Phosphorylation of Maize and *Arabidopsis* HMGB Proteins by Protein Kinase CK2α†

Christian Stemmer,‡,§ Diana J. Leeming,‡ Lars Franssen,‡ Rudi Grimm,|| and Klaus D. Grasser*,‡

Institute of Life Sciences, Aalborg University, Sohngaardsholmsvej 49, DK-9000 Aalborg, Denmark, and Agilent Technologies, Hewlett-Packard-Strasse, D-76337 Waldbronn, Germany

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ABSTRACT: In plants, a variety of chromatin-associated high mobility group (HMG) proteins belonging to the HMGB family have been identified. We have examined the phosphorylation of the HMGB proteins from the monocotyledonous plant maize and the dicotyledonous plant *Arabidopsis* by protein kinase CK2α. Maize CK2α phosphorylates the maize HMGB1 and HMGB2/3 proteins and the *Arabidopsis* HMGB1, HMGB2/3, and HMGB4 proteins. Maize HMGB4 and HMGB5 and *Arabidopsis* HMGB5 are not phosphorylated by CK2α. Depending on the HMGB protein up to five amino acid residues are phosphorylated in the course of the phosphorylation reaction. The HMGB1 proteins from both plants are markedly more slowly phosphorylated by CK2α than the other HMGB substrate proteins, indicating that certain HMGB proteins are clearly preferred substrates for CK2α. The rate of the phosphorylation reaction appears to be related to the ease of interaction between CK2α and the HMGB proteins, as indicated by chemical cross-linking experiments. MALDI/TOF mass spectrometry analyses demonstrate that the HMGB1 and HMGB2/3 proteins occur in various phosphorylation states in immature maize kernels. Thus, HMGB1 exists as monophosphorylated, double-phosphorylated, triple-phosphorylated, and tetraphosphorylated protein in kernel tissue, and the tetraphosphorylated form is the most abundant version. The observed in vivo phosphorylation states indicate that protein kinase(s) other than CK2α contribute(s) to the modification of the plant HMGB proteins. The fact that the HMGB proteins are phosphorylated to various extents reveals that the existence of differentially modified forms increases the number of distinct HMGB protein variants in plant chromatin that may be adapted to certain functions.

High mobility group (HMG)[1] (*1*) proteins represent a heterogeneous class of small and relatively abundant chromatin-associated proteins of eukaryotes (*2*, *3*). Proteins belonging to the subgroup of the HMGB proteins[2] have in common a distinctive DNA-binding motif termed the HMG-box domain, which confers non-sequence-specific interaction with linear DNA and structure-specific binding to distorted DNA sites. Upon binding to linear DNA the HMGB proteins bend the double helix by over 90° (*4*). They act as architectural factors facilitating the assembly of nucleoprotein complexes, which contribute, for instance, to the regulation of transcription and recombination (*3*, *4*).

Chromosomal HMGB proteins have been identified and characterized from a variety of plants, revealing similarities and dissimilarities to their vertebrate counterparts (*5*−*8*). Higher plants have ≥5 different HMGB proteins, which have a single HMG-box domain flanked by a basic N-terminal domain and an acidic C-terminal domain. While the HMG-box domain of the various plant HMGB proteins is relatively conserved, the basic and acidic flanking regions are variable in length and sequence (*9*). The regions flanking the HMG-box domain modulate the DNA interactions of the HMGB proteins (*10*). The various plant HMGB proteins differ in their chromatin association (*11*), in their expression pattern (*12*), in some of their DNA interactions (*6*, *13*), and in their interaction with the transcription factor Dof2 (*14*). Therefore, the various plant HMGB proteins may be adapted to fulfill a variety of architectural functions in the nucleus.

Vertebrate HMGB proteins have been reported to be posttranslationally modified by acetylation, methylation, ADP-ribosylation, and glycosylation (*15*, *16*), but in most instances [apart from acetylation (*17*)] the functional significance of these modifications is unknown. Insect HMGB proteins are phosphorylated by protein kinases C and CK2, altering the DNA-binding properties of the HMGB proteins (*18*, *19*). HMG proteins from the dicotlydonous plant broccoli are in vitro substrates for protein kinase CK2 (*20*). The maize HMGB1 and HMGB2/3 proteins are phosphorylated by CK2α in vitro, and the same sites have been found to be phosphorylated in vivo in HMGB proteins isolated from maize suspension cultured cells. CK2α phosphorylates residues within the acidic C-terminal domain of the HMGB1 and HMGB2/3 proteins, modulating the stability of the proteins and their DNA-binding properties (*21*).

Previously, we have observed that the maize HMGB2/3 proteins (89% amino acid sequence identity) are significantly

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* To whom correspondence should be addressed. Tel: +45 9635 9126. Fax: +45 9814 1808. E-mail: kdg@bio.auc.dk.

