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Characterization of the human immunodeficiency virus type 1 enhancer-binding proteins from the human T-cell line Jurkat

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The transcription of the human immunodeficiency virus type 1 (HIV-1) is under the control of cellular proteins that bind to the viral long terminal repeat (LTR). Among the protein-binding regions of the HIV-1 LTR is the transcription-enhancer region. We show that at least one inducible, C1, and one constitutive, C2, protein can bind to the HIV enhancer in Jurkat cells. The two proteins differ in their surface charge, since they are separable by anion-exchange chromatography. Bivalent cations such as Mg^{2+} and Zn^{2+} differentially affect their binding to oligonucleotides which contain the HIV-enhancer domain. Both C1 and C2 proteins also bind to a similar sequence found in the interleukin-2-receptor α-subunit enhancer. The inducible C1 protein was partially purified by three chromatographic steps and characterized by u.v. cross-linking as a 47 kDa protein.

INTRODUCTION

Human immunodeficiency virus 1 (HIV-1) is the etiological agent of AIDS (acquired immunodeficiency syndrome) [1,2]. The virus typically infects cells expressing the CD4 receptor [3,4]. However, the virus is able to express its gene products even when transfected into cells that do not express the CD4 receptor, such as HeLa cells [5]. These observations imply that HIV is able to use host-cell transcriptional factors common to many cell types for its transcriptional regulation.

Deletion analysis of chloramphenicol acetyltransferase constructs (CAT assays) and DNAase footprinting assays of the HIV long terminal repeat (LTR) have revealed that at least five distinct regions are involved in the regulation of the transcription of the viral genes by interacting with cellular proteins [6]. Among these regions, a negative regulatory element (−173 to −159) and a positive regulatory element or enhancer (−104 to −78) have been identified [6–8]. The enhancer element is composed of two direct repeats of the sequence GGGACTTTCC [8,9]. This sequence is identical with the κ-light-chain-enhancer sequence or κB site, except that the sequence is not repeated [10]. The κ-light-chain-enhancer site is bound by a constitutively expressed protein, NF-κB, in mature B-cells. In pre-B-cells, NF-κB is induced by lipopolysaccharide [10]. NF-κB was recently purified from bovine spleen and shown to be a 42–44 kDa protein [11]. Furthermore, Nabel & Baltimore [9] showed that phorbol ester plus mitogen induced a protein that is specific to a human T-cell line (Jurkat) and binds to the HIV enhancer element. In their original report those authors postulated that the HIV-enhancer-specific binding protein in Jurkat cells is identical with B-cell NF-κB. Stimulation of Jurkat cells with lectin and phorbol esters induces the expression of both interleukin-2 (IL2) and its receptor α-subunit (IL2Rα). Purified NF-κB binds to both IL2 and IL2Rα enhancer domains [11]. However, another protein, HIVEN 86A, characterized in the same cells, was able to bind both the HIV enhancer element [12] and the IL2Rα enhancer [13]. Two other groups have purified HIV enhancer-binding proteins from phorbol-ester-treated HeLa cells [14] and the Namalwa B-cell line [15]. The protein purified from HeLa-cell extracts had an apparent M_r of 55000–60000, whereas the M_r of the protein from the Namalwa cells was reported to be 51000. Therefore both lymphoid and non-lymphoid cells express proteins capable of specifically binding to the HIV enhancer domain, but only mature B-cells express the NF-κB protein constitutively. The relationship between NF-κB and other HIV enhancer-binding proteins is not yet elucidated.

Here we show that stimulation of Jurkat cells with phorbol 12-myristate 13-acetate (PMA) and phytohaemagglutinin (PHA) induces at least two proteins capable of binding specifically to the HIV enhancer element. We describe the separation of these proteins and the partial purification and characterization of the major 47 kDa protein.

EXPERIMENTAL

Cell culture

The Jurkat T-cell line was cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal-calf serum, glutamine and penicillin/streptomycin. The cells were stimulated for 3 h with 2 μg of PHA (Burroughs Wellcome) and 50 ng of PMA (Sigma).

Protein extraction

Whole cell extracts were obtained as described previously [16]. The final protein extract contained 5–25 mg

Abbreviations used: LTR, long terminal repeat; HIV(-1), human immunodeficiency virus type 1; AIDS, acquired immunodeficiency syndrome; IL2, interleukin 2; IL2Rα, IL2-receptor α-subunit; PMSF, phenylmethanesulphonyl fluoride; PMA, phorbol 12-myristate 13-acetate; PHA, phytohaemagglutinin; CRE, cyclic AMP-responsive element; BrdUTP, 5-bromo-2′-deoxyuridine 5′-triphosphate; DTT, dithiothreitol.

of protein/ml of buffer A50 [50 mM-$(NH_4)_2SO_4$/1 mM-EDTA/10% (v/v) glycerol/1 mM-dithiothreitol (DTT)/20 mM-Tris/HCl (pH 8.0)/1 mM-PMSF]. The extraction procedure included an $(NH_4)_2SO_4$ precipitation at 50% saturation. Cell extracts were stored at −80 °C.

