Implications of adenylate kinase-governed equilibrium of adenylates on contents of free magnesium in plant cells and compartments

Abir U. IGAMBERDIEV[1] and Leszek A. KLECZKOWSKI[2]
Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, 901-87 Umeå, Sweden

On the basis of the equilibrium of adenylate kinase (AK; EC 2.7.4.3), which interconverts MgATP and free AMP with MgADP and free ADP, an approach has been worked out to calculate concentrations of free magnesium (Mg^{2+}), based on concentrations of total ATP, ADP and AMP in plant tissues and in individual subcellular compartments. Based on reported total adenylate contents, [Mg^{2+}] in plant tissues and organelles varies significantly depending on light and dark regimes, plant age and developmental stage. In steady-state conditions, [Mg^{2+}] in chloroplasts is similar in light and darkness (in the millimolar range), whereas in the cytosol it is very low in the light and increases to about 0.4 mM in darkness. During the dark-to-light transition (photosynthetic induction), the [Mg^{2+}] in chloroplasts falls to low values (0.2 mM or less), corresponding to a delay in photosynthetic oxygen evolution. This delay is considered to result from lower activities of Mg-dependent enzymes in the Calvin cycle. In mitochondria, the changes in [Mg^{2+}] are similar but smoother. On the other hand, when the transition from light to darkness is considered, an initial increase in [Mg^{2+}] occurs in both chloroplasts and mitochondria, which may be of importance for the control of key regulatory enzymes (e.g. mitochondrial malic enzyme and pyruvate dehydrogenase complex) and for processes connected with light-enhanced dark respiration. A rationale is presented for a possible role of [MgATP]/[MgADP] ratio (rather than [ATP_{total}]/[ADP_{total}]) as an important component of metabolic cellular control. It is postulated that assays of total adenylates may provide an accurate measure of [Mg^{2+}] in plant tissues/cells and subcellular compartments, given that the adenylates are equilibrated by AK.

Key words: adenylate energy charge, ATP/ADP ratio, cell energetics, photosynthesis, respiration.

INTRODUCTION

Magnesium is one of the most important cations in living systems. Its total concentration in plant tissues is in the order of tens of mM (with most metabolically inactive magnesium located in vacuole) [1,2]. However, a significant amount of magnesium is bound to different metabolites, especially nucleoside tri- and di-phosphates, and the free magnesium (Mg^{2+}) concentration can vary significantly. Nucleoside triphosphates bind magnesium very tightly, and their actions as cofactors and energy-rich compounds are realized in complexes with Mg. Nucleoside diphosphates bind Mg^{2+} less firmly, and the enzymes that use MgADP are usually strongly inhibited by free ADP [3]. The uncomplexed forms of both ADP and ATP may cause appreciable inhibition of the kinase-type phosphotransferase enzyme systems [3,4]. Nucleoside monophosphates, particularly AMP that allosterically regulates many key enzymes, bind Mg^{2+} very weakly [5], and they participate in metabolism usually as magnesium-free forms [3].

Attempts to estimate [Mg^{2+}] in tissues/cells and subcellular compartments have proved difficult, especially in plant tissues where the presence of cell walls requires careful and frequently time-consuming cell-fractionation procedures. In some cases, ionophores binding Mg^{2+} were used for such a purpose, but they could be provided only after isolation of organelles and could also displace the equilibrium between free and complexed cations [2,6]. Other methods, e.g. NMR, also proved unreliable due mainly to interference by other cations [7,8].

It has been demonstrated that, in erythrocytes, contents of Mg^{2+} can be accurately estimated from contents of total adenylates (ATP, ADP, AMP) provided that the adenylates are equilibrated *in vivo* via adenylate kinase (AK; ATP:AMP phosphotransferase, EC 2.7.4.3) reaction [9]. This was possible because the apparent equilibrium constant (K_{app}) of AK, based on concentrations of total adenylates, varies significantly depending on [Mg^{2+}] (see below). The reaction of AK is freely reversible and can be generally presented as $ATP + AMP \leftrightarrow 2ADP$. The enzyme utilizes Mg-complexed ATP and free AMP (reverse reaction) and one Mg-complexed and one free ADP (forward reaction) as true substrates. This has been demonstrated, for example, for AKs from yeast [10], animal tissues [9,11] and plants [12]. Maize chloroplastic AK was also demonstrated to have considerable activity towards other non-adenylate nucleoside phosphates (e.g. deoxyadenylates and cytidylates), suggesting that it may equilibrate the total pool of nucleoside phosphates in chloroplasts [13,14]. Plant tissues may contain several isoenzymes of AK, e.g. six isoenzymes have been found in tobacco cells [15]. Chloroplasts usually contain the bulk of total AK activity (40–90 % in different species), which corresponds to up to three chloroplastic isoforms of AK, including one membrane-bound isoform [16]. In addition to chloroplastic isoenzyme(s), one AK isoenzyme has been found in mitochondria (intermembrane space) [17,18] and one or two in the cytosolic/nuclear fraction [15]. The intermembrane AK most probably equilibrates adenylates in the cytosol, given the general permeability of the outer membrane to metabolites [19], but can also be involved (to some extent), together with adenine nucleotide translocator, in regulating matrix adenylates [17,18,20]. Its specific activity in mitochondria exceeds that of ATP synthase by 2–4 times [18]. In plant mitochondrial matrix, contrary to animals, AK is probably absent. In animals, AK (as GTP:AMP phosphotransferase) participates in the Krebs (tricarboxylic acid) cycle,

Abbreviations used: AK, adenylate kinase; ICDH, isocitrate dehydrogenase; PDC, pyruvate dehydrogenase complex; PEP, phosphoenolpyruvate.

