1050–1055 *Nucleic Acids Research, 1997, Vol. 25, No. 5*

Basal transcription factors TBP and TFIIB and the viral coactivator E1A 13S bind with distinct affinities and kinetics to the transactivation domain of NF-κB p65

Klaus Paal, Patrick A. Baeuerle+ and M. Lienhard Schmitz*

Institute of Biochemistry and Molecular Biology, Albert-Ludwigs University, Hermann-Herder Strasse 7, D-79104 Freiburg, Germany

Received October 22, 1996; Revised and Accepted January 9, 1997

ABSTRACT

Transactivation domains (TADs) are able to contact several components of the basal transcription apparatus and co-activator molecules. In order to study these interactions in biophysical detail, binding of the well-characterized TAD from the human transcription factor NF-κB p65 (RelA) to the basal transcription factors TBP and TFIIB and the viral co-activator protein E1A 13S was chosen as a model system to investigate the kinetics and affinities of such protein–protein interactions by surface plasmon resonance analysis. The TAD of NF-κB p65 showed remarkably different affinities and kinetics in binding to the various proteins. The real-time kinetic measurements revealed an association rate constant (k_{ass}) of 2.3×10^6/M/s for the interaction between the p65 TAD and TBP. The association rate constants of the p65 TAD were much weaker for TFIIB (6.8×10^4/M/s) and for the E1A 13S protein (4.9×10^4/M/s). The dissociation rate constants (k_{diss}) were determined to be 7.9×10^{-4}/s for TBP, 1.6×10^{-3}/s for TFIIB and 1.3×10^{-3}/s for the E1A protein. Accordingly, the calculated dissociation constants (K_d) differed between 3.4×10^{-10} M for the strongly binding TBP protein and 2.3×10^{-8} M and 2.6×10^{-8} M for the weaker binding TFIIB and E1A 13S proteins respectively. Non-linear analysis of the appropriate part of the sensorgrams revealed monophasic association and dissociation kinetics for binding between the p65 TAD and all three interaction partners. The remarkable differences in protein affinities add another aspect to a more detailed understanding of formation of the transcription preinitiation complex. The co-transfection of TBP and E1A 13S stimulated NF-κB p65-dependent gene expression, showing the biological significance of these interactions.

INTRODUCTION

Transcription initiation at eukaryotic genes requires the assembly of a preinitation complex (PIC) on the promoter DNA. The PIC consists of RNA polymerase II and at least seven basal transcription factors: TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH and TFIIG/J (1). The human PIC contains at least 10 distinct polypeptide subunits in the RNA polymerase II complex plus a minimum of another 35 polypeptides (2). A key step in transcription initiation is binding of TFIID to the TATA box, which in most promoters is located close to the transcription start site. The multisubunit TFIID complex consists of the TATA binding protein (TBP) and at least eight additional proteins, termed TBP-associated factors (TAFs) (3). TBP binds to the TATA box in a sequence-specific fashion and the TBP–promoter complex is subsequently recognized by TFIIB. This complex nucleates the subsequent stepwise association of TFIIA, RNA polymerase II, TFIIF and further factors (4).

In addition to a properly assembled PIC, sequence-specific DNA binding proteins are required for activated transcription. These are typically composed of several domains minimally mediating DNA binding, nuclear translocation and transactivation. Transactivation domains (TADs) from various transcription factors have been found to exert their stimulatory effects on transcription even over large distances by directly contacting general transcription factors, such as TBP and TFIIB, TAFs or co-activator proteins. These multiple protein–protein interactions might either facilitate binding of the general transcription factors to the promoter, result in covalent modifications of promoter-associated proteins or lead to conformational changes in the PIC (5). Contact between the TADs and its binding partners finally results in initiation of transcription and increases the efficiency of transcription elongation (6). Co-activators, including TAF and non-TAF proteins, constitute another group of proteins participating in transcription (7,8). A well-studied example of a non-TAF co-activator is the adenovirus-encoded E1A 13S protein, which stimulates transcription of several host transcription factors, including ATF-2, Oct-4, c-Jun, USF, Sp1 and NF-κB p65 (9,10).