Oligonucleotides and gel-retardation assay

Two synthetic double-stranded oligonucleotides were used as probes for detection of the HIV enhancer-binding proteins. A 31-mer oligonucleotide or 'Tandem', which contains the two direct repeats of the HIV enhancer sequence from the position −107 to −80 [6] (5′-ACAAGGGACTTTCCGCTGGGGACTTTCCAGG-3′), and a 16-mer oligonucleotide or 'Mono', containing only the 5′ site from the position −107 to −92. Two other double-stranded oligonucleotides were also synthesized: a 16-mer containing the sequence of the IL2Rα promoter from the position −270 to −255 [17] (5′-GCAGGGAATCTCCCT-3′) and a 33-mer oligonucleotide containing the cyclic AMP-responsive element (CRE) of the human pro-enkephalin from the position −108 to −76 [18] (5′-GGGCTGGCGTAGG-GCCTGCGTCAGCTGCAGCCC-3′). In the u.v. cross-linking experiments, an oligonucleotide containing the AP1/c-Jun binding site [19] was also used (5′-GATCCGGCTGACTCATG-3′). The oligonucleotide probes were end-labelled with phage-T_4 kinase.

The gel-retardation assays were performed as follows: 5 μl of protein solution (0.4–200 μg) was incubated on ice in a final volume of 20 μl of 20 mM-Tris/HCl (pH 8.0)/10% glycerol/1 mM-DTT/60 mM-KCl/poly-(dI·dC) (2 μg). After 15 min incubation, the ^{32}P-end-labelled oligonucleotide was added (100000–500000 c.p.m.; 0.1 ng) and the binding reaction was allowed to proceed at room temperature for 15 min. The bound oligonucleotide was separated from the free by electrophoresis on a 6% (w/v) polyacrylamide gel in 0.25X TBE [22.5 mM-Tris/borate (pH 8.0)/0.5 mM-EDTA]. The specific activity was determined as described by Yano *et al.* [20], except that the radioactivity of the bands was counted by the Čerenkov rather than the liquid-scintillation method, and the control DNA was the unbound free oligonucleotide. The specific activity is expressed as pg of retarded radiolabelled probe/mg of protein. Total activity was obtained by multiplying the specific activity by the total amount of protein and is therefore also expressed in pg.

H.p.l.c. and affinity-chromatographic fractionation

The whole-cell extract from 25×10^9 cells (400 mg of protein) was diluted 3-fold with the column buffer [20 mM-Tris/HCl (pH 8.0)/1 mM-EDTA] and loaded on to a Bio-Gel TSK-DEAE-5-PW column at a flow rate of 5 ml/min. The column was washed with twice its volume of column buffer, and the bound proteins were eluted with a linear gradient of KCl (0–0.5 M). The 5 ml fractions were dialysed against the A50 buffer and tested for their HIV enhancer-binding activity by the DNA gel-retardation assay. The fractions containing the PMA + PHA-induced proteins were pooled (pool 1; see Fig. 3 below) and further fractionated by affinity chromatography by using a trimeric repeat of the mono oligonucleotide (a 48-mer) covalently bound to a CNBr-activated Sepharose (Pharmacia) as described in [21]. The density of the oligonucleotide was approx. 140 μg/g of dry gel. The proteins were loaded at a rate of 0.5 ml/min. The unbound material was washed out with 2.5 column volumes of the A50 buffer. The bound proteins were eluted in 2 ml fractions by a discontinuous gradient of KCl (0.2, 0.5 and 1.0 M) in the presence of a 0.05% Nonidet P40. After dialysing the fractions against the A50 buffer, the DNA-binding activity was assayed by the DNA gel-retardation assay. The active dialysed fractions were pooled, and the affinity chromatography was repeated a second time.

U.v. cross-linking

The probe used for the u.v. cross-linking experiment was prepared by following the instructions of the manufacturer (Boehringer-Mannheim); the Tandem single-stranded oligonucleotide was added to the Boehringer-Mannheim random primed DNA labelling kit mixture. A 100 μCi portion of [^{32}P]dCTP and 0.5 nmol of BrdUTP were added to the reaction mixture. After 30 min incubation at 37 °C, 0.5 nmol of dCTP were added. The labelled probe was purified from the free nucleotides by two passages through Sepharose G-25. Its radioactivity averaged 500000 c.p.m./μl. The u.v. cross-linking of proteins with the ^{32}P-labelled BrdUr-substituted Tandem oligonucleotide was performed as described [22], except that after irradiation the samples were immediately boiled in 2.5% SDS/1 mM-EDTA/20% glycerol/1% 2-mercaptoethanol. The electrophoretic separation was performed on 10%-polyacrylamide gels as described by Porzio & Pearson [23].