‡ Aalborg University.

§ Present address: Greenovation Biotech GmbH, Bötzinger Str. 29b, D-79111 Freiburg, Germany.

|| Agilent Technologies.

[1] Abbreviations: HMG, high mobility group; AU−PAGE, acetic acid−urea−polyacrylamide gel electrophoresis; BMS, Black Mexican Sweet.

[2] The nomenclature of the HMG proteins has been revised recently (see ref *1* and http://www.informatics.jax.org/mgihome/nomen/gene-families/hmgfamily.shtml).

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more vigorously phosphorylated by CK2α in radioactive phosphorylation assays than HMGB1 and that HMGB4 and HMGB5 are not phosphorylated by maize CK2α (*21*). Therefore, we have studied here the differential phosphorylation of the maize HMGB proteins by CK2α, and we have examined the phosphorylation of the *Arabidopsis* HMGB proteins.

EXPERIMENTAL PROCEDURES

Preparation of Proteins. The recombinant maize and *Arabidopsis* HMGB proteins were expressed in *Escherichia coli* as 6 × His-tagged fusion proteins and purified by three-step column chromatography as described previously (*6*, *12*, *22*). Recombinant maize CK2α was expressed in *E. coli* and purified by three-step column chromatography as described previously (*21*). Native HMGB proteins were purified from immature maize kernels (10−12 days after pollination) by 2% TCA extraction, ion-exchange chromatography, and reversed-phase HPLC as described previously (*12*, *22*).

Radioactive CK2α Phosphorylation Assays. In the standard phosphorylation assay, the different HMGB proteins (1 μM) were incubated in a total volume of 20 μL at 37 °C for 1 h with 40 ng of recombinant CK2α in the presence of 100 nCi of [γ-^{32}P]ATP (Hartmann Analytic) in CK2 buffer (25 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, 1 mM DTT). The phosphorylation reactions (which were stopped by addition of SDS loading buffer and heating to 95 °C) were monitored by separation of the proteins by SDS−PAGE in 18% polyacrylamide gels and analysis with a Typhoon 8600 phosphorimager (Amersham Biosciences).

Nonradioactive CK2α Phosphorylation Assays. The HMGB proteins (2 μg) were incubated as described above in a total volume of 20 μL with 100 ng of recombinant CK2α in the presence of 0.3 mM unlabeled ATP, and the reactions were stopped by addition of AU loading buffer (8 M urea, 5% acetic acid, 5 mg/mL protamine sulfate, 0.02% Pyronin Y) and heating to 60 °C. The different phosphorylation stages of the proteins were separated in 18% polyacrylamide gels by acetic acid−urea (AU) PAGE (*23*) and visualized by Coomassie staining.

Chemical Cross-Linking. The interaction of CK2α with the HMGB proteins was examined by cross-linking with glutaraldehyde, which was essentially performed as described previously (*14*). Adding glutaraldehyde (final concentration of 0.025%) to the protein samples started the cross-linking reaction, which was stopped after various times by addition of SDS loading buffer and heating of the samples for 5 min at 95 °C. The proteins were separated by SDS−PAGE in 18% polyacrylamide gels, which were stained with silver.

Mass Spectrometry. MALDI/TOF mass spectrometry of native HMGB proteins was performed on a Voyager-DE PRO system equipped with a nitrogen laser (Applied Biosystems) using the linear and the reflector mode. The system was calibrated with standards provided by the supplier. As matrix sinapinic acid (20 mg/mL in 50% acetonitrile, 0.1% TFA) was used. Samples were mixed 1:1 with the matrix on the target plate and air-dried.

