

Stability Constants of Mg-Complexes with ATP, Phosphoenolpyruvate, and Sulphate*

JENS GR. NØRBY

Fysiologisk Institut, Aarhus Universitet, DK-8000 Aarhus, Denmark

The object of this investigation was to determine stability constants for MgATP^{2-} , MgPEP , and MgSO_4 at varying ionic strengths by a spectrophotometric titration method using 8-quinolinol or 8-quinolinol-5-sulphonate as indicators for Mg^{2+} . The following results are reported:

1. A theoretical treatment of the method including a discussion of the influence of various parameters on the stability constants calculated.

2. Molar absorbance coefficients of the quinolinols and their Mg-complexes.

3. Acid dissociation constants of the quinolinols and stability constants for their Mg-complexes at 37°C and at ionic strength from 0.06 to 0.24 M (tris-buffer).

4. Apparent stability constants for MgATP^{2-} at 37°C, pH 7.4 or 8.4, ionic strength from 0.06 to 0.24 M (tris-buffer). The "true" stability constant for MgATP^{2-} was found to decrease with increasing ionic strength being approx. 35000 M^{-1} at an ionic strength equal to 0.06 M and 3500 M^{-1} at 0.24 M. The results were independent of whether 8-quinolinol or 8-quinolinol-5-sulphonate was used as indicator.

5. Mg-complexes with PEP and SO_4^{2-} could be demonstrated with certainty only at ionic strengths lower than 0.1 M. The stability constants were: MgPEP , 200 M^{-1} ; MgSO_4 , 20 M^{-1} (ionic strength 0.06–0.09 M).

The quantitative interpretation of kinetic studies on Mg-activated, ATP-hydrolyzing enzymes depends on accurate values for the stability constants of MgATP -complexes and other Mg- or ATP-complexes present. Unfortunately, there is some disagreement among various groups of workers as to the value of these constants, even if the effect of deviation in temperature and ionic strength is taken into consideration.^{1,2}

* Abbreviations: ATP, adenosine-5'-triphosphate; PEP, phosphoenolpyruvate; $[\text{Ox}^-]$, $[\text{HOx}]$, and $[\text{H}_2\text{Ox}^+]$ denote concentration of the various forms of 8-quinolinol; $[\text{Oxs}^{2-}]$, $[\text{HOxs}^-]$, and $[\text{H}_2\text{Oxs}]$ denote concentration of the various forms of 8-quinolinol-5-sulphonate; a suffix t (e.g. $[\text{Ox}]_t$) means "total"; A, absorbance (1 cm light-path); ϵ_i , molar absorbance coefficient of substance i.

In connection with studies on Mg-activated and Mg+Na+K-activated ATP-ases performed in this laboratory, an attempt to measure the MgATP stability constants under conditions corresponding to those of the kinetic studies was therefore performed. Experiments on the complexing of Mg with phosphoenolpyruvate (PEP) and SO_4^{2-} were included in the investigation, since these substances are present in the coupled enzyme assay³ involving pyruvate kinase used in some of the kinetic studies.

The stability constants were determined by a spectrophotometric technique similar to that used by Burton.⁴ This technique takes advantage of the spectral changes of 8-quinolinol and 8-quinolinol-5-sulphonate induced by formation of Mg-complexes with these metal indicators.^{5,6} The present paper is, therefore, also concerned with a characterization of these substances and their Mg-complexes.

THEORY

The spectrophotometric determination of Mg^{2+} with 8-quinolinol is based on the following theoretical considerations (for 8-quinolinol-5-sulphonate the theory is similar):

For aqueous solutions containing only 8-quinolinol and Mg we have (the notation of Näsänen *et al.*^{7,8} is used):

$$\frac{[\text{H}^+][\text{HOx}]}{[\text{H}_2\text{Ox}^+]} = K_1' \quad (1)$$

$$\frac{[\text{H}^+][\text{Ox}^-]}{[\text{HOx}]} = K_2' \quad (2)$$

$$\frac{[\text{MgOx}^+]}{[\text{Mg}^{2+}][\text{Ox}^-]} = k_1 \quad (3)$$

$$[\text{Ox}]_t = [\text{H}_2\text{Ox}^+] + [\text{HOx}] + [\text{Ox}^-] + [\text{MgOx}^+] \quad (4)$$