The ubiquitous transcription factor NF-κB regulates expression of a plethora of immunologically relevant genes (11). In most cell types the dimeric DNA binding form of this transcription factor is retained in the cytoplasm by association with the inhibitory IκB molecule (12,13). Exposure of cells to a variety of pathogenic agents leads to the degradation of IκB and nuclear translocation of the released DNA binding subunits (14,15). The NF-κB p65 subunit displays the strongest transactivation potential of the five distinct DNA binding subunits and contains an acidic TAD in its

*To whom correspondence should be addressed at present address: Department of Immunochemistry, Deutsches Krebsforschungszentrum (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. Tel: +49 6221 423725; Fax: +49 6221 423746

+Present address: Tularik Inc., 2 Corporate Drive, South San Francisco, CA 94080, USA

C-terminal 80 amino acids (16–18). This domain is related to the TAD of herpes simplex protein VP16 and was found to be unstructured under physiological conditions (19). Under conditions mimicking protein–protein interactions the NF-κB p65 TAD can adopt an α-helical conformation. Among the proteins specifically binding to the p65 TAD are the general transcription factors TFIIB and TBP, as well as the co-activating E1A 13S protein (10,20). This viral protein activates NF-κB by a dual mechanism: in a first step E1A activates cytoplasmic NF-κB by induced degradation of IκB-α and subsequently binds to the TADs of NF-κB p65, thereby co-activating its transcriptional activity in the cell nucleus (10). The various protein–protein interactions between NF-κB p65 and its ligands were identified by functional and biochemical studies (10,20). Here we show that TBP binds two orders of magnitude more strongly to the TAD of NF-κB p65 than E1A 13S and TFIIB. All three ligands of the TAD displayed a monophasic association and dissociation behavior. The biological significance of these interactions was evident from co-transfection experiments in COS cells, where TBP and E1A 13S stimulated NF-κB p65-dependent gene expression.

MATERIALS AND METHODS

Protein expression and purification

The vector encoding a His-tagged human TFIIB protein was cloned by PCR using primers A (5′-GGGATCCCATGGCGTCTACC-AGCCG-3′) and B (5′-GGGATCCTTATAGCTGTGGTAGT-TT-3′) and a vector containing the full-length cDNA of human TFIIB as a template. The annealing nucleotides are underlined. The amplified 963 bp fragment was recut with *Bam*HI and cloned into the pQE10 vector (Qiagen Inc.) previously opened by digestion with *Bam*HI. The resulting plasmid, pHis-TFIIB, bears an N-terminal hexahistidine tag, allowing purification of the expressed protein on Ni–NTA–agarose. This expression plasmid was introduced into *Escherichia coli* strain M15 and grown in LB medium containing ampicillin and kanamycin until an OD_{600} of 0.7 was reached. Subsequently, IPTG (Boehringer Inc.) was added to a final concentration of 1 mM. Protein expression was allowed for 6 h at 30°C. Cells were collected by 10 min centrifugation and the pellet was dissolved in 6 ml buffer A [50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM 2-mercaptoethanol] per liter LB. Cells were lyzed by three cycles of freeze–thawing, the addition of lysozyme and sonification. Cell debris was removed by centrifugation at 15 000 *g* for 20 min at 4°C. The His-tagged TFIIB protein was precipitated from the supernatant in 40% ammonium sulfate. After another centrifugation the protein pellet was dissolved in 5 ml BC200 (20 mM Tris–HCl, pH 7.9, 200 mM KCl, 20% glycerol, 10 μM $ZnCl_2$, 1 mM 2-mercaptoethanol, 1 mM PMSF) and TFIIB was allowed to bind to the Ni–NTA–agarose (Qiagen Inc.) for 1 h. After washing the column with BC200 containing 5 mM imidazole the TFIIB protein was eluted in BC200 buffer containing 100 mM imidazole and directly applied to an SP-Sepharose column (Pharmacia Biotech Inc.). After washing this column with BC200, the TFIIB protein was eluted in BC400 buffer (20 mM Tris–HCl, pH 7.9, 400 mM KCl, 2 mM EDTA, 20% glycerol, 10 μM $ZnCl_2$, 1 mM 2-mercaptoethanol, 1 mM PMSF). Recombinant GAL4–$p65^{471–551}$ protein was expressed in *E.coli* and purified on Ni–NTA–agarose and heparin–agarose columns essentially as described (21). Bovine serum albumin (BSA) was from Boehringer Mannhein Inc. and the recombinant purified E1A 13S and human TBP proteins were obtained from Santa Cruz Inc. and Promega Inc.

