Stimulation of Transcription in Cultured Cells by High Mobility Group Protein 1: Essential Role of the Acidic Carboxyl-Terminal Region†

Shigemi Aizawa,‡ Hiroaki Nishino,§ Kouhei Saito,‡ Koichi Kimura,§ Hitoshi Shirakawa,‡ and Michiteru Yoshida*,‡

Department of Biological Science and Technology and Department of Applied Biological Science, Science University of Tokyo, Yamazaki, Noda-shi, Chiba 278, Japan

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ABSTRACT: Several *in vitro* studies have suggested that high mobility group (HMG) protein 1 has a role in gene regulation as a trans activator or quasi-transcription factor. However, data on the molecular functions of HMG1 protein in these reactions are contradictory or obscure. In order to assess whether HMG1 protein does, in fact, have transcriptional activation potential, two *assay* systems in cultured cells were employed. HMG1 protein introduced into COS-1 cells as a complex with a reporter plasmid carrying the *lacZ* gene enhanced the level of the gene expression. Cotransfection of an expression plasmid carrying HMG1 cDNA into the cells with the reporter plasmid enhanced the activity of β-galactosidase 2−3-fold in comparison with that of the control effector plasmid. The enhancement was proved to be dependent not on the replication but on the transcription of the reporter plasmid. In the cotransfection experiments, an expression plasmid encoding the HMG1 molecule lacking the acidic carboxyl terminus repressed the expression of the reporter gene. The binding of an HMG1 protein variant lacking the acidic carboxyl terminus to DNA gave an extremely large shift of gel retardation in comparison with the complete HMG1 molecule. Together, these results indicate that HMG1 protein can enhance expression in cells in culture at the step of gene transcription and that the DNA binding domains comprising two-thirds of the HMG1 protein molecule are responsible for the inhibition property. Also, the acidic terminus of the HMG1 molecule is essential for the enhancement of gene expression in addition to elimination of the repression caused by the DNA binding. Thus it has been directly demonstrated that HMG1 protein can function as a quasi-transcription factor in the process of gene transcription. The repressive property of the HMG box in gene expression and the stimulation induced by the acidic region may help to shed light on the functional mechanism of the many transcription factors containing a DNA-binding motif and/or an acidic domain.

High mobility group (HMG)[1] proteins 1 and 2 are the most prevalent non-histone components in chromatin of relatively low molecular weight. HMG1 and HMG2 proteins have been implicated in DNA replication and cellular differentiation [for reviews, see Bustin et al. (1990) Einck and Bustin (1985)]. HMG1 protein may be associated with the actively transcribed regions of chromatin (Vidali et al., 1977). Microinjection of antibodies against HMG1 protein into living oocyte nuclei has been shown to cause the retraction of transcription loops (Kleinschmidt et al., 1983). HMG1 and HMG2 proteins stimulate *in vitro* transcription (Tremethick & Molloy, 1986) by facilitating the formation of effective initiation complexes (Tremethick & Molloy, 1988) or by stimulating the binding of a specific transcription factor to the adenovirus major late promoter (Watt & Molloy, 1988). HMG1 and HMG2 proteins function as general class II transcription factors (Singh & Dixon, 1990). These observations suggest that the HMG proteins play a direct functional role in gene transcriptional events. However, it is also probable that HMG proteins can play an active role in gene regulation by functioning as quasi-transcription factors (Landsman & Bustin, 1991). The proteins have structural roles in chromatin related to their ability to unwind double-stranded DNA (Yoshida & Shimura, 1984; Makiguchi et al., 1984; Javaherian et al., 1978), bind preferentially to single-stranded DNA (Bidney & Reeck, 1978; Isackson et al., 1979; Yoshida & Shimura, 1984; Hamada & Bustin, 1985), suppress nucleosome assembly at physiological ionic strength (Waga et al., 1989), and remove the transcriptional blocks caused by left-handed Z-form DNA (Waga et al., 1988) or cruciform DNA (Waga et al., 1990), resulting in stimulation of *in vitro* transcription (Waga et al., 1988, 1990; Tremethick & Molloy, 1986, 1988; Singh & Dixon, 1990). One basic but effective approach for elucidating the putative roles of HMG proteins in transcriptional events would be to test whether the proteins indeed function in cells in culture. Landsman and Bustin (1991) have reported that HMG1 protein did not function as a transcription activator in yeast cells.

