BINDING OF CHROMOSOMAL NON HISTONE PROTEINS TO DNA AND TO NUCLEOHISTONES. EFFECT OF IN VITRO PHOSPHORYLATION

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1. Introduction

The possibility that chromosomal non-histone proteins (NHP) could be regulators of gene expression in eukaryotic cells is suggested by chromatin reconstitution experiments and by DNA/RNA hybridation analyses [1–2]. These experiments showed that NHP modified transcription in a manner characteristic of the tissue of origin. But the mechanisms by which the NHP was able to interact and to modify the transcription are not completely clear. However, several studies have implied that NHP is able to stimulate the template activity of nucleohistones, at least partly, through the presence of phosphoproteins and of protein kinases [3]. Similarly, NHP modifications are associated with the stage of the cell cycle and with the differentiation of cells during development and aging [4–8].

A direct role for NHP cannot be excluded since some NHP does bind to DNA [9,10]. In chromatin however, large parts of DNA are covered with histones [11,12]. Histones have been demonstrated to play a role in the structure of DNA and in the chromatin conformation [13,14]. They have an inhibitory effect on the transcription availability of DNA. This inhibitory action can be partially suppressed by histone-phosphorylation [15].

In this work we have studied comparatively the binding of NHP to DNA and to formaldehyde-treated nucleohistones, this last technique allowing a covalent binding of histones to DNA [12,16]. DNA and formaldehyde-treated nucleohistones (FNH) were fixed on a cellulose matrix and poured into a column. A small NHP fraction remained bound and could be eluted by increasing ionic strength. Protein peaks eluted from the column were analysed on polyacrylamide gel electrophoresis. The results were identical with DNA, with FNH and with phosphorylated nucleohistones. However, when NHP was phosphorylated by endogenous protein kinases, a smaller proportion remained bound to the column.

2. Materials and methods

NHP was prepared from rat liver nuclei as previously described [17]. A sepharose 6 B column was used to make sure that NHP was free from any DNA contamination. Nucleohistones were recovered during NHP preparation when DNA-histone coprecipitation occurs, by decreasing the ionic strength of the nuclear extract.

DNA-cellulose was prepared according to Alberts et al. [18]. It contained 3 mg of DNA per g of cellulose powder.

Nucleohistones were treated by 0.1% formaldehyde at 4°C overnight and dialyzed against 10 mM Tris-HCl pH 8.0, 10 mM β-mercaptoethanol, mixed with extensively washed cellulose powder and dried under nitrogen flow at room temperature. It was then lyophilized and washed with 50 mM Tris–HCl pH 8, 10 mM β-mercaptoethanol, 2 M NaCl until all unbound material was removed. The column was then equilibrated with 50 mM NaCl, 10 mM Tris–HCl pH 8.0 starting buffer. This material will be referred to as formaldehyde-treated nucleohistones (FNH). It contained 3 mg of DNA per g of dry powder and has a protein DNA ratio ranging from 0.6 to 0.9.

Histone and NHP phosphorylations were performed as previously described [19,20].

A sample of 1.65 mg of NHP was loaded either on DNA cellulose or on FNH cellulose columns containing 3 mg of DNA. In each case, NHP and cellulose were previously equilibrated with the starting buffer. The columns were then washed with the same buffer. Stepwise elution was processed with the same buffer containing 0.15, 0.6 and 2 M NaCl. Optical density was automatically recorded at 230 and 280 nm. In control experiments with cellulose alone, there was no binding of NHP to the column.

NHP and the protein peaks from the columns were submitted to polyacrylamide gel electrophoresis for 5 hr according to Panyim and Chalkley [21]. P_{32} radioactivity was determined in gel slice as previously described [4].

3. Results

3.1. *NHP binding to DNA cellulose and FNH cellulose*

The same amounts of NHP were loaded on both types of columns. The overall recovery was 70–80%. The columns were further washed with the starting Tris-buffer containing 0.05 M NaCl. The eluted peak constituted 93% of the recovered material. The proteins were then eluted by raising the NaCl concentrations in two separate steps of 0.15 M NaCl and 0.60 M NaCl. Higher NaCl concentrations (2.0 M NaCl) did not remove any significant amount of material. This elution pattern was highly reproducible (fig.1).