$$[\text{Mg}]_t = [\text{Mg}^{2+}] + [\text{MgOx}^+] \quad (5)$$

if $\text{pH} > \text{p}K_1' + 2$, which is the case in this investigation, $[\text{H}_2\text{Ox}^+]$ can be neglected in eqn. (4):

$$[\text{Ox}]_t = [\text{HOx}] + [\text{Ox}^-] + [\text{MgOx}^+] \quad (6)$$

Combination of (2) and (6) gives

$$[\text{Ox}]_t = [\text{HOx}] \left(\frac{1 + K_2'}{[\text{H}^+]} \right) + [\text{MgOx}^+] \quad (7)$$

$$[\text{HOx}] = ([\text{Ox}]_t - [\text{MgOx}^+]) / \left(\frac{1 + K_2'}{[\text{H}^+]} \right) \quad (8)$$

and combination of (2) and (3) leads to

$$\frac{[\text{MgOx}^+][\text{H}^+]}{[\text{Mg}^{2+}][\text{HOx}]} = K_2' k_1 = k_1' \quad (9)$$

which by substitution of $[\text{HOx}]$ from eqn. (8) becomes

$$\frac{[\text{MgOx}^+][\text{H}^+] + K_2'}{[\text{Mg}^{2+}][\text{Ox}]_t - [\text{MgOx}^+]} = k_1' \quad (10)$$

Finally substitution of $[\text{Mg}^{2+}]$ from eqn. (5) gives

$$\frac{[\text{MgOx}^+][\text{H}^+] + K_2'}{([\text{Mg}]_t - [\text{MgOx}^+])([\text{Ox}]_t - [\text{MgOx}^+])} = k_1' \quad (11)$$

$[\text{MgOx}^+]$ is determined from the general equation

$$A = \varepsilon_{\text{HOx}}[\text{HOx}] + \varepsilon_{\text{Ox}^-}[\text{Ox}^-] + \varepsilon_{\text{MgOx}^+}[\text{MgOx}^+] \quad (12)$$

which by combination with eqns. (2) and (6) gives

$$[\text{MgOx}^+] = (A - A_0)/(\varepsilon_{\text{MgOx}^+} - \varepsilon_{\text{start}}) = \Delta A/(\varepsilon_{\text{MgOx}^+} - \varepsilon_{\text{start}}) \quad (13)$$

(where A_0 is the absorbance of the solution without Mg and $\varepsilon_{\text{start}} = A_0/[\text{Ox}]_t$).

The molar absorbance coefficients (ε) can be obtained as outlined by Näsänen *et al.*,⁷ and so can the value for K_2' (see RESULTS). The value of k_1' is then obtained from simultaneous measurements of ΔA and $[\text{Mg}]_t$ (titration of 8-quinolinol with Mg) according to eqns. (13) and (11).

When the solution besides 8-quinolinol contains another substance, *e.g.* ATP, which complexes with Mg^{2+} , $[\text{Mg}]_t$ is given by:

$$[\text{Mg}]_t = [\text{Mg}^{2+}] + [\text{MgOx}^+] + [\text{MgATP}^{2-}] + [\text{MgATPH}^-] \quad (14)$$

and furthermore we have:

$$[\text{ATP}]_t = [\text{ATP}^{4-}] + [\text{ATPH}^{3-}] + [\text{MgATP}^{2-}] + [\text{MgATPH}^-] \quad (15)$$

Mg-titration of this solution and combination of eqns. (13), (10), (14), and (15) now permits the calculation of an apparent stability constant, K_{MA}' :

$$K_{\text{MA}}' = \frac{[\text{MgATP}']}{[\text{Mg}^{2+}] \cdot [\text{ATP}']} \quad (16)$$

where $[\text{MgATP}'] = [\text{Mg}]_t - [\text{Mg}^{2+}] - [\text{MgOx}^+]$ (*cf.* eqn. (14))

and $[\text{ATP}'] = [\text{ATP}]_t - [\text{MgATP}']$ (*cf.* eqn. (15))

The theoretical aspects concerning the formation of ATPH^{3-} and MgATPH^- relevant for the present study will be considered in the discussion of the results.

The calculations of K_{MA}' from titration data were performed on a desk computer (Olivetti Programma 101, programmed by magnetic cards).