In this study, we attempted to assess the transcriptional activation potential of HMG1 protein using COS-1 cells. The results showed that HMG1 protein can stimulate transcription to a certain extent. In addition, the acidic carboxyl-terminal region of the HMG1 protein molecule was found to be necessary for the stimulation to overcome the repressive effect of the DNA-binding regions.

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* To whom correspondence should be addressed.

‡ Department of Biological Science and Technology.

§ Department of Applied Biological Science.

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[1] Abbreviations: bp, base pair(s); CAT, chloramphenicol acetyltransferase; cDNA, complementary DNA; FCS, fetal calf serum; HMG, high mobility group; SDS, sodium lauryl sulfate; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

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MATERIALS AND METHODS

Preparation of Protein. HMG1 protein was prepared from pig thymus chromatin as described previously (Yoshida, 1987). An HMG1 protein variant (1−184 amino acid residues) with a truncated acidic carboxyl-terminal region was overexpressed in *Escherichia coli* BL21 cells transfected with the plasmid pGEM-1ABL carrying the corresponding cDNA sequence downstream from the T7 promoter and purified to homogeneity (Saito et al., in preparation).

Preparation of Plasmid. A reporter plasmid pCH110 carrying the *lacZ* gene downstream from the SV40 origin/early promoter was obtained from Pharmacia. An HMG protein expression plasmid, pcD-MK02, carrying the HMG1 cDNA sequence from pig thymus was constructed in our laboratory (Tsuda et al., 1988). A control expression plasmid lacking the HMG1 cDNA sequence (pcD-cont) was constructed by removal of the cDNA sequence from pcD-MK02 by digestion with *Bam*HI and purification by agarose gel electrophoresis, followed by ligation.

In some experiments, an expression plasmid vector pMIK-neo (constructed and supplied by Dr. K. Maruyama) carrying the SRα promoter upstream from the multicloning site, the SV40 origin/early promoter, and the *neo* gene was used for the construction. The *neo* gene located downstream from the SV40 origin/early promoter in the plasmid was exchanged with the chloramphenicol acetyltransferase (CAT) gene. Then, the HMG1 cDNA sequence (for construction of pMSC-1F) or a sequence encoding the DNA-binding regions (amino acid residues 1−178) obtained by PCR (for construction of pMSC-1ABL) was ligated into the multicloning site. A sequence encoding the *lacZ* gene fragment was also ligated into the site, but in the reverse direction, for construction of a control plasmid (pMSC-cont) of similar DNA size. The nucleotide sequence of each construct was confirmed by the dideoxy sequencing method using Sequenase (USB). The plasmids were prepared by the alkaline−SDS method, followed by purification by equilibrium density gradient centrifugation in cesium chloride (Seyedin & Kistler, 1979).

Linearized pBR322 DNA was prepared for gel retardation assay by digestion of supercoiled plasmid DNA with *Hin*dIII.

DNA Transfection into COS-1 Cells. The transfection of plasmid DNA into COS-1 cells by the liposome method was carried out according to Felgner et al. (1987) with a slight modification. For standard transfection of plasmid DNA complexed with HMG1 protein, 10 μg of reporter plasmid (pCH110) DNA was mixed with 40 μg of HMG1 protein in 20 mM Hepes, pH 7.4, and 150 mM NaCl containing 50 μg of bovine serum albumin and incubated for 30 min at 37 °C. The plasmid DNA or DNA−HMG1 protein complex was then mixed gently with the transfection reagent, Lipofectin (BRL), according to the manufacturer's manual, followed by transfection into COS-1 cells in the Hepes-buffered saline. For cotransfection of the reporter and effector plasmids, 0.75 μg of each respective plasmid and 15 μg of Lipofectin were mixed in the Hepes-buffered saline and kept for 15 min, followed by transfection into COS-1 cells. After incubation for 3 h, the medium was exchanged for Dulbecco's modified Eagle's medium (DMEM, Nissui) supplemented with 10% fetal calf serum (FCS, Gibco BRL) and 0.06 mg/mL kanamycin, and the cells were further incubated for various intervals.

Determination of β-Galactosidase Activity. The harvested COS-1 cells were suspended in 0.25 M Tris-HCl, pH 7.8, followed by three cycles of freezing and thawing. The supernatant was obtained by centrifugation at 12000*g* for 10 min. An aliquot of the cell extract was measured for β-galactosidase activity (Maniatis et al., 1990), which was expressed as units per milligrams of protein.

