  represents a stability constant for the M(AMPS) complexes from which the M<sup>2+</sup>/N7 interaction is eliminated (Table 3, column 4).

Now a further stability difference can be defined according to eq 16a:

$$\Delta \log \Delta_{M(\text{AMPS})} = \log K_{M(\text{AMPS})_{\text{calc}}}^M - \log K_{M(\text{AMPS})_{\text{op}}}^M \quad (16a)$$

$$= \log K_{M(\text{AMPS})}^M - \log \Delta_{M(\text{AMP})} - \log K_{M(\text{AMPS})_{\text{op}}}^M \quad (16b)$$

$$= (\log K_{M(\text{AMPS})}^M - \log K_{M(\text{AMPS})_{\text{op}}}^M) - (\log K_{M(\text{AMP})}^M - \log K_{M(\text{AMP})_{\text{op}}}^M) \quad (16c)$$

$$= \log \Delta_{M(\text{AMPS})}^* - \log \Delta_{M(\text{AMP})} \quad (16d)$$

Equations 16b,c reveal further connections between various equilibrium constants (cf. also eqs 13 and 15) and indicate how

**Table 3.** Measured<sup>a</sup> Stability Constants,  $K_{M(AMPS)}^M$ , and Calculated<sup>b</sup> Stability Constants,  $K_{M(AMPS)_{op}}^{M*}$ , for M(AMPS) Complexes and Their Isomers with a Sole Thiophosphate Coordination of  $M^{2+}$ , Respectively, as Well as the Resulting Values for  $\log \Delta_{M(AMPS)}^*$ .<sup>c</sup> Given Are Also the Stability Constants,  $K_{M(AMPS)_{calc}}^M$ , in Which the Contribution from the  $M^{2+}/N7$  Interaction Is Eliminated (eq 15) as Well as the Derived Stability Difference,  $\Delta \log \Delta_{M(AMPS)}$ , Which Reflects the Effect of the Sulfur in the Thiophosphate Group on the Stability of the M(AMPS) Complexes. All Values Refer to Aqueous Solution at 25 °C and  $I = 0.1$  M (NaNO<sub>3</sub>)

$M^{2+}$	$\log K_{M(AMPS)}^M$ <sup>a</sup> (eq 5)	$\log \Delta_{M(AMP)}^d$ (eq 13)	$\log K_{M(AMPS)_{calc}}^M$ (eq 15)	$\log K_{M(AMPS)_{op}}^{M*}$ <sup>b</sup>	$\log \Delta_{M(AMPS)}^{*c,e}$	$\Delta \log \Delta_{M(AMPS)}^e$ (eq 16)
Mg <sup>2+</sup>	1.28 ± 0.04	0.06 ± 0.05	1.22 ± 0.06	1.25 ± 0.03	0.03 ± 0.05	-0.03 ± 0.07
Ca <sup>2+</sup>	1.27 ± 0.06	0.03 ± 0.06	1.24 ± 0.08	1.25 ± 0.05	0.02 ± 0.08	-0.01 ± 0.10
Ba <sup>2+</sup>	0.99 ± 0.03	0.02 ± 0.06	0.97 ± 0.07	1.03 ± 0.04	-0.04 ± 0.05	-0.06 ± 0.08
Mn <sup>2+</sup>	2.03 ± 0.10	0.07 ± 0.05	1.96 ± 0.11	1.80 ± 0.05	0.23 ± 0.11	0.16 ± 0.12
Co <sup>2+</sup>	2.03 ± 0.10	0.36 ± 0.07	1.67 ± 0.12	1.60 ± 0.06	0.43 ± 0.12	0.07 ± 0.14
Ni <sup>2+</sup>	2.35 ± 0.07	0.61 ± 0.06	1.74 ± 0.09	1.58 ± 0.05	0.77 ± 0.09	0.16 ± 0.10
Zn <sup>2+</sup>	2.52 ± 0.18	0.25 ± 0.09	2.27 ± 0.20	1.61 ± 0.06	0.91 ± 0.19	0.66 ± 0.21
Cd <sup>2+</sup>	4.62 ± 0.12	0.30 ± 0.07	4.32 ± 0.14	1.95 ± 0.05	2.67 ± 0.13	2.37 ± 0.15

<sup>a</sup> From column 3 in Table 1. The error limits given throughout correspond to *three times* the standard error of the mean value ( $3\sigma$ ). <sup>b</sup> Calculated with  $pK_{AMPS-H}^{AMPS} = 4.71$  (section 3.1) and the reference-line equations listed in refs 29b or 39c (see also Figures 2 and 3). <sup>c</sup> Defined in analogy to eq 13, i.e.,  $\log \Delta_{M(AMPS)}^* = \log K_{M(AMPS)}^M - \log K_{M(AMPS)_{op}}^{M*}$ . <sup>d</sup> From column 4 in Table 2. <sup>e</sup> The errors given here ( $3\sigma$ ) were calculated according to the error propagation after Gauss by using the errors listed in the appropriate columns to the left.

one arrives at eq 16d. The values calculated now for  $\Delta \log \Delta_{M(AMPS)}$  are listed in column 7 of Table 3.

Evidently, the stability difference  $\Delta \log \Delta_{M(AMPS)}$  (eq 16) solely reflects the effect of the sulfur atom in the thiophosphate group on the stability of the M(AMPS) complexes and it is graphically represented in the vertical differences (dotted lines) in Figures 2 and 3 between the data points due to the M(AMPS) complexes (full circles) and the straight reference lines. A positive value (Table 3, column 7) can only mean that the sulfur participates in the binding of the metal ion to the thiophosphate group. The effect is very dramatic for Cd(AMPS), quite remarkable for Zn(AMPS), and less pronounced for Mn(AMPS) and Ni(AMPS). For Co(AMPS) the value of  $\Delta \log \Delta_{Co(AMPS)}$  is slightly positive, yet also zero within its error limits; hence, in this case so far, no final conclusion can be drawn.

**3.5. Correlations with Other Sulfur Donors and Further Evidence That the Sulfur Participates in Metal Ion Binding in the M(AMPS) Complexes of Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, and Cd<sup>2+</sup>.** The order of the  $\Delta \log \Delta_{M(AMPS)}$  values (eq 16; Table 3, column 7), attributed to the metal ion–sulfur binding, reflects the well-known larger affinity of Cd<sup>2+</sup> for sulfur binding sites if compared, e.g., with Mn<sup>2+</sup> and Ni<sup>2+</sup>. Indeed, this general behavior is also seen in the much lower solubility of cadmium sulfide compared with the sulfides of the other metal ions considered. Therefore, we endeavored to correlate the  $\Delta \log \Delta_{M(AMPS)}$  values determined in this study with the solubility products of the corresponding metal sulfides, i.e. M<sup>I</sup>S.

