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CHARACTERIZATION OF HIGH MOBILITY GROUP NONHISTONE PROTEINS FROM DIFFERENTIATED NEUROPHILIC GRANULOCYTES

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Abstract

The quantitative changes of the high mobility group (HMG) nonhistone chromosomal proteins in nondividing, differentiated peritoneal exudate neutrophils were identified by their solubility, electrophoretic mobility on both SDS and acetic acid gels, densitometric traces and elution profile on CM-sephadex. The results indicated that in neutrophils, HMG1 undergoes a considerable reduction (80%) compared to thymus proteins, However, HMG2 and HMG17 remain constant. Also, HMG14 was not detectable in neutrophils implying the possible role of the HMG proteins in genome function.

Introduction

The high mobility group (HMG) of nonhistone chromatin proteins are heterogeneous and consist of four major proteins HMG1, HMG2, HMG14, and HMG17 [1]. These proteins constitute two groups; HMG1 and 2 are similar proteins with a considerable sequence homology and show a preferential binding to single stranded DNA [2,3]. HMG14 and HMG17, on the other hand, have lower molecular weights and bind to similar or identical sites on the nucleosomes [4]. Although the function of these proteins *in vivo* is not clear yet, it has been postulated that they probably have an important role in the cellular processes including replication and transcription [5,6].

HMG proteins have been characterized in a wide variety of tissue and organisms implying their widespread occurance in eukaryotic cells [1]. Tissue specific alterations of protein content during differentiation might occur in which the cells achieve their specific set of structural and functional characteristics [7]. Neutrophilic granulocytes, morphologically characterized by a very dense and segmented nucleus, are a good example of nondividing highly differentiated end cells. It has also been assumed that these cells have little, if any, capacity for protein synthesis [8]. The present paper describes a dramatic quantitative change of HMG proteins in neutrophils.

Key words: High-Mobility-Group, Neutrophilic granulocytes; Non-histone proteins.

Materials and Methods

(Razi Institute, Hesarak)

Wistar Albino rats 150-250g of either sex were used throughout the experiments. All processes were carried out at 4°C except when otherwise stated.

Rats were intraperitonealy injected with 1% starch or glycogen in saline (0.14M NaCl,1% glucose, pH 7.4). After 4-5 hrs animals were anaesthetized by ether and the cells were collected from the peritoneal cavity by rinsing out with 30ml saline. The cells were pelleted by centrifugation for 15 min at 2000g, washed once with saline and counted by hemocytometer. The morphology and purity of the cells were examined using cytocentrifuged slides stained with Wright-Gimsa stain.

Approximately (5×10^6) cells were directly extracted three times each with three volume of 0.74N perchloric acid (PCA) and HMG proteins separated from histone H1 by fractional acetone precipitation as described previously [9]. HMG proteins were also isolated from calf thymus using the same procedure

as a control. PCA extracted proteins from neutrophils were analysed on both 20% acetic acid [10] and 15% sodium dodecyl sulfate (SDS) [11] polyacrylamide gels against thymus HMG proteins. The acid gels were stained with 0.2% procion navy and SDS gels with comassie B.B. as described.

Stained cylindrical acid gels were scanned at 600nm using gel scanner model GSC-260 for Shimadzu spectrophotometer and the quantity of the proteins were calculated by comparison of areas under the gel scan protein peaks with the area of a known amount of thymus HMG proteins.

Elution profile was determined using CM-Sephadex column (1×5cm) and NaCl salt gradient [9]. Conductivity of the fractions was measured at 25°C. The content of protein was determined by the method of Lowry [12].

Results and Discussion

Total number of peritoneal exudate neutrophils obtained from one rat was an average of $3-4\times10^8$ cells. Examination of the cell morphology of stained cytocentrifuged preparations showed that the cells were intact and nearly 80–90% neutrophilic granulocytes.

For isolation of total HMG proteins, PCA extraction procedure was used to minimize degradation products of other nuclear proteins instead of isolating chromatin and extracting with 0.35M NaCl. Proteins thus obtained were analysed on acid and SDS gels. Calf or rat thymus HMG proteins were also prepared with the same procedure and used as standard. Fig la shows the comparison of HMG proteins from thymus and neutrophils. It is apparent that proteins with an electrophoretic mobility identical to thymus HMG2 and 17 exists in neutrophils, but only a very faint band is visible on HMG1 position (laB). No band was also identified for HMG14 protein.

