ENHANCED EXPRESSION OF HMG-Y PROTEINS IN PROLIFERATING TISSUES

Dinesh Singh and M.R. Rajeswari*

Department of Biochemistry, All India Institute of Medical Sciences, New Delhi-110029, India

ABSTRACT

The high mobility group (HMG) proteins I and Y are well characterised non-histone chromosomal proteins which bind to A-T rich regions of DNA and regulate gene expression and / or DNA replication. A correlation has been demonstrated between the increased expression of HMG-Y proteins and malignancy. However, it is not known whether the expression of HMGs particularly, the Y group, is a function of proliferation rate. In the present study, we have used normal tissues of calf testes, thymus and liver. The results show distinctly high expression of HMG- Y proteins in testes than in thymus and the expression was practically undetectable in liver. The results suggest that even in normal tissues there is a direct correlation between the proliferation rate and the expression of the HMG-Y proteins, which can partly explain its increased expression in cancer.

KEY WORDS : HMG- Y proteins, Cancer, Cell Proliferation

INTRODUCTION

The high mobility group (HMG) proteins are a class of low molecular weight non-histone chromosomal proteins that bind to DNA and function as architectural transcription factors (1). These are named as HMG proteins because of their rapid migration during acid urea polyacrylamide gel electrophoresis (2). HMG proteins are classified into three distinct families: HMG-1/2, HMG-14/17 and HMG I family. HMG 1/2 proteins participate in the regulation of chromatin structure and act as positive or negative regulatory factors in various aspects of DNA replication (3). HMG 14 / 17 proteins associate non-specifically with nucleosomes at transcriptionally active sites. HMG-I family is composed of HMG I, HMG Y and HMG C proteins. The HMG-I and HMG-Y are isoforms and are generally referred as HMG I(Y) proteins while, HMG-C is a product of a separate gene. The HMG I(Y) proteins contain multiple copies of A-T hook motif which bind to the A-T rich regions of DNA and presumably alter the architecture of chromatin by binding to the base- unpairing regions [BUR], (4) and

enhance transcription (3,5). Recently there is a sudden and increased interest in the HMG Y proteins because of their role in tumorigenesis and its strong correlation with the metastatic potential of cancer (6-10). There are also reports suggesting HMG Y as a potential marker for malignancy and aggressiveness of various tumours like colorectal carcinomas (11), thyroid tumors (10), uterine cervix (12). Recently HMG Y gene at 6p21 has also been shown to be rearranged in lipomas and chondroid hamartomas (13). Adenovirus mediated suppression of HMG Y protein synthesis is also tried as a potential therapy of neoplasias (14).

In our laboratory, we have been working on DNA-Protein interactions and are presently engaged in the work related to the relationship between HMG Y and proliferation rate in skin carcinomas (15). There is considerable evidence to support the fact that HMG Y expression is higher in the malignant tissues, however, it is not clear whether the proliferation rate (usually elevated in malignancy) contributes to the increased expression of HMG Y proteins. Testes and thymus were chosen as tissues with higher proliferation rate and high fraction of cells undergoing cell division while, liver was chosen as tissue with lower fraction of cells undergoing division. Here, we report the results on the expression of HMG proteins

***Author for correspondence :**

Dr. M.R. Rajeswari, Associate Professor, at the above address. Fax : 91-11-6862663,
Email : mrraji@hotmail.com

(particularly I, Y) as a function of proliferation rate in various normal tissues of calf. These results are expected to help in our understanding of the role of HMG- Y in normal proliferating tissues.

MATERIALS AND METHODS

Acrylamide, bisacrylamide, urea, coomassie blue G-250, BSA were purchased from Biorad Co. Ltd. USA. All other reagents used in making buffers were of AR grade from Qualigens, Glaxo. Calf thymus, testes and liver tissues were obtained locally.