Unfortunately, the available data<sup>42–44</sup> for the solubility products of the metal ion sulfides of Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, and Cd<sup>2+</sup> vary widely. Therefore, we decided to use two “extreme” data sets for the solubility product constants,  $SP$  (eq 17):

(42) (a) Sillén, L. G.; Martell, A. E. *Stability Constants of Metal-Ion Complexes*; Special Publication 17; the Chemical Society: London, 1964. (b) Sillén, L. G.; Martell, A. E. *Stability Constants of Metal-Ion Complexes, Supplement No. 1*; Special Publication 25; The Chemical Society: London, 1971. (c) Högföldt, E. *Stability Constants of Metal-Ion Complexes, Part A: Inorganic Ligands*; IUPAC Chemical Data Series 21; Pergamon Press: Oxford, U.K., and New York, 1982. (d) Smith, R. M.; Martell, A. E. *Critical Stability Constants, Vol. 4: Inorganic Complexes*; Plenum Press: New York and London, 1976. (e) Vogel, A. I. *Macro and Semimicro Qualitative Inorganic Analysis*, 4th ed.; Longmans, Green and Co.: London, New York, and Toronto, 1957; p 40. (f) Wiberg, N. *Holleman-Wiberg: Lehrbuch der Anorganischen Chemie*, Aufl. 91–100; Walter de Gruyter: Berlin and New York, 1985; p 213.

(43) Treadwell, W. D. *Tabellen zur Qualitativen Analyse*; 18. Aufl.; Franz Deuticke: Wien, Austria, 1947; p 9.

(44) Lide, D. R., Ed. *Handbook of Chemistry and Physics*, 74th ed.; CRC Press: Boca Raton, FL, Ann Arbor, MI, London, and Tokyo, 1993; pp 8–49.

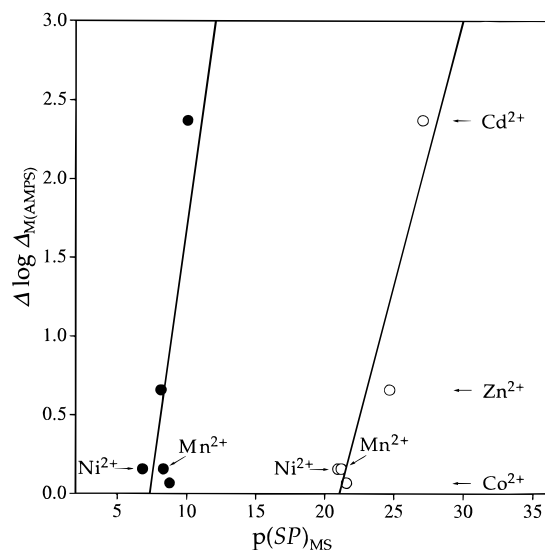
$$(SP)_{MS} = [M^{2+}][S^{2-}] \quad (17)$$

In the first case we calculated the  $p(SP)_{MS}$  values from the solubility data (mol/L) given in ref 43; these data refer to freshly prepared precipitates and the connected concentrations of the reactants in solution. However, metal ion sulfides have a strong tendency to age,<sup>43</sup> becoming thus even less soluble with time and giving also rise to various forms of the solids. Our second set, which contains relatively large  $p(SP)_{MS}$  values, mostly from ref 44, refers to aged precipitates. Because in ref 44 no value for Co<sup>II</sup>S is listed, an average from values in ref 42a is used,<sup>45a</sup> and because the  $p(SP)_{MS}$  value for Mn<sup>II</sup>S from ref 44 appears as too small, again the value from ref 42a is employed which refers to the “green modification” of MnS.<sup>45b</sup>

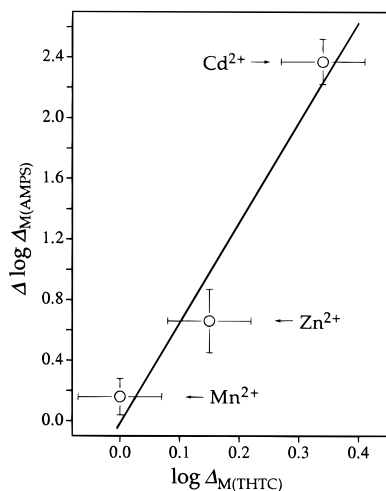
In Figure 4, our  $\Delta \log \Delta_{M(AMPS)}$  values (Table 3, column 7) are plotted versus the two mentioned data sets for the  $p(SP)_{MS}$  values. Even though the differences between the two sets of the  $p(SP)_{MS}$  data correspond to more than 10 log units, in both instances, a rather good linear correlation is obtained. Hence, despite all shortcomings, the clear message from Figure 4 is that at least an approximate correlation between  $\Delta \log \Delta_{M(AMPS)}$  and  $p(SP)_{MS}$  exists. Because in the sulfides only a sulfur–metal ion interaction is possible, the data of Figure 4 provide further evidence that in the M(AMPS) complexes of Cd<sup>2+</sup> and Zn<sup>2+</sup> the metal ion is mainly coordinated to the sulfur atom of the thiophosphate group and that also in the other M(AMPS) complexes the sulfur participates in metal ion binding at least to a certain extent.

This result (Figure 4) led to a further search<sup>42</sup> for a set of self-consistent stability constants for metal ion complexes formed with a sulfur ligand. Unfortunately, there are only few data available; the only ones allowing also further evaluations (see section 3.6) refer to the Mn<sup>2+</sup>, Zn<sup>2+</sup>, and Cd<sup>2+</sup> complexes of tetrahydrothiophene-2-carboxylate (THTC<sup>-</sup>).<sup>46</sup> With this ligand, metal ions bind not only to the carboxylate group but to a certain extent also to the thioether unit, giving rise to a five-membered chelate and an intramolecular equilibrium (analogous to eq 9). The extent of the thioether–metal ion interaction is reflected in an increased complex stability, compared to the one expected for a sole carboxylate–metal ion

(45) (a) Average of four  $p(SP)_{CoS}$  values (22.51, 21.3, 22.10, 20.4), which refer to 25 °C and which are listed in ref 42a. (b) The value  $p(SP)_{MnS} = 21.21$  (20 °C) is listed in ref 42a; it is based on refs 45c,d. (c) Kolthoff, I. M. *J. Phys. Chem.* **1931**, 35, 2711–2721. (d) Moser, L.; Behr, M. *Z. Anorg. Chem.* **1924**, 134, 49–74.



**Figure 4.** Correlation between the  $\Delta \log \Delta_{M(\text{AMPS})}$  values (eq 16; Table 3, column 7) of this study and two data sets for the negative logarithms of the solubility product constants,  $p(\text{SP})_{\text{MS}}$ , of the sulfides of  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cd}^{2+}$ . The data for the small  $p(\text{SP})_{\text{MS}}$  values (●) are from ref 43, those for the larger  $p(\text{SP})_{\text{MS}}$  values (○) from refs 44–46; see also text in section 3.5.