MATERIALS AND METHODS

8-Quinolinol (Merck *p.a.*) and 8-quinolinol-5-sulphonic acid, $2\text{H}_2\text{O}$ (Merck) were dissolved in redistilled H_2O , equimolar amounts of NaOH being added in the case of the sulphonic acid. Tris-buffers were prepared from TRIZMA-base (Sigma) and HCl. Preliminary experiments at high ionic strength revealed the presence of an unidentified substance in Tris 7-9 (Sigma) which complexed with the quinolinols. This substance was not present in tris-buffers prepared from TRIZMA-base. The other reagents used were: $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (Baker or Merck *p.a.*), $(\text{NH}_4)_2\text{SO}_4$ (Merck *p.a.*), phosphoenolpyruvate, tricyclohexylammonium salt (Boehringer), and redistilled water. $\text{Na}_2\text{H}_2\text{ATP} \cdot 3\text{H}_2\text{O}$ (Boehringer) was purified and converted to tris-ATP by chromatography on a Dowex 1-X2, Cl^- , column (200-400 mesh, Bio-Rad laboratories).

The Mg-content of the reagents was measured by atomic absorption. ATP was found to contain 0.5–1.5 mole % Mg. The other reagents contained insignificant amounts of Mg.

The ionic strength was calculated from the Cl^- content of the tris-buffer and the ATP-solution, and by assuming that PEP has 3 and ATP 4 negative charges per molecule at the pH's used.^{9,2} $[\text{Cl}^-]$ was determined on a Marius "chlor-o-counter" (Bruxelles).

Magnesium titrations were performed in the following manner. Five cuvettes containing 8-quinolinol (0.33 mM) or 8-quinolinol-5-sulphonate (0.5 mM), tris-buffer to adjust pH and ionic strength and other desired additions (Mg, ATP, etc.) were placed in the thermostated cell holder of a Zeiss PMQII spectrophotometer. When temperature equilibrium ($37 \pm 0.1^\circ\text{C}$) was reached, MgCl_2 solution was added in 25 μl aliquots and changes in absorbance, ΔA , were read directly at the galvanometer. Maximally 100 μl MgCl_2 was added to each cuvette and ΔA was corrected for the volume change. At the termination of an experiment pH was measured in each cuvette at 37°C (pH-meter 26 with micro glass electrode, Radiometer, Copenhagen).

RESULTS

Characterization of 8-quinolinol, 8-quinolinol-5-sulphonic acid and their Mg-complexes. The molar absorbance coefficients (ϵ) at 360 nm (8-quinolinol) and 357 nm (8-quinolinol-5-sulphonate) for the different forms of the metal-indicators were obtained by measuring absorbance (A) as a function of pH as described by Näsänen *et al.*⁷ and so were the values for K_2' . The ϵ -values of the Mg-quinolinol complexes were determined by measurements of A for solutions containing 0.26–0.5 mM quinolinol and 16–42 mM Mg. In these solutions $[\text{MgOx}^+] \approx [\text{Ox}]_t$, $[\text{Mg}^{2+}] \approx [\text{Mg}]_t - [\text{Ox}]_t$, and $[\text{MgOx}^+]/[\text{HOx}]$ varies from 70 to 27. The A -values were subsequently corrected for the influence of small amounts of uncomplexed quinolinol, the correction being between +1 % and +4 % of the value read. All measured ϵ -values were independent of ionic strength.

The ϵ -values are shown in Table 1 together with those found by Näsänen and coworkers.^{5-7,10} The close agreement between the results is obvious. In Fig. 1, $\text{p}K_2' (= -\log K_2')$ for 8-quinolinol and 8-quinolinol-5-sulphonate is plotted as a function of ionic strength. For comparison the results of Näsänen and coworkers^{7,10,11} are also plotted.

Table 1. Molar absorbance coefficients (1 cm light path) of quinolinols and their Mg-complexes.

Substance	8-Quinolinol (360 nm)		Substance	8-Quinolinol-5-sulphonic acid (357 nm)	
	Present investigation $\epsilon \times 10^{-3}$	Ref. 5, 7 ^a $\epsilon \times 10^{-3}$		Present investigation $\epsilon \times 10^{-3}$	Ref. 6, 10 ^a $\epsilon \times 10^{-3}$
H_2Ox^+	1.72	1.7	H_2Oxs	2.67	2.7
HOx	0.15	0.13	HOxs^-	0.25	0.25
Ox^-	2.77	2.75	Oxs^{2-}	5.22	5.3
MgOx^+	2.33	2.3	MgOxs	4.3	4.3

^a Values read from published spectral curves.