RESULTS

Induction of HIV enhancer-binding proteins

We first examined the capacity of proteins extracted from Jurkat cells to bind to the synthetic oligonucleotides which represented either a single copy (Mono) or two copies (Tandem) of the κB site of the HIV enhancer domain. Both oligonucleotides formed two protein–DNA complexes, C1 and C2, with distinct electrophoretic mobilities (Fig. 1). The C1 complex was greatly increased by the stimulation of Jurkat cells by PMA and PHA. This is in agreement with previously described induction of HIV enhancer-binding proteins in Jurkat cells by Nabel & Baltimore [9]. In their study the radiolabelled probe used was much larger (90 bp) compared with the Tandem oligonucleotide and included an upstream 50 bp extension. The single κB site (Mono)-retarded migration was undistinguishable from that of the Tandem, suggesting that the two oligonucleotides bind the same proteins. It is also very likely that the two potential κB sites in the Tandem oligonucleotide cannot bind proteins simultaneously, since no slower migrating complex than that of C1 was seen with the Tandem. However the length of the probe is critical, since we did not see any retardation of Mono oligonucleotides shorter than 14 bp. This observation may be attributable to the high dissociation rate of the protein–DNA complex with short DNA probes. The C1 complex was not affected by cycloheximide and actinomycin D treatment of Jurkat cells, suggesting that its induction is not due to protein synthesis *de novo* (results not shown). The C2-complex in contrast with the C1-complex proteins appear to be constitutively active in Jurkat cells (Fig. 1).

Ionic requirement of the C1 and C2 complexes

We further characterized the DNA binding of C1 and

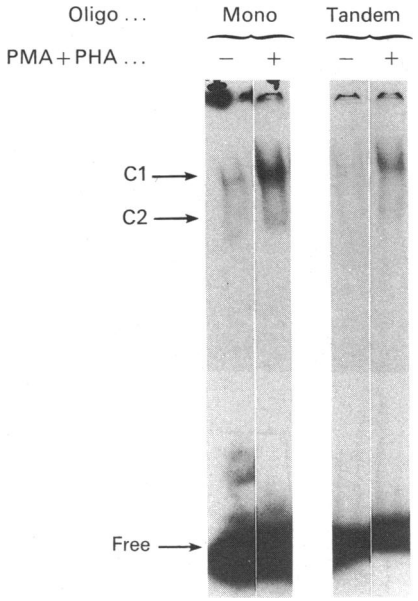

Fig. 1. Induction of HIV enhancer-binding proteins in Jurkat cells

Gel retardation of ^{32}P-labelled Mono and Tandem oligonucleotides (Oligo) by proteins (15 μg) extracted from PMA+PHA-stimulated (+) or unstimulated (−) Jurkat cells was performed as described in the Experimental section. C1 and C2 indicate the migration of the oligonucleotides complexed to the proteins. 'Free' refers to the free oligonucleotides.

C2 by studying the effect of ions on their interaction with the mono probe. Bivalent cations such as Zn^{2+}, Ca^{2+} and Mg^{2+} had differential effects on the stability of C1 and C2 complexes. At 1 mM, Zn^{2+} inhibited the formation of the C1 complex (Fig. 1, lane 2), whereas Mg^{2+} and Ca^{2+} did not (Fig. 2, lanes 5 and 8). At higher Zn^{2+} concentrations (2 and 5 mM) C1 complex was dissociated, whereas the C2 complex was greatly augmented (Fig. 2, lanes 3 and 4). At the same concentrations Ca^{2+} had no effect on the stability of either C1 or C2, whereas Mg^{2+} (5 mM) had a dissociating effect on the C1 complex without affecting the C2 complex (Fig. 2). EDTA (10 mM) had no effect on the C1 complex, favoured partial dissociation of C2 complex (Fig. 2, lane 11) and efficiently abolished the dissociating effect of Mg^{2+} on the C1 complex (results not shown). C1 and C2 also differed in their sensitivity to high ionic strength, since C1 was more easily dissociated by 0.4 M-KCl than was C2 (Fig. 2, lane 12). Therefore it is possible to favour selectively the binding of either C1 or C2 proteins to the HIV enhancer by manipulating the ionic composition of the reaction medium.

Isolation of the C1-complex protein

In order to isolate the inducible C1-complex proteins we performed the fractionation and oligonucleotide-binding assays in the absence of Mg^{2+} ions. The first chromatographic fractionation was performed with DEAE-5PW h.p.l.c. About 70% of the loaded proteins did bind to the column (Fig. 3*b*). The oligonucleotide-binding activity was found exclusively in the KCl-eluted fractions, suggesting that all the active proteins bound to the DEAE (Fig. 3*a*). The proteins migrating as C2′ were