[1] Present address: Risø National Laboratory, Plant Research Department, 4000 Roskilde, Denmark.
[2] To whom correspondence should be addressed (e-mail leszek.kleczkowski@plantphys.umu.se).

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whereas in plants succinyl-CoA synthetase forms ATP not GTP, so AK is not necessary.

In the present paper, we derived an equation allowing estimation of [Mg^{2+}] from levels of total adenylates, and then analysed literature data on the tissue and subcellular distribution of adenylates in plants to estimate corresponding cellular and subcellular [Mg^{2+}]. We show that [Mg^{2+}] in chloroplasts varies significantly during light–dark transitions, smaller variations are observed in mitochondria, whereas the cytosolic [Mg^{2+}] is low, particularly in the light. We connect these changes with activation/inactivation patterns of key enzymes of the Calvin cycle, oxidative penthose phosphate pathway and the tricarboxylic acid cycle, and compare them with current knowledge on [Mg^{2+}] in plant compartments. A rationale for estimation of [Mg^{2+}] in plant tissues/compartments is underlined based on equilibrium between total adenylate levels.

ADENYLATE KINASE EQUILIBRIUM AND ITS APPLICATION FOR ESTIMATING [Mg^{2+}]

Relationship between K_{true} and K_{app} of AK

Several reports have noted the dependency of an apparent equilibrium constant of AK on [Mg^{2+}] [3,9,11,12], reflecting the use of defined Mg-bound and free adenylate species by the enzyme. In Figure 1, the relationship between [Mg^{2+}] and equilibrium constants of AK is presented, using experimental points obtained for rabbit muscle AK [11] and plant AK [12]. Equilibrium concentrations of Mg-bound and free adenylates were calculated using stability constants as in [21] (see below). The apparent equilibrium constant (K_{app}), defined as $[ATP_{total}][AMP_{total}]/[ADP_{total}]^2$, strongly depended on [$Mg^{2+}$], resulting in a bell-shaped curve. On the other hand, the true equilibrium constant (K_{true}), set as $[MgATP][AMP_{free}]/([ADP_{free}][MgADP])$, was constant at 5.6 ± 0.7, regardless of [Mg^{2+}]. For both muscle and leaf AK, the experimental points nearly overlapped; when calculations were done separately for the animal and plant AKs, the K_{true} values were 5.3 ± 0.6 and 5.9 ± 0.3, respectively. K_{app} had an optimum of 1.5, corresponding to an [Mg^{2+}] of approx. 0.2 mM, whereas any other value of K_{app} corresponded to two values of [Mg^{2+}] (Figure 1).

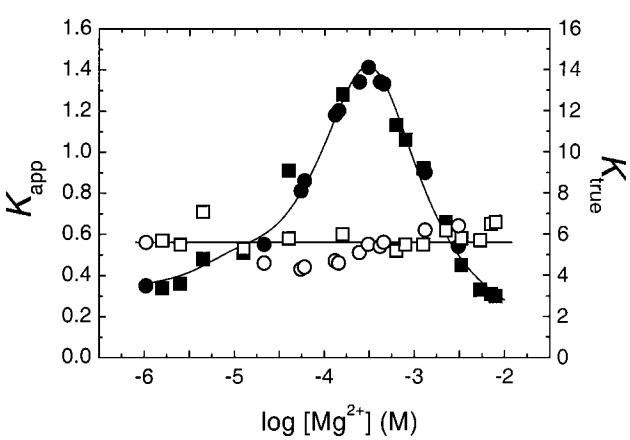

Figure 1 The dependence of K_{app} of AK on [Mg^{2+}] based on experimental data for AK from rabbit muscle [11] (●, ○) and maize leaves [12] (■, □)

The K_{app} and K_{true} values are shown by filled (●, ■) and open (○, □) symbols, respectively. The K_{true} values were calculated from eqn (2) and the [Mg^{2+}] estimations according to [21]. The mean K_{true} value ($\pm$S.D.) was 5.6 ± 0.7. Please note that, whereas the points refer to experimental data, the bell-shaped curve is theoretical and was drawn according to eqn (4).

Derivation of the equation linking K_{app} and K_{true} of AK with [Mg^{2+}]

For practical utilization of the information provided in Figure 1, we have derived an equation where known contents of total adenylates were linked to [Mg^{2+}] under conditions governed by AK equilibrium. To do so, we needed to define the relationship between K_{app} and [Mg^{2+}] for the AK reaction. For the calculations we used the following abbreviations: $K_{app} = K$; $K_{true} = C$; t = MgATP stability constant (K_{MgATP}); d = MgADP stability constant (K_{MgADP}); m = MgAMP stability constant (K_{MgAMP}); x = [Mg^{2+}]. We skipped the stability constants for minor species, such as $MgHATP^-$ or MgHADP, which are low compared with stability constants for the main species [22]. Also, monoprotonated species can make a significant contribution only in the pH range 4.5–6.0 [21], which is below pH values reported for plant compartments that are considered in the present study (chloroplasts, mitochondria and cytosol).