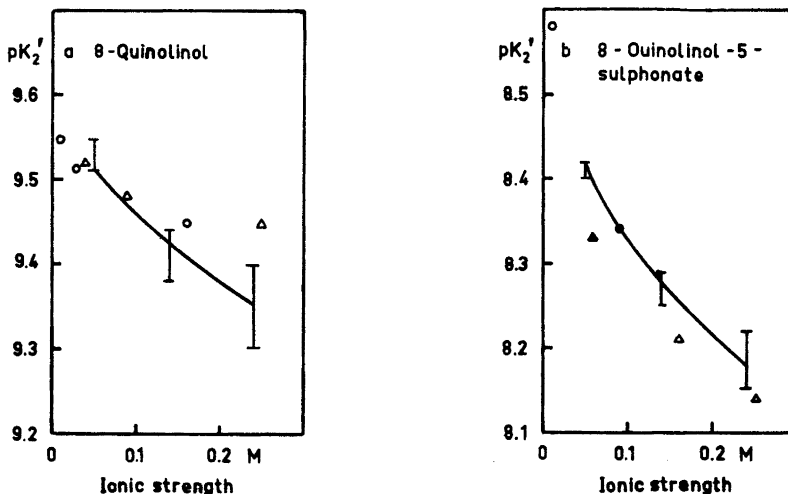


Fig. 1. pK_2' for the quinolinols as a function of ionic strength at 37°C. Vertical lines represent the range of results obtained in the present investigation and the curves show the values used in the calculation of stability constants (eqn. (10), THEORY). The other symbols indicate: Fig. 1a, 8-quinolinol, (O) Näsänen *et al.*,⁷ potentiometric; (Δ) Näsänen *et al.*,⁷ spectrophotometric. Fig. 1b, 8-quinolinol-5-sulphonate, (O) Näsänen *et al.*;¹⁰ (Δ) Näsänen *et al.*¹¹ The values of Näsänen and coworkers were all corrected to 37°C.

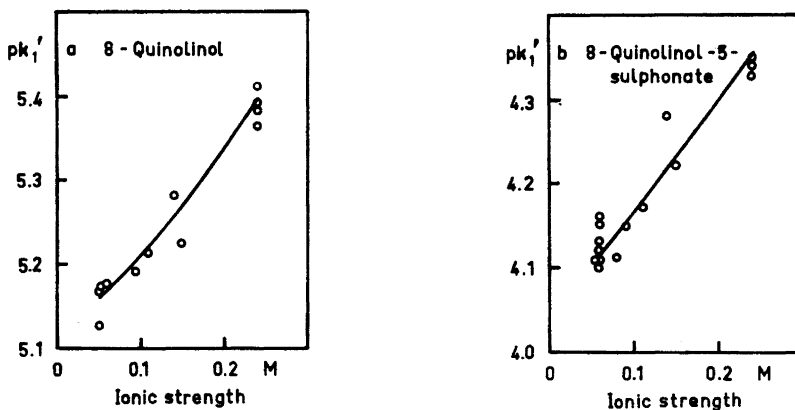


Fig. 2. The influence of ionic strength on pK_1' (eqn. (10), THEORY) for the Mg-complexes of 8-quinolinol (Fig. 2a) and 8-quinolinol-5-sulphonate (Fig. 2b) at 37°C. The values primarily chosen for further calculations are represented by the two curves.

On the basis of the ϵ_{MgOx^+} and ϵ_{MgOx} values in Table 1 and the pK_2' 's chosen from Fig. 1, k_1' was now determined by titration of the quinolinols with Mg as mentioned in the theory section. Fig. 2 shows pK_1' as a function

of ionic strength (typical titration curves are given in Fig. 3). Näsänen *et al.*^{5,11} determined pk_1' -values for Mg-complexes with 8-quinolinol and 8-quinolinol-5-sulphonate at 20°C and 25°C, respectively, whereas the experiments reported here were performed at 37°C. As no information concerning the influence of temperature on pk_1' has been published a direct comparison of the two sets of results is not possible in this case. The pk_1' -values published here are, however, of the same magnitude as those given by Näsänen *et al.*^{5,11}