Surface plasmon resonance analysis

Binding kinetics were determined using a BIAcore™ biosensor system (Pharmacia Biosensor) (22). The GAL4–$p65^{471–551}$ protein was immobilized on research grade CM5 sensor chips in 10 mM sodium acetate, pH 5.5, using the manufacturer's amine coupling kit. Unreacted residues on the surface were blocked by two washes with 1 M ethanolamine, pH 8.5. Measurements were peformed in binding buffer (10 mM HEPES, pH 7.9, 5 mM $MgCl_2$, 150 mM KCl, 10 μM $ZnCl_2$, 0.005% v/v P20) at 25°C at a flow rate of 20 μl/min. Detailed methods for using this device are reported elsewhere (23). Data processing was performed with the BIAevaluation software (Pharmacia, version 2.1). The dissociation rate constants were measured in flow buffer according to the equation $R_t = R_0\, e^{-k\mathrm{diss}(t - t0)}$. In this equation k_{diss} is the dissociation rate constant, R_t is the relative response at time t and R_0 is the relative response at the starting time t_0. The association rate constants were calculated from the measured k_{diss} according to the equation $R_t = R_{eq}[1–e^{(k_{diss}C + k_{diss})(t–t0)}]$, where R_{eq} is the steady-state response level and C is the molar concentration of the non-immobilized interacting partners. The dissociation constants were calculated by dividing k_{diss} by k_{ass}.

Cell culture and transient transfection assays

Monkey COS7 cells were grown at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS) and 1% penicillin and streptomycin (all from Gibco-BRL, Eggenstein, Germany). Approximately 5×10^5 exponentially growing COS7 cells were transfected in suspension as described (24). The amounts of reporter plasmids and expression vectors used are given in the figure legends. The eukaryotic expression vectors pHβAPr-E1A 13S (25), CMV-TBP, CMV-TFIIB (20) and CMV-p65 (16) have been described previously. The NF-κB-dependent luciferase reporter plasmids used were HIV-1 $(κB)_{wt}$ LTR luciferase and HIV-1 $(κB)_{mut}$ LTR luciferase (26). Cells were harvested and analyzed for activity of the reporter genes 36 h post-transfection. Cells were washed once with ice-cold phosphate-buffered saline (PBS) and harvested by scraping with a rubber policeman and transferred to Eppendorf tubes. After centrifugation for 3 min at 2000 *g* the pellet was lyzed by addition of 150 μl 1% (v/v) Triton X-100, 25 mM glycylglycine, pH 7.8 (adjusted with KOH), 15 mM $MgSO_4$, 4 mM EGTA, pH 8 (adjusted with KOH), and 1 mM DTT. The lysates were centrifuged at 4°C and 50 μl supernatant assayed for luciferase activity. This was performed by adding 150 μl reaction buffer (25 mM glycylglycine, pH 7.8, 15 mM $MgSO_4$, 30 mM potassium phosphate, pH 7.6, 4 mM EGTA, 1 mM DTT and 3 mM ATP) and measuring the light emission in a Microlumat LB96 P luminometer (Berthold). The luminometer was programed to inject 100 μl 0.3 mg/ml luciferin (Sigma) and to measure light emission for 30 s after injection.