The stability constants define equilibrium between Mg and adenylates, e.g. for K_{MgATP} this equilibrium is described by the equation $ATP^{4-} + Mg^{2+} \leftrightarrow MgATP^{2-}$. Thus $K_{MgATP} = [MgATP^{2-}]/[Mg^{2+}][ATP^{4-}]$ or $t = [MgATP]/(x[ATP_{free}])$. Similarly, $d = [MgADP]/(x[ADP_{free}])$ and $m = [MgAMP]/(x[AMP_{free}])$. We have linked K_{true} (C) to free adenylate concentrations, K_{MgATP} (t) and K_{MgADP} (d) via eqn (1):

$$C = [MgATP][AMP_{free}]/([MgADP][ADP_{free}]) = t[ATP_{free}][AMP_{free}]/(d[ADP_{free}]^2) \quad (1)$$

We have further defined K_{app} (K) as a function of free adenylate content and [Mg^{2+}]:

$$\begin{aligned} K &= ([ATP_{free}] + [MgATP])([AMP_{free}] + [MgAMP])/([ADP_{free}] + [MgADP])^2 \\ &= ([ATP_{free}] + tx[ATP_{free}])([AMP_{free}] + mx[AMP_{free}])/([ADP_{free}] + dx[ADP_{free}])^2 \\ &= ([ATP_{free}][AMP_{free}]/[ADP_{free}]^2)(1+tx)(1+mx)/(1+dx)^2 \\ &= C(d/t)(1+tx)(1+mx)/(1+dx)^2 \end{aligned}$$

Without abbreviations this equation can be simplified to eqn (2):

$$K_{app} = K_{true}K_{MgADP}(1 + K_{MgATP}[Mg^{2+}])(1 + K_{MgAMP}[Mg^{2+}])/\{K_{MgATP}(1 + K_{MgADP}[Mg^{2+}])^2\} \quad (2)$$

Eqn (2) defines K_{app} as a function of [Mg^{2+}]. Taking the stability-constant values as recommended by O'Sullivan and Smithers [21] ($K_{MgATP} = 73\ mM^{-1}$, $K_{MgADP} = 4\ mM^{-1}$ and $K_{MgAMP} = 0.07\ mM^{-1}$), the maximum value of K_{app} corresponds to an [Mg^{2+}] of 0.23 mM. From eqn (2), concentration of the Mg^{2+} (x) can be determined as shown in eqn (3):

$$x = \frac{Cd(t+m) - 2Ktd \pm [t^2d^2(2K-C)^2 - 4td(Kd-Cm)(Kt-Cd)]^{1/2}}{2td(Kd-Cm)} \quad (3)$$

Substituting the numerical values for the constants (see above), eqn (4) was obtained:

$$[Mg^{2+}] = [0.7 - 0.25K_{app} \pm 0.57(1.5 - K_{app})^{1/2}]/(K_{app} - 0.1) \quad (4)$$

Graphical presentation of this equation results in a bell-shaped curve, where [Mg^{2+}] has a single value of about 0.2 mM at a maximum K_{app} of approx. 1.5 (Figure 1). Otherwise, for a given K_{app} value, [Mg^{2+}] has two values: one below and one above the 0.2 mM optimum. In plants, contrary to most animal tissues [9], the [Mg^{2+}] is mostly in the millimolar range, particularly in leaves [2]. Thus given that K_{app} in all cases but one (when it equals 1.5) corresponds to two [Mg^{2+}] values, a given calculated K_{app} would

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most likely correspond to an $[Mg^{2+}]$ higher than 0.2 mM (see Figure 1). On the other hand, any experimentally obtained values of K_{app} higher than 1.5 can be assumed to result from an error in total adenylate content estimations or indicate the absence of AK equilibrium in the studied tissue.

The values of stability constants may depend on pH and other factors, which could theoretically affect the K_{true} and K_{app} values and thus bear effects on estimates of $[Mg^{2+}]$. However, in accordance with numerous investigations [5,9,22] the following assumptions could be made: the values of K_{MgATP}, K_{MgADP} and K_{MgAMP} do not depend significantly on pH, temperature and other parameters. Equations and curves showing the dependence on pH and on the concentration of potassium ions revealed a very low effect of these parameters [9,11]. The effect of pH was pronounced only below pH 7 [21], but in plant cell compartments only vacuole and some intermembrane spaces exhibit acidic pH values. To test the extent to which changes in stability constants and K_{true} may affect the accuracy of $[Mg^{2+}]$ determinations, we modulated their values and substituted them into eqn (3) (Figure 2). Differences in K_{true} led only to modulations in the height of the curve. Thus at $[Mg^{2+}]$ values higher and lower than the critical point there were insignificant changes that did not alter the whole picture (Figure 2A). Differences in K_{MgATP} led to almost no changes at $[Mg^{2+}]$ values higher than the critical point (Figure 2B), whereas changes in K_{MgADP} resulted in the most significant shifts of the critical point and in wide variation in the estimation of $[Mg^{2+}]$ (Figure 2C). However, in different estimations [5,21,22] this latter constant varies rather insignificantly (between 3.2 and 4.3 mM^{-1}) and thus the possible errors are not as dramatic. Differences in K_{MgAMP} also led to some changes in the shape of the curve (Figure 2D), but they were less significant than those for K_{MgADP}.