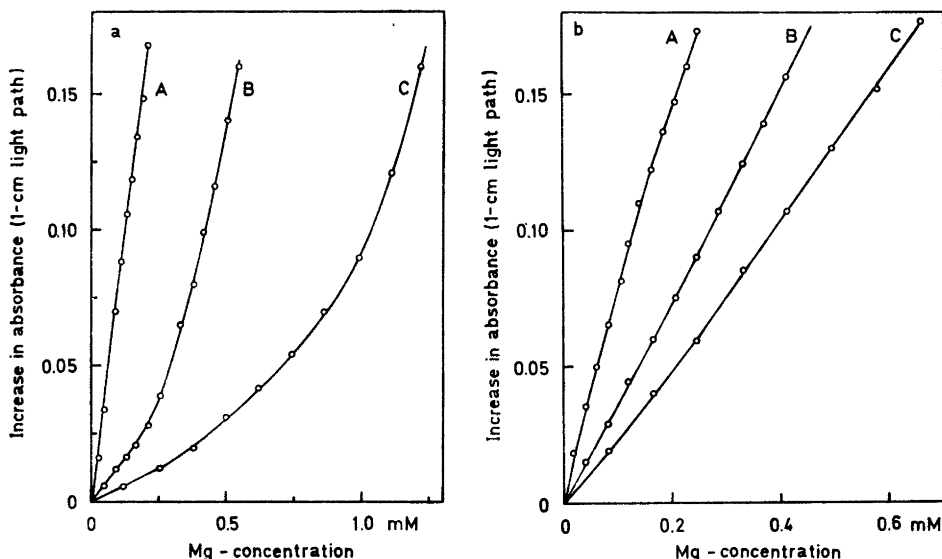


Fig. 3 a. Mg-titration curves at 37°C, ionic strength=0.058 M.

A: 0.33 mM 8-quinolinol, pH 8.37

B: 0.33 mM 8-quinolinol + 0.40 mM ATP, pH 8.34

C: 0.33 mM 8-quinolinol + 1.20 mM ATP, pH 8.32.

b. Mg-titration curves at 37°C, ionic strength=0.24 M.

A: 0.40 mM 8-quinolinol-5-sulphonate, pH 7.41

B: 0.50 mM 8-quinolinol-5-sulphonate + 0.51 mM ATP, pH 7.41

C: 0.50 mM 8-quinolinol-5-sulphonate + 1.02 mM ATP, pH 7.41.

Determination of the apparent stability constant of MgATP. Mg-titrations of solutions containing quinolinol and ATP were carried out at ionic strength from 58 mM to 240 mM, the concentration of ATP being varied from 0.4 mM to 2.9 mM. Titrations were performed with both 8-quinolinol at pH 8.3–8.5 and 8-quinolinol-5-sulphonate at pH 7.4–7.5, the temperature being 37°C in all experiments. Four typical titration curves are shown in Fig. 3. Each curve provided a number of corresponding values of ΔA and $[Mg]_t$ from which the apparent stability constant, K_{MA}' , was calculated (see THEORY). Table 2 gives a survey of the results obtained.

It appears from Table 2 that the constants determined by the two indicators at a given ionic strength are of the same magnitude, but whereas K_{MA}' deter-

Table 2. The apparent stability constant, K_{MA}' , of MgATP.

Ionic strength M	[ATP] mM	n^a	[MgATP']/[ATP'] range	$K_{MA}' (M^{-1})$ $\times 10^{-3}$ range	K_{MA}' as a function of increasing [MgATP']/[ATP']
8-Quinolinol, pH 8.3–8.5					
0.058	0.4	2	0.2–4	31–36	constant
	0.8	1	0.2–3	32–39	slight increase
	1.2	1	0.2–5	25–36	slight increase
0.085	0.5	2	0.2–3	20–23	slight increase, constant
0.100	0.8	1	0.2–4	18–20.5	constant
	0.150	2	0.1–1.5	8–9.5	constant
0.240	1.0	1	0.1–1.5	8.5–9.2	constant
	0.5	1	0.1–1	3.2–3.4	constant
	1.0	1	0.1–1.5	3.0–3.3	constant
8-Quinolinol-5-sulphonate, pH 7.4–7.5 ^b					
0.058	0.5	2	0.2–3	30–38	increase, constant
	0.8	3	0.2–4	30–38	increase, constant
	1.4	2	0.2–5	30–38	increase, constant
0.065	0.8	2	0.2–4	28–34	slight increase, constant
	2.9	1	0.2–4	24–38	increase
0.085	0.5	2	0.2–3	19–25	increase
0.100	0.8	4	0.2–3	16–22	increase
0.150	0.5	2	0.2–2	8.5–12	increase, constant
0.240	0.5	1	0.1–1	3.2–4.0	increase
	1.0	1	0.1–1.5	3.2–3.9	increase

^a Number of experiments.^b These K_{MA}' -values are probably too high, see DISCUSSION.