RESULTS

Differential Phosphorylation of Maize HMGB1 and HMGB2/3 by CK2α. Protein kinase CK2 (also known as casein kinase II) is an ubiquitous enzyme catalyzing the phosphorylation of certain Ser/Thr residues in acidic regions of the substrate protein (*24*−*26*). In plants, CK2 has been found in two different forms (*27*), the heterotetrameric CK2A (composed of two catalytic α and regulatory β subunits each) and the monomeric CK2B (consisting of the catalytic α subunit). Our previous studies have revealed that the maize HMGB2/3 proteins were more vigorously phosphorylated than HMGB1 by recombinant maize CK2α, although in all three proteins two or three residues could be phosphorylated by CK2 (*21*). To examine whether HMGB2 and HMGB3 are preferred substrates of CK2α relative to HMGB1, we have analyzed the phosphorylation of the HMGB proteins in competitive phosphorylation assays. Equal amounts of HMGB1 and HMGB2 or HMGB1 and HMGB3 were reacted with CK2α for various times in the presence of [^{32}P]ATP. The phosphorylated proteins were separated by SDS−PAGE, and the ^{32}P incorporation into the proteins was determined using a phosphorimager (Figure 1). In these competitive phosphorylation reactions, HMGB2 and HMGB3 were more readily phosphorylated than HMGB1. Whereas radiolabeled bands of HMGB2 and HMGB3 were detectable after 15 s, a HMGB1 band of comparable intensity was detectable only after ∼5 min, demonstrating that the phosphorylation of HMGB2/3 proceeded ∼20 times more rapidly than that of HMGB1. The time course of the phosphorylation reaction was examined by reacting the three HMGB proteins individually with CK2α for various times and by monitoring the phosphorylation state of the proteins by AU−PAGE (Figure 2). This electrophoresis system allows resolving the different phosphorylation stages of the HMGB proteins. In the course of the phosphorylation reaction, in addition to the initial nonphosphorylated protein band, new bands with reduced electrophoretic mobility appeared, which correspond to the individual phosphorylation states of the protein. HMGB2 and HMGB3 were completely double-phosphorylated by CK2α after ∼15 min, whereas HMGB1 was still partially monophosphorylated after 60 min. Markedly more extended CK2α reactions (data not shown) resulted also in triple-phosphorylated forms of the HMGB proteins (see HMGB1 after 60 min). The number of phosphorylated amino acid residues detected by AU−PAGE corresponds well with the results of mass spectrometry [data not shown (*21*)]. These experiments demonstrate that relative to HMGB1, HMGB2 and HMGB3 are significantly more rapidly phosphorylated by CK2α.

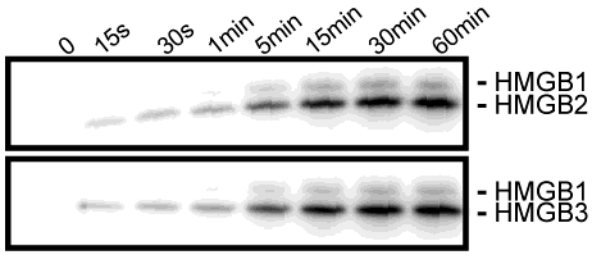

FIGURE 1: Competitive phosphorylation of the maize HMGB1 and HMGB2/3 proteins by CK2α. Equal amounts of HMGB1 and HMGB2 (top panel) and of HMGB1 and HMGB3 (bottom panel) were phosphorylated for the indicated times by CK2α in the presence of [^{32}P]ATP. The proteins were separated by SDS−PAGE, and the ^{32}P incorporation was examined using a phosphorimager. The electrophoretic migration positions of the HMGB proteins are indicated.

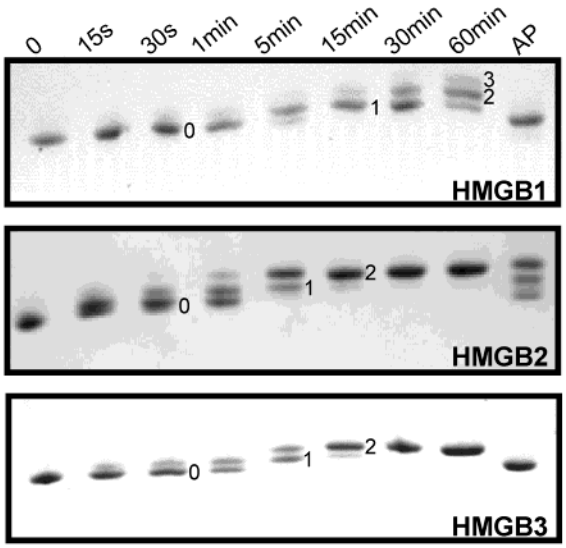

FIGURE 2: Time course of the phosphorylation of the HMGB proteins by CK2α. The HMGB1, HMGB2, and HMGB3 proteins were reacted for the indicated times with CK2α in the presence of unlabeled ATP. The proteins were separated by AU−PAGE (resolving the different phosphorylation stages of the proteins) and stained with Coomassie. In the course of the phosphorylation reaction, the initially nonphosphorylated proteins are increasingly phosphorylated by CK2α and display, depending on the number of phosphorylations, a reduced electrophoretic mobility relative to the nonphosphorylated proteins. The number of protein phosphorylations corresponding to the differently migrating protein bands is indicated next to the bands in the gel. As control, proteins, which were phosphorylated for 60 min, were subsequently dephosphorylated with alkaline phosphatase and separated on the same gel, indicated by AP.