**Figure 5.** Correlation between the  $\Delta \log \Delta_{M(\text{AMPS})}$  values (eq 16; Table 3, column 7) of this study and the  $\log \Delta_{M(\text{THTC})}$  values (which are defined in analogy to eq 13)<sup>46a</sup> for the  $\text{M}(\text{THTC})^+$  complexes of  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cd}^{2+}$ .<sup>46a</sup> The  $\log \Delta_{M(\text{THTC})}$  values quantify the extent of the metal ion–thioether interaction in the  $\text{M}^{2+}$  complexes of tetrahydrothiophene-2-carboxylate ( $\text{THTC}^-$ ). The vertical error bars correspond to the error limits given in column 7 of Table 3; the horizontal error bars (corresponding to  $\pm 0.07$  log units) are estimates based on experience<sup>39c</sup> with this kind of data.

binding, i.e., in analogy to eq 13, values for  $\log \Delta_{M(\text{THTC})}$  can be calculated.<sup>46a</sup>

The available three data pairs of  $\Delta \log \Delta_{M(\text{AMPS})}$  versus  $\log \Delta_{M(\text{THTC})}$  are plotted in Figure 5; they fit within their error limits on a straight line. This result demonstrates that the intensity of the metal ion–sulfur interaction in the thiophosphate group of  $\text{AMPS}^{2-}$  as quantified by  $\Delta \log \Delta_{M(\text{AMPS})}$  (eq 16) can be correlated not only with data for the 2-fold negatively charged  $\text{S}^{2-}$  ion but also with those for the neutral thioether sulfur. In other words, the values for  $\Delta \log \Delta_{M(\text{AMPS})}$  (Table 3, column 7) reflect well the *relative* binding intensities of various kinds of

sulfur ligands independently of the charge of the sulfur site. This conclusion is meaningful for the comparisons to be made in section 4 involving rate constants.

### 3.6. Estimates for the Extent of the Sulfur–Metal Ion Interaction in the $\text{M}(\text{AMPS})$ Complexes of $\text{Cu}^{2+}$ and $\text{Pb}^{2+}$ .

Application of the correlations seen in Figures 4 and 5 evidently allows estimation of values of  $\Delta \log \Delta_{M(\text{AMPS})}$  for unknown  $\text{M}(\text{AMPS})$  complexes, provided values for  $p(\text{SP})_{\text{MS}}$  and/or  $\log \Delta_{M(\text{THTC})}$  are available. Therefore, the straight-line equations corresponding to the lines seen in Figures 4 and 5 are given below in eqs 18, 19, and 20; they refer to the left and right parts of Figure 4, and to Figure 5, respectively:

$$\Delta \log \Delta_{M(\text{AMPS})} = (0.630 \pm 0.309)[p(\text{SP})_{\text{MS}}] - (4.613 \pm 2.618) \quad (18)$$

$$\Delta \log \Delta_{M(\text{AMPS})} = (0.336 \pm 0.075)[p(\text{SP})_{\text{MS}}] - (7.088 \pm 1.748) \quad (19)$$

$$\Delta \log \Delta_{M(\text{AMPS})} = (6.61 \pm 1.61)[\log \Delta_{M(\text{THTC})}] - (0.02 \pm 0.34) \quad (20)$$

Application of these equations is demonstrated for two examples, i.e., the  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  complexes of  $\text{AMPS}^{2-}$ . We selected these two metal ions because (i) the  $\text{Cu}^{2+}/\text{AMPS}^{2-}$  system cannot be studied due to formation of a precipitate resulting most likely from a redox reaction (see section 3.2) and (ii) the  $\text{Pb}^{2+}/\text{AMPS}^{2-}$  system was not studied up to now because all the background information (i.e., reference-line equation, etc.; cf., e.g., Figure 2) needed for a detailed evaluation of its experimental data is lacking. Both ions, however, are of general interest and especially  $\text{Pb}^{2+}$  is presently receiving increasing attention in ribozyme<sup>22a,f</sup> research.

For  $\text{Pb}^{2+}$  we applied to eq 18 the (low) solubility product,  $p(\text{SP})_{\text{PbS}} = 10.96$ , from ref 43 and obtained  $\Delta \log \Delta_{\text{Pb}(\text{AMPS})} = 2.29$ ; from eq 19 and the (high) solubility products,  $p(\text{SP})_{\text{PbS}} = 27.52$  and  $28.04$ , from refs 44 and 47 follows  $\Delta \log \Delta_{\text{Pb}(\text{AMPS})} = 2.16$  and  $2.33$ , respectively. In ref 46a  $\log \Delta_{\text{Pb}(\text{THTC})} = 0.40$  is given, which leads with eq 20 to  $\Delta \log \Delta_{\text{Pb}(\text{AMPS})} = 2.62$ . The agreement between the four results is good, and as an average, one obtains  $\Delta \log \Delta_{\text{Pb}(\text{AMPS})} = 2.35 \pm 0.29$  ( $3\sigma$ ).

For cupric sulfide, eq 18 could not be used because the (low) solubility product of ref 43 is not applicable. The (high) solubility products,  $p(\text{SP})_{\text{CuS}} = 36.22$  and  $36.13$ , given in refs 44 and 48, respectively, lead with eq 19 to  $\Delta \log \Delta_{\text{Cu}(\text{AMPS})} = 5.08$  and  $5.05$ . Equation 20 gives with  $\log \Delta_{\text{Cu}(\text{THTC})} = 1.16$  of ref 46a  $\Delta \log \Delta_{\text{Cu}(\text{AMPS})} = 7.65$ . The agreement between these three results is much poorer than for  $\text{Pb}(\text{AMPS})$ ; this is not surprising as with  $\text{Cu}^{2+}$  the extrapolation takes place far outside of the data ranges which led to eqs 18–20 and which are seen in Figures 4 and 5. However, one may still estimate that  $\Delta \log \Delta_{\text{Cu}(\text{AMPS})} \approx 6 \pm 2$ .

To conclude, calculations based on eqs 18–20 are expected to yield reliable results if the applied values for  $p(\text{SP})_{\text{MS}}$  and/or  $\log \Delta_{M(\text{THTC})}$  are within or close to the data range defined by Figures 4 and 5. If the applied constants are beyond this range the results are less reliable but expected to be still in the approximate order; in the absence of any other more reliable information this may still be helpful (cf. also Table 4 in the next section).

(47) Average of 11  $p(\text{SP})_{\text{PbS}}$  values (27.47, 28.3, 29.04, 29.15, 29.37, 28.17, 28.15, 27.10, 26.6, 27.9, 27.15) determined close to 25 °C and as listed in ref 42a.

(48) Average of six  $p(\text{SP})_{\text{CuS}}$  values, i.e., for cupric sulfide (37.46, 37.49, 36.10, 35.10, 35.2, 35.40), which refer to 25 °C and are listed in ref 42a.

(46) (a) Sigel, H.; Scheller, K. H.; Rheinberger, V. M.; Fischer, B. E. *J. Chem. Soc., Dalton Trans.* **1980**, 1022–1028. (b) Sigel, H. *Angew. Chem., Int. Ed. Engl.* **1982**, 21, 389–400.