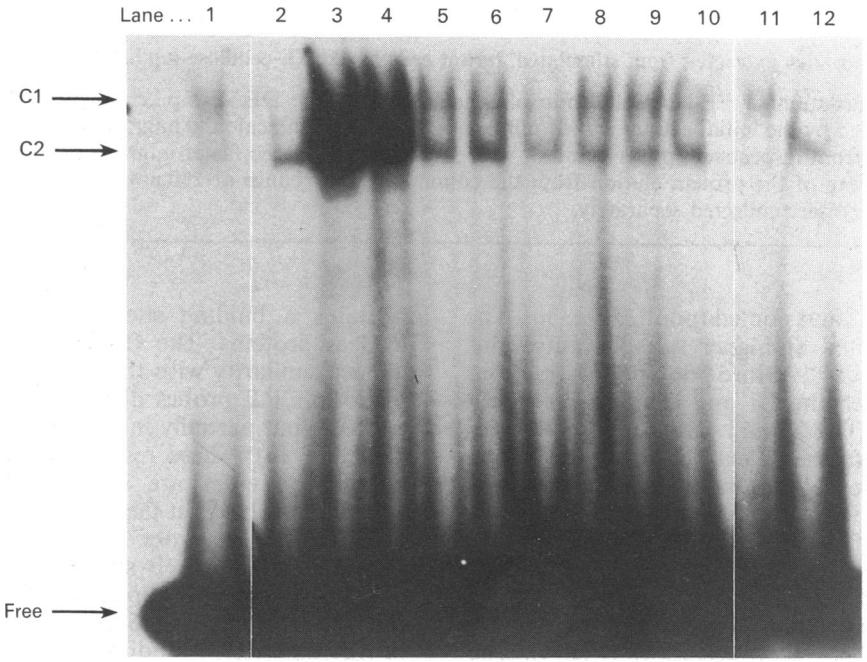

Fig. 2. Ion-dependence of oligonucleotide–protein interaction

Gel retardation of the ^{32}P-labelled mono oligonucleotide was performed with 15 μg of stimulated Jurkat-cell extracts, either under the standard conditions described in the Experimental section (lane 1) or in the presence of 1, 2 or 5 mM-$ZnSO_4$ (lanes 2, 3 and 4); 1, 2 or 5 mM-$MgCl_2$ (lanes 5, 6 and 7); 1, 2 or 5 mM-$CaCl_2$ (lanes 8, 9 and 10); 10 mM-EDTA (lane 11) and 40 mM-KCl (lane 12). 'Free' refers to the free oligonucleotides. 'C1' and 'C2' refer to the complexes.

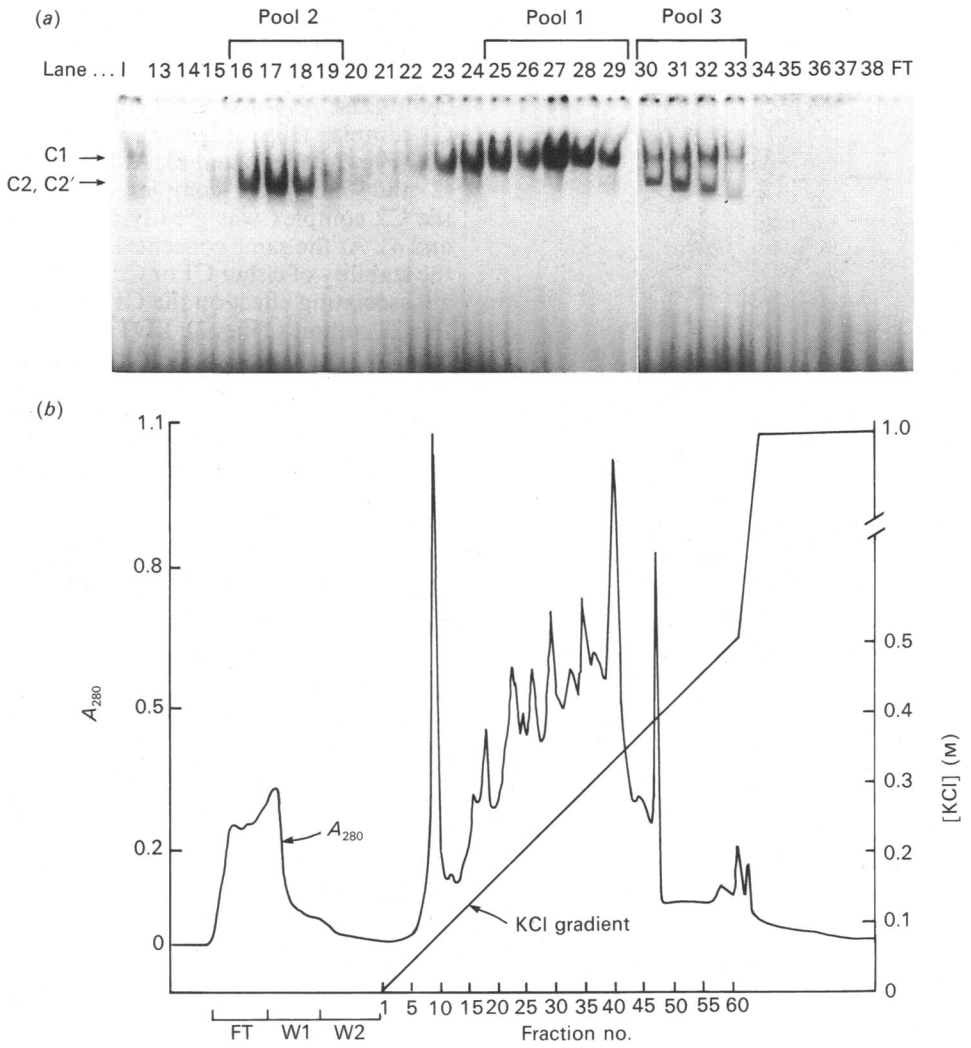

Fig. 3. Fractionation of proteins extracted from stimulated Jurkat cells by DEAE-cellulose h.p.l.c.

