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Autoimmune antigen Ku is enriched on oligonucleotide columns distinct from those containing the octamer binding protein DNA consensus sequence

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During purification of the AP1 complex from the T cell line MLA144 we enriched for a complex which bound to an oligonucleotide column containing the AP1 DNA consensus sequence and co-eluted with a fraction required for AP1 binding activity. This complex although co-eluting with AP1 binding activity had previously been determined to be non-specific in its DNA binding properties. Further investigation determined that the complex was a heterodimer of 85 and 70 kDa which was antigenically related to the autoimmune antigen Ku. It is important to be aware of the abundance and avidity of the Ku complex to bind oligonucleotide columns when purifying sequence specific binding proteins.

Autoimmune antigen Ku; Sequence specific DNA binding protein

1. INTRODUCTION

The autoimmune antigen Ku is a DNA binding protein with two subunits of 85 and 70 kDa. The Ku antigen has been purified using immunoaffinity chromatography with sera obtained from patients with scleroderma-polymyositis and demonstrated to bind double stranded DNA in vitro [1,2]. It has also recently been demonstrated that Ku or Ku related antigens purified by oligonucleotide affinity chromatography will bind to the octamer binding protein DNA consensus sequence [3,4]. In this paper, we demonstrate that Ku is enriched significantly with passage over an AP1 oligonucleotide column but in this case it is non-specific in its DNA binding properties and has no effect in generation of the specific AP1 complex.

2. RESULTS AND DISCUSSION

It was observed that in several cell lines, gel retardation using an AP1 oligonucleotide gave rise to a specific complex and a faster migrating non-specific complex. In those cell lines which did not demonstrate a specific complex, the faster migrating non-specific complex was still observed [5–7]. In our previous studies on specific DNA binding proteins which interact with the AP1 consensus sequence of the Gibbon Ape Leukemia Virus enhancer in the T-Cell line MLA144, we enriched for this non-specific DNA binding complex which co-purified with one of the proteins which participated in formation of the specific AP1 complex [7]. For this reason we attempted to define the components of this non-specific DNA binding complex from the enriched fraction we had obtained from MLA144 cells.

MLA144 cells were grown as previously described [7] and nuclear extract was prepared by the method of Dignam et al. [8]. Nuclear extract was fractionated as described previously [7]. Partial purification of the specific AP1 binding complex in MLA144 gave rise to two fractions which had to be complemented with one another to give rise to the specific AP1 complex. One of these fractions, the material retained on an AP1 oligocolumn which contained a monomer of the sequence GCAGAAATAGATGAGTCAACAGC and referred to as core also gave rise without complementation to a non-specific complex we had previously observed when using nuclear extract.

This fraction (core) which was enriched for this non-specific DNA binding complex contained several protein species including major bands of 85 and 70 kDa, Fig. 1a. A two-dimensional gel analysis which consisted of gel retardation in the first dimension followed by SDS PAGE in the second dimension demonstrated that the non-specific complex was associated with the proteins of 85 and 70 kDa, Fig. 1b.

This complex of 85 and 70 kDa proteins is similar to that which the autoimmune antigen Ku forms on DNA (observation by R. Morimoto). Sera were obtained from patients with scleroderma-polymyositis, which recognised the Ku complex (a generous gift of J. Kraft, Yale) and used to confirm the identity of the non-