RESULTS AND DISCUSSION

Binding assays using either the DNA binding domain of the yeast transcription factor GAL4 or a fusion protein beween GAL4 and the p65 TAD showed exclusive binding of TFIIB, TBP and E1A 13S to the p65 TAD portion. The GAL4 protein alone displayed

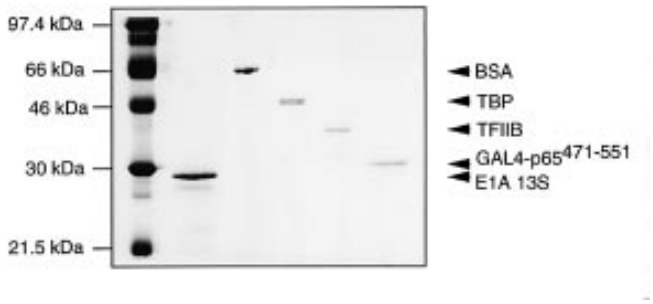

Figure 1. Reducing SDS–PAGE of the purified proteins. The indicated proteins were produced in *E.coli*, purified and analyzed on a 15% SDS gel. The gel was stained with Coomassie blue. Arrowheads indicate the positions of the proteins. The molecular masses of the pre-stained protein markers are given in kDa.

no binding activity with any of the p65 TAD binding partners (10,20,27). In order to study these interactions by plasmon surface resonance analysis, a GAL4–p65 TAD fusion protein containing amino acids 471–551 of NF-κB p65 fused to GAL4 and TBP, TFIIB and E1A 13S were expressed and purified from *E.coli*. These proteins and the control protein BSA were analyzed by electrophoresis on a reducing SDS–polyacrylamide gel stained with Coomassie blue. All proteins migrated roughly according to their predicted size and were sufficiently pure to subject them to further analysis using a BIAcore® device (Fig. 1).

The GAL4–p65 TAD protein, which is also a potent activator of transcription in intact cells, was coupled to the surface of a sensor chip solely via its GAL4 portion, since the p65 TAD does not contain any basic amino acids, which are necessary for covalent attachment of the protein to the sensor chip. Three different concentrations of GAL4–p65 [719, 872 and 3800 response units (RU)] were immobilized on the gold-coated surface of three sensor chips. The amount of protein which associates with the immobilized protein is quantified by measurement of the surface plasmon resonance signal, from which parameters of the binding can be calculated. In order to define the conditions allowing measurement of true and specific protein–protein interactions, the control protein BSA was dialyzed against binding buffer containing either 100, 150 or 200 mM salt respectively. Various concentrations of these dialysates were injected into flow cells with immobilized GAL4–p65 TAD and the interactions were measured. In accordance with previous results obtained from column binding assays (20), no significant binding of BSA could be measured in binding buffer containing 150 or 200 mM KCl (data not shown). In order to ensure the specificity of the recorded data, the binding proteins TBP, TFIIB and E1A 13S were therefore dialyzed against binding buffer containing 150 mM KCl prior to measurement of the protein–protein interaction. The injection of binding buffer containing TFIIB to a sensor chip coated with immobilized GAL4–p65 TAD protein showed a typical increase in RUs indicative of a binding reaction (Fig. 2A). Replacement of the TFIIB solution by plain buffer was followed by rapid dissociation of most of the bound TFIIB. In a control experiment, no binding of TFIIB was observed when a sensor chip was used that lacked the GAL4–p65 TAD protein during the coating process (data not shown). Concentration-dependent binding studies were conducted to allow calculation of kinetic paramenters. The time- and concentration-dependent increase in RUs observed during the binding of TFIIB (Fig. 2A) is indicative of a primarily monophasic reaction, which