(*a*) shows the gel retardation of ^{32}P-labelled Mono oligonucleotide by the DEAE h.p.l.c. fractions. The migration of the oligonucleotide retarded by the initial extract is shown in lane I. C1 and C2 indicate the migration of the oligonucleotide in the presence of fractions from respectively pools 1 and 3, whereas C2′ indicates the faster-migrating complexes from pool 2. (*b*) Represents the recording of the protein elution from the column by absorbance at 280 nm. The flow-through (FT) and the washings (W1 and W2) were collected separately.

eluted with 0.15 M-KCl and pooled (pool 2; Fig. 3*a*). The C1 proteins were eluted at higher salt concentrations (0.22–0.25 M-KCl) (pool 1). A third pool (pool 3, fractions 30–33), contained both the C1 and C2 proteins eluted with 0.25–0.3 M-KCl. The C2 and C2′ proteins either co-migrated in the unfractionated extract or one of the two activities was generated by the fractionation procedure. Indeed, the fractionation could possibly have eliminated an inhibitor of one of the two binding activities.

After pooling the fractions containing the same binding activities, we analysed the specificity of their binding to the ^{32}P-labelled Mono probe by competition with a 100-fold excess of unlabelled oligonucleotides (Fig. 4). The unlabelled Mono and Tandem oligonucleotides competed for binding to C1 and C2′, but not C2. The IL2Rα enhancer probe partially inhibited the binding of C1 and C2′ and not C2 (lanes 5, 10 and 15). To check the selectivity of binding of the three pools to the κB site, we used the CRE oligonucleotide as a competitor, since it contains a binding site for the cyclic AMP-induced nuclear proteins. The CRE oligonucleotide has no sequence similarity with the κB site (see the Experimental section). CRE probes did not compete for binding C1 and C2′, but partially inhibited the binding of C2 (lanes 4, 9 and 14). These results indicate that C1 and C2′ binding activities are specific for binding the HIV enhancer probes, but the C2 protein complex is not. The IL2Rα enhancer probe sequence is similar to, but not identical with, the κB site, which may explain why it appears to have a lower affinity for C1 and C2′ binding activities (see also Fig. 6 below). The sensitivity to Mg^{2+} of fractionated C1 binding activity remained unchanged compared with the C1 binding activity from the initial extract. C2′ complex was also inhibited by 5 mM-Mg^{2+}, whereas C2 was not, suggesting that the C2 binding activity in the initial extract was mainly due to proteins from pool 3 (results not shown).

The C1 binding activity from pool 1, which represents

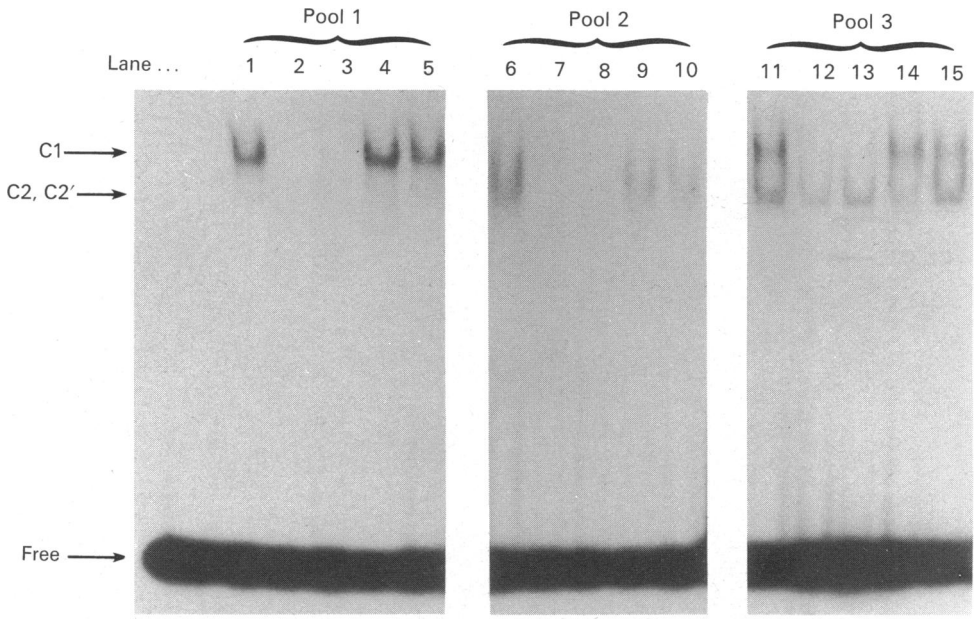

Fig. 4. Gel retardation of ^{32}P-labelled Mono oligonucleotide in the presence of competitor DNA

Lanes 1–5 show the result of the assay performed with fractions from the pool 1. Lanes 6–10 and 13–15 represent respectively the assay with pools 2 and 3. The assay performed in the absence of competitor DNA is shown in lanes 1, 6 and 11. The other lanes represent the retardation in the presence of 100-fold excess of Mono (lanes 2, 7 and 12), Tandem (lanes 3, 8, and 13), CRE (lanes 4, 9 and 14) and IL2Rα enhancer (lanes 5, 10 and 15) oligonucleotides. 'Free' refers to the free oligonucleotide.

the major induced proteins, was further purified by two-step affinity chromatography. The activity was eluted with 0.5 M-KCl and co-migrated with the C1 complex from the unfractionated pool 1 (Fig. 5). A single migrating activity was generated by this chromatographic step; however, recovery after this step was superior to that obtained after DEAE h.p.l.c. (see Table 1). One possible explanation for this enhanced yield is the elimination of an inhibitor during this chromatographic step. Similar increases in activity were recently described during the purification of the NF-κB from bovine spleen [11] (see the Discussion section). The overall enrichment after this first affinity chromatography was 800-fold (see Table 1). The active fractions were pooled and refractionated by the same chromatographic procedure. C1 activity was eluted in two fractions with 0.5 M-KCl (results not shown). This second step resulted in a 1000-fold overall purification (Table 1), but recovery decreased from 70 to 43% of the initial activity. C2′ binding activity from pool 2 was also analysed by affinity chromatography. However, the recovery of activity after this step was too low to allow further analysis.