Direct Detection of β-Galactosidase Activity in COS-1 Cells. The COS-1 cells transfected as described above were incubated in DMEM supplemented with 10% FCS and 0.06 mg/mL kanamycin for 24 h. Then DMEM (supplemented with 10% FCS) containing the chromogenic substrate 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal) at 1 mg/mL was added, and the mixture was incubated for another 24 h. The β-galactosidase activity was visualized by the X-gal staining.

Southern Hybridization. Plasmid DNA was prepared from COS-1 cells by the method of Hirt (1967). The DNA was separated by electrophoresis on 0.7% (w/v) agarose gel, transferred to a GeneScreen Plus membrane (Du Pont), hybridized with a [^{32}P]DNA probe of 1-kbp fragment excised from the *lacZ* gene in pCH110 by double digestion with *Eco*RI and *Sca*I, and labeled with a random primer DNA labeling kit (Takara). The autoradiogram was scanned using a laser densitometer (Molecular Dynamics, PD110).

Northern Hybridization. Whole RNA was prepared from the cells by the guanidine thiocyanate method (Strohman et al., 1977). The RNA was fractionated on 1.1% (w/v) agarose gel, transferred to a Zeta-Probe membrane (Bio-Rad), hybridized with the [^{32}P]DNA fragment from the *lacZ* gene described above according to the method of Thomas (1983), processed for autoradiography, and densitometrically traced.

Gel Retardation Assay. Agarose gel electrophoresis of the complexes of pBR322 DNA with HMG proteins or the variant protein was performed as a function of protein/DNA at various molar ratios. An aliquot of protein solution was mixed with 0.5 μg of DNA in a reaction solution (20 μL) containing 10 mM Tris-HCl, pH 7.8, 100 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 1 mM dithiothreitol, 10% glycerol, and 20 μg of bovine serum albumin. After incubation at 25 °C for 60 min, the solution was electrophoresed on a 1.2% agarose gel in 40 mM Tris−acetate, pH 8.0, containing 1 mM EDTA. After electrophoresis, the gel was stained with ethidium bromide and photographed under UV transillumination.

RESULTS

Stimulation of Transcription in COS-1 Cells by HMG1 Protein When Transfected as a DNA−HMG1 Protein Complex into the Cells. In order to investigate whether HMG1 protein can stimulate transcription in cells in culture, we preliminarily examined an assay system that exploited the intrinsic DNA-binding property of HMG1 protein. HMG1 protein was complexed with reporter plasmid DNA (pCH110) carrying the *lacZ* gene downstream from the SV40 origin/early promoter. The complex was transfected by the cationic liposome method into COS-1 cells, which allow for amplification of the plasmid DNA. Cell extracts were prepared from the transfected cells at regular intervals after transfection, and the effect of HMG1 protein on β-galactosidase activity in the cell extract was determined (Figure 1A). No β-galactosidase activity was detected in an extract