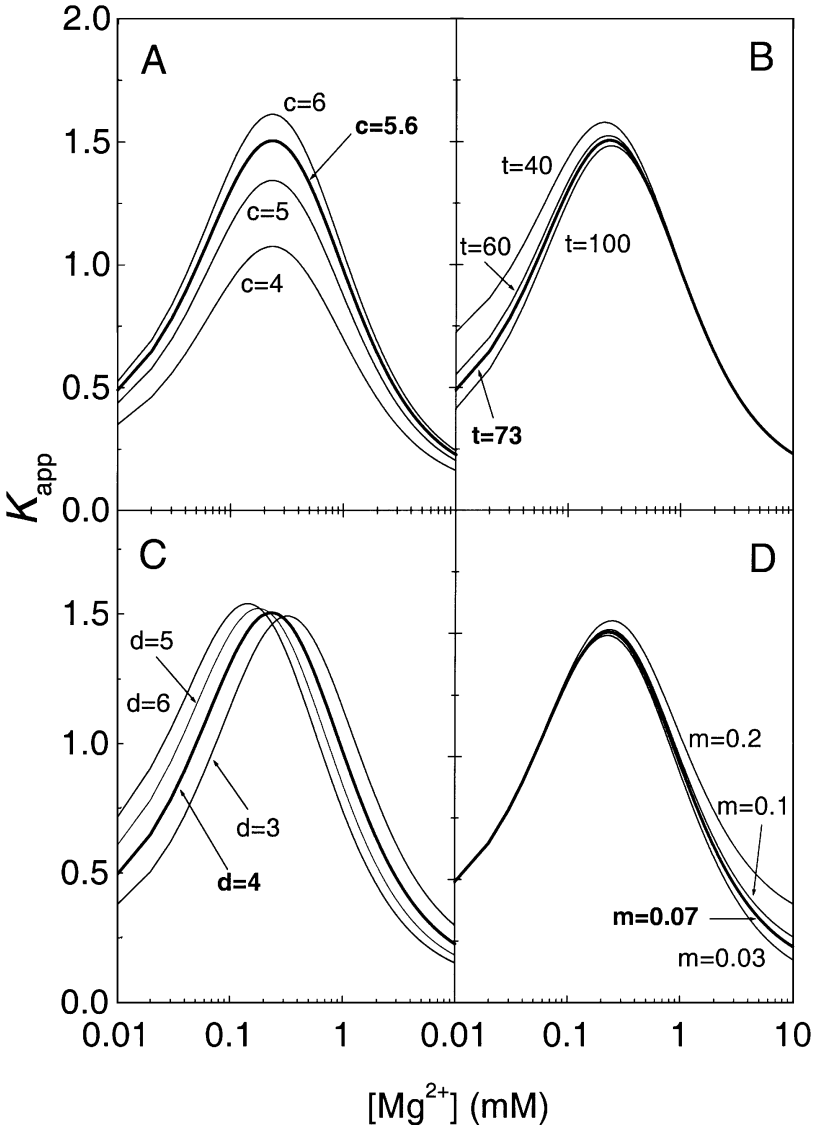

Figure 2 Effects of variations of (A) K_{true}, (B) K_{MgATP}, (C) K_{MgADP} and (D) K_{MgAMP} on the AK-dependent relationship between K_{app} and $[Mg^{2+}]$

In each panel, the curve that is based on the parameters taken for calculations of $[Mg^{2+}]$ in Figure 1 is shown in bold. $c = K_{true}$, $t = K_{MgATP}$, $d = K_{MgADP}$ and $m = K_{MgAMP}$; values for t, d and m are given in mM^{-1}.

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ESTIMATION OF [Mg^{2+}] IN WHOLE TISSUES

We used eqn (4) to re-evaluate literature reports where contents of total ATP, ADP and AMP were determined in order to estimate [Mg^{2+}]. Based on data for adenylate distribution in bean leaves [23], it can be inferred that both dark conditions and salt stress decreased total [Mg^{2+}], especially in young leaves. Similarly, young pepper leaves had lower [Mg^{2+}] than old leaves (3 and 6 mM, respectively), whereas salt stress decreased [Mg^{2+}] in sunflower buds (from 10 to 4 mM) [24]. Generally, total adenylate pools in leaves, when expressed as K_{app} of AK, fall within the expected 1.5 limit (Figure 1), suggesting that the bulk of leaf adenylates is indeed equilibrated via the AK reaction. A similar situation apparently exists in soya bean nodules (comprised of bacteroid and plant compartments) where active nitrogen fixation via the nitrogenase complex needs a high energy supply. Estimations of adenylate levels by different authors indicate that in bacteroids the [Mg^{2+}] is significantly higher than in the plant compartment. The data of Tajima and Kouzai [25] correspond to [Mg^{2+}] values of 1.0–1.6 mM and 0.2–0.3 mM in bacteroids and nodules, respectively. Nitrogenase activity depends on MgATP as a substrate, whereas MgADP is a potent inhibitor, having a K_i value that is lower than the K_m value for MgATP [26]. Thus nitrogenase activity may be under the control of the [MgATP]/[MgADP] ratio. In contrast with actively metabolizing tissues, such as leaves and nodules, adenylate levels in roots are less strongly linked to AK equilibrium. For instance, in bean roots, the calculated K_{app} values were 3.5 and 34 for plants grown under normal and phosphate-deficiency conditions, respectively [27], suggesting that AK does not equilibrate the total adenylate pool in this tissue, at least in phosphate-deficient plants.