Molecular-mass analysis of C1 proteins by u.v. cross-linking

To determine the molecular mass of the C1 proteins, we loaded the initial extract directly on the affinity-chromatography column in the presence of 1 mM-EDTA. The C1 binding activity was eluted with 0.5 M-KCl (results not shown). This single fractionation step permitted a 200-fold enrichment of the C1 activity. The active fractions were then u.v.-cross-linked with ^{32}P-labelled BrdUr-substituted Tandem probe as described in the Experimental section. Two proteins of 47 kDa (p47) and 60 kDa (p60) were prominently labelled (Fig. 6, lane 1). The addition of 500-fold molar excess of unlabelled competitors differentially affected the two proteins complexed with the oligonucleotide probe. Unlabelled Tandem probe completely inhibited the labelling of p47 and only partially inhibited p60 complex, whereas two sequence-unrelated oligonucleotides, corresponding to the CRE and AP1 binding sites, did not inhibit the labelling of p47, although they partially inhibited the labelling of the p60 (Fig. 6, lanes 3 and 5). The IL2Rα enhancer probe partially inhibited the labelling of both p47 and p60 (Fig. 6, lane 4). When u.v. cross-linking was performed with the initial extract, many proteins were labelled, including p47 and p60 (results not shown). We also performed u.v. cross-linking of 1000-fold-purified C1 binding activity by both the DEAE h.p.l.c. and the two affinity-chromatographic steps (see Table 1). A labelled protein migrated with an apparent molecular mass of 40 kDa. This difference in molecular mass is probably due to partial proteolysis occurring during the three-step purification. We deduce from the u.v.-cross-linking data that the protein responsible for C1 complex-formation has a molecular mass of 47 kDa.

DISCUSSION

In the present study we have shown that several proteins extracted from mitogen-stimulated Jurkat cells can interact with the κB sites that are contained in the HIV enhancer domain. These proteins differ in the extent of their migration during non-denaturing gel electrophoresis after binding with oligonucleotides. They also differ in their ionic requirements for stable interaction with the oligonucleotides. The effect of Mg^{2+} on C1 and C2 activities is summarized in Table 2. The

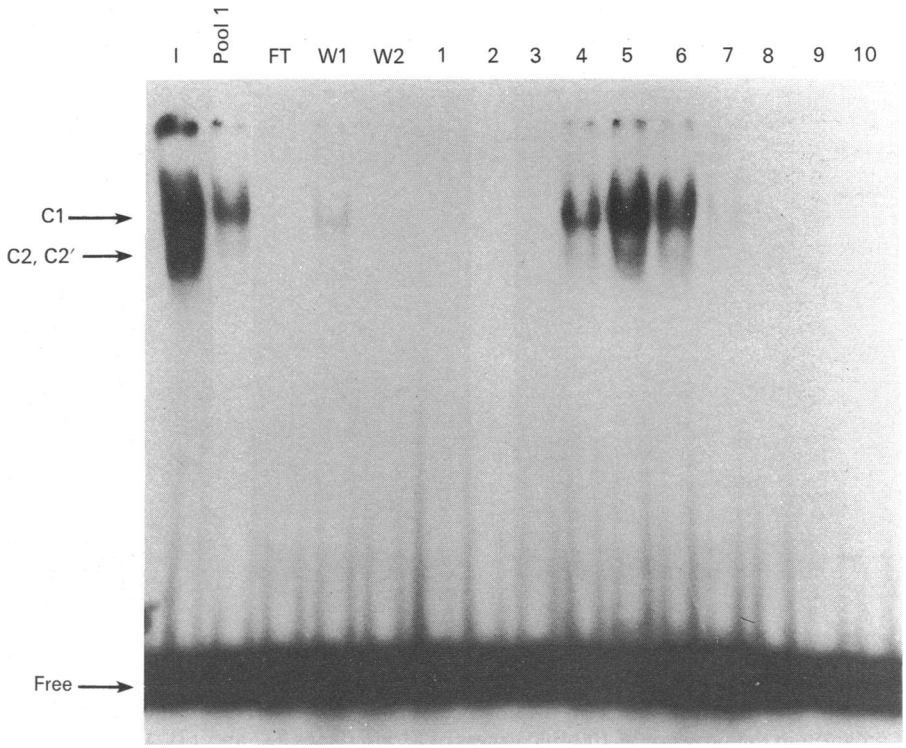

Fig. 5. Affinity chromatography of the pool-1 proteins

The Figure shows the gel retardation of ^{32}P-labelled Mono oligonucleotide by proteins from fractions eluted from the DNA–Sepharose column. The retardation by the initial extract is shown in lane I. The pool-1 lane shows the retardation by proteins before affinity-chromatographic fractionation. The flow-through (FT) and column washings (W1 and W2) correspond to unbound material. Bound proteins were eluted in ten fractions (lanes 1–10) with increasing concentration of KCl: 0.2 M-KCl (lanes 1, 2 and 3), 0.5 M-KCl (lanes 4, 5 and 6) and 1 M-KCl (lanes 7, 8, 9 and 10). 'Free' refers to the free oligonucleotide.

