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A nuclear protein associated with actively transcribed nucleosomes exhibits Zn^{2+}-binding activity

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The presence of the zinc-finger motif in nuclear protein structure often confers specific DNA-binding capabilities and, consequently, may implicate such proteins as being involved in gene regulation. We measured the zinc-binding capacity of nuclear proteins by using a zinc (Zn^{2+}) blotting technique. This assay revealed that some non-histone proteins are selectively associated with this metal ion. Of particular interest, a nuclear protein, B_2 (M_r 68000; pI 7.3), associated with actively transcribed nucleosomes and possibly involved with premessenger splicing, exhibits Zn^{2+}-binding activity. Moreover, this activity can be achieved with as little as 2 μg of nuclear protein and the zinc-binding capacity is, on average, 2 ions per molecule of the protein B_2.

Nuclear phosphoprotein complex; Binding domain, Zn^{2+}

1. INTRODUCTION

Metalloproteins are known to be involved in several important biological processes. These include oxidation-reduction reactions, enzyme catalysis, metal transport and storage. Miller et al. [1] demonstrated that a small tandemly repeated peptide envelopes a Zn^{2+} atom within each repeat to form separate domains in transcription factor IIIA of *Xenopus laevis*. Subsequently, several groups of nucleic acid binding proteins were found to contain general amino acid sequences of Zn^{2+} finger motifs: Cys-X_{2-4}-Cys-X_{2-15}-a-X_{2-4}-a or a-X_{2-4}-a-X_{2-15}-Cys-X_{2-4}-Cys, where 'a' represents either cysteine or histidine and 'X' may be any amino acid, as reported by Berg [2]. Miller et al. [1] suggested that the Zn^{2+}-binding domains of the polypeptides interact with the nucleic acids via hydrophilic residues. To date, the retroviral low molecular weight nucleic acid binding proteins, adenovirus EIA gene products, the mammalian retrovirus outer capsid protein, δ3, aminoacyl tRNA synthetases, large T-antigens, yeast and bacteriophage proteins, and many hormonal receptors have all been found to exhibit Zn^{2+}-binding domains [3,4]. The discovery of Zn^{2+}-binding loop-like domains in nuclear proteins such as the *Xenopus* transcription factor IIIA, Sp-1, estrogen receptor, etc. has led to the hypothesis that the transcription factor interacts with DNA by binding with its Zn^{2+} finger domains [5]. Furthermore, Kadonaga et al. [6] have demonstrated that in the presence of the chelating agent EDTA, the purified transcription factor Sp-1 failed to bind to GC boxes in the SV40 21 bp repeat sequence.

In this report we demonstrate that the nuclear protein (M_r 68 000, pI 7.3) exhibits specific Zn^{2+}-binding domains as revealed by two dimensional polyarylamide gel electrophoresis. The characteristic Zn^{2+}-binding properties of B_{2a} suggest that this nuclear protein is associated with DNA in actively transcribed regions.

2. MATERIALS AND METHODS

Nuclear proteins were extracted from the highly purified rat liver nuclei as described previously [7]. Enriched nonhistone nuclear proteins were collected from the carboxyl-methyl cellulose (CM-23) column [8]. These proteins (250 μg) were analyzed by two-dimensional polyacrylamide gel electrophoresis and stained with Coomassie blue. Proteins from an identical gel were transferred to nitrocellulose paper, probed with radioactive $^{65}ZnCl_2$, and subsequently identified by autoradiography as described by Mazen et al. [9].

To quantitate the binding of Zn^{2+} ion to highly purified nuclear protein isolated from two-dimensional polyacrylamide gel electtrophoresis, 2, 4, 8 μg of the protein was slot-blotted onto nitrocellulose membrane; bovine serum albumin (1, 2, 4 μg) was used as a positive control. The nitrocellulose membrane was probed with 1 mCi/ml $^{65}ZnCl_2$ for 15 min according to the method of Mazen et al. [9]. After autoradiography, the nitrocellulose filter was cut and the individual slots were counted using a gamma-counter.

3. RESULTS

An enriched fraction of the nuclear phosphoprotein

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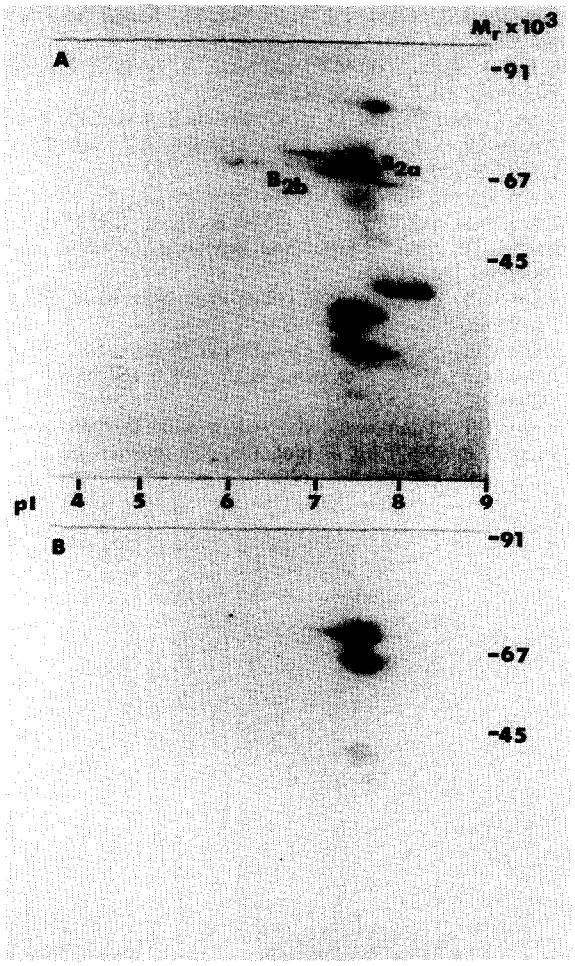