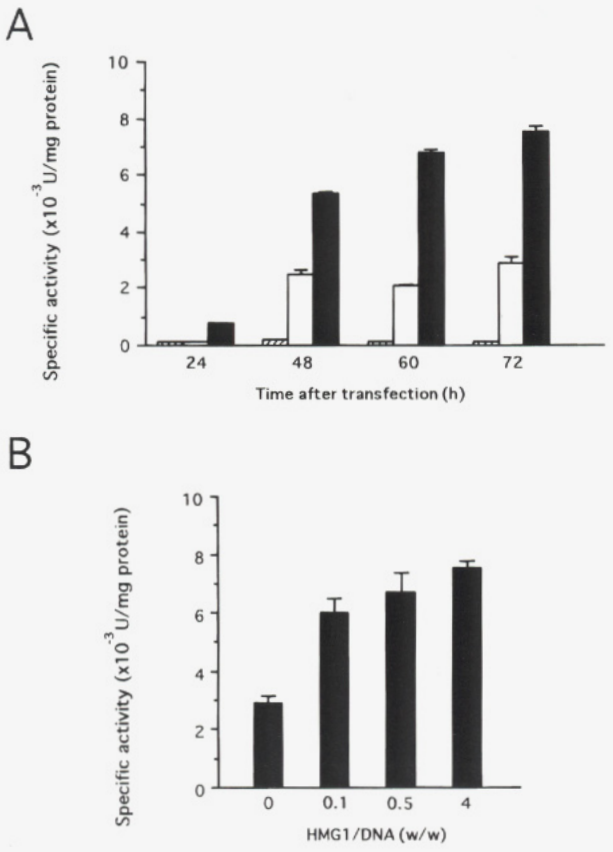

FIGURE 1: Expression of β-galactosidase activity from the reporter plasmid (pCH110) transfected into COS-1 cells as a complex with HMG1 protein. The specific activities, expressed as units per milligram of protein in the cell extract, are the means of five independent determinations, and bars represent standard deviations. (A) The β-galactosidase activity in extracts from COS-1 cells transfected with the reporter plasmid complexed with HMG1 protein (filled columns), reporter plasmid alone (open columns), or HMG1 protein (hatched columns) was determined at 24, 48, 60, and 72 h after transfection. (B) The β-galactosidase activity in extracts from COS-1 cells at 72 h after transfection with the reporter plasmid complexed with the various amounts of HMG1 protein, expressed as w/w.

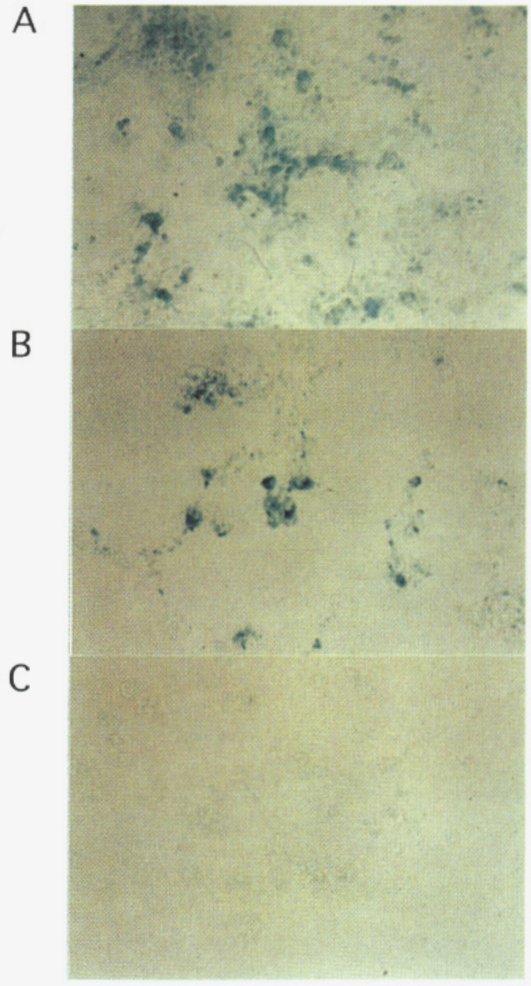

FIGURE 2: Direct detection of β-galactosidase activity expressed from the reporter plasmid in COS-1 cells transfected with the HMG1 expression plasmid (pCH110). The COS-1 cells were incubated for 24 h after transfection with the plasmids and then incubated in the presence of the chromogenic substrate 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal) for another 24 h. Panels: (A) cells transfected with the plasmid complexed with HMG1 protein; (B) cells transfected with the reporter plasmid alone; (C) cells transfected with HMG1 protein alone.

of nontransfected cells or an extract of cells transfected solely with HMG1 protein. By transfection of the reporter plasmid in the presence of HMG1 protein, β-galactosidase activity was enhanced 2−3-fold in comparison with that in the absence of HMG1 protein. The enhancement was dependent on the relative amount of HMG1 protein complexed with the plasmid DNA (Figure 1B). On the other hand, the expression level of β-galactosidase was examined by staining the transfected cells with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal) (Figure 2). The cells that had been transfected with reporter plasmid DNA complexed with HMG1 protein showed higher β-galactosidase activity than those that had been transfected with plasmid alone. The control cells transfected with HMG1 protein showed no β-galactosidase activity. These results suggested that HMG1 protein introduced into the cells with the reporter plasmid DNA had enhanced the expression level of the *lacZ* gene. However, other possible reasons for the enhancement can be considered. First, HMG1 protein complexed with plasmid DNA may accelerate the transfer of plasmid DNA into the nucleus through the intrinsic nuclear localization activity contained in the protein (Kaneda et al., 1989). Second, HMG1 protein may stabilize the plasmid DNA structure against nuclease attack in the transfected cells. Third, HMG1 protein may enhance the replication of plasmid DNA in the transfected cells, resulting in an increase of the plasmid copy number. In order to examine or rule out these possibilities, an additional assay system was employed.