Table 3. The stability constant of Mg-phosphoenolpyruvate.

Ionic strength M	[PEP] mM	n^a	$K_{MgPEP^-} (M^{-1})$ range	K_{MgPEP^-} as function of increasing $[Mg^{2+}]$
8-Quinolinol, pH 8.3–8.5				
0.058	3.2	2	160–250	slight increase
0.085	1.1	1	160–250	slight increase
0.150	1.1	1	negative–60	increase
8-Quinolinol-5-sulphonate, pH 7.4–7.5				
0.058	3.2	1	170–250	slight increase
0.085	1.1	1	100–280	increase
	3.2	1	120–200	slight increase
0.150	1.1	1	70–150	slight increase
	3.2	1	50–100	slight increase
0.240	2.1	1	50–80	constant

^a Number of experiments.

mined with 8-quinolinol in most cases is practically independent of the calculated ratio $[\text{MgATP}']/[\text{ATP}']$, it shows a general tendency to increase with increasing values of this ratio in the experiments where 8-quinolinol-5-sulphonate was used (Table 2). The possible explanations for this, and the expected lowering effect of decreasing pH on the apparent K_{MA}' (due to formation of ATPH^{3-}) will be discussed later in this paper.

Determination of stability constants for MgPEP and MgSO₄. Titrations with Mg of solutions containing quinolinol and PEP (1.1–3.2 mM) or $(\text{NH}_4)_2\text{SO}_4$ (7.5–14.7 mM) gave curves which were very similar, but in most cases lower (ΔA smaller for a given $[\text{Mg}]_t$), than the curves for pure quinolinol solutions shown in Fig. 3. The stability constants calculated from these titrations are given in Tables 3 and 4.

Table 4. The stability constant of MgSO_4 .

Ionic strength M	$[\text{SO}_4^{2-}]$ mM	n^a	K_{MgSO_4} (M^{-1}) range	K_{MgSO_4} as a function of increasing $[\text{Mg}^{2+}]$
8-Quinolinol, pH 8.3–8.5				
0.095	14.7	2	8–18	slight increase
0.150	14.7	1	negative–13	increase
8-Quinolinol-5-sulphonate, pH 7.4–7.5				
0.085	7.5	1	20–35	slight increase
	14.7	2	6–24	increase
0.100	14.7	3	negative–23	increase, slight increase
0.150	14.7	1	9–13	constant

^a Number of experiments.

DISCUSSION

The apparent stability constant of MgATP. It is seen from Table 2 that the values for K_{MA}' obtained with 8-quinolinol-5-sulphonate tend to increase with increasing $[\text{MgATP}']/[\text{ATP}']$ ratio whereas those determined with 8-quinolinol are to a much smaller extent dependent upon this ratio. A theoretical analysis of the equations used in the calculation of K_{MA}' (eqns. (10), (13), (14), (15), and (16), THEORY section) reveals that this can be due to the use of incorrect values for one or more of the many parameters involved. Since the increasing trend seems to be specific for one of the indicators, systematic errors in $[\text{ATP}]_t$, $[\text{Mg}]_t$, and probably also pH and ΔA may be ruled out. Furthermore, the values for ϵ_{MgOxs} used in the present work agree with those published elsewhere (Table 1). K_2' is only about 10 % of $[\text{H}^+]$ (eqn. (10)), which means that rather large deviations in K_2' have only a small influence on the calculated K_{MA}' . One possible cause of the increase in K_{MA}' with $[\text{MgATP}']/[\text{ATP}']$ in the experiments with 8-quinolinol-5-sulphonate is that the values of k_1' (eqn. (10)) chosen from the results reported in Fig. 2b are