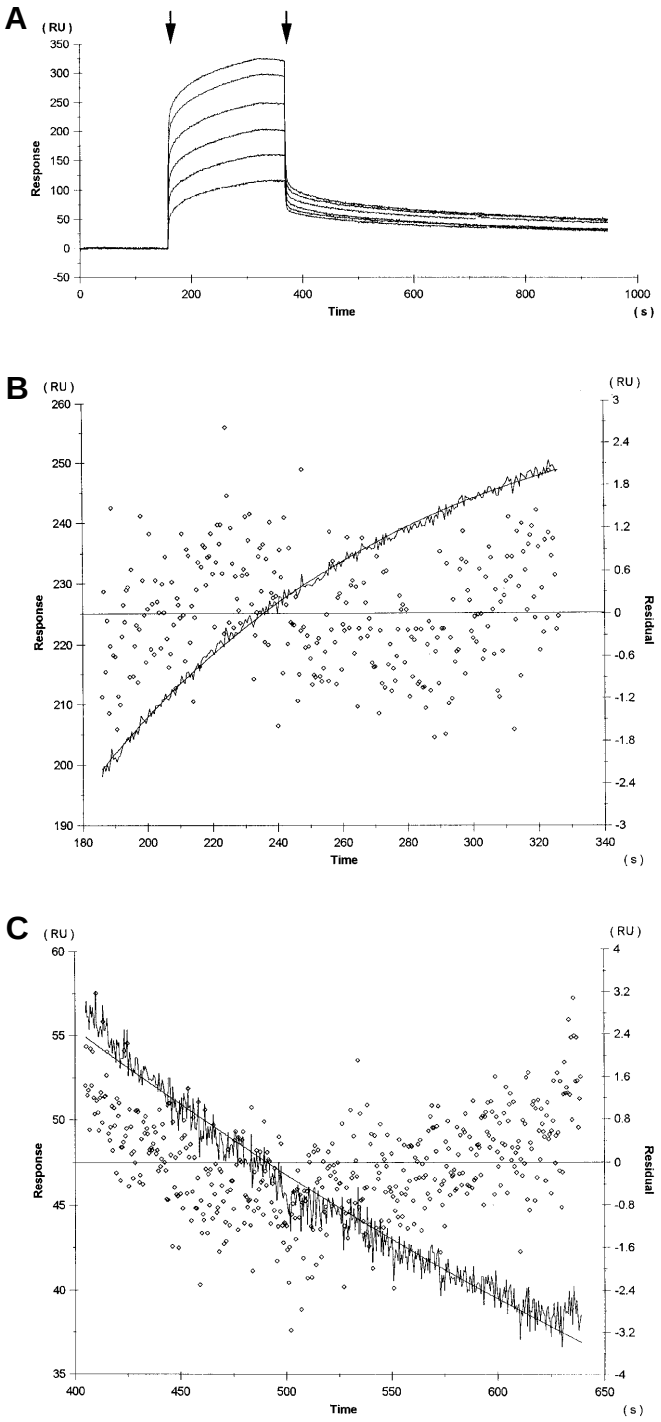

Figure 2. Analysis of TFIIB binding to the immobilized GAL4–p65 TAD protein. (**A**) Real-time kinetic analysis of binding. For the recording of the displayed sensogram the following concentrations of TFIIB were used (from bottom to top): 100, 150, 200, 250, 300 and 330 nM. The two arrows indicate the beginning and end of injections. (**B**) Overlay plot of the fitted association curve and the residual plot of TFIIB protein according to the BIAevaluation homogeneous A + B ↔ AB model. The dots are displayed by plotting the statistical residual value on the *y*-axis against time on the *x*-axis of the graph. The randomly scattered residual values around the *x*-axis are indicative of a good curve fitting. The curved line represents the experimentally determined values after plotting time versus RUs, the line representing the calculated ideal fit. (**C**) Curve fitting for dissociation of the TFIIB protein according to the BIAevaluation A + B ↔ AB homogeneous model. Details of the figure legend are as explained in (B).