**Table 4.** Stability Increase,  $\Delta\log \Delta_{M(\text{AMPS})}$  (Eq 16), Attributed to a Sulfur–Metal Ion Coordination in M(AMPS) Complexes and Extent of this Interaction as Quantified by  $K_{\text{I/S}}$  (Eq 21) and the Percentage of the Sulfur-Coordinated Species, (PS·M), for Aqueous Solutions at 25 °C and  $I = 0.1 \text{ M}$  ( $\text{NaNO}_3$ )

M(AMPS)	$\Delta\log \Delta_{M(\text{AMPS})}$	$K_{\text{I/S}}$	% (PS·M)
Mg(AMPS)	$-0.03 \pm 0.07^a$	~0	~0
Ca(AMPS)	$-0.01 \pm 0.10^a$	~0	~0
Ba(AMPS)	$-0.06 \pm 0.08^a$	~0	~0
Mn(AMPS)	$0.16 \pm 0.12^a$	$0.45 \pm 0.40$	$31 \pm 19$
Co(AMPS)	$0.07 \pm 0.14^a$	$0.17 \pm 0.38$	~15 ( $\leq 38$ )
Ni(AMPS)	$0.16 \pm 0.10^a$	$0.45 \pm 0.33$	$31 \pm 16$
Zn(AMPS)	$0.66 \pm 0.21^a$	$3.57 \pm 2.21$	$78 \pm 11$
Cd(AMPS)	$2.37 \pm 0.15^a$	$233 \pm 81$	$99.6 \pm 0.15$
Pb(AMPS)	$2.35 \pm 0.29^b$	$223 \pm 149$	$99.6 \pm 0.30$
Cu(AMPS)	$6 \pm 2^b$	~ $10^6$	~100

<sup>a</sup> From column 7 in Table 3. Regarding the error limits see footnotes *a* and *e* in Table 3. <sup>b</sup> Estimates as derived in section 3.6.

**3.7. Extent of Sulfur–Metal Ion Binding in the M(AMPS) Complexes.** From the evaluations in sections 3.4 and 3.5 it has become clear that the sulfur atom of the thiophosphate group in  $\text{AMPS}^{2-}$  participates in metal ion binding. Therefore, the question arises: Which is the formation degree of the sulfur-coordinated species?

Neglecting outer-sphere species, the M(AMPS) complexes may occur at least in four forms: the two “open” isomers (eq 9) in which the metal ion either is bound to an oxygen or to the sulfur of the thiophosphate, and the corresponding two “closed” or macrochelated species in which the thiophosphate-coordinated metal ion interacts also with N7 of the adenine residue (eq 9). The latter-mentioned interaction was accounted for by eq 15 and the calculation of the values for  $\log K_{M(\text{AMPS})}^{\text{M}}$  (column 4 of Table 3); from these constants the increments,  $\Delta\log \Delta_{M(\text{AMPS})}^{\text{alc}}$ , were derived (eq 16) which solely quantify the stability increase, if any, due to the participation of the sulfur atom in metal ion binding (column 7 in Table 3).

If we define the sulfur-coordinated species as (PS·M) and the oxygen-bound ones as (PO·M), we may further define the intramolecular equilibrium constants  $K_{\text{I/S}}$  (eq 21). The second term of eq 21 may be derived in analogy to the situation described in section 3.3, i.e., especially to eq 12. Hence, one obtains

$$K_{\text{I/S}} = [(\text{PS}\cdot\text{M})]/[(\text{PO}\cdot\text{M})] = 10^{\Delta\log \Delta_{M(\text{AMPS})}} - 1 \quad (21)$$

Application of eq 21 to the data listed for  $\Delta\log \Delta_{M(\text{AMPS})}$  in column 7 of Table 3 and estimated in section 3.6 leads to the results summarized in Table 4.

From column 4 in Table 4 it is evident that in Cd(AMPS), Pb(AMPS) and Cu(AMPS) the metal ions are nearly completely S-coordinated to the thiophosphate. In Zn(AMPS) about four-fifths of all species are also sulfur-coordinated. This contrasts with the other extreme, i.e., the M(AMPS) complexes of  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Ba}^{2+}$ , in which the metal ions are overwhelmingly oxygen-coordinated to the thiophosphate group. For the complexes with  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ni}^{2+}$  it is evident, despite the rather large error limits, that significant amounts of the sulfur-coordinated isomers occur in the corresponding equilibria.

**3.8. Some Comments on the Formation of the Zn(AMPS)(OH)<sup>-</sup> Species.** As indicated already in section 3.2, it was surprising to observe that Zn(AMPS) forms a hydroxo complex, i.e.,  $\text{Zn}(\text{AMPS})(\text{OH})^-$ , in a pH range, where  $\text{Zn}(\text{aq})^{2+}$  itself does not yet form a hydroxo species. Consequently, according to equilibrium 6a, the following acidity constant was determined:  $\text{p}K_{\text{Zn}(\text{AMPS})(\text{aq})}^{\text{H}} = 6.8 \pm 0.4$  (cf. eq 6b; Table 1).

It has previously been concluded<sup>49</sup> that a reduction of the coordination number from 6 to 4 makes water bound to  $\text{Zn}^{2+}$  considerably more acidic; hence, such a reduction of the coordination number may also be assumed for the formation of  $\text{Zn}(\text{AMPS})(\text{OH})^-$ . From the following examples, taken from ref 49, the effect of the varying coordination number can nicely be seen: for the six-coordinated  $\text{Zn}(\text{H}_2\text{O})_6^{2+}$   $\text{p}K_{\text{a}} = 8.95$  ( $\pm 0.15$ ), for the four-coordinated  $\text{Zn}(\text{imidazole})_3(\text{H}_2\text{O})^{2+}$   $\text{p}K_{\text{a}} = 8.0$ , and for the also four-coordinated  $\text{Zn}(\text{L})(\text{H}_2\text{O})^{2+}$  (L = cyclo-1,5,9-triazadodecane)  $\text{p}K_{\text{a}} = 7.3$ .

Aside from steric effects, the formation of 4-fold coordinated  $\text{Zn}^{2+}$  is apparently driven<sup>49</sup> by the Lewis basicity of the donor atoms: if the coordinating ligand is a strong Lewis base, the coordination number of  $\text{Zn}^{2+}$  drops and the bond length shortens, i.e., the metal–ligand bond becomes more of the covalent type. This hypothesis would explain why an S-donor promotes the formation of 4-fold coordinated  $\text{Zn}^{2+}$  more effectively than an O-donor. In any case, the above example regarding the lowering of the  $\text{p}K_{\text{a}}$  value for a  $\text{Zn}^{2+}$  complex containing a sulfur binding site is certainly meaningful for biological systems.