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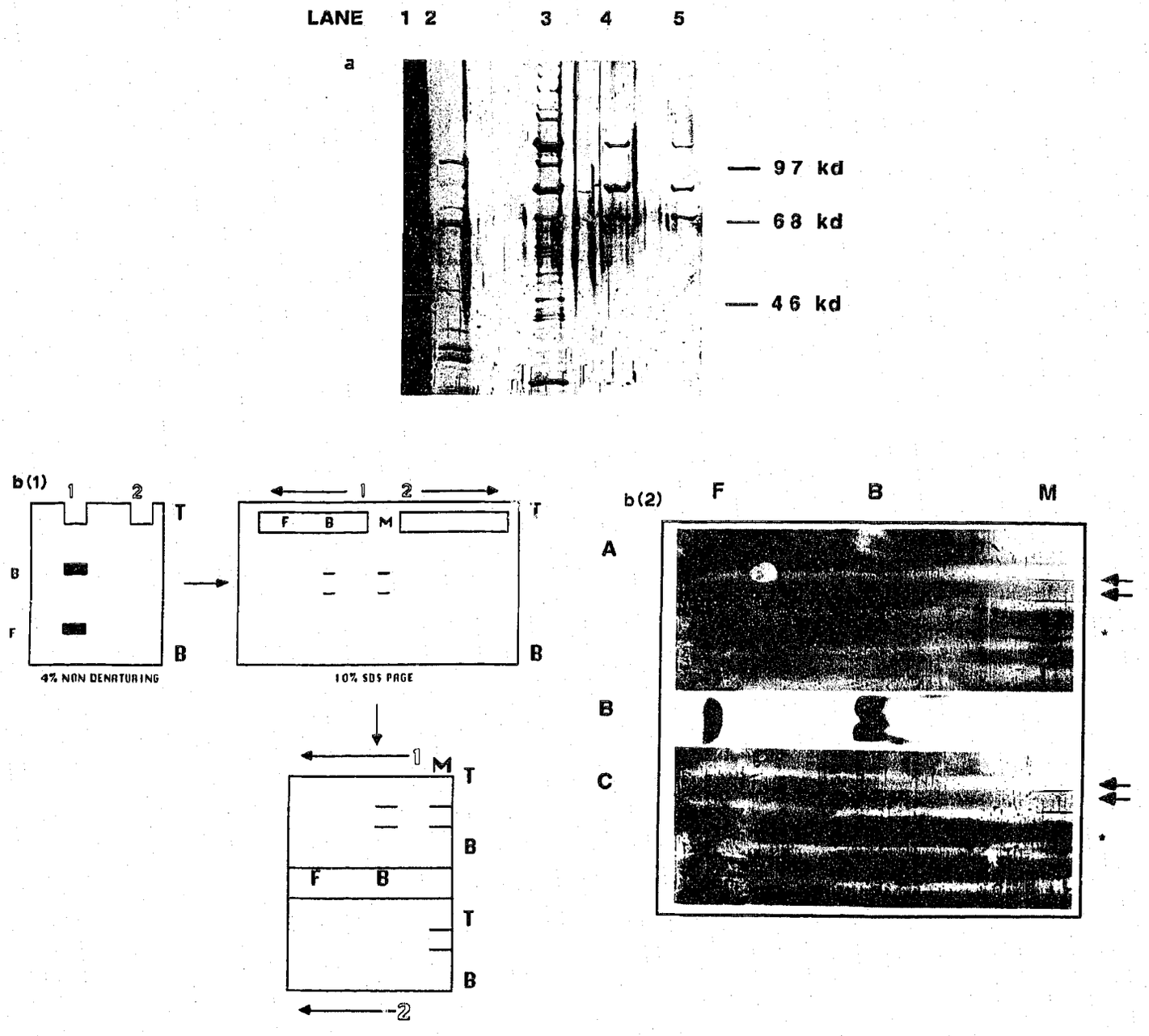

Fig. 1. (a) Proteins of 85 and 70 kDa are enriched by oligonucleotide affinity chromatography. MLA144 nuclear extract was prepared and chromatographically enriched for an AP1 binding activity [7]. This enriched AP1 binding activity was passed over an AP1 oligonucleotide column. Lane 1, MLA144 nuclear extract; lane 2, partially purified fraction containing AP1 binding activity; lane 3, the non-binding flow-through of the AP1 oligonucleotide affinity column; lane 4, material from the 0.5 M NaCl elution of the AP1 oligonucleotide column (core); lane 5, the 0.5 M NaCl elution of the material in lane 4 passed over the oligonucleotide column a second time. AP1 binding activity is reconstituted by addition of flow-through from the column to the material eluted from the column (core) [7].