Stimulation of Expression by Cotransfection of Reporter Plasmid with HMG1 Expression Plasmid. An expression plasmid, pcD-MK02, carrying the whole cDNA sequence for pig HMG1 (Tsuda et al., 1988) and the reporter plasmid (pCH110) were cotransfected into COS-1 cells. A plasmid (pcD-cont) lacking the HMG1 cDNA sequence was used as a control effector plasmid. Cell extracts were prepared from the cotransfected cells after various intervals, and their β-galactosidase activity was determined (Figure 3A). The cotransfection of the expression plasmid carrying the HMG1 cDNA enhanced the β-galactosidase activity 2−3-fold relative to that of the control effector plasmid. The maximal β-galactosidase activity was detected at 72 h after cotransfection, as observed in the previous assay (Figure 1A).

To examine the relative amounts of reporter plasmid DNA (pCH110) in the cells cotransfected with the HMG1 expression or control plasmid, total DNA prepared from the cells

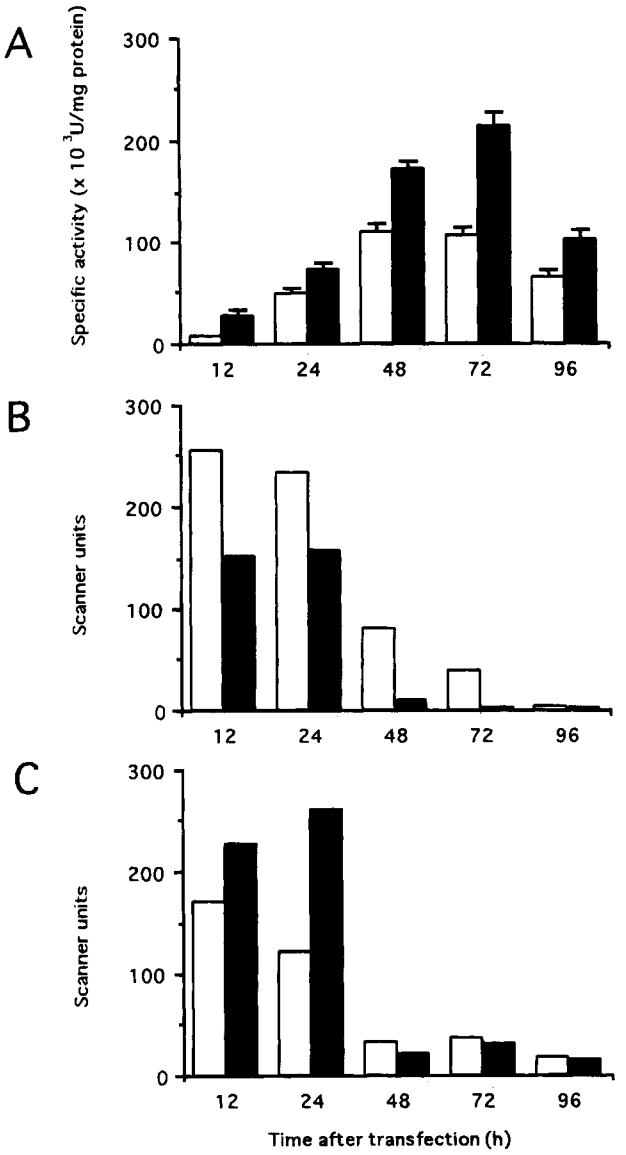

FIGURE 3: (A) Expression of β-galactosidase activity from the reporter plasmid in COS-1 cells cotransfected with the HMG1 expression plasmid. The specific activity of β-galactosidase in the cell extract from COS-1 cells cotransfected with the HMG1 expression plasmid (pcD-MK02) (filled columns) or control plasmid (pcD-cont) (open columns) was determined at 12, 24, 48, 72, and 96 h after cotransfection. The specific activities are means of five independent determinations, and bars represent standard deviations. (B and C) Determination of the amounts of the reporter plasmid DNA and β-galactosidase mRNA in COS-1 cells cotransfected with the HMG1 expression plasmid. Total DNA and RNA prepared from the COS-1 cells of five independent preparations at 12, 24, 48, 72, or 96 h after cotransfection with the HMG1 expression plasmid (pcD-MK02, filled columns) or control plasmid (pcD-cont, open columns) were electrophoresed on agarose gels (2 μg of DNA/lane and 1 μg of RNA/lane), blotted onto membranes, and hybridized with a [^{32}P]DNA fragment from the *lacZ* gene. Relative amounts of plasmid DNA (B) and β-galactosidase mRNA (C) were determined by scanning the autoradiograms using a laser densitometer and expressed as scanner units.