The protein extraction was done 1 hr. after the slaughter of the animal. However, the tissues were stored at 4°C during the transport from the slaughterhouse to the laboratory. The protein stability is not affected during this period. The entire process of extraction of HMG proteins was done at 4°C. The total HMGs were extracted using the method of Goodwin et al., (16) using 5% perchloric acid (PCA). The thawed tissue was homogenized with 2 volumes of 5% Perchloric acid (PCA) for 3 minutes and was left on ice for 10 minutes. This was centrifuged for 15 minutes at 5000 rpm and the supernatant was stored on ice. The pellet was re-extracted with two more volumes of 5% PCA for 3 minutes and was centrifuged for 15 minutes. The supernatant and the previously stored supernatant were pooled and the combined supernatant was centrifuged at 20,000-x g to discard the suspending particles. The supernatant obtained was filtered through 4 layers of cheesecloth to get a clear solution. Ice cold trichloroacetic acid (TCA) was added to the extract to a final concentration of 20%. This was left on ice for 16 hours for precipitation of HMG proteins. This suspension was centrifuged at 10,000-x g for 30 minutes. The HMG protein pellet obtained was washed with ice cold solution of acidified acetone (acetone:HCl 400:1, v/v) and thrice with ice cold acetone alone. The washed pellet was dried under vacuum.

The extraction of histone 1 was done by the method described by Elgin and Bonner (17). The total HMG protein was estimated by Lowry's method (18). Approximately 0.5mg of the total HMG was obtained

from a tissue of 30 gm.

Reverse Phase-HPLC [RP- HPLC] analysis of different fractions of calf testes, thymus and liver HMG proteins was carried out on a Waters HPLC system using a Vydac C-4 column of dimensions 250mm x 4.6 mm (I.D.); pore size 300Å and 5µm particle size. The total HMG sample of 100µg was dissolved in solvent A [containing 95% water 5% acetonitrile, 0.1% trifluoroacetic acid (TFA)] and injected onto the column and eluted with a 15-55% linear gradient of solvent B (containing 95% acetonitrile, 5% water and 0.1% TFA) for a period of 40-50 minutes at a flow rate of 1ml/min.

Acid urea polyacrylamide gel electrophoresis was carried out on a Biorad Mini Protean II Electrophoresis apparatus at 4°C using a Cooling Module, Biorad. The total HMG from calf thymus, testes and liver were separated by a procedure given by Elton and Reeves (19). However, their procedure was modified and the following experimental conditions so as to give better resolution. The acrylamide concentration was 18% and had a monomer to cross-linking ratio of 30:0.375. The resolving gel was prepared by mixing acrylamide, N,N' methylene bisacrylamide mix, 9 M urea, acetic acid and 0.5% ammonium persulphate (APS). Polymerisation was initiated by tetramethylenediamine (TEMED). After the resolving gel was polymerised, a 7.5% stacking gel was polymerised over it.

The pre-electrophoresis was done for 6 hours at 150 V with 0.9 M acetic acid as the electrophoresis buffer. The vacuum dried HMG pellets solubilized in 7M urea, 3% mercapto ethanol and 0.9 M acetic acid were loaded on the gel and calf thymus HMG proteins were loaded as markers. Electrophoresis was carried out at 80 V till the bands moved up to the end of stacking gel. Afterwards electrophoresis was carried out at 120 V. The electrophoresis was allowed to run for half an hour after the tracking dye was eluted. This usually took three hours for a gel of size 8 x 8 cm. After electrophoresis, the gel was stained with 0.1% coomassie brilliant blue R-250 in a mixture of methanol, acetic acid and water (50:10:40 v/v/v) for an hour and destained with methanol, acetic acid, water (10:10:80

v/v) once the background became clear, a photograph was taken using a yellow filter.

RESULTS AND DISCUSSION

Reverse phase high performance liquid chromatography (RP-HPLC) using a multi-step acetonitrile gradient is capable of resolving all the major groups of HMG proteins in addition to histone H1. We did the separation of HMGs and the RP-HPLC profile of the total HMG proteins of calf testes, thymus and liver (Fig. 1).

Fig 1. RP-HPLC analysis of different fractions of PCA soluble total HMG proteins from calf testes (A), thymus (B) and liver (C). Proteins sample (100µg) was dissolved in solvent A (0.1% TFA) injected onto the C-4 Vydac column and eluted with a 15-55% linear gradient of solvent 8 (95% acetonitrile, 5% H₂O, 0.1% TFA) in 40 minutes at a flow rate of 1ml / min. Absorbance was monitored at 214 nm. Elution peaks 1 to 7 were identified as HMG 17, HMG-Y, HMG-14, HMG-I, histone H1, HMG-2 and HMG-1 respectively, however, in panel C, the peak 2 corresponding to HMG Y is absent.