too high (equivalent to pk_1' being chosen too low). It is seen from eqn. (10) (THEORY) that $[Mg^{2+}]$ for a constant $[MgOx^+]$ (equal to a given ΔA) is inversely proportional to k_1' . Thus if k_1' is decreased, K_{MA}' is also decreased, and calculations will furthermore disclose that the decrease is higher the higher the $[MgATP']/[ATP']$ ratio. A 10 % decrease in k_1' (corresponding to an increase in pk_1' of 0.04–0.05 units, cf. Fig. 2b) will tend to make K_{MA}' independent of $[MgATP']/[ATP']$ in the experiments with 8-quinolinol-5-sulphonate and the constants determined with this indicator are now 10–20 % lower than those found with 8-quinolinol at the same ionic strength.

Since the apparent stability constant of MgATP in the present paper is determined both at pH 8.3–8.5 and at pH 7.4–7.5 (cf. Table 2) it is necessary to take into consideration the formation of $ATPH^{3-}$ and $MgATPH^-$ when comparing the K_{MA}' values obtained with the two indicators. In this context the following equations are relevant:

$$\begin{aligned} \frac{[ATP^{4-}][H^+]}{[ATPH^{3-}]} &= K_4 & \frac{[MgATPH^-]}{[Mg^{2+}][ATPH^{3-}]} &= K_{MAH} \\ \frac{[MgATP^{2-}][H^+]}{[MgATPH^-]} &= K_{4M} & \frac{[MgATP^{2-}]}{[Mg^{2+}][ATP^{4-}]} &= K_{MA} \\ [Mg]_t &= [Mg^{2+}] + [MgOx^+] + [MgATP^{2-}] + [MgATPH^-] \\ [ATP]_t &= [ATP^{4-}] + [ATPH^{3-}] + [MgATP^{2-}] + [MgATPH^-] \end{aligned}$$

Analysis of these equations and the program used in the calculation of stability constants from Mg-titration data (see THEORY section) shows that the apparent stability constant, K_{MA}' , is pH-dependent:

$$K_{MA}' = K_{MA} \frac{1 + ([H^+]/K_{4M})}{1 + ([H^+]/K_4)}$$

According to Phillips *et al.*,² K_{4M} is about 6.5×10^{-6} M which means that $[H^+]/K_{4M}$ in the pH region 7.4–8.5 is smaller than 0.5×10^{-2} . This reduces the expression for K_{MA}' to

$$K_{MA}' = K_{MA} \frac{K_4}{K_4 + [H^+]} \quad (17)$$

Phillips, George and Rutman¹² have determined pK_4 at 37°C as a function of ionic strength and found the following relation: $pK_4 = 7.73 - 3.64\sqrt{I} + 4.25I$. This equation together with eqn. (17) permitted the calculation of K_{MA}'/K_{MA} for varying values of pH and ionic strength. The results (Table 5) indicate that the apparent stability constant determined at pH 8.4 (8-quinolinol) theoretically should be close to the true stability constant K_{MA} for $MgATP^{2-}$, whereas that determined at pH 7.4–7.5 (8-quinolinol-5-sulphonate) would be expected to be about 80 % of K_{MA}' at pH 8.4.

The conclusion of this part of discussion must be that the values for k_1' applied in the calculation of K_{MA}' at pH 7.4–7.5 (8-quinolinol-5-sulphonate) given in Table 2 are probably too high. A lowering of k_1' by approx. 10 % will make K_{MA}' independent of the $[MgATP']/[ATP']$ ratio, and furthermore,

Table 5. The effect of ionic strength (I) and pH on the ratio between the apparent stability constant (K_{MA}') and the true stability constant (K_{MA}) of MgATP²⁻.

I M	pH=7.4	K_{MA}'/K_{MA} pH=7.5	pH=8.4	K_{MA}' (pH 7.5) K_{MA}' (pH 8.4)
0.058	0.68	0.74	0.95	0.78
0.085	0.70	0.76	0.96	0.79
0.150	0.74	0.79	0.96	0.82
0.240	0.74	0.79	0.96	0.82

it will bring the ratio (K_{MA}' , pH 7.5)/(K_{MA}' , pH 8.4) close to the theoretical value given in Table 5. It should finally be pointed out that the complex [MgATPH⁻] amounts to less than 1 % of [MgATP²⁻] at the pH's used in the present investigation since pK_{4M} is about 5.3.²