was analyzed by Southern hybridization using the *lacZ* gene as a probe. The amount of reporter plasmid DNA decreased quickly after 48 h in the cells cotransfected with the HMG1 expression plasmid as well as in those transfected with the control plasmid, and the amount of the plasmid DNA in the former cells was somewhat lower than in the latter (Figure 3B). Northern hybridization using the *lacZ* gene as a probe

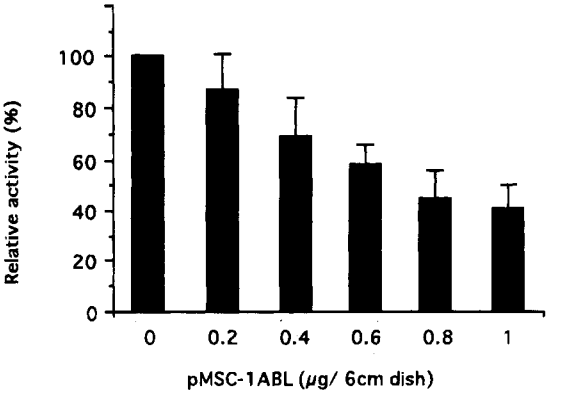

FIGURE 4: Expression of β-galactosidase activity from the reporter plasmid in COS-1 cells cotransfected with the acidic carboxyl-terminal-truncated HMG1 expression plasmid. In this experiment, the reporter plasmid (pCH110) was cotransfected with an increasing amount of acidic carboxyl-terminal-truncated HMG1 expression plasmid (pMSC-1ABL) supplemented with the control plasmid (pMSC-cont) to unify the total amount (1 μg) of effector plasmid. The specific activity of β-galactosidase in the extract from COS-1 cells was determined at 72 h after cotransfection. The activities relative to that in the cells cotransfected with the control effector plasmid are presented. The relative values are means of four independent determinations, and bars represent standard deviations.

showed that the expression of β-galactosidase mRNA in the cells cotransfected with the HMG1 expression plasmid was enhanced in comparison with that of cells cotransfected with the control plasmid, although the expression in both cells decreased rapidly during culture after 48 h of cotransfection (Figure 3C). The fluctuation in the expression of β-galactosidase mRNA paralleled that of HMG1 mRNA. Together, the cotransfection experiments ruled out the several possibilities described above and indicated that HMG1 protein is capable of stimulating expression in COS-1 cells at the step of reporter gene transcription.

Requirement of the Acidic Carboxyl-Terminal Region of HMG1 Protein for Stimulation. Two-thirds of the HMG1 protein molecule consists of two DNA-binding regions each of about 70 amino acid residues, known as HMG boxes (Jantzen et al., 1990). The residual acidic carboxyl-terminal region consists of a serial array of 30 acidic amino acid residues (Tsuda et al., 1988), and this is expected to be involved in the stimulation of gene expression. To examine the requirement for the acidic carboxyl-terminal region in the stimulation, an expression plasmid (pMSC-1ABL) encoding the acidic terminal-truncated HMG1 molecule was constructed. β-Galactosidase activity was examined by cotransfection of the plasmid with the reporter plasmid. As the amount of the effector plasmid increased, the β-galactosidase activity decreased depending on the relative amount of the effector plasmid (Figure 4). The amount of reporter plasmid in the cells cotransfected with the effector plasmid was similar to that in the cells cotransfected with the HMG1 expression plasmid (pMSC-1F) or the control one (pMSC-cont). These results demonstrate that the DNA-binding domains comprising two-thirds of the HMG1 protein molecule inhibit gene expression and also that the acidic terminus of the HMG1 molecule is necessary for stimulation of the expression.

Possible Reduction of DNA-Binding Activity of HMG Boxes by the Acidic Carboxyl-Terminal Region of HMG1 Protein. In order to estimate the effect of the acidic carboxyl-terminal region on the binding of HMG1 protein