We have compared the electrophoretic patterns of proteins recovered from DNA-cellulose column (fig.2). It can be seen in figs.2B-2D that the electrophoretic patterns of the proteins in the beginning and the end

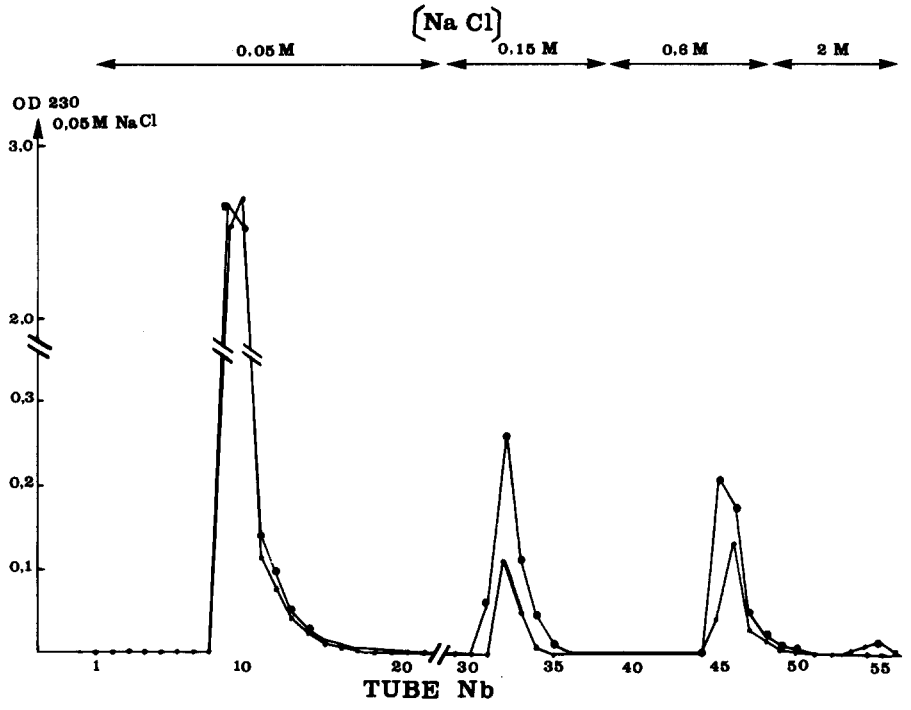

Fig.1. Chromatography of NHP on FNH-cellulose column OD 230 nm profiles for untreated NHP (●—●) and in vitro phosphorylated NHP (•—•).