Table 1. Isolation of p47 protein

Step	Protein (mg)	Volume (ml)	Specific activity* (pg/mg)	Total activity (pg)	Purification factor	Recovery (%)
Extraction	400	15	38	15200	1	100
DEAE h.p.l.c.	11.5	25	260	2990	6.8	20
First affinity chromatography	0.328	6	32000	10496	842	70
Second affinity chromatography	0.160	4	41000	6560	1078	43

* The specific activity was defined as described in the Experimental section as pg of retarded radiolabelled probe/mg of protein.

negative effect of Mg^{2+} on the interaction of the inducible C1 DNA-binding activity suggests a regulatory mechanism for this protein, because Mg^{2+} has been also shown to inhibit transcription assays *in vitro* with the SV 40 enhancer which contains the κB site [24]. The inhibitory effect of Mg^{2+} is not understood, but it could cause conformational changes of DNA-binding proteins.

Using two different chromatographic fractionation procedures we partially isolated an inducible DNA-binding activity C1 and identified it as a 47 kDa protein (p47). The C2′ protein(s) were eluted from DEAE at lower salt molarity than was C1, but exhibited the same Mg^{2+}-sensitivity as C1 (Table 2). It is not clear whether the protein(s) responsible for C2′ electrophoretic mobility are related to p47. C2′ could be a proteolytic product of p47 or it may be a modified form of p47 (perhaps due to phosphorylation of C1).

The induction of active NF-κB in T-cells has been attributed to dissociation from a cytoplasmic inhibitor, IκB [28], which allowed the translocation of NF-κB into the nucleus and subsequent activation of transcription of IL2 and IL2Rα genes. P47 also might be associated with an inhibitor, since its fractionation from the whole-cell extract by affinity chromatography increased binding activity (see the Results section, in particular Table 1).

Several cellular and viral genes, including the κ light chain, SV 40, β-microglobulin and IL2rα, have been shown to contain κB sites in their regulatory regions [10,11,13,20]. In HIV-1, SV 40, κ light chain and β-microglobulin, the κB site was shown to regulate gene

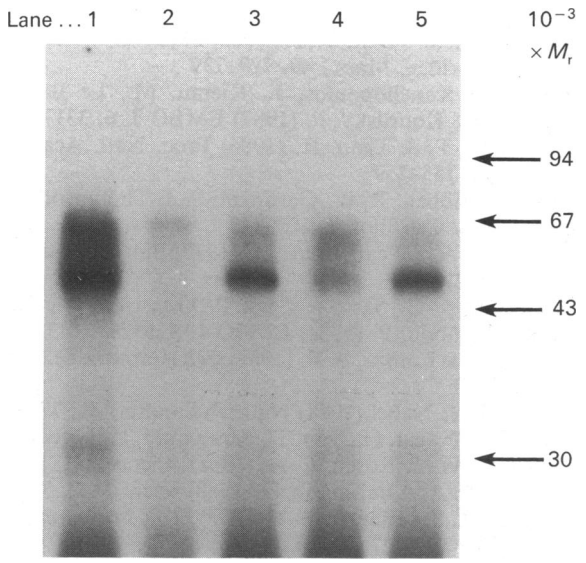

Fig. 6. SDS/polyacrylamide-gel electrophoresis of u.v.-cross-linked proteins to ^{32}P-labelled BrdUrd-substituted Tandem oligonucleotide

Affinity-chromatographically purified fraction (0.4 μg) was incubated with the Tandem oligonucleotide (500000 c.p.m.) alone (lane 1) or with a 500-fold excess of competitor DNA: Tandem (lane 2), CRE (lane 3), IL2Rα enhancer (lane 4) and AP1 (lane 5).

Table 2. Effect of Mg^{2+} on stability of protein–Tandem oligonucleotide complexes

The protein–oligonucleotide complexes were analysed by the gel-retardation assay. The $MgCl_2$ concentration was 5 mM.

Stage	PMA+PHA-induced	PMA+PHA-non-induced	Mg^{2+}-dissociated
Initial extract	C1	C2	C1
DEAE h.p.l.c.	C1	C2	C1
	C2′ (?)		C2′

transcription by interacting with cellular proteins. Some κB-site-binding proteins were isolated and partially characterized. For example NF-κB, a 42–44 kDa protein, was purified from bovine spleen cells [11], HIVEN 86A, a 86 kDa protein, was found in mitogen-stimulated Jurkat cells [12], EBP-1, a 55–60 kDa protein, was purified from phorbol-ester-treated HeLa cells [14], and H2TF1, a 48 kDa protein, was purified from murine thymoma cells [20]. NF-κB is constitutively expressed only in mature B-cells, where it regulates the transcription of κ light chain, but it is also inducible in T-cells, where it might regulate transcription of IL2 and IL2Rα [11,13,26,27]. At present we have not identified with certainty a cellular enhancer for p47, since it binds IL2Rα enhancer probe with much lower affinity than HIV-1 enhancer probes, at least in the assays *in vitro* used in the present study.