[Mg^{2+}] IN SUBCELLULAR COMPARTMENTS OF PLANT LEAF

Chloroplasts

Surprisingly, there have been only few studies where concentrations of all three adenylate species (ATP, ADP and AMP) in a given cell compartment were determined [28–30]. As shown in Figure 3, we applied eqn (4) to estimate subcellular [Mg^{2+}] in the chloroplast stroma from leaf protoplasts of spinach, beet [28] and oat [29]. Comparisons were made between derived [Mg^{2+}] values depending on the light–dark transition. According to determinations of total adenylates in chloroplast stroma, in the dark, Mg^{2+} was present at 2.5 and 3 mM in spinach and beet, respectively [28]. These data are in agreement with values of 1–3 mM for Mg^{2+} in darkened chloroplasts using an ionophore [2]. When the light was turned on, the derived [Mg^{2+}] decreased drastically to 0.2 mM or less (Figure 3A). Within a few seconds of the exposure to light, K_{app} values were higher than those allowed by AK equilibrium, suggesting that the adenylates were not equilibrated by AK under these conditions. Alternatively, experimental errors were possible in assays of low contents of adenylate species, especially AMP. After the drastic decrease following the onset of illumination, stromal [Mg^{2+}] increased to 1 mM after 3 min of light. When the illuminated chloroplasts were transferred to the dark, [Mg^{2+}] increased to more than 4 mM in beet (after 15 s) and to 2.5 mM in spinach (after 1 min) and then equilibrated at 2–3 mM [28].

A similar situation with respect to changes in [Mg^{2+}] was observed when the values for adenylates from oat chloroplasts fractionated in seconds by silicone oil centrifugation [29] were substituted into eqn (4) (Figure 3B). When assays of adenylates were carried out under steady-state conditions in wheat chloroplasts (after 9 min in either the light or dark), after rapid fractionation using membrane filtration (less than 1 s) [30], the calculated steady-state [Mg^{2+}] values were 5 and 4 mM for dark and light conditions, respectively. Rapid separation of organelles and fixation into acid media (to prevent turnover and hydrolysis of ATP) appear essential for determinations of intracellular adenylate status. Despite some intrinsic difficulties and shortcomings associated with these methods, they are especially valuable when comparisons are made between different metabolic situations [31].

The fluctuations in stromal [Mg^{2+}], as inferred from the levels of total adenylates (Figures 3A and 3B), are consistent with the

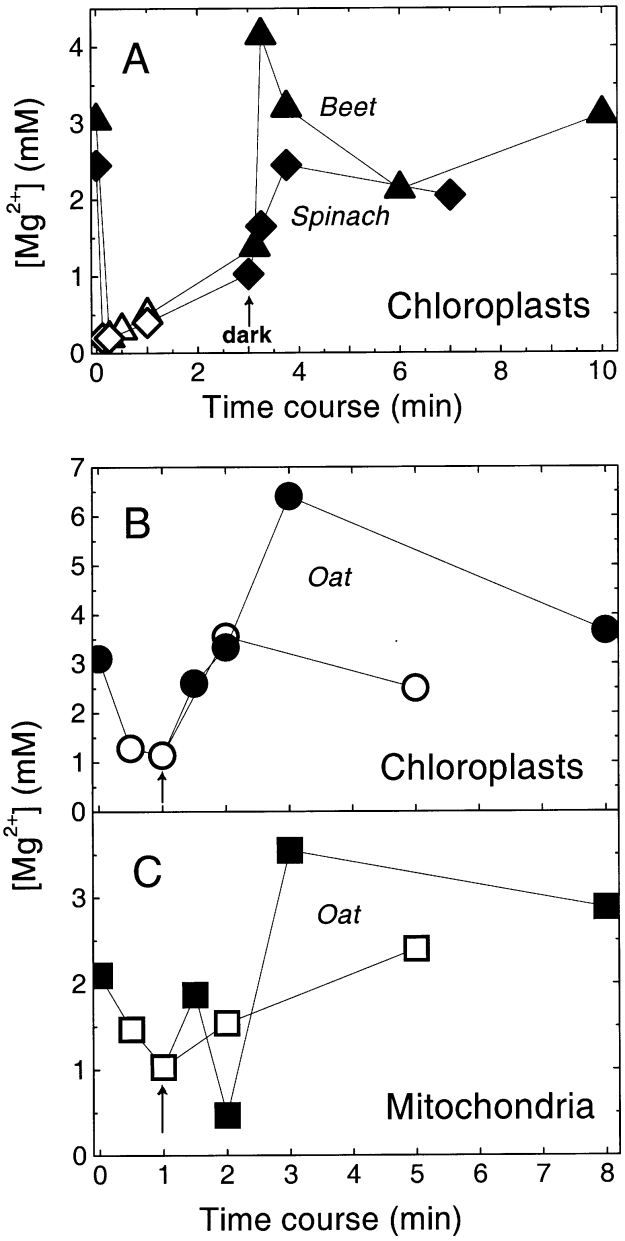

Figure 3 Calculated values of [Mg^{2+}] based on reported contents of adenylates in chloroplasts and mitochondria