**3.9. Considerations on the Structure of the Monoprotonated M(H;AMPS)<sup>+</sup> Complexes.** As mentioned in section 3.2, for  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Cd}^{2+}$ , also the monoprotonated species,  $\text{M}(\text{H};\text{AMPS})^+$ , form; the corresponding stability constants are collected in column 2 of Table 1. Cd(H;AMPS) is the most stable of these complexes, and therefore, its properties will be discussed first.

The question whether  $\text{Cd}^{2+}$  in Cd(H;AMPS) is bound to the adenine residue or to the thiophosphate group may be treated according to the microconstant scheme shown in Figure 6, which was developed in analogy to a similar problem discussed in ref 50.  $(\text{Cd}\cdot\text{AMPS}\cdot\text{H})^+$  represents the species where  $\text{Cd}^{2+}$  is coordinated either to N1 or N7 of the adenine residue (an ambiguity which has been repeatedly discussed and shall not be considered here further)<sup>51</sup> and the proton is located at the thiophosphate group.<sup>27</sup> The symbol  $\text{H}(\text{AMPS}\cdot\text{Cd})^+$  represents all those complexes where  $\text{Cd}^{2+}$  is located at the thiophosphate group and the proton either at N1 or also at the thiophosphate. Hence,  $\text{H}(\text{AMPS}\cdot\text{Cd})^+$  encompasses the following isomers:

(1)  $(\text{AMPS}\cdot\text{H}\cdot\text{Cd})^+$ ;  $\text{Cd}^{2+}$  and  $\text{H}^+$  both are bound to the thiophosphate group and the metal ion does not form a macrochelate with N7 (=open isomer)

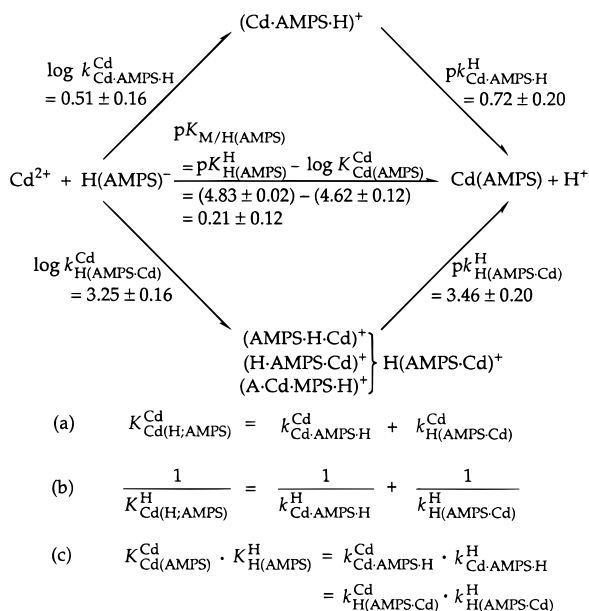
(2)  $(\text{A}\cdot\text{Cd}\cdot\text{MPS}\cdot\text{H})^+$ ;  $\text{Cd}^{2+}$  and  $\text{H}^+$  both are bound to the thiophosphate group but  $\text{Cd}^{2+}$  forms a macrochelate with N7 (=closed isomer)

(3)  $(\text{H}\cdot\text{AMPS}\cdot\text{Cd})^+$ ; the proton is now at N1 and  $\text{Cd}^{2+}$  at the thiophosphate group. One might think that this species also forms a macrochelate with N7 leading to

(4)  $(\text{H}\cdot\text{A}\cdot\text{Cd}\cdot\text{MPS})^+$ . However, such a species is expected to be negligible because it is known, e.g., from  $\text{Cu}^{2+}$  (cf. ref 52a),  $\text{Pd}^{2+}$  (cf. ref 52b), and  $\text{Pt}^{2+}$  (cf. ref 53), that binding of  $\text{M}^{2+}$  to N7 of the base moiety acidifies a proton at the N1 site by about 1–2 log units.

Therefore,  $(\text{H}\cdot\text{A}\cdot\text{Cd}\cdot\text{MPS})^+$  is not considered further, but

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**Figure 6.** Equilibrium scheme showing the interrelation between the monoprotonated Cd(H;AMPS)<sup>+</sup> species where the metal ion may either be coordinated at the adenine residue (upper part of the scheme), i.e., (Cd·AMPS·H)<sup>+</sup>, or at the thiophosphate group (lower part of the scheme), i.e., H(AMPS·Cd)<sup>+</sup>, together with the other species in equilibrium with these two complexes (each of which may still consist of various isomers; see text). The scheme defines also microconstants (*k*) and gives their interrelations with the macroconstants (*K*); the arrows indicate the directions for which the constants are defined. The macroconstants are from section 3.1 and Table 1; the microconstants have been derived by applying the above equations a, b, and c together with the assumption described in the text in section 3.9. The error limits of the various constants were calculated according to the error propagation after Gauss; the limits correspond to three times the standard error.

equilibria involving the above-mentioned first three species have to be kept in mind.

The equilibrium scheme in Figure 6 shows the reaction between Cd<sup>2+</sup> and H(AMPS)<sup>-</sup> leading to Cd(AMPS) and H<sup>+</sup>, proceeding either via (Cd·AMPS·H)<sup>+</sup> (upper part) and/or H(AMPS·Cd)<sup>+</sup> (lower part). The various microconstants (*k*) and their interrelation with the corresponding macroconstants (*K*) (eqs 3 and 5) also are defined and given. There are three independent equations a, b, and c, but four unknown microconstants; hence, as soon as one of them is determined or estimated, the other three can be calculated. A value for log  $k_{Cd·AMPS·H}^{Cd}$  may be estimated in the following way: The stability constant of the Cd(adenosine)<sup>2+</sup> complex is known, log  $K_{Cd(Ado)}^{Cd} = 0.11 \pm 0.06$  (cf. ref 51a), and by assuming that the charge effect of the -P(S)(O)<sub>2</sub>(H)<sup>-</sup> group corresponds to 0.4 ± 0.15 log units, as is known from various other cases where the distances between the positive or negative charges are of a comparable size,<sup>30b</sup> one obtains log  $k_{Cd·AMPS·H}^{Cd} = 0.51 \pm 0.16$ . This value with its (estimated) error limit is given on the arrow seen in the upper left part of Figure 6. The other three microconstants can now be calculated, and the corresponding results are given on the various arrows in the scheme.

An analogous analysis is also possible for the M(H;AMPS)<sup>+</sup> complexes of Mn<sup>2+</sup>, Co<sup>2+</sup>, or Ni<sup>2+</sup>, and the resulting constants are collected in Table 5, where the values from Figure 6 for the Cd(H;AMPS)<sup>+</sup> system are again listed. The latter should facilitate comparisons between Figure 6 and Table 5 and also help to identify the meaning of the various microconstants which appear in Table 5, i.e., Cd<sup>2+</sup> in Figure 6 has to be replaced by M<sup>2+</sup> to obtain the constant symbols of Table 5.