(b) The non-specific DNA binding complex which interacts with the AP1 oligonucleotide is composed of a heterodimer of 70 and 85 kDa. (1) Diagrammatic outline of the experiment. The core fraction was subjected to 4% native PAGE in the first dimension in the presence (lane 1) or absence (lane 2) of an AP1 oligonucleotide labeled with ^{32}P. The gel was subjected to autoradiography, F indicates the migration of the free probe and B that of the complex. The relevant lanes were cut out and analysed by 10% SDS-PAGE in the second dimension. The core material (M) was used as a control for migration of the proteins in the second dimension. After electrophoresis the SDS-PAGE was transferred to a nitrocellulose filter (550 mA, 4°C for 90 min), the filter was then stained by aurodye (Janssen). By alignment of the autoradiograph from the migration of the labeled oligonucleotide in the native PAGE with the SDS-PAGE we could determine the proteins which constituted the complex. (2) The result of the experiment outlined above is shown. Shown is a composite of three panels. In panel B is the autoradiograph of lane 1 from the electrophoresis in the first dimension. This is bracketed by A, the pattern generated from SDS-PAGE of lane 1 in the second dimension and panel C, the pattern generated from SDS-PAGE of lane 2. The arrows represent the position of the 85 and 70 kDa proteins and the * represents artifacts generated from keratins which are seen on the nitrocellulose after silver staining. Note that the 85 and 70 kDa are seen only in panel A and are migrating with the B position obtained in lane 1, corresponding to the migration of the non-specific complex.

specific complex. As demonstrated in Fig. 2a, these sera recognised proteins of 85 and 70 kDa in HeLa, MLA144, CV1, CV1 infected with SV40 and also the partially purified fraction (core). Addition of these sera to a gel retardation assay using the AP1 oligonucleotide with MLA144 nuclear extract did not affect the mobility or intensity of the specific complex but removed totally the lower non-specific complex (Fig. 2b). Finally Western blot analysis of a gel retardation using these sera only identified the lower non-specific complex both