(**A**) Isolated chloroplasts of beet and spinach during dark–light–dark transitions [28]. (**B**) Isolated chloroplasts of oat [29] and (**C**) mitochondria of oat [29]. Filled symbols, darkness; open symbols, light. Arrows correspond to the moment when light was turned off for some samples, whereas others remained in the light.

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current knowledge about the so-called photosynthetic induction phenomenon, where a delay in photosynthesis occurs, lasting for 0.5–3 min depending on species and experimental conditions [32,33]. Part of this phenomenon may result from the depletion of Mg^{2+} immediately following illumination, even though net influx of Mg^{2+} into the stroma is known to occur at the thylakoid membrane during light conditions [1,2]. The Mg^{2+} would be utilized mostly for complexation with ATP formed in the light reactions of photosynthesis and, to some extent, with phosphorylated sugars arising throughout the Calvin cycle. The depletion of $[Mg^{2+}]$ would cause a delay in the activation of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), fructose-1,6-bisphosphatase and other enzymes that require Mg^{2+} for activity [34–36]. Portis and Heldt [1] showed inhibition of photosynthesis under exhaustion of Mg^{2+} from isolated chloroplasts caused by application of an ionophore. Using a similar technique, Krause [37] demonstrated substantial changes in $[Mg^{2+}]$ in chloroplasts during the transition between darkness and light. Our analysis gives evidence that stromal $[Mg^{2+}]$ in the steady state is relatively high and does not differ significantly in the dark and light. However, during the transitions between light and dark, $[Mg^{2+}]$ may fluctuate drastically (Figures 3A and 3B).

Cytosol

The $[ATP][AMP]/[ADP]^2$ value in the cytosol of plant cells [30] is quite high (approx. 1.4 in darkness and higher in light, with large S.D. values), so a major proportion of total magnesium may be bound to ATP in this compartment. For wheat leaves [30], the calculated cytosolic $[Mg^{2+}]$ values (eqn 4) were 0.4 and probably approx. 0.2 mM under dark and light conditions, respectively. This is consistent with the cytosolic ATP/ADP ratio exceeding that in chloroplasts and mitochondria, especially in the light [31,32]. Operation of vacuolar Mg^{2+} transporter may also affect $[Mg^{2+}]$ in the cytosol [38].

The low cytosolic $[Mg^{2+}]$ may have important consequences for the operation of gluconeogenesis/glycolysis in this compartment. Fructose-1,6-bisphosphatase is one example of several enzymes that are dependent on $[Mg^{2+}]$; its cytosolic form has a lower K_m for Mg^{2+} than the chloroplastic isoenzyme [35], consistent with lower $[Mg^{2+}]$ in the cytosol. The MgATP-dependent phosphofructokinase is allosterically regulated by free adenylates, with MgATP acting as an inhibitor of fructose 6-phosphate binding to the enzyme; its inhibitory effect is relieved by free adenylates or inorganic phosphate [39]. The magnesium pyrophosphate-dependent phosphofructokinase, which is unique for plants [40], needs higher $[Mg^{2+}]$ because of the much lower affinity that Mg^{2+} has for complexation with pyrophosphate than with ATP ($K_{MgPPi} = 1.2\ mM^{-1}$ compared with $K_{MgATP} = 73\ mM^{-1}$) [21].Thus, its operation in the light is doubtful. Yet another example of a cytosolic activity prone to regulation by $[Mg^{2+}]$ is NADP-isocitrate dehydrogenase (ICDH), which requires Mg^{2+} or Mn^{2+} for activity [41]. The bivalent ions are necessary to complex isocitrate, which then serves as a substrate for the enzyme. Thus at low $[Mg^{2+}]$ values, as in the cytosol, in the light there is a possibility of NADP-ICDH being inhibited by the deficiency of Mg^{2+}. The very high abundance of this enzyme in the cytosol [41] may be necessary to overcome its incomplete saturation by Mg^{2+} in the light to sustain its role in providing 2-oxoglutarate for refixation of photorespiratory ammonia [33]. Aconitase, an enzyme supplying isocitrate for ICDH's reaction, is also located in both cytosol and mitochondria and regulated by $[Mg^{2+}]$; magnesium markedly displaces the equilibrium between citrate and isocitrate because of an almost 10-fold difference in the stability constants for magnesium citrate and magnesium isocitrate [42]. Low $[Mg^{2+}]$ in the cytosol may have also important implications for regulation of phosphoenolpyruvate (PEP) carboxylase. This enzyme is strongly dependent on Mg^{2+} because it uses MgPEP as a true substrate, whereas the stability constant for MgPEP is very low ($0.18\ mM^{-1}$) when compared with those of MgATP and MgADP [43]. The Mg^{2+}-limitation of PEP carboxylase activity may play a similar role in C_4 plants to that of Rubisco limitation by CO_2 in C_3 plants.