The elution of HMGs obtained with time were in the order HMG-17, HMG-Y, HMG-14, HMG-I, histone H1, HMG-2 and HMG-1 respectively correspond to the peaks 1 to 7 in panels, A to C. The identification of various peaks were done using calf thymus as the

standard reference and these assignments of HMGs were in good agreement with those reported by Elton & Reeves (19). However, the minor fractions of the isoforms and probably the modified forms of some of the HMGs can be seen as unresolved peaks closer to the baseline. The RP-HPLC profile of HMGs from the testes, thymus and liver are in general similar except for the absence of HMG-Y corresponding to peak 2 in the panel C (liver) of Fig. 1.

However, the peak intensity of HMG-Y in testes (panel A) is higher than that is seen with thymus (see peak 2 panel B, Fig 1.) It may also be noticed that there is a slight decrease in the concentrations of HMG 14 and HMG I in thymus and liver as compared to testes. (compare peaks 3 and 4 in in Fig. 1.)

Acid urea gel electrophoresis of the total HMG proteins extracted from calf thymus, testes and liver are shown in Fig. 2.

Fig 2. Acid urea polyacrylamide gel electrophoresis of the total HMG proteins extracted from various tissues of calf. Total HMG proteins of 10 µg each were dissolved in sample buffer (9M urea, 0.9M acetic acid, 1% mercaptoethanol, Pyronin-Y 0.5mg/ml) and loaded on to a 18% polyacrylamide gel and detected by coomassie blue. Lanes 1 to 4 represent histone H1 (marker) and total HMG proteins from calf testes, thymus and liver respectively.

A total HMG of 10 µg each from calf thymus, testes and liver was loaded on lanes 2, 3 and 4 respectively. The histone 1 (lane 1) and HMGs from calf thymus (lane 2) were used as protein markers (Fig. 2). It may be mentioned that the standard protein markers used for the routine SDS-PAGE analysis can not be used for the acid urea electrophoresis. The most prominent band is about 22 KD of histone H1 that remains in the extract as a contaminant and is generally used as an internal marker. HMGs 1 and 2 (25 KD) migrate slightly behind the histone H1 (22 KD). The HMG I, Y and 14 with very low masses move like a group and can be seen at the center of the gel profile. The HMG-I (12 KD), HMG-14 (11.9 KD) migrate closely, while HMG-Y with about 10.6 KD moves ahead. HMG-17, with lowest molecular weight of 10 KD is the fastest migrating protein (19). A common feature that can be noticed from the Fig. 2 is that the gel pattern in general from all three sources is similar. The expression of HMG 1, 2 and 17 are practically unchanged while, the HMG-14 is only marginally increased, which is in agreement with the RP HPLC profile.

Significant feature in Fig. 2 is that the expression of HMG-Y in testes is higher than in thymus while it is practically undetectable in the liver, and this is in support of the HPLC analysis as given in Fig. 1. The expression of HMG-I is also slightly higher in the testes and thymus as compared to liver. These findings suggest that the expression of HMG-Y and to some extent that of HMG-I is constitutively higher in tissues with higher proliferation rate like testes and thymus.

It is known from the previous studies that the expression of HMG I(Y) is greater in carcinomas and

was correlated with the stage of the tumor (6,7,8,10). However, the present results indicate a differential expression of HMG I(Y) proteins even in normal tissues as a function of proliferation rate. In prostate (20), thyroid (10) and cervical (12) carcinomas, it is shown that there is an enhanced expression of both the isoforms of HMG I(Y) proteins; the individual HMG isoforms I and Y were, however, not clearly identified. In colon carcinomas, it has been shown that there is a preference for the translation of HMG-I, the longer isoform of the HMG I(Y) proteins (11). The variation in the levels of HMG-I and HMG-Y as observed in the present study suggests that these two proteins have different biological functions although both have structural similarities.

It has been recently shown that NF-κB which is a transcription factor can not perform its function unless an additional co-activator is present and this transcriptional co-activator was later identified as HMG-Y (20). The HMG-Y proteins are also reported to interact with certain other sequence specific transcription factors that bind to DNA major groove (e.g., NF-κB, C-Jun, C-Rel, ATF-2, Elf-1, Ets, SP-1 and Oct-6) (1).

In view of the above facts, high levels of HMG-Y proteins are expected in the transcriptionally active tissues like testes, placenta and thymus. As elevated expression of HMG-Y protein is found in various tumors, we suggest that the proliferation rate, which is usually high in majority of tumors, do play a role in elevated expression of HMG I(Y).

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