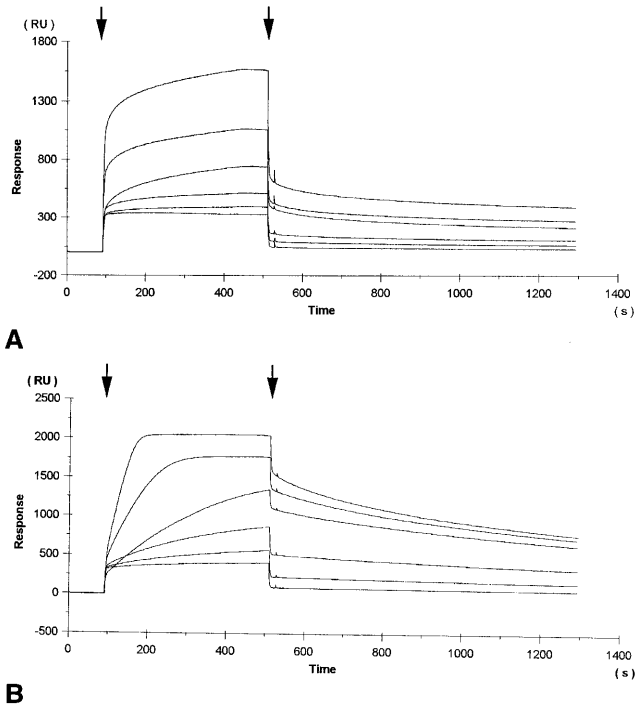

Figure 3. Real-time kinetic analysis of the binding of E1A 13S and TBP to immobilized GAL4–p65 TAD protein. (**A**) Sensogram obtained after injection of 10, 50, 100, 150, 200 and 250 nM (from bottom to top) E1A 13S protein. (**B**) Sensogram obtained after injection of increasing amounts (1, 5, 10, 20 and 30 nM) of TBP protein. The two arrows indicate the beginning and end of injections.

was confirmed by residual plotting. Non-linear analysis of the appropriate part of the sensorgrams showed good curve fitting to the homogeneous association model according to the equation A + B ↔ AB (Fig. 2B). Similarly, dissociation of TFIIB from the GAL4–p65 TAD protein revealed a monophasic reaction (Fig. 2C). Using the type 1 association software (BIAevaluation 2.1) the association rate constant of the interaction between TFIIB and the GAL4–p65 TAD protein was calculated to be $6.8 \pm 0.6 \times 10^4$/M/s and the dissociation rate constant to be $1.6 \pm 0.2 \times 10^{-3}$/s, resulting in a K_d value of 2.3×10^{-8} M.

The parameters for binding to the immobilizd GAL4–p65 TAD protein were subsequently recorded for E1A 13S and TBP respectively (Fig. 3A and B). Both proteins dose-dependently bound to the TAD of NF-κB p65. Analysis of the binding parameters of the E1A 13S protein revealed a k_{ass} of $4.9 \pm 0.5 \times 10^4$/M/s and a k_{diss} of $1.3 \pm 0.4 \times 10^{-3}$/s ($K_d = 2.6 \times 10^{-8}$ M). The binding parameters recorded for the interaction between the TBP protein and immobilized GAL4–p65 TAD differed strongly in comparison with the other two protein ligands. After omission of TBP in the binding buffer a significant amount of TBP remained bound to its substrate protein (Fig. 3B). The stronger interaction between TBP and its GAL–p65 TAD binding partner was also evident from the calculated association rate constant of $2.3 \pm 0.4 \times 10^6$/M/s and the dissociation rate constant of $7.9 \pm 0.4 \times 10^{-4}$/s. The resulting K_d of 3.4×10^{-10} M revealed a two orders of magnitude higher affinity of TBP for the p65 TAD when compared with the binding affinities of TFIIB and E1A 13S. Like TFIIB, E1A and the TBP also showed monophasic association and dissociation kinetics, as verified by residual plotting (data not shown). The stronger affinity for TBP is apparently not a feature of all acidic TADs, since the C-terminal TAD of the yeast GAL4 protein showed a comparable affinity for yeast TBP and yeast TFIIB (28).