Mitochondria

Plants do not have matrix AK, and this may lead to incomplete equilibration of nucleotides in mitochondria. On the other hand, AK activity in the intermembrane space of plant mitochondria is high; in potato mitochondria it is two to four times higher than the maximum activity of ATP synthase [17,18]. It was shown that respiration sustains a limited but significant net formation of ATP, even in the absence of any added ADP. A model involving the combined activities of the adenylate carrier, the intermembrane AK and the inner-membrane-associated ATP synthase was proposed to account for the ATP synthesized under these conditions [20]. Furthermore, it was suggested that the cycling of mitochondrial ADP and ATP via this model may exert a major regulatory influence on mitochondrial respiration under conditions of ADP-limitation, a condition likely to reflect the situation in plant cells *in vivo*.

Details on total ATP, ADP and especially AMP concentrations in mitochondria are difficult to obtain since this compartment occupies only a small part of the cellular volume [44]. Based on measurements of adenylate contents in oat [29], we calculated that mitochondrial $[Mg^{2+}]$ in darkness was 2 mM, whereas after 1 min of illumination it decreased to 1 mM, and after an additional 5 min of light it increased to 2 mM (Figure 3C). During the transition from light to darkness, $[Mg^{2+}]$ increased to 3 mM after 2 min, and then slowly decreased. Thus the phenomenon of a post-illumination increase in respiration (light-enhanced dark respiration) [33] could be, at least in part, connected with the increase of $[Mg^{2+}]$. As a result the NAD-malic enzyme is activated (its activity strongly dependent on Mn^{2+} or Mg^{2+}), similarly to the pyruvate dehydrogenase complex (PDC). The mitochondrial PDC is activated by Mg^{2+} with a K_a of approx. 0.04 and 0.36 mM for maize and pea, respectively (for plastid PDC the K_a for Mg^{2+} was approx. 1 mM). This may be connected, in part, to activation of PDC by PDC phosphatase, which is strongly affected by magnesium [45].

Interestingly, mitochondrial adenylate translocator uses free adenylates rather than Mg-bound adenylates [17,46]: the same was also demonstrated for plastidial adenylate translocator [47]. Its action was shown not to be inhibited by Mg-bound adenylates, which otherwise could seriously impair the ATP/ADP exchange [17,46]. The action of the translocator may contribute to low $[Mg^{2+}]$ in the cytosol (by importing ATP in exchange for ADP) and, by the same token, to higher $[Mg^{2+}]$ in the mitochondria.

Mg-BOUND ADENYLATES AS A DRIVING FORCE OF METABOLISM?

The $[ATP_{total}]/[ADP_{total}]$ ratio is frequently considered as a driving force in metabolism, e.g. during CO_2 fixation driving 3-phosphoglycerate reduction [48]. However, most enzymes utilizing MgATP are inhibited by MgADP in a competitive way (see e.g. [26]),and thus they are dependent on the [MgATP]/[MgADP] ratio, which can differ from $[ATP_{total}]/[ADP_{total}]$ (see below). For some enzymes, free AMP and/or free ADP may act as allosteric

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effectors [3], providing strong additive effects to activation by a high [MgATP]/[MgADP] ratio.

Using the abbreviations of K_{true} (C) and K_{app} (K) of AK (eqns 1 and 2), we can present the relationship between [MgATP]/[MgADP] and $[ATP_{total}]/[ADP_{total}]$ as:

$$([MgATP]/[MgADP])/([ATP_{total}]/[ADP_{total}]) = C([ADP_{free}]/[AMP_{free}])/(K[ADP_{total}]/[AMP_{total}])$$

Taking $[AMP_{total}]$ and $[ADP_{total}]$ as sums of free and Mg-bound adenylates respectively, and given concentrations of free Mg^{2+} (x), we get:

$$\begin{aligned}([MgATP]/[MgADP])/([ATP_{total}]/[ADP_{total}]) &= C([ADP_{free}]/[AMP_{free}])/\{K([ADP_{free}]+dx[ADP_{free}])/([AMP_{free}]+mx[AMP_{free}])\} \\ &= (C/K)(1+mx)/(1+dx)\end{aligned}$$

Inserting the equation for K_{true} (eqn 1), and after some simple rearrangements, we get eqn (5):

$$([MgATP]/[MgADP])/([ATP_{total}]/[ADP_{total}]) = (t/d)(1+dx)/(1+tx) \quad (5)$$

At low $[Mg^{2+}]$ values, the [MgATP]/[MgADP] ratio is higher than $[ATP_{total}]/[ADP_{total}]$ (Figure 4). This is due to more efficient binding of Mg by ATP than by ADP, so the concentration of ADP_{free} becomes high under these conditions. Provided that the [MgATP]/[MgADP] ratio, rather than that of $[ATP_{total}]/[ADP_{total}]$, is a driving force in many energy-linked processes, e.g. nitrogenase reaction (see above), the rate of metabolic flux may be even higher under depletion of $[Mg^{2+}]$.