Interaction of CK2α with the HMGB Proteins. To examine whether the differential efficacy of the phosphorylation seen with the various maize HMGB proteins correlates with different protein interactions between CK2α and the HMGB proteins, we have performed chemical cross-linking experiments. CK2α and the HMGB proteins were mixed and treated for various times with glutaraldehyde, before the cross-linked samples were analyzed by SDS−PAGE (Figure 3). While no cross-linked products could be detected, when the individual proteins were treated with glutaraldehyde, HMGB1 and HMGB3 could be readily cross-linked with CK2α (top panels). The formation of the CK2α/HMGB complex was slightly more efficient (~1.5-fold) with HMGB3 than with HMGB1. In contrast, HMGB4 and HMGB5 [which are not phosphorylated by CK2α (*21*)] are hardly cross-linked with CK2α. The presence of both CK2α and the HMGB protein in the protein band corresponding to the cross-linked complex was confirmed by immunoblot analyses using antisera against CK2α and HMGB (data not shown). Therefore, the efficacy of the CK2α-mediated phosphorylation of the HMGB proteins appears to be related to the protein interaction between CK2α and the HMGB protein.

Phosphorylation States of HMGB Proteins Purified from Maize Kernels. The HMGB1, HMGB2, and HMGB3 proteins were purified from immature maize kernels to determine their native phosphorylation states. The purified proteins were analyzed by MALDI/TOF mass spectrometry (Table 1), revealing that the majority of the maize HMGB proteins occur as phosphoproteins. Each of the three HMGB proteins

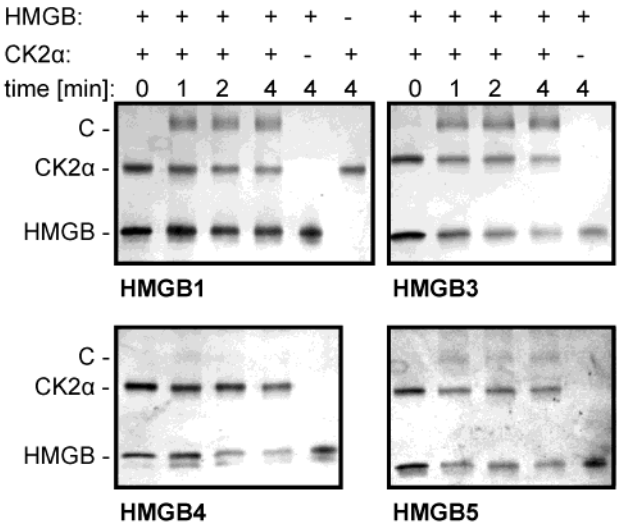

FIGURE 3: Chemical cross-linking of CK2α and HMGB proteins. CK2α and the HMGB proteins were reacted individually or as a mixture with glutaraldehyde. Aliquots of the reactions were taken at the indicated times, and the proteins were analyzed by SDS−PAGE and silver staining. The electrophoretic migration positions of the HMGB proteins, CK2α, and the cross-linked complex (C) are indicated.

Table 1: Masses of the HMGB Proteins from Immature Maize Kernels and BMS Suspension Cultured Cells Determined by Mass Spectrometry

	calc mass[a]	BMS[b]	BMS[c]	kernel[d]	kernel[e]
HMGB1	17145.9	17531	4	17229 + 17310	1 + 2
				17387 + 17468	3 + 4
HMGB2	15315.7	15556	3	15557 + 15637	3 + 4
HMGB3	15007.3	15325 + 15406[f]	2 + 3	15328 + 15410[f]	2 + 3

[a] The mass values (in kDa) were calculated on the basis of the known protein sequences. [b] The masses (in kDa) of the HMGB proteins from BMS cells were determined by nanospray mass spectrometry (*21*). [c] Number of phosphorylations calculated from the mass difference of BMS cell proteins and dephosphorylated proteins (*21*). [d] The masses (in kDa) of the HMGB proteins from kernels were determined by MALDI/TOF mass spectrometry. [e] Number of phosphorylations calculated from the mass difference of kernel proteins and the calculated masses. [f] The HMGB3 contains additional modification(s) other than phosphorylation (~162 Da) that is (are) not removed by dephosphorylation with alkaline phosphatase (*21*).

exists in different phosphorylation states in immature maize kernels. Thus, HMGB1 occurs predominantly in the tetraphosphorylated form but also in the triple-phosphorylated and monophosphorylated form and to a minor extent in the double-phosphorylated form, whereas the nonphosphorylated species of HMGB1 was not detectable (Figure 4A). A similar distribution of the different phosphorylation states of HMGB1 was determined by AU−PAGE of the native HMGB1 (Figure 4B). Treatment of native HMGB1 with alkaline phosphatase could remove the phosphate groups, resulting in the dephosphorylated protein. For HMGB2 and HMGB3 from kernels two masses of each were obtained, corresponding to the triple-phosphorylated/tetraphosphorylated and double-phosphorylated/triple-phosphorylated proteins, respectively (Table 1). Comparison of these results with the masses obtained for HMGB proteins isolated from maize BMS suspension cultured cells (*21*) indicates that the extent of HMGB protein phosphorylation varies depending on the plant tissue (Table 1).