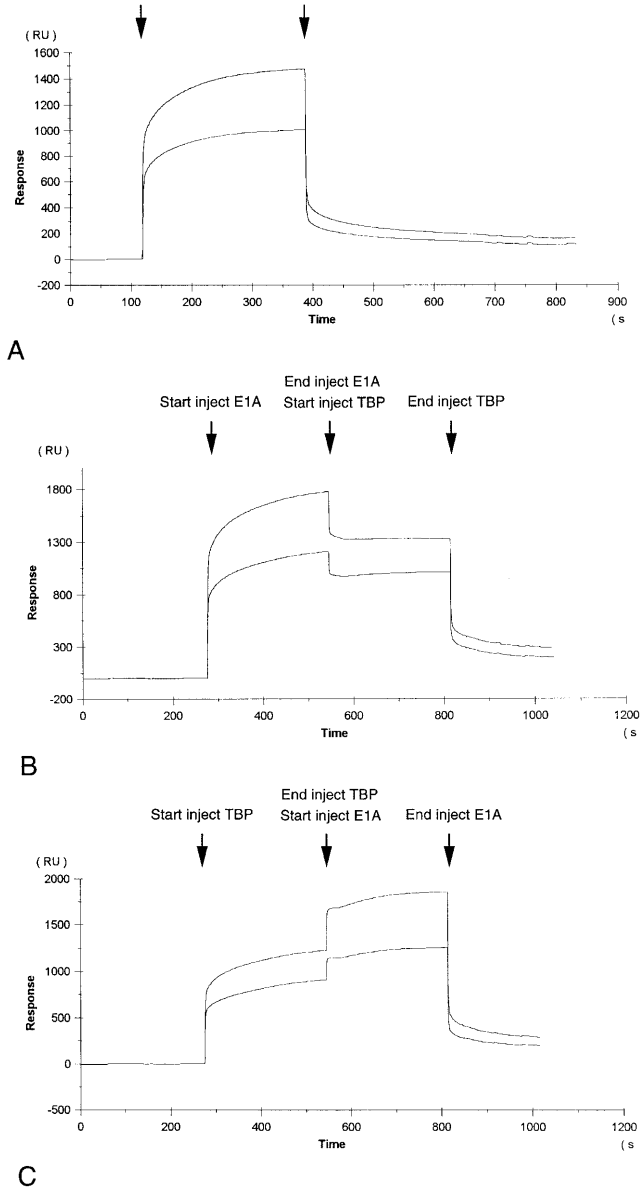

Figure 4. Binding analysis of E1A 13S and TBP protein mixes to immobilized GAL4–p65 TAD protein ligand. (**A**) For the recording of the displayed sensogram two mixtures of E1A and TBP proteins were analyzed for binding: 100 nM E1A and 20 nM TBP (lower) and 200 nM E1A and 20 nM TBP (upper). The two arrows indicate the beginning and end of injections. (**B**) Analysis of TBP binding to a preformed E1A–GAL4–p65 complex. 100 (lower) and 200 nM E1A protein (upper) were injected onto a sensor chip containing the immobilized GAL4–p65 protein. After the end of E1A injection 20 (lower) and 30 nM (upper) TBP was injected as indicated on the sensogram. (**C**) Analysis of E1A binding to a preformed TBP–GAL4–p65 complex. Twenty (lower) and 30 nM TBP protein (upper) were injected onto a sensor chip containing the immobilized GAL4–p65 protein, followed by injection of 100 (lower) and 200 nM (upper) E1A protein as shown on the sensogram.