The results of Table 5 allow now to make an estimate of the ratio  $R_{M/AMPS/H}$  (eq 22) of the species (M·AMPS·H)<sup>+</sup> versus H(AMPS·M)<sup>+</sup> (see also Figure 6), which carry M<sup>2+</sup> at the adenine residue or the thiophosphate group, respectively:

$$R_{M/AMPS/H} = \frac{[(M·AMPS·H)^+]}{[H(AMPS·M)^+]} = \frac{k_{M·AMPS·H}^M}{k_{H(AMPS·M)}^M} \quad (22)$$

Application of eq 22 to the microconstants of Table 5 provides for the various metal ion systems the following results:

$$R_{Mn/AMPS/H} = \frac{[(Mn·AMPS·H)^+]}{[H(AMPS·Mn)^+]} = \frac{10^{-(0.42 \pm 0.43)}}{10^{(1.24 \pm 0.20)}} = 10^{-(1.66 \pm 0.47)} = \frac{1}{46} \left( \frac{1}{15}, \frac{1}{135} \right) \approx \frac{2}{98} \quad (23)$$

$$R_{Co/AMPS/H} = \frac{[(Co·AMPS·H)^+]}{[H(AMPS·Co)^+]} = \frac{10^{(0.37 \pm 0.54)}}{10^{(0.88 \pm 0.37)}} = 10^{-(0.51 \pm 0.65)} = \frac{1}{3.2} \left( \frac{1.4}{1}, \frac{1}{14} \right) \approx \frac{24}{76} \quad (24)$$

$$R_{Ni/AMPS/H} = \frac{[(Ni·AMPS·H)^+]}{[H(AMPS·Ni)^+]} = \frac{10^{(0.72 \pm 0.19)}}{10^{(0.87 \pm 0.53)}} = 10^{-(0.15 \pm 0.56)} = \frac{1}{1.4} \left( \frac{2.6}{1}, \frac{1}{3.6} \right) \approx \frac{41}{59} \quad (25)$$

$$R_{Cd/AMPS/H} = \frac{[(Cd·AMPS·H)^+]}{[H(AMPS·Cd)^+]} = \frac{10^{(0.51 \pm 0.16)}}{10^{(3.25 \pm 0.16)}} = 10^{-(2.74 \pm 0.23)} = \frac{1}{550} \left( \frac{1}{320}, \frac{1}{930} \right) \approx \frac{0.2}{99.8} \quad (26)$$

The values given in parentheses in the third rows of the eqs 23–26 represent the upper (first value) and lower limits (second value) of the ratios of the isomeric species; the final value in the third row is always the ratio of the approximate percentages of the two species.

From the results given in eq 26 it is evident that the H(AMPS·Cd)<sup>+</sup> species are strongly dominating with more than 99%. Consequently, we can conclude that the concentration of the monoprotonated Cd(H;AMPS)<sup>+</sup> complexes, where Cd<sup>2+</sup> is located at the adenine residue, is negligible and also that the reaction between Cd<sup>2+</sup> and H(AMPS)<sup>-</sup> proceeds overwhelmingly via the lower path of the microconstant scheme of Figure 6. Since Cd<sup>2+</sup> coordinated at the thiophosphate group compensates the 2-fold negative charge of this group and  $pK_{H(AMPS·Cd)}^H = 3.46 \pm 0.20$  (see Figure 6 and column 7 in Table 5), a value rather similar to the acidity constant of H(adenosine)<sup>+</sup>,  $pK_{H(Ado)}^H = 3.61 \pm 0.03$ ,<sup>24b</sup> one may conclude that in H(AMPS·Cd)<sup>+</sup> the proton is mainly at N1. Conse-

**Table 5.** Results of the Analysis Regarding the Microconstants for the Reaction of  $M^{2+}$  with  $H(\text{AMPS})^-$  to  $M(\text{AMPS})$  and  $H^+$  via the Isomers of  $M(\text{H};\text{AMPS})^+$ <sup>a</sup>

$M^{2+}$	$\log K_{M(\text{Ado})}^M$ <sup>b</sup>	$\log k_{M\text{-AMPS-H}}^M$ <sup>c</sup>	$\log k_{H(\text{AMPS-M})}^M$ <sup>d</sup>	$pK_{M/H(\text{AMPS})}^e$	$pK_{M\text{-AMPS-H}}^H$ <sup>f</sup>	$pK_{H(\text{AMPS-M})}^H$ <sup>f</sup>
$\text{Mn}^{2+}$	$-0.82 \pm 0.4$	$-0.42 \pm 0.43$	$1.24 \pm 0.20$	$2.80 \pm 0.10$	$2.38 \pm 0.44$	$4.04 \pm 0.22$
$\text{Co}^{2+}$	$-0.03 \pm 0.52$	$0.37 \pm 0.54$	$0.88 \pm 0.37$	$2.80 \pm 0.10$	$3.17 \pm 0.55$	$3.68 \pm 0.38$
$\text{Ni}^{2+}$	$0.32 \pm 0.12$	$0.72 \pm 0.19$	$0.87 \pm 0.53$	$2.48 \pm 0.07$	$3.20 \pm 0.20$	$3.35 \pm 0.53$
$\text{Cd}^{2+}$	$0.11 \pm 0.06$	$0.51 \pm 0.16$	$3.25 \pm 0.16$	$0.21 \pm 0.12$	$0.72 \pm 0.20$	$3.46 \pm 0.20$

<sup>a</sup> All constants listed are defined analogously to the constants given on the various arrows in Figure 6 (25 °C;  $I = 0.1 \text{ M}$ ,  $\text{NaNO}_3$ ). The error limits are either generously estimated or they correspond to *three times* the standard error of the mean value ( $3\sigma$ ). The error propagation was calculated according to Gauss. <sup>b</sup> For these data  $t$  is close to 25 °C but  $t$  varies; see ref 51a, from which the  $\log K_{M(\text{Ado})}^M$  values are taken. <sup>c</sup> See arrow at the left in the upper part of Figure 6;  $\log k_{M\text{-AMPS-H}}^M = \log K_{M(\text{Ado})}^M + (0.4 \pm 0.15)$ ; see text in section 3.9. <sup>d</sup> See arrow at the left in the lower part of Figure 6. The constants were calculated via eq (a) of Figure 6 with the values given above in the third column and those for  $\log K_{M(\text{H};\text{AMPS})}^M$  of column 2 in Table 1. <sup>e</sup> Calculated according to the definition given on the horizontal arrow in Figure 6 with the macroconstants given in section 3.1 and Table 1. <sup>f</sup> With the other constants known, the values for this microconstant follow now from the properties of cyclic systems (see also Figure 6).

quently, the dominating isomer is  $(\text{H}\cdot\text{AMPS}\cdot\text{Cd})^+$  using the definition given above.