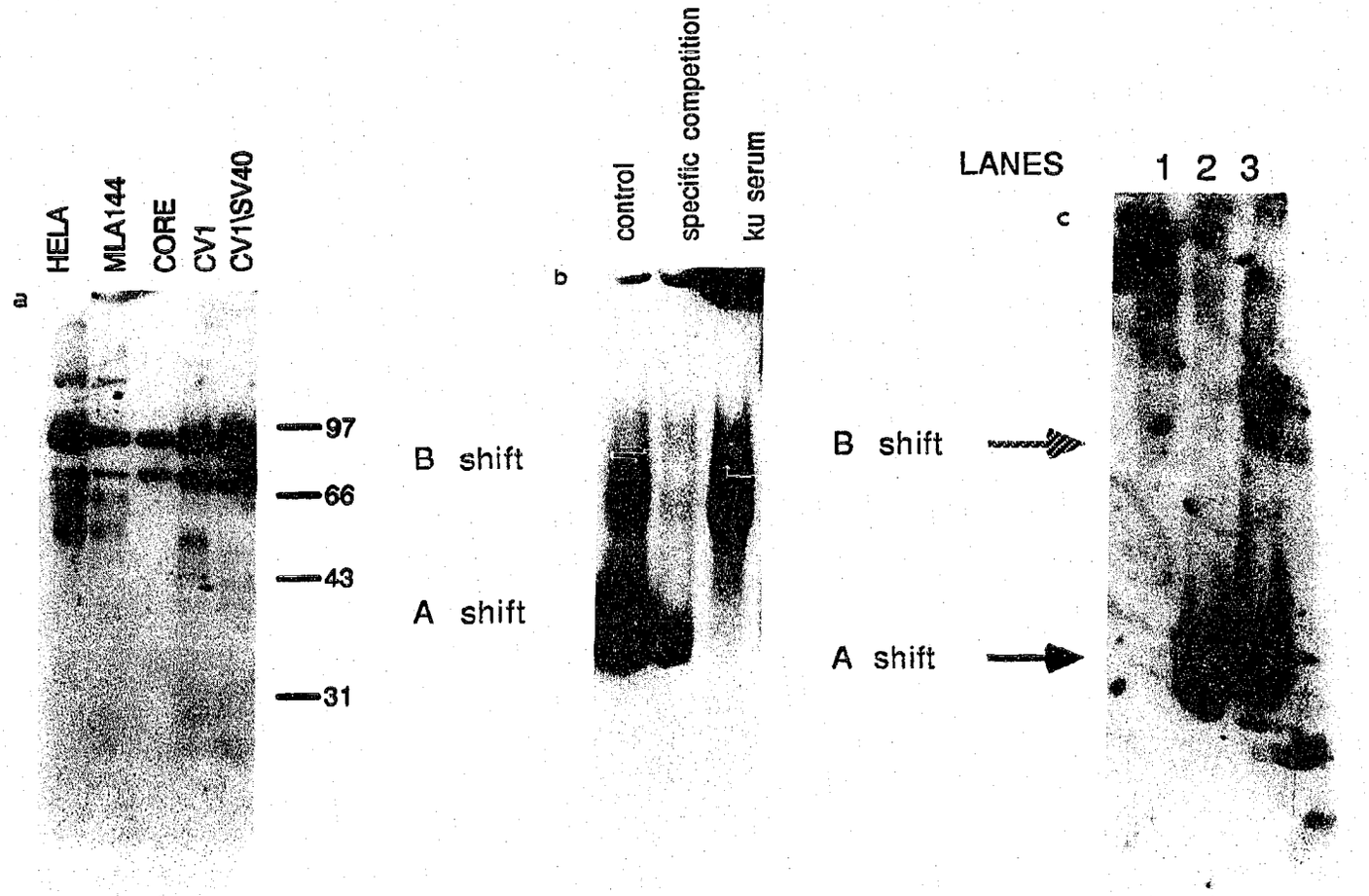

Fig. 2. The 85 and 70 kDa proteins are antigenically related to Ku. (a) Ku serum recognises proteins of 85 and 70 kDa in several cell extracts and our enriched oligonucleotide column fraction (core). Protein extracts were separated by 10% SDS-PAGE and transferred to nitrocellulose filter (550 mA, 4°C for 90 min). The filter was blocked with blotto (5% carnation non-fat milk powder, 50 mM Tris, pH 7.5, 50 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol) for 90 min at room temperature, and probed with Ku serum at 1:500 dilution. Anti-rabbit Ig, ^{125}I-labelled F(ab') (Amersham) was allowed to react with the filter in blotto at room temperature prior to autoradiography. Lane 1, HeLa (human); lane 2, MLA144 (primate); lane 3, the core fraction from MLA144; lane 4, the primate cell line CV1; and lane 5, CV1 cells infected with SV40.

(b) Ku serum recognises the non-specific complex which forms on the AP1 oligonucleotide in vitro. MLA144 nuclear extract was analysed by gel retardation using a documented AP1 binding site [6,7]. Gel retardation analysis was performed as described previously [13–15]. Briefly, binding reactions contained a ^{32}P-3' end-labelled double-stranded oligonucleotide probe previously demonstrated to bind the AP1 complex in the gibbon ape leukemia virus enhancer, GALV (GCAGAAATAGATGAGTCAACAGC: [6,7]). This oligonucleotide gives rise to two complexes: (B) a specific complex and (A) a non-specific complex which has been demonstrated previously [6]. Lane 1, the normal pattern seen with this oligonucleotide; lane 2, specific oligonucleotide competition removes only the B complex; lane 3, addition of Ku serum removes the lower non-specific complex A.