Since E1A can also bind to the TBP protein (27), we next investigated whether E1A and TBP may form a ternary complex

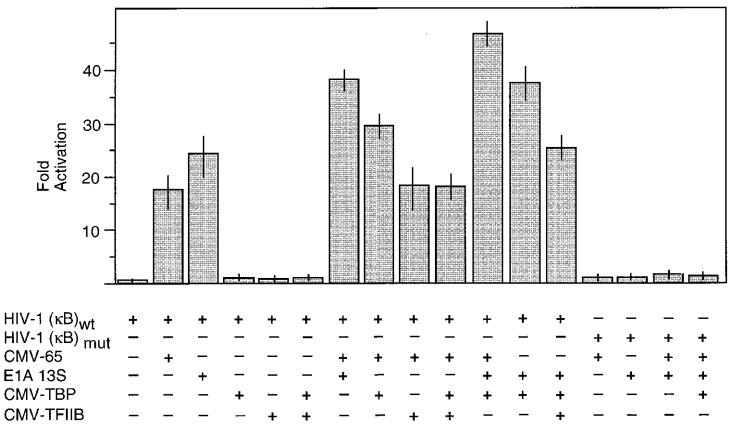

Figure 5. Functional interaction of TBP, TFIIB and E1A 13S with NF-κB p65 in transcription activation. COS cells were co-transfected with 1.5 pmol HIV-1 LTR–*luc* reporter gene constructs, 1 pmol p65 expression vector and 1 pmol expression vectors encoding binding partners as indicated. Thirty-six hours after transfection cells were harvested and gene expression was determined. The transcriptional activities are given as fold induction, which was calculated by comparison with the basal level of transcription of the *luc* reporter gene alone. The standard deviations obtained from four experiments are given by error bars.

with the GAL4–p65 protein. Therefore, the GAL4–p65 protein was immobilized on a sensor chip and binding of co-injected E1A and TBP or a mixture of both proteins was recorded. There was no significant difference in the binding of a mixture of E1A and TBP to the immobilized GAL4–p65 protein when compared with binding of the individual proteins (Fig. 4A). This excludes formation of a ternary complex being highly favored over formation of a bimolecular complex. We next tested the binding of TBP to an already existing complex between GAL4–p65 and E1A (Fig 4B). Addition of TBP prevented the reduction in RUs after the end of E1A injection. In a further experiment an already preformed complex between GAL4–p65 and TBP was not a preferred target for E1A binding (Fig. 4C). Again, these results exclude the predominance of a ternary complex over the bimolecular complex. On the other hand, some ternary complex formation on the sensor cannot been ruled out, since the binding characteristics are indistinguishable from a bimolecular model in which displaced TBP (or E1A) is replaced by available E1A (or TBP).

The regulatory effects of the three interacting proteins on p65-dependent transcription were directly compared in co-transfection experiments. Monkey COS cells were transiently transfected with *luc* reporter gene constructs and expression vectors for NF-κB p65 and the respective interacting proteins (Fig. 5). Expression of NF-κB p65 and E1A induced transcription of the κB-dependent *luc* reporter gene. The transcriptional activity of p65 was stimulated to comparable amounts by co-expression of either TBP or E1A 13S. These transcriptional activations were dependent on integrity of the two κB binding sites in the reporter gene construct, showing that the observed effects are due to interactions of the various interacting proteins with NF-κB rather than with other transcription factors (Fig. 5). The superactivation of p65-dependent transcription by E1A 13S is due to its co-activating function. It has previously been shown that the 13S splice variant of E1A specifically enhanced the transcriptional activity of a GAL4–p65$^{286–551}$ fusion protein on a Gal4-dependent reporter gene (29). Co-expression of TBP also stimulated transcriptional activity of the GAL4–p65$^{286–551}$ fusion protein (data not shown). The stimulatory role of TBP expression on NF-κB p65-dependent transcription might be simply explained