For the  $\text{Mn}^{2+}/\text{H}(\text{AMPS})^-$  system also, the  $\text{H}(\text{AMPS}\cdot\text{Mn})^+$  species (about 98%; eq 23) and consequently the lower pathway in Figure 6 are dominating. However, with  $pK_{H(\text{AMPS-Mn})}^H = 4.04 \pm 0.22$  [which is identical with  $pK_{M(\text{H};\text{AMPS})}^H = 4.05 \pm 0.23$  (Table 1, column 4) but higher than  $pK_{H(\text{Ado})}^H = 3.61 \pm 0.03$ ], it appears that the dominating isomer is  $(\text{AMPS}\cdot\text{H}\cdot\text{Mn})^+$ , which carries both the proton and the metal ion at the thiophosphate group.

For the  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}/\text{H}(\text{AMPS})^-$  systems the results of eqs 24 and 25, respectively, reveal that the species  $(\text{M}\cdot\text{AMPS}\cdot\text{H})^+$  and  $\text{H}(\text{AMPS}\cdot\text{M})^+$  occur in comparable amounts, i.e., their ratio being 1:1 within the error limits, though the lower pathway in Figure 6 appears to be slightly favored. In agreement with this conclusion is that the values for the acidity constants,  $pK_{M\text{-AMPS-H}}^H$  and  $pK_{H(\text{AMPS-M})}^H$ , are within their error limits identical (see columns 6 and 7 of Table 5, respectively). Hence, by also taking into account the size of these acidity constants, one may postulate that  $(\text{H}\cdot\text{AMPS}\cdot\text{M})^+$  and  $(\text{M}\cdot\text{AMPS}\cdot\text{H})^+$  are the dominating isomers.

To conclude, despite the in part rather large error limits of some of the constants listed in Table 5, the results still demonstrate quite nicely how different metal ions may favor the formation of different isomeric complexes.

#### 4. General Conclusions

The evaluations carried out with the stability constants measured in this study have shown that  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  coordinate to the thiophosphate group of  $\text{AMPS}^{2-}$  in a phosphate-type manner. This means that the presence of the sulfur atom does not affect the kind of binding of these two alkaline earth ions, except that the stability of the  $\text{M}(\text{AMPS})$  complexes is somewhat smaller, if compared with that of the parent  $\text{M}(\text{AMP})$  complexes, but this is simply the result of the reduced basicity of the thiophosphate group if compared to that of the phosphate residue.<sup>27</sup>

On the contrary, metal ions, like  $\text{Zn}^{2+}$  or  $\text{Cd}^{2+}$ , show a significant binding affinity for the S atom of a thiophosphate group, i.e., the stability of their  $\text{M}(\text{AMPS})$  complexes is even larger than that of the corresponding  $\text{M}(\text{AMP})$  complexes despite the lower basicity of  $\text{AMPS}^{2-}$  compared to that of  $\text{AMP}^{2-}$ . Metal ions, like  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ , or  $\text{Ni}^{2+}$ , own intermediate properties; they bind partially to the sulfur atom, but this becomes apparent only if the different acid–base properties of the two ligands are taken into account. It is evident that applications of  $\text{AMPS}^{2-}$  as well as of other thionucleotide analogues as probes in enzymic studies in the presence of metal ions, like  $\text{Mn}^{2+}$  or  $\text{Zn}^{2+}$ , have to be made with great care, as

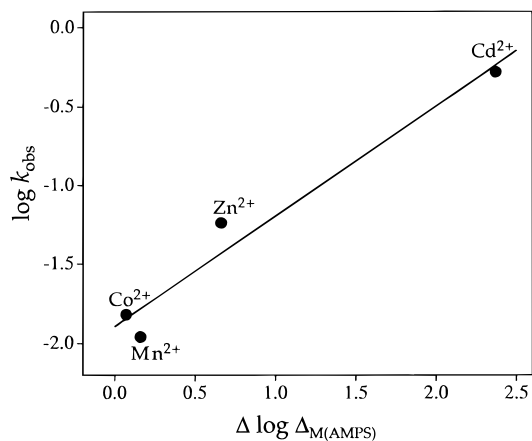
the metal ion-binding properties differ significantly from those of the parent nucleotides.

Apparently often initiated (cf., e.g., ref 13c) by Pearson's "hard–soft" principle there are now many examples available<sup>22a</sup> in which a phosphorothioate linkage is used in studies of ribozymes to identify metal ion binding sites via so-called "rescue" experiments. For example,<sup>22f</sup> replacement of the phosphate at the substrate cleavage site by a phosphorothioate linkage, containing a sulfur atom in place of a nonbridging phosphoryl oxygen, in the hammerhead ribozyme dramatically diminished reactivity under normal conditions which include  $\text{Mg}^{2+}$ ; replacement of  $\text{Mg}^{2+}$  by  $\text{Mn}^{2+}$  restored reactivity.<sup>22a,f</sup> Similarly, replacement of the bridging 3'-oxygen by sulfur at the cleavage site of a DNA substrate for the *Tetrahymena* ribozyme led in the presence of  $\text{Mg}^{2+}$  to an unreactive system,<sup>13b</sup> whereas addition of  $\text{Mn}^{2+}$  or  $\text{Zn}^{2+}$  (to some extent also of  $\text{Co}^{2+}$  and  $\text{Cd}^{2+}$ ) restored catalytic activity.<sup>13b,22a</sup> These examples indicate that the metal ion directly participates in the reactions.

Of interest in this context is a very recent study<sup>54</sup> about the effect of metal ions on the hydrolytic cleavage properties of an oligonucleotide containing a bridged internucleotide 5'-phosphorothioate RNA linkage. The oligonucleotide 5'-ApCpGpGpTpCpTprCpsApCpGpApGpC-3' is cleaved in the presence of metal ions into the 5'-thiol-containing (6-mer) and 2',3'-cyclic phosphate-containing (8-mer) fragments. The pseudo-first-order rate constants,  $k_{\text{obs}}$  ( $\text{min}^{-1}$ ; given in parentheses), for cleavage at pH 7.5 (50 mM Tris-HCl; ambient temperature) in the presence of  $M^{2+}$  are as follows: none ( $1.5 \times 10^{-4}$ ),  $\text{Mg}^{2+}$  ( $1.5 \times 10^{-3}$ ),  $\text{Ca}^{2+}$  ( $3.7 \times 10^{-3}$ ),  $\text{Mn}^{2+}$  ( $1.1 \times 10^{-2}$ ),  $\text{Co}^{2+}$  ( $1.5 \times 10^{-2}$ ),  $\text{Zn}^{2+}$  ( $5.7 \times 10^{-2}$ ), or  $\text{Cd}^{2+}$  ( $5.3 \times 10^{-1}$ ). It was concluded<sup>54</sup> that the rate enhancements correlate well with Pearson's principle, suggesting that cleavage is mediated in part by coordination of the metal to the 5'-mercapto leaving group.