(c) Ku serum recognises the proteins which constitute the non-specific complex by Western blot analysis. Gel retardation analysis as in (b) was transferred to nitrocellulose and probed with Ku serum (1:500 dilution). Non-radioactive GALV oligonucleotide was incubated with the following fractions and extracts as follows: lane 1, flow-through of the oligonucleotide column; lane 2, contains core extract; lane 3, MLA144 extract.

in MLA144 cells and in the core fraction (Fig. 2c). Thus, this non-specific complex identified as composed predominantly of proteins of 85 and 70 kDa was antigenically related to Ku.

The Ku complex is highly abundant in many cells and has the ability to recognise DNA with great affinity; it binds to an AP1 oligonucleotide column and elutes at greater than 0.4 M NaCl [7]. It would also appear to bind numerous oligonucleotides we have used in our laboratory and show little sequence specificity in cross competition (data not shown). The Ku complex in our oligonucleotide affinity preparation can be removed without affecting the specificity of the AP1 complex (data not shown).

Speculation that Ku was a transcriptional activator was based on DNA sequence analysis of the cloned gene products of the 70 kDa species [9] which demonstrated a putative leucine zipper and acidic domains which are found in numerous transcription factors [10]. This role for Ku in gene regulation has recently been highlighted by Knuth et al. [3] and Gunderson et al. [11], who have shown, by in vitro transcription reactions using partially purified components that proteins antigenically related to Ku and of similar mol. wt to Ku activate transcription in vitro. This activation is correlated with a fraction which is shown to bind to an octamer binding DNA consensus site. May et al. [4], have also purified an octamer DNA consensus binding protein antigenically related to Ku. Therefore, although recent evidence indicates that Ku or Ku-related proteins bind the octamer DNA consensus sequence and activate transcription it is important to be aware that our data demonstrate that Ku will bind non-specifically to other oligonucleotides and will be enriched with passage over oligonucleotide columns quite distinct from those containing the DNA consensus sequence for octamer binding protein.

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REFERENCES

[1] Mimori, T. and Hardin, J.A. (1986) J. Biol. Chem. 261, 10375–10379.
[2] Mimori, T., Hardin, J.A. and Steitz, J.A. (1986) J. Biol. Chem. 261, 2274–2278.
[3] Knuth, M.W., Gunderson, S.I., Thompson, N.E., Strasheim, L.A. and Burgess, R.R. (1990) J. Biol. Chem. 265, 17911–17920.
[4] May, G., Sutton, C. and Gould, H. (1991) J. Biol. Chem. 266, 3052–3059.
[5] Quinn, J.P., Holbrook, N. and Levens, D. (1987) Mol. Cell. Biol. 7, 2735–2744.
[6] Quinn, J.P., Takimoto, M., Iadarola, M., Holbrook, N. and Levens, D. (1989) J. Virol. 633, 1737–1742.
[7] Quinn, J.P., Farina, A.R., Gardiner, K., Krutzsch, H. and Levens, D. (1989) Mol. Cell Biol. 9, 4713–4721.
[8] Dignam, J.D., Martin, P., Shastry, B.S. and Roeder, R.G. (1983) Methods Enzymol. 101, 582–598.
[9] Reeves, W.H. and Sthoeger, Z.M. (1989) J. Biol. Chem. 264, 5047–5052.
[10] Mitchell, P.J. and Tjian, R. (1989) Science 245, 371–377.
[11] Gunderson, S.I., Knuth, M.W. and Burgess, R.R. (1990) Genes Dev. 4, 2048–2059.
[12] Prabhaker, B.S., Allaway, G.P., Srinivasappa, J. and Notkins, A.L. (1990) J. Clin. Invest. 86, 1301–1305.
[13] Fried, M. and Crothers, D.M. (1981) Nucleic Acids Res. 9, 6505–6525.
[14] Strauss, F. and Varshavsky, A. (1984) Cell 37, 889–901.
[15] Singh, H., Sen, R., Baltimore, D. and Sharp, P.A. (1986) Nature 319, 154–158.

Note added in proof

Antibodies raised against the gel-purified 85 and 70 kDa proteins were prepared. The antibody against the 70 kDa has been shown to recognise baculovirus expressed Ku 70 kDa species and recognise a 70 kDa species in HeLa cells [12].