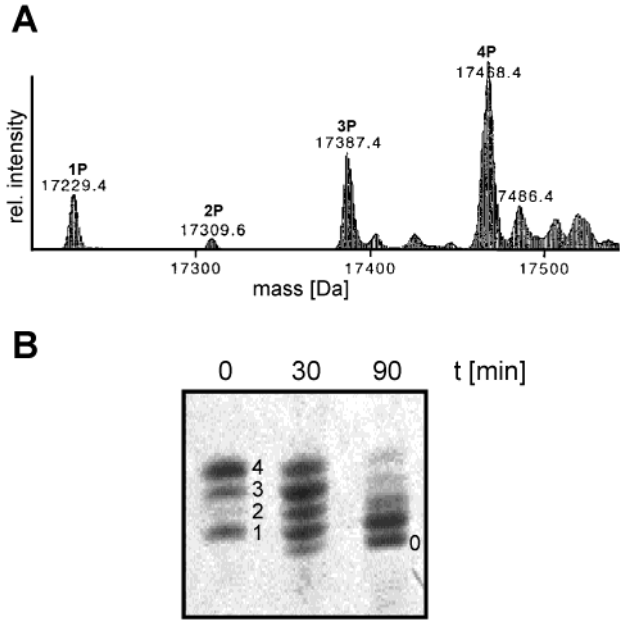

FIGURE 4: Phosphorylation state of HMGB1 from maize kernels determined by mass spectrometry and AU−PAGE. (A) HMGB1 was purified from immature maize kernels and analyzed by MALDI/TOF mass spectrometry. The protein was found in four differently phosphorylated forms with masses that differ by ~80 Da from each other. The diagram displays the relative intensities of the different forms of HMGB1 found in kernel tissue. The number of protein phosphorylations (1P, 2P, 3P, 4P) is indicated along with the different masses determined for HMGB1. (B) HMGB1 from maize kernels was examined by AU−PAGE. Untreated HMGB1 and HMGB1 treated for 30 or 90 min with alkaline phosphatase was analyzed by AU−PAGE and Coomassie staining. The number of phosphorylations corresponding to the differently migrating protein bands is indicated next to the bands in the gel.

Phosphorylation of Arabidopsis HMGB Proteins by CK2. Five HMGB proteins have been characterized from the dicotyledonous plant *Arabidopsis*, which are structurally similar to the HMGB proteins of the monocotyledonous plant maize (*28*). Despite their general amino acid sequence similarity, there is no clear homology between certain maize and *Arabidopsis* HMGB proteins (*9*). Using the PhosphoBase 2.0 software (*29*), the amino acid sequences of the five *Arabidopsis* HMGB proteins were predicted to contain four to six CK2 phosphorylation sites. Therefore, we have examined whether the *Arabidopsis* HMGB proteins are substrates of the recombinant maize CK2α (which shares 86% amino acid sequence identity with *Arabidopsis* CK2α). Equal amounts of the five *Arabidopsis* HMGB proteins were incubated with CK2α in the presence of [^{32}P]ATP. The protein samples were separated by SDS−PAGE, and the ^{32}P incorporation into the proteins was visualized using a phosphorimager (Figure 5). The HMGB2/3 proteins (which exhibit 91% amino acid sequence identity) and HMGB4 are phosphorylated by CK2α to comparable extents, while HMGB1 is phosphorylated less vigorously. For HMGB5 no CK2α-mediated ^{32}P incorporation could be detected. Despite the presence of predicted CK2 phosphorylation sites, not all *Arabidopsis* HMGB proteins are phosphorylated by CK2α, as seen with the maize HMGB proteins (*21*). The finding that HMGB proteins from both the monocot plant maize and the dicot plant *Arabidopsis* are phosphorylated by CK2α indicates that CK2 generally plays a role in the modification

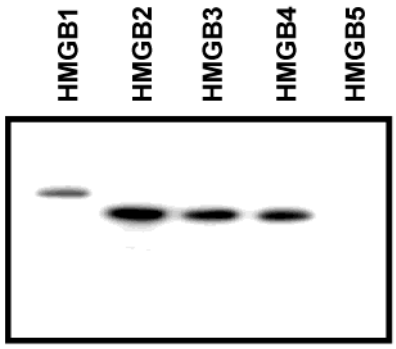

FIGURE 5: Phosphorylation of *A. thaliana* HMGB proteins by CK2α. Equal amounts of the recombinant *Arabidopsis* HMGB proteins were phosphorylated by CK2α in the presence of [^{32}P]ATP. The proteins were separated by SDS−PAGE, and the ^{32}P incorporation was examined using a phosphorimager.

of HMGB proteins from higher plants [and insects (*19*)], whereas vertebrate HMGB proteins lack Ser/Thr amino acid residues within their acidic domains that could be phosphorylated (*2*).