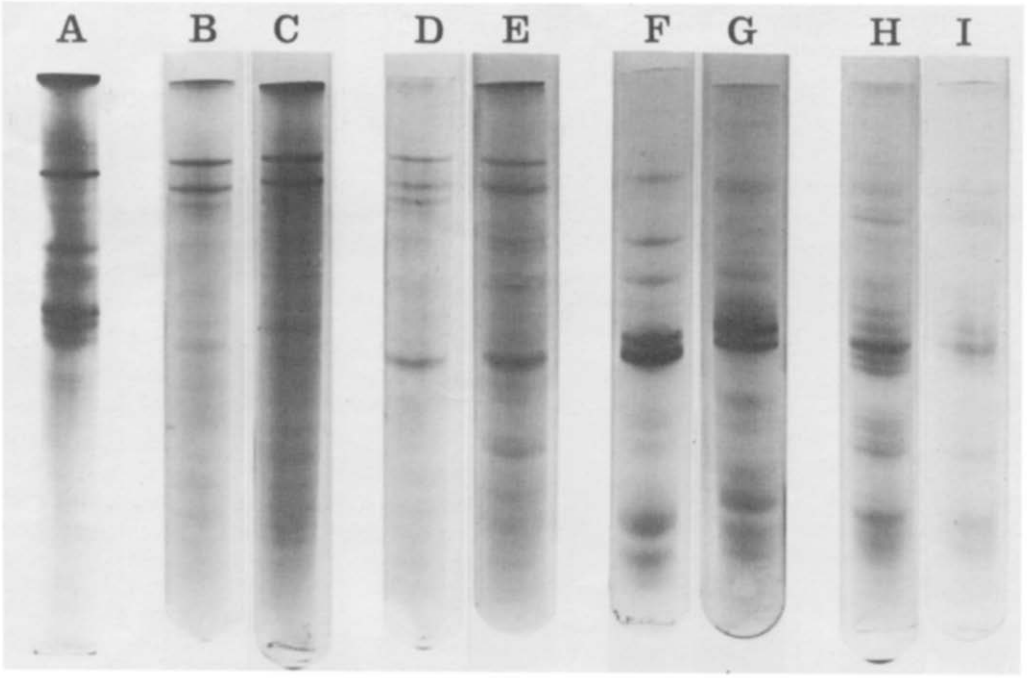

Fig.2. Patterns of 15% polyacrylamide (2.5 M Urea, pH 2.7) gel electrophoresis of rat liver NHP.
Before chromatography. (2-A). After chromatography on DNA cellulose for the following NaCl concentrations: 2 B at 0.05 M, 2 D at he end of the peak eluted by 0.05 M, 2 F at 0.15 M and 2 H at 0.6 M NaCl. After chromatography on FNH-cellulose. 2 C at 0.05 M, 2 E at the end of the peak eluted by 0.05 M, 2 G at 0.15 M, 2 I at 0.6 M NaCl.

of the first peak are not identical, i.e. the main band which migrated half way in the gel was proeminent in gel 2D and was barely detectable in gel 2 B. This result means that some NHP of this peak have some affinities for DNA, even at very low ionic strength. The pattern of proteins eluted in the two following peaks (0.15 NaCl and 0.6 M NaCl) were clearly different as observed previously by Van der Broek et al. [9]. The same procedure was used with FNH column. No significant difference was observed either in the amount of bound NHP nor in the electrophoretic pattern of the various fractions, when compared with results obtained with DNA cellulose.

In separate experiments, nucleohistones were treated with lower concentrations of formaldehyde, 0.01% and 0.04% in order to reduce the cross-links. In these cases, fewer histones remained bound to DNA [12]. The results obtained with NHP were the same as in the previous experiments.

Since phosphorylation of histones is involved in the control of cell growth [22] and transcription, we have repeated the experiments using fully phosphorylated histones in FNH. The elution pattern was identical to the pattern observed with untreated histones (results not shown).

3.2. *Effect of in vitro phosphorylation of NHP on their binding.*

Experiments were performed, as described above, but using NHP previously phosphorylated in vitro by endogenous protein kinases. The radioactivity was found in most of the electrophoretic bands. No significant variation of migration of the various bands was observed after phosphorylation. As shown in fig.1 the phosphorylation reduced the amount of bound NHP. Amou of NHP eluted by 0.15 M and 0.6 M NaCl were respectively 30% and 50% lower than the amounts found with non phosphorylated NHP. These results are in agreement with the observations made by Kleinsmith

in in vivo experiments [23]. However the electrophoretic patterns of phosphorylated proteins, recovered in the different peaks were similar to that found in the previous experiments.

4. Discussion

Firstly we can conclude from these experiments that few NHP have affinity towards DNA, as already mentioned by others [9,24]. The binding of NHP to DNA could be in favour of a possible regulatory role of these proteins.

Secondly, it was shown that NHP binds similarly to DNA and to nucleohistones. It cannot be excluded however that formaldehyde blocks all the available binding sites of histones towards NHP, although the formaldehyde concentration was very low. Nonetheless, the experimental results favor a direct binding of some NHP to DNA, independently of histones; although a binding to histones does occur in chromatin [25,26], it could not be detected in our findings. According to our experiments, the NHP which had the highest affinity towards DNA is the less acidic, since the comparison of the successive electrophoretic patterns shows a progressive disparition of the slowest migrating proteins which are the most acidic in the gel system used.

Several authors have reported the presence of stretches of DNA not bound to histones in chromatin. These fragments could theoretically be available for a binding to NHP. Nevertheless we cannot exclude that in our experimental conditions histones are not bound to DNA as they are in chromatin.

Lastly phosphorylated NHP has less affinity for DNA. But in our experimental conditions we failed to find any specific effect of the phosphorylation. It is then difficult to correlate this phosphorylation to some specific regulatory role. It does not rule out the possibility that a control mechanism, through protein kinase mediated NHP phosphorylation, could occur in vivo.

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