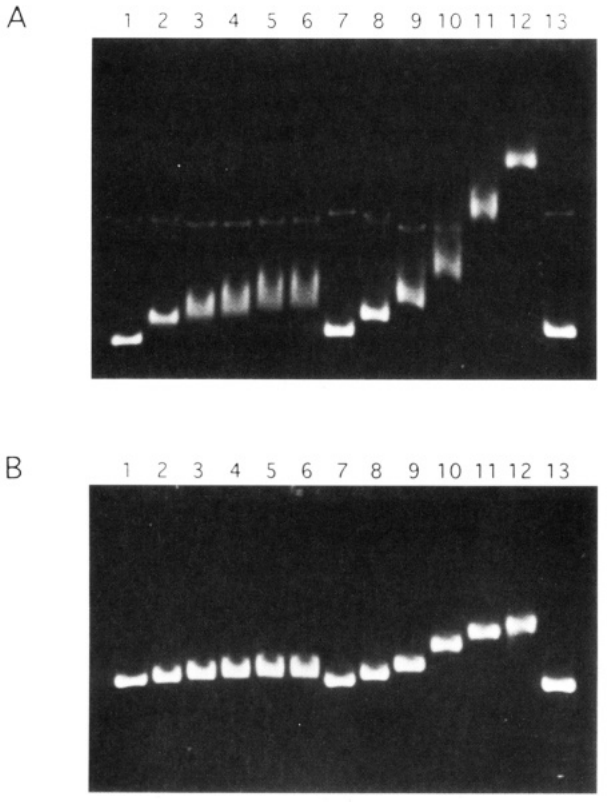

FIGURE 5: Gel retardation of DNA by HMG1 protein and its variant. Different concentrations of HMG1 protein (lanes 1−6) or the variant (lanes 7−12) were complexed with negatively supercoiled pBR322 DNA (panel A) or the linearized DNA (panel B) at various molar ratios and separated by agarose gel electrophoresis. The molar ratios of protein to pBR322 DNA in lanes 1−6 and 7−12 were 0, 60, 120, 180, 240, and 300, respectively. Lane 13 contained DNA alone.

to DNA, an HMG1 protein variant (1−184 amino acid residues) lacking the acidic carboxyl-terminal region was overexpressed in *E. coli* cells and purified. The ability of the variant to bind to DNA was examined using the gel retardation assay (Figure 5). The band shifts of DNA shown by the variant were markedly larger than those shown by the complete molecule when compared at equimolar ratios of DNA-binding region to DNA. If it is assumed that the relative band shift mainly correlates with the amount of protein bound to DNA, then the acidic carboxyl-terminal region of the HMG1 protein may decrease the DNA-binding ability of the HMG boxes in HMG1 protein.

DISCUSSION

Although several *in vitro* experiments have suggested that HMG1 protein may stimulate gene transcription (Waga et al., 1988, 1990; Tremethick & Molloy, 1986, 1988; Singh & Dixon, 1990), data on the molecular functions of HMG1 protein in these reactions are contradictory or obscure. The assessment in yeast cells has shown that HMG1 protein did not function as a transcription activator (Landsman & Bustin, 1991). In the present study, two systems in cells in culture were employed in order to examine the effect of HMG1 protein on transcription. HMG1 protein introduced into COS-1 cells as a complex with a reporter plasmid stimulated the enzyme activity derived from the reporter gene (Figures 1 and 2). In addition, HMG1 protein expressed in COS-1 cells stimulated the enzyme activity from the reporter gene in the plasmid cotransfected with the effector plasmid (Figure 3). The assay systems as well as the Southern and Northern analyses proved that the stimulatory effect of HMG1 protein was due to an enhanced level of transcription of the reporter gene. In the present assay, the expression of the reporter gene was measured by enzyme activity for β-galactosidase. The maximum β-galactosidase activity was expressed at 72 h after transfection (Figure 1A) or cotransfection of the reporter plasmid (Figure 3A), although the amount of β-galactosidase mRNA in the cells decreased quickly after 48 h of cotransfection (Figure 3C). A similar result was obtained with a reporter plasmid harboring a gene for chloramphenicol acetyltransferase (data not shown). These results may suggest that β-galactosidase is relatively stable protein in COS-1 cells, although mRNA for the enzyme is unstable. Therefore, the enzyme activity at various hours after transfection of the reporter plasmid may represent the amount of enzyme protein synthesized and accumulated in the cells before preparation of the cell extract and may be not proportional to the amount of mRNA in cells at the time of the preparation.