AU−PAGE was used to reveal the number of amino acid residues in the different *Arabidopsis* HMGB proteins that are phosphorylated by CK2α. The HMGB proteins were reacted with CK2α for various times in the presence of unlabeled ATP, and the phosphorylated proteins were resolved by AU−PAGE. The CK2α-mediated phosphorylation resulted in the appearance of protein bands with a reduced electrophoretic mobility (relative to the initially nonphosphorylated protein), which correspond to the individual phosphorylation stages of the proteins (Figure 6). In case of HMGB1, up to two (a third is appearing at 60 min) phosphorylation states could be resolved, while with HMGB2 and HMGB3 five and with HMGB4 three phosphorylations were detected. With HMGB1 the reaction clearly proceeds more slowly (double-phosphorylation not completed after 60 min) than with the other three *Arabidopsis* HMGB proteins (majority of protein in the triple-phosphorylated or penta-phosphorylated state after 60 min) and reflects the situation with maize HMGB1 (Figures 1 and 2). The fact that the phosphorylation reaction with HMGB1 proceeds more slowly explains the difference in phosphorylation efficacy between HMGB1 on one hand and HMGB2/3 and HMGB4 on the other hand seen in the radioactive CK2α phosphorylation experiment (Figure 5).

DISCUSSION

Protein kinase CK2 is a eukaryotic Ser/Thr kinase, which is localized in the nucleus and cytoplasm. In mammals, CK2 occurs in various forms composed of different catalytic α and regulatory β subunits (*24*−*26*). The biological significance of the different isoforms is not clear, but they differ in their expression and enzyme activity (*25*, *26*). CK2 from plants has been characterized from various species, revealing many similarities to the mammalian enzymes (*27*). Like the mammalian enzyme, plant CK2 exists in various heterotetrameric ($\alpha_2\beta_2$) forms, but monomeric, catalytically active CK2α has been also isolated from maize, broccoli, and *Arabidopsis* (*20*, *30*−*35*). Numerous substrate proteins have been reported for CK2, and many of them are nuclear proteins (*24*, *27*, *36*). CK2 is involved in cell growth and proliferation but also in stress response and cell survival (*37*−*40*).

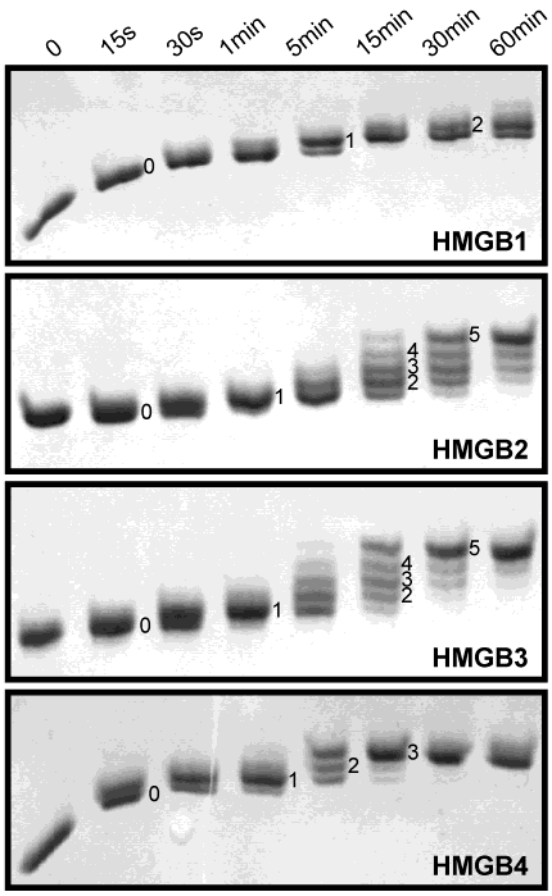

FIGURE 6: Time course of the phosphorylation of the *Arabidopsis* HMGB proteins by CK2α. The HMGB1, HMGB2, HMGB3, and HMGB4 proteins were reacted for the indicated times with CK2α in the presence of unlabeled ATP. The proteins were separated by AU−PAGE and stained with Coomassie. The number of protein phosphorylations corresponding to the differently migrating protein bands is indicated next to the bands in the gel.