The $[ATP_{total}]/[ADP_{total}]$ ratios have frequently been used for estimation of the energy state of cells and their compartments, or the 'adenylate energy charge' value $([ATP_{total}]+\frac{1}{2}[ADP_{total}])/([ATP_{total}]+[ADP_{total}]+[AMP_{total}])$, and attempts have been made to correlate enzyme activity with this parameter [49]. It was also stated that, under AK equilibrium, the $[AMP_{total}]$ changes non-linearly with changing $[ATP_{total}]/[ADP_{total}]$ ratio and this may be important for regulation of enzymes that respond to the $[ATP_{total}]/[ADP_{total}]$ ratio and that are influenced by $[AMP_{total}]$ as the allosteric effector [50]. However, many enzymes respond also to $[Mg^{2+}]$ acting as an essential activator; thus they are regulated via changes in the ratio between Mg-bound and Mg-free adenylates. Other enzymes use Mg-complexed substrates which bind magnesium weakly, like PEP or isocitrate; thus the $[Mg^{2+}]$ parameter is critical for their operation. The dependence of K_{app} of AK upon $[Mg^{2+}]$ has been considered as the major factor that affects cellular energy models drawn upon the adenylate energy charge concept [3].

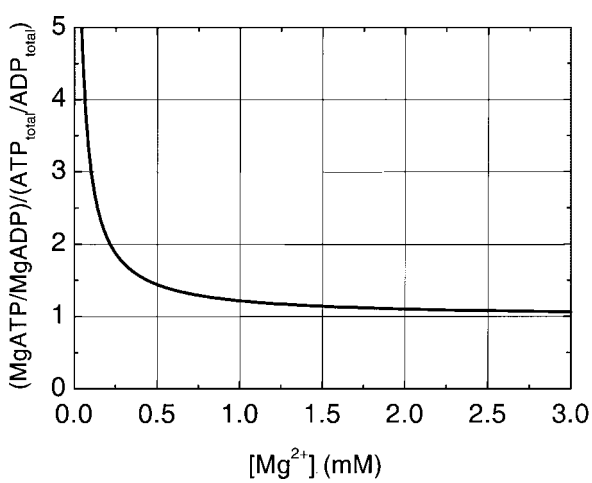

Figure 4 The AK-governed dependence of [MgATP]/[MgADP] on $[Mg^{2+}]$, compared with that of $[ATP_{total}]/[ADP_{total}]$

The line was drawn according to eqn (5).

CONCLUSIONS AND PERSPECTIVES

The practical aspect of this work involves a simple and reproducible method for calculation of $[Mg^{2+}]$ from experimentally determined contents of adenylates, using eqn (4). Rapid changes of $[Mg^{2+}]$ in different subcellular compartments can be determined by this method using, for example, rapid fractionation techniques that allow the separation of plant protoplasts into organellar fractions in 0.2–0.3 s [31,51]. With such a high resolution, assays of total adenylates can be done on samples collected every few seconds and, given that adenylates are under the AK equilibrium, provide a convenient measure of the dynamics of $[Mg^{2+}]$ in a given compartment (Figure 3). Since the turnover of adenylates is quite rapid, occurring within a few seconds, changes in concentration of $[Mg^{2+}]$ will also be fast, e.g. during transitions between light and darkness. Determination of $[Mg^{2+}]$ from concentrations of total adenylates may be essential for understanding subcellular regulation of photosynthetic metabolism and its interaction with respiration, especially during dark–light transitions, but it will also be important in any tissue or cell type where changes in $[Mg^{2+}]$ are supposed to play a major role. Also, the application of inhibitors of photosynthetic and mitochondrial electron transport may drastically change $[Mg^{2+}]$ via changes in adenylate concentration, and such an effect and its consequences have only seldom been considered. Another area of immediate interest concerns studies on magnesium state in transgenic plants with some major metabolic modification, where magnesium balance may have direct effects on the rationalization of metabolic and enzymic activity profiles.

From a theoretical point of view, AK represents an important mechanism regulating $[Mg^{2+}]$ in the cell. Without equilibration by AK, the $[Mg^{2+}]$ would be rapidly exhausted in the cell under intensive ATP synthesis, which would lead to rapid inactivation of key photosynthetic (e.g. Rubisco) and respiratory (e.g. ICDH) enzymes. In the presence of AK, they will be gently regulated by buffered changes of $[Mg^{2+}]$ as a feedback signal of AK. This important role of $[Mg^{2+}]$ in cellular homoeostatic control is frequently overlooked, especially in studies concerned with cell energetics which usually consider only total ATP, ADP and AMP species as determinants of cellular regulation [49]. All these species bind magnesium with quite different stability constants, and Mg-bound and Mg-free species have very different, sometimes opposite, effects on enzymes. Our present study provides a link between total adenylate content and $[Mg^{2+}]$ via AK equilibrium, which should constitute a convenient measure for re-evaluation of data in the literature and for designing new approaches to studying cell energetics.

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