Quantitative analysis of the procion navy stained gels were measured by densitometric scanning (Fig 1b). Calculation of the area under the peaks indicated that HMG1 protein shows 80% reduction in neutrophils and no peak was observed in HMG14 position. The amount of HMG2 and 17 were the same in both tissues. Analysis of the proteins on SDS gel was also in agreement with results obtained above (Fig 2).

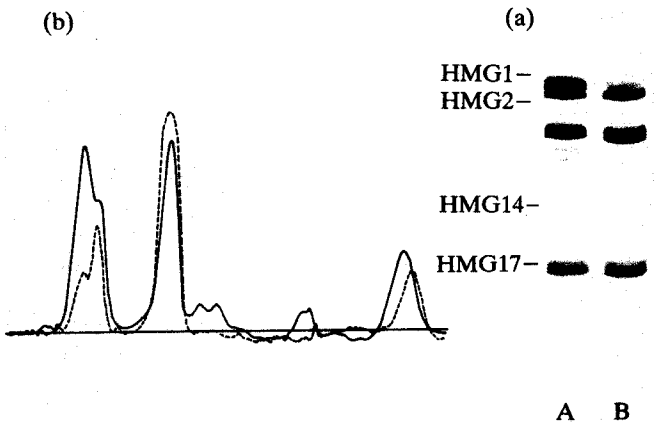

Fig 1: (a) acid-polyacrylamide gel electrophoresis pattern of total HMG proteins from neutrophils and thymus A: thymus B: neutrophils
(b) densitometer scan of both proteins at 600nm thymus HMG proteins ... neutrophils HMG proteins

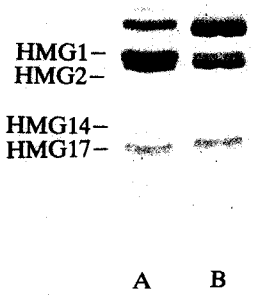

Fig 2: 15% SDS polyacrylamide gel patterns of HMG proteins form A; Thymus B;Neutrophils

CM-Sephadex chromatogram of total HMG proteins from neutrophils in comparison with thymus proteins are given in Fig 3. Eluted proteins were detected by their absorbance at 230 and 280 nm. HMG1 and 2 eluted at the beginning of the pattern, the quantitative changes of HMG1 in granulocytes is clearly shown by (peaks A and B). However, HMG17 and H1 are eluted with salt gradient (peaks C and D) respectively.

As far as tissue specificity is concerned, it has become apparent that, although thymus and neutrophils have HMG proteins they do differ in number and composition. Seydin and Kistler have reported that in different rat organs, the loss of proliferative activity is associated with a depletion of HMG2, whereas the level of HMG1 remains unchanged [15]. Bucci et al. have also reported a substantially high level of HMG2 in testis and absence of HMG proteins from late spermatides [7]. Although no direct evidence for the specific roles of HMG1 and HMG2 exist, it was observed that the level of both proteins are decreased in terminally differentaited cells [14].

HMG1 protein shows a preferential binding to single stranded DNA and unwinds it [15,16]. On the

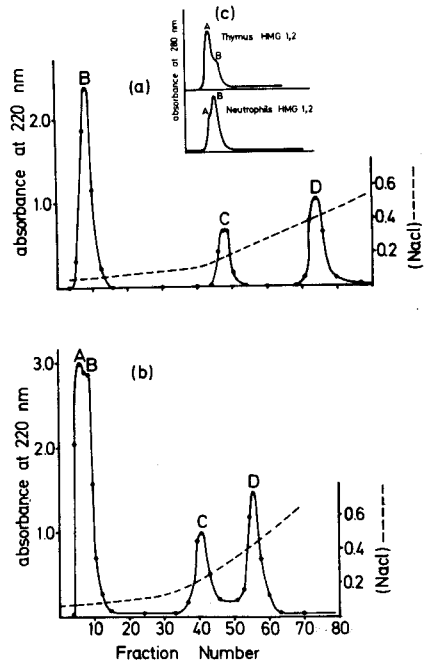

Fig 3: Chromatography of thymus and neutrophils total HMG proteins on CMC-52 column. (a) Neutrophil HMG proteins, (b) Thymus HMG proteins, (c) Comparisons of patterns measured at 280nm.

other hand, it has also been reported that actively transcribed genes in chromatin become sensitized to DNasel by binding of HMG14 and 17 to nucleosomes [6].

From these results outlined above it is concluded that reduction in HMG proteins content in a fully differentiated cells with no capacity for dividing and proliferation is an indication of their role in genome activity such as replication and transcription. Further studies will be necessary to prove this possibility.

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