CK2 phosphorylates preferentially Ser/Thr residues in an acidic environment of the substrate proteins (*25*, *26*), and accordingly, the CK2 phosphorylation sites that have been identified in plant and insect HMGB proteins are localized within the highly acidic C-terminal domain of these proteins (*19*, *21*). All the maize and *Arabidopsis* HMGB proteins that are phosphorylated by CK2α contain consensus phosphorylation sites in the C-terminal domain. The acidic tail of the HMGB proteins plays an important role in modulating the DNA interactions of the proteins. Thus, the acidic domain reduces substantially the affinity for various DNA substrates, and in some cases it has been demonstrated that the negative effect of the acidic tail correlates with its length and number of negative charges (*10*, *41*−*46*). Phosphorylation of the acidic domain by CK2 further increases the negative charge and, hence, its negative effect on certain DNA interactions (*19*, *21*). Moreover, the functional interaction of the maize HMGB1 and HMGB2/3 proteins with the transcription factor Dof2 is abolished by CK2α-mediated phosphorylation of the acidic tails of the HMGB proteins (*14*).

The HMGB1 proteins from maize and *Arabidopsis* are phosphorylated by CK2α at a markedly lower rate than HMGB2/3 and *Arabidopsis* HMGB4, which explains the striking difference seen in the efficiency of CK2α phosphorylation of the HMGB proteins in standard phosphorylation assays [Figure 5 (*21*)]. The remarkably more rapid phosphorylation of the maize HMGB2/3 proteins (Figures 1 and 2) coincided with an only slightly more efficient cross-linking with CK2α, relative to that of HMGB1. This indicates that the protein kinase favorably interacts with the preferred substrate proteins, but the ease of protein interaction between the protein kinase and the HMGB substrate presumably does not fully explain the significant differences seen in the phosphorylation efficiencies. CK2α forms only inefficient complexes with the maize HMGB4 and HMGB5 proteins (Figure 3), which are not phosphorylated by CK2α (*21*). A significant fraction of the HMGB proteins isolated from immature maize kernels (and BMS suspension cultured cells) contains a greater number of phosphorylated amino acid residues (Table 1) than have been found to be phosphorylated by CK2α in vitro. Therefore, it is likely that protein kinases other than CK2α are involved in the phosphorylation of the HMGB proteins in plants. Possibly other forms of CK2, for instance, tetrameric CK2 isoforms, could catalyze these phosphorylations, since the different forms of CK2 have various enzymatic activities and to some extent different substrate specificities (*33*, *35*, *40*). In maize HMGB1, up to three residues within the acidic domain can be phosphorylated by CK2 (and the same sites are phosphorylated in HMGB1 isolated from BMS cells), but the fourth phosphorylated residue is probably not situated in the acidic region (*21*) and may be phosphorylated by a protein kinase other than CK2. CK2α can phosphorylate up to five amino acid residues in *Arabidopsis* HMGB2/3 (Figure 6), but only four serine residues are present in the acidic tail, indicating that at least one amino acid residue outside the acidic domain is phosphorylated. The finding that the maize HMGB1 and HMGB2/3 proteins occur in different phosphorylation states demonstrates that numerous HMGB protein variants exist in plants. Since the CK2-mediated phosphorylation modulates DNA and protein interactions of the HMGB proteins (*13*, *14*, *19*, *21*), the various posttranslationally modified versions increase the repertoire of architectural HMGB proteins in plant chromatin, which have distinct properties and could act in different processes.

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REFERENCES

1. Bustin, M. (2001) *Trends Biochem. Sci. 26*, 152−153.
2. Bustin, M., and Reeves, R. (1996) *Prog. Nucleic Acids Res. 54*, 35−100.
3. Bustin, M. (1999) *Mol. Cell. Biol. 19*, 5237−5246.
4. Thomas, J. O., and Travers, A. A. (2001) *Trends Biochem. Sci. 26*, 167−174.
5. Pedersen, T. J., Arwood, L. J., Spiker, S., Guiltinan, M. J., and Thompson, W. F. (1991) *Plant Mol. Biol. 16*, 95−104.
6. Ritt, C., Grimm, R., Fernández, S., Alonso, J. C., and Grasser, K. D. (1998) *Plant J. 14*, 623−631.
7. Spiker, S. (1984) *J. Biol. Chem. 259*, 12007−12013.
8. Webster, C. I., Packman, L. C., Pwee, K.-H., and Gray, J. C. (1997) *Plant J. 11*, 703−715.
9. Grasser, K. D. (1998) *Trends Plant Sci. 3*, 260−265.
10. Ritt, C., Grimm, R., Fernández, S., Alonso, J. C., and Grasser, K. D. (1998) *Biochemistry 37*, 2673−2681.
11. Lichota, J., and Grasser, K. D. (2001) *Biochemistry 40*, 7860−7867.