In our experiment we found that p47 differs in size from the previously described Jurkat-cell HIV enhancer-binding protein, HIVEN 86A [12], as well as from the HeLa-cell HIV enhancer-binding protein, EBP-1 [14]. However, its size is comparable with NF-κB isolated either from bovine spleen cells (42–44 kDa) [11] or a human B-cell line (51 kDa) [15], as well as with the 48 kDa H2TF1 or KBF1 [20]. It is not clear whether p47, NF-κB and H2TF1 are products of the same gene with different post-translational modifications or whether they result from the expression of different genes. It is not unusual for several different proteins to recognize the same DNA sequence. Indeed, the mammalian transcription factor c-Jun/AP1, the yeast GCN4 and several other mammalian and yeast proteins all bind to the same consensus sequence ATGA(C/G)TCAT (see [25] for review).

H2TF1 and NF-κB differ by their κB-site-binding characteristics [11,29]. However, the relation between p47, NF-κB, H2TF1, EBP-1 and HIVEN 86 A is not clear. In spite of several described differences such as molecular mass, affinities for κB site and modulation of the binding to κB site by nucleotides, NF-κB, H2TF1 and p47 could be structurally related. The specificity of their transcriptional activity could be due to different post-translational modifications or to interaction with other, cell or signal, specific proteins.

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REFERENCES

1. Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauquet, C., Alxer-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983) Science **220**, 867–888
2. Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G. M., Keplen, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P. & Markhan, P. D. (1984) Science **224**, 500–503
3. Dalgleish, A. G., Beverley, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. A. (1984) Nature (London) **312**, 763–766
4. Klatzman, D., Barre-Sinoussi, F., Nugeyre, M. T., Dauguet, C., Vilmer, E., Griscelli, C., Vezinet-Brun, F., Rouzioux, C., Gluckman, J., Chermann, J. & Montagnier, L. (1984) Science **225**, 59–63
5. Levy, J. A., Cheung-Meyer, C., Dina, D. & Luciw, P. A. (1986) Science **232**, 998–1001
6. Garcia, J. A., Wu, F. K., Mitsuyasu, R. & Gaynor, R. B. (1987) EMBO J. **6**, 3761–3770
7. Rosen, C. A., Sodroski, J. & Haseltine, W. A. (1985) Cell (Cambridge, Mass.) **41**, 813–823
8. Muesing, M., Smith, D. H. & Capon, D. J. (1987) Cell (Cambridge, Mass.) **48**, 691–701
9. Nabel, G. & Baltimore, D. (1987) Nature (London) **326**, 711–713

10. Sen, R. & Baltimore, D. (1986) Cell (Cambridge, Mass.) **47**, 921–928
11. Lenardo, M. J., Kuang, A., Gifford, A. & Baltimore, D. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 8825–8829
12. Franza, B. R., Josephs, S. F., Gilman, M. Z., Ryan, W. & Clarkson, B. (1988) Nature (London) **330**, 391–395
13. Bohlein, E., Lowenthal, J. W., Siekevitz, M., Ballard, D. W., Franza, B. R. & Greene, W. C. (1988) Cell (Cambridge, Mass.) **53**, 827–836
14. Wu, F. K., Garcia, J. A., Harrich, D. & Gaynor, R. B. (1988) EMBO J. **7**, 2117–2129
15. Kawakami, K., Schleidereit, C. & Roeder, R. G. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 4700–4704
16. Sorger, P. K. & Pelham, H. R. B. (1987) EMBO J. **6**, 3035–3041
17. Cross, S. L., Feinberg, M. B., Wolf, J. B., Holbrook, N. J., Wong-Staal, F. & Leonard, W. J. (1987) Cell (Cambridge, Mass.) **49**, 47–56
18. Comb, M., Birnberg, N. C., Seasholdz, A., Herbert, E. & Goodman, H. M. (1986) Nature (London) **323**, 353–356
19. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Ramsorf, H. J., Jonat, C., Herrlich, P. & Karin, M. (1987) Cell (Cambridge, Mass.) **49**, 729–739
20. Yano, O., Kanellopoulos, J., Kieran, M., Le Bail, O., Israel, A. & Kourilsky, P. (1987) EMBO J. **6**, 3317–3324
21. Kadona, J. T. & Tjian, R. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 755–759
22. Safer, B., Cohen, R. B., Garfinkel, S. & Thompson, J. A. (1988) Mol. Cel. Biol. **8**, 105–113
23. Porzio, M. A. & Pearson, A. M. (1977) Biochem. Biophys. Acta **490**, 27–34
24. Wildeman, A. G., Sassone-Corsi, P., Gunstrom, T., Zenke, M. & Chambon, P. (1984) EMBO J. **3**, 3129–3133
25. Curran, T. & Franza, B. R. (1988) Cell (Cambridge, Mass.) **55**, 395–397
26. Leung, K. & Nabel (1988) Nature (London) **33**, 776–778
27. Ruben, S., Poteat, H., Tan, T., Kawakami, K., Roeder, R., Haseltine, W. & Rosen, C. A. (1988) Science **241**, 89–92
28. Baeuerle, P. A. & Baltimore, D. (1988) Science **242**, 540–546
29. Baldwin, A. S. & Sharp, P. A. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 723–727

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