Fig.1. Two-dimensional polyacrylamide gel electrophoresis of enriched nonhistone nuclear proteins stained with Coomassie Blue (A) or probed with ^{65}Zn (B).

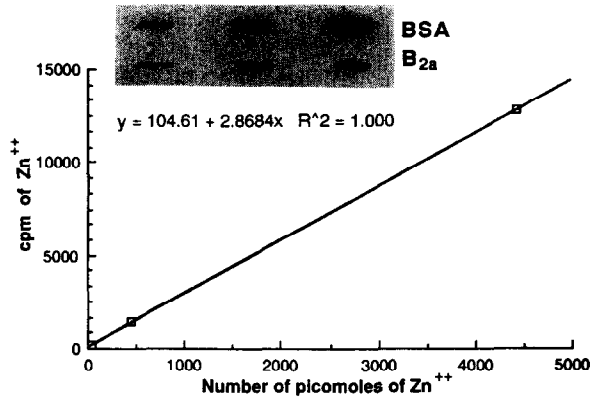

Fig.2. Autoradiograph of highly purified nuclear protein B_{2a} slot-blotted onto nitrocellulose with bovine serum albumin serving as a control (see insert) and probed with ^{65}Zn. A standard linear curve was derived from the counts per minute of a known amount of ^{65}Zn which was blotted onto the nitrocellulose filter and was used to estimate the number of Zn^{2+} ions bound per molecule of B_{2a}.

was obtained by the phenol-buffer extraction method and subsequently eluted by 0.3 M NaCl in CM-cellulose column as described previously [5]. This enriched nuclear phosphoprotein fraction was then analyzed by two-dimensional polyacrylamide gel electrophoresis as shown in fig.1A. At least 9 proteins can be identified with molecular weights ranging from 30 to 90 kDa. Following the transfer of the proteins from the two-dimensional polyacrylamide gel to nitrocellulose membrane, isotopically labelled $^{65}Zn^{2+}$ was equilibrated with the filter. As shown in fig.1B, only two distinctive spots have Zn^{2+}-binding activity. Other proteins (as in fig.1A), although very prominently stained with Coomassie blue, did not bind Zn^{2+} ions and served as internal negative controls.

As shown in fig.2, the nuclear protein B_{2a} and bovine serum albumin were slot-blotted at different concentrations onto nitrocellulose paper prior to equilibration with $^{65}ZnCl_2$ solution, according to the method of Mazen et al. [9]. The number of Zn^{2+}-binding domains was measured by plotting the known specificity of Zn^{2+} and extrapolating for the different concentrations of the nuclear protein. The binding of radioactive Zn^{2+} ion can be observed with as little as 1 μg of bovine serum albumin or 2 μg of nuclear proteins. It was also found that the Zn^{2+}-binding activity was linearly related to the amount of nuclear protein, and that an average 2 Zn^{2+} ions were bound per molecule of nuclear protein B_{2a}.

DISCUSSION

We have characterized a nuclear protein which is associated with actively transcribed nucleosomes and which may participate in premessenger RNA splicing [8,10,11]. Monoclonal antibodies have been produced to this protein and have been used in Western blot analyses. We have also shown that the antigen is present in the nuclear matrix [12]. This nuclear antigen has a molucular weight of 68 kDa and exhibits heterogeneity due to a pI ranging from pH 6.5 to 8.2. This heterogeneity may be due to different degrees of phosphorylation, a proposal which is supported by in vivo labelling of nuclear proteins with inorganic phosphate ^{32}P [8]. We defined this nuclear protein as the nuclear phosphoprotein complex B_2, which is subdivided into the phosphorylated part (B_{2b}) and the non-phosphorylated part (B_{2a}). Upon furter analysis, we found that the phosphorylation site was mainly on serine residues with very few on threonine [8]. We have also demonstrated that this nuclear antigen is distinct from the lamina proteins as demonstrated by Western blot analysis [12]. We have postulated that this nuclear protein is dynamically shuttled between the actively transcribed nucleosomes and the nuclear matrix [13]. This report describes a unique feature of this nuclear protein which consists of Zn^{2+}-binding domains. Schiff et al. [4] suggested that the ability to detect a Zn^{2+}- binding protein using a zinc blot depends on the nature of

the Zn^{2+}-binding sites. Only the Zn^{2+}-binding sites with residues lying within a short stretch of primary amino acid sequence can be detected [4]. Our results, therefore, demonstrate the existence of a Zn^{2+} -finger motif in the nuclear protein B_{2a}.

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