12. Stemmer, C., Grimm, R., and Grasser, K. D. (1999) *Plant Mol. Biol. 41*, 351−361.
13. Stemmer, C., Fernández, S., Lopez, G., Alonso, J. C., and Grasser, K. D. (2002) *Biochemistry 41*, 7763−7770.
14. Krohn, N. M., Yanagisawa, S., and Grasser, K. D. (2002) *J. Biol. Chem. 277*, 32438−32444.
15. Einck, L., and Bustin, M. (1985) *Exp. Cell Res. 156*, 295−310.
16. van Holde, K. E. (1989) in *Chromatin*, Springer, Heidelberg.
17. Ugrinova, I., Pasheva, E. A., Armengaud, J., and Pashev, I. G. (2001) *Biochemistry 40*, 14655−14660.
18. Wisniewski, J. R., Schulze, E., and Sapetto, B. (1994) *Eur. J. Biochem. 225*, 687−693.
19. Wisniewski, J. R., Szewczuk, Z., Petry, I., Schwanbeck, R., and Renner, U. (1999) *J. Biol. Chem. 274*, 20116−20122.
20. Klimczak, L. J., and Cashmore, A. R. (1994) *Plant Physiol. 105*, 911−919.
21. Stemmer, C., Schwander, A., Bauw, G., Fojan, P., and Grasser, K. D. (2002) *J. Biol. Chem. 277*, 1092−1098.
22. Grasser, K. D., Grimm, R., and Ritt, C. (1996) *J. Biol. Chem. 271*, 32900−32906.
23. Lennox, R. W., and Cohen, L. H. (1989) *Methods Enzymol. 170*, 532−549.
24. Allende, J. E., and Allende, C. C. (1995) *FASEB J. 9*, 313−323.
25. Guerra, B., Boldyreff, B., Sarno, S., Cesaro, L., Issinger, O.-G., and Pinna, L. A. (1999) *Pharmacol. Ther. 82*, 303−313.
26. Pinna, L. A., and Ruzzene, M. (1996) *Biochim. Biophys. Acta 1314*, 191−225.
27. Riera, M., Peracchia, G., and Pagès, M. (2001) *Mol. Cell. Biochem. 227*, 119−127.
28. Stemmer, C., Ritt, C., Igloi, G. L., Grimm, R., and Grasser, K. D. (1997) *Eur. J. Biochem. 250*, 646−652.
29. Kreegipuu, A., Blom, N., and Brunak, S. (1999) *Nucleic Acids Res. 27*, 237−239.
30. Dobrowolska, G., Meggio, F., and Pinna, L. A. (1987) *Biochim. Biophys. Acta 931*, 188−195.
31. Dobrowolska, G., Meggio, F., Szczegielniak, J., Muszynska, G., and Pinna, L. A. (1992) *Eur. J. Biochem. 204*, 299−303.
32. Espunya, M. C., and Martínez, M. C. (1997) *Plant Sci. 124*, 131−142.
33. Klimczak, L. J., Collinge, M. A., Farini, D., Giuliano, G., Walker, J. C., and Cashmore, A. R. (1995) *Plant Cell 7*, 105−115.
34. Li, H., and Roux, S. J. (1992) *Plant Physiol. 99*, 686−692.
35. Riera, M., Peracchia, G., Nadal, E., Ariño, J., and Pagès, M. (2001) *Plant J. 25*, 365−374.
36. Guo, C., Davis, A. T., Yu, S., Tawfic, S., and Ahmed, K. (1999) *Mol. Cell. Biochem. 191*, 135−142.
37. Ahmed, K., Gerber, D. A., and Cochet, C. (2002) *Trends Cell Biol. 12*, 226−230.
38. Guerra, B., and Issinger, O.-G. (1999) *Electrophoresis 20*, 391−408.
39. Litchfield, D. W. (2003) *Biochem. J. 369*, 1−15.
40. Pinna, L. A. (2002) *J. Cell Sci. 115*, 3873−3878.
41. Lee, K.-B., and Thomas, J. O. (2000) *J. Mol. Biol. 304*, 135−149.
42. Payet, D., and Travers, A. A. (1997) *J. Mol. Biol. 266*, 66−75.
43. Sheflin, L. G., Fucile, N. W., and Spaulding, S. W. (1993) *Biochemistry 32*, 3238−3248.
44. Stros, M., Stokrova, J., and Thomas, J. O. (1994) *Nucleic Acids Res. 22*, 1044−1051.
45. Wisniewski, J. R., and Schulze, E. (1994) *J. Biol. Chem. 269*, 10713−10719.
46. Yoshida, K., Saito, K., Tanabe, T., Yamamoto, A., Ando, Y., Nakamura, Y., Shirakawa, H., and Yoshida, M. (1999) *Biochemistry 38*, 589−595.

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