The enhancement of expression by HMG1 protein was 2−3-fold in comparison with the control (Figures 1 and 3). In addition, we have found that the stimulation and its level may not be dependent on the species of the promoter and the gene (unpublished data). The reporter plasmid DNA in the COS-1 cells cotransfected with the HMG1 expression plasmid appeared to be destabilized because the plasmid DNA was more susceptible, although only to a slight extent, to micrococcal nuclease digestion than that in cells cotransfected with the control expression plasmid (Ogawa et al., in preparation). HMG1 protein affects several processes that involve the unwinding of DNA (Yoshida & Shimura, 1984; Makiguchi et al., 1984; Javaherian et al., 1978), including repression of nucleosome assembly (Waga et al., 1989) and inhibition of the formation of Z-DNA and cruciform structures in negatively supercoiled plasmid DNA (Waga et al., 1988, 1990). These properties suggest that HMG1 protein, which binds to DNA in a non-sequence-specific manner (Waga et al., 1990), modulates the gene structure by altering the conformation of the entire plasmid DNA. HMG proteins are relatively abundant in the nucleus, 10^6 copies per cell nuclei (Goodwin et al., 1978). Calculation based on the copy number suggests that endogenous HMG1 protein in the cells may bind with DNA at a ratio of one molecule of the protein to several thousand nucleotides. Thus, the amount of HMG1 protein in the nucleus seems not sufficient for the structural modulation of nucleosome with which HMG1 protein binds. The apparent enhancement of transcription by HMG1 protein cotransfected with the reporter plasmid (Figures 1 and 2) or expressed from the effector plasmid (Figure 3) in COS-1 cells may suggest that additional HMG1 molecules are needed to modulate chromatin structure. The structural modulation produced by HMG1 may cause a limited change in the level of gene expression independent of the species of the promoter and the gene. The change of the relative amount of proteins in chromatin in the cells by overproduction of HMG1 protein may support the possibility (Ogawa et al., in preparation).

The complete primary sequences of HMG1 protein from pig (Tsuda et al., 1988), rat (Paonessa et al., 1987), calf (Kaplan & Duncan, 1988), and human (Wen et al., 1989) have been deduced from the nucleotide sequences of their cDNAs. The amino-terminal two-thirds of the molecule

contains two DNA-binding domains with similar sequences, each about 70 amino acid residues long. The sequence known as the "HMG box" is also present in many eukaryotic transcription factors (Laudet et al., 1993). The carboxyl-terminal domain is an array of 30 consecutive acidic amino acids (Tsuda et al., 1988). The structure of HMG1 protein is reminiscent of certain transcription factors such as GAL4, GCN4, and VP16 (Landsman & Bustin, 1991). The charged amino acids are distributed asymmetrically along the polypeptide chains. The negative residues are clustered toward the amino-terminal region. If it is assumed that the amino-terminal two-thirds of the HMG1 molecule participates only in binding to DNA, then the remaining carboxyl-terminal domain may be involved in the stimulatory effect on the expression of the reporter gene. The DNA-binding activity of the carboxyl-terminal-truncated HMG1 molecule seems to be stronger than that of the complete HMG1 molecule (Figure 5), suggesting that the carboxyl-terminal domain decreases the DNA-binding activity. The binding of HMG boxes with an extremely negative net charge to DNA may stabilize the DNA structure, as in the case of histones as shown in primary experiments (van Holde, 1988). Also, the carboxyl-terminal-truncated HMG1 repressed the expression of the reporter gene, depending on the amount cotransfected (Figure 4). These results strongly suggest the necessity of the acidic domain for stimulation of gene expression. Considering that the carboxyl-terminal domain of the HMG1 protein has activity to lower the melting temperature of DNA in the presence of divalent cation (Yoshida, 1987), this domain may destabilize the structure of DNA under the stress caused by its supercoiling and/or nucleosome organization (Waga et al., 1988), leaving the DNA structure necessary for efficient transcription. These findings demonstrate that the transcription-activating domain of HMG1 is localized to the acidic terminal domain, similar to transcription factors such as GAL4, GCN4, and VP16 (Landsman & Bustin, 1991). However, the acidic carboxyl-terminal domain of HMG1 protein may function in a manner different from those of the transcription factors, because it cannot form an amphipathic α-helix.

The present data represent first and direct evidence that HMG1 protein can function in cells in culture as a quasi-transcription factor in the process of gene transcription, different from the more intensively studied transcription factors. The binding of the HMG box to DNA and the subsequent repression of gene transcription may be important for helping to clarify the functional mechanism of the many transcription factors containing the DNA-binding motif (Laudet et al., 1993) as well as that of HMG1 protein. The newly revealed role of the acidic carboxyl-terminal domain of HMG1 protein in overcoming the repressive effect of the HMG box may open a new avenue of investigation for clarifying the function of the protein. In addition, the nature of the modulatory alteration of DNA and chromatin structure by HMG1 protein in the cell nucleus is currently under investigation.

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