Assuming that in the transition state of the rate-determining step of the above reaction metal ion binding occurs to the sulfur atom, then the reaction rate should be proportional to the corresponding complex stability. Of course, we cannot know the absolute stability constant for such a transition state complex, but from the results discussed in section 3.5 in connection with Figures 4 and 5, the stability of such a complex for a given  $M^{2+}$  is expected to be proportional to the sulfur–metal ion binding constants now determined. Therefore, we have plotted in Figure 7 the logarithms of the above-given observed pseudo-first-order rate constants,  $\log k_{\text{obs}}$ , in dependence on the  $\Delta\log \Delta_{M(\text{AMPS})}$  values (Table 3, column 7), which not only reflect the intensity of the sulfur–metal ion binding in the  $\text{M}(\text{AMPS})$  complexes (see eq 16 and section 3.4) but also represent in a general way *relative* complex stabilities (section 3.5). Indeed,

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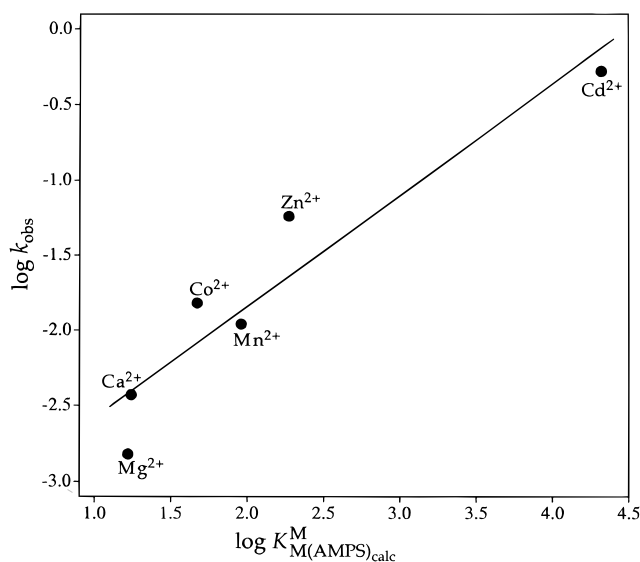


**Figure 7.** Relationship between the logarithms of the observed pseudo-first-order rate constants ( $\text{min}^{-1}$ ) for the hydrolytic cleavage of an oligonucleotide containing an internucleotide 5'-phosphorothioate bridge,  $\log k_{\text{obs}}$  (see text for the values),<sup>54</sup> and the stability differences,  $\Delta \log \Delta_{\text{M(AMPS)}}$  (eq 16; Table 3, column 7), which reflect the effect of the sulfur atom of a thiophosphate group on complex stabilities and also the *relative* stabilities of sulfur–metal ion interactions. Straight-line equation ( $y = mx + b$ ):  $\log k_{\text{obs}} = (0.70 \pm 0.10) \Delta \log \Delta_{\text{M(AMPS)}} - (1.90 \pm 0.13)$ ; correlation coefficient  $R = 0.98$ .

the four available data sets nicely fit on a straight line, indicating a sulfur–metal ion interaction during the rate-determining step of the transition state of the reaction for the systems with  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cd}^{2+}$ .

However,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  also enhance the mentioned cleavage reaction, though their rate enhancements (with factors of 10 and 24, respectively; see the data given above)<sup>54</sup> are much smaller than those of the divalent transition ions (with factors of  $>70$ ). The present study shows that these alkaline earth ions have no remarkable affinity toward the sulfur of a thiophosphate group, but they do have a rather pronounced binding tendency toward the oxygens in a phosphate group. Therefore, we constructed a second plot, this time of  $\log k_{\text{obs}}$  versus  $\log K_{\text{M(AMPS)}_{\text{calc}}}^{\text{M}}$  (see column 4 in Table 3). The latter values quantify the interaction toward the thiophosphate group as a whole, i.e., the metal ion affinity of  $\text{AMPS}^{2-}$  is corrected for the effect of N7 (eq 15), which gives rise to macrochelate formation (see section 3.3), but it encompasses the metal ion affinity of the sulfur *as well as* of the oxygen atoms of the thiophosphate group.<sup>55</sup> Figure 8 shows the corresponding plot for the six available metal ion systems.<sup>55</sup> A remarkable correlation is observed which suggests that on top of the sulfur–metal ion interaction during the transition state of the rate-determining step of the hydrolytic cleavage reaction also an oxygen–metal ion interaction occurs and that these two effects are “additive”. In this context and regarding ribozymes it warrants to be mentioned that the sulfur affinity of  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  is comparable (Table 4) but that  $\text{Pb}^{2+}$  has a larger affinity for O sites than  $\text{Cd}^{2+}$ .

(55) It should be pointed out that the construction of a plot with  $\log k_{\text{obs}}$  versus  $\log K_{\text{M(AMPS)}_{\text{op}}}^{\text{M}}$  (Table 3, column 5; this latter stability constant quantifies only the oxygen-binding capacity of a phosphate group) results for the six metal ion systems in a poor fit regarding a straight line (correlation coefficient  $R = 0.85$ ).



**Figure 8.** Relationship between the logarithms of the observed pseudo-first-order rate constants ( $\text{min}^{-1}$ ) for the hydrolytic cleavage of an oligonucleotide containing an internucleotide 5'-phosphorothioate bridge,  $\log k_{\text{obs}}$  (see text for the values),<sup>54</sup> and the stability constants,  $\log K_{\text{M(AMPS)}_{\text{calc}}}^{\text{M}}$  (eq 15; Table 3, column 4), corrected for the effect of a possible N7–metal ion interaction. Straight-line equation ( $y = mx + b$ ):  $\log k_{\text{obs}} = (0.74 \pm 0.13) \log K_{\text{M(AMPS)}_{\text{calc}}}^{\text{M}} - (3.32 \pm 0.30)$ ; correlation coefficient  $R = 0.95$ .

The correlations in Figures 7 and 8 appear as very satisfying when one takes the experimental errors of both data sets into account, as well as the fact that the cleavage reactions<sup>54</sup> were measured in Tris<sup>23</sup> buffer, which is known to form rather stable complexes (especially) with divalent transition metal ions<sup>56</sup> and also to inhibit the metal ion-promoted triphosphate hydrolysis.<sup>57</sup> Indeed, the use of buffers, especially of Tris, in many of the experiments regarding ribozymes is quite common, but this constitutes a serious handicap in the interpretation of such data for the effect of metal ions which are varyingly sequestered by the buffer. However, despite this caveat, both plots in Figures 7 and 8 suggest that the data points for the  $\text{Zn}^{2+}$  system are truly somewhat above the straight lines, i.e., the reaction with  $\text{Zn}^{2+}$  is “too fast”. As  $\text{Zn}^{2+}$  is the only one of the considered metal ions that can form hydroxo complexes already at pH 7.5, a property which is facilitated by sulfur coordination (see section 3.8), this observation may indicate, in accord with related suggestions (e.g., refs 22a and 57a), that an intramolecular  $\text{OH}^-$  attack plays also some role during the transition state of the  $\text{Zn}^{2+}$ -facilitated hydrolytic cleavage reaction.

**Acknowledgment.** The competent technical assistance of Mrs. Rita Baumbusch and a research grant of the Swiss National Science Foundation (H.S.) are gratefully acknowledged.

JA962970L

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