A comparison between the stability constants for MgATP²⁻ reported here and those published by other workers is complicated by the dissimilarities in experimental conditions. The studies of Burton⁴ and the thorough investigation of Phillips *et al.*² show in agreement with the present paper that the stability constant decreases with increasing ionic strength. The ionic strength effect is, however, in some cases difficult to evaluate since the supporting electrolytes may complex with ATP⁻⁴ or Mg²⁺. The influence of the supporting electrolytes is probably responsible for part of the deviation among the reported values and may also be the reason why the results of the present paper are in the lower range of those mentioned in the literature. Although there is no information on the formation of a tris-ATP complex, the study of O'Sullivan and Perrin¹³ indicates that the stability constant was four times lower in tris-buffer than in triethanolamine-buffer.

The stability constants of MgPEP⁻ and MgSO₄. The results given in Tables 3 and 4 clearly demonstrate the limited accuracy obtained in the determination of relatively small stability constants by the present method. The lowering of k_1' for 8-quinolinol-5-sulphonate mentioned in the discussion of the MgATP²⁻-complex leads to negative values for K_{MgPEP^-} and K_{MgSO_4} at ionic strength higher than 0.1 M and it must be concluded that complexes of Mg²⁺ with PEP³⁻ and SO₄²⁻ could only be demonstrated at ionic strengths below this value.

For MgPEP⁻ the stability constant at low ionic strength is about 200 M⁻¹ which is in good agreement with the value of 180 M⁻¹ determined by Wold and Ballou.⁹ No information seems to be available in the literature as to the stability constant for MgSO₄ at conditions similar to those in the present study. Dunsmore and James¹⁴ found K_{MgSO_4} to be about 50 M⁻¹ at 25°C in 0.5 M glycine, whereas Kenttämää,¹⁵ who investigated the ionic association in saturated aqueous solutions at about 0°C, obtained values of 36 M⁻¹ ($I=0.05$ M) to 12 M⁻¹ ($I=0.25$ M) for the stability constant. The results of the present study, although rather inaccurate, are of the same order of magnitude.

As mentioned in the introduction to this paper, a coupled enzyme assay involving pyruvate kinase was used in studies on the ATP-ase reaction. In

this assay PEP and SO_4^{2-} are both present. The quantitative importance of the MgPEP and MgSO_4 complex formation for the interpretation of such studies will be discussed in forthcoming papers³ dealing with the above mentioned assay and the kinetics of the ATP-ase reaction.

Acknowledgements. I thank Mrs. A. Sand and Ing. T. Nørby for excellent technical assistance and help in preparing the computer programme, respectively. I am also indebted to Dr. J. Jensen and Professor J. C. Skou for helpful discussions.

REFERENCES

1. Bock, R. M. In Boyer, P. D., Lardy, H. and Myrbäck, K. *The Enzymes*, Academic, New York 1957, Vol. 2 A, p. 10.
2. Phillips, R. C., George, P. and Rutman, R. J. *J. Am. Chem. Soc.* **88** (1966) 2631.
3. Nørby, J. G. *To be published.*
4. Burton, K. *Biochem. J.* **71** (1959) 388.
5. Näsänen, R. *Acta Chem. Scand.* **6** (1952) 352.
6. Näsänen, R. and Uusitalo, E. *Acta Chem. Scand.* **8** (1954) 835.
7. Näsänen, R., Lumme, P. and Mukula, A.-L. *Acta Chem. Scand.* **5** (1951) 1199.
8. Näsänen, R. *Acta Chem. Scand.* **5** (1951) 1293.
9. Wold, F. and Ballou, C. E. *J. Biol. Chem.* **227** (1957) 301.
10. Näsänen, R. and Ekman, A. *Acta Chem. Scand.* **6** (1952) 1384.
11. Näsänen, R. and Uusitalo, E. *Acta Chem. Scand.* **8** (1954) 112.
12. Phillips, R. C., George, P. and Rutman, R. J. *Biochemistry* **2** (1963) 501.
13. O'Sullivan, W. J. and Perrin, D. D. *Biochemistry* **3** (1964) 18.
14. Dunsmore, H. S. and James, J. C. *J. Chem. Soc.* **1951** 2925.
15. Kenttämää, J. *Acta Chem. Scand.* **12** (1958) 1323.

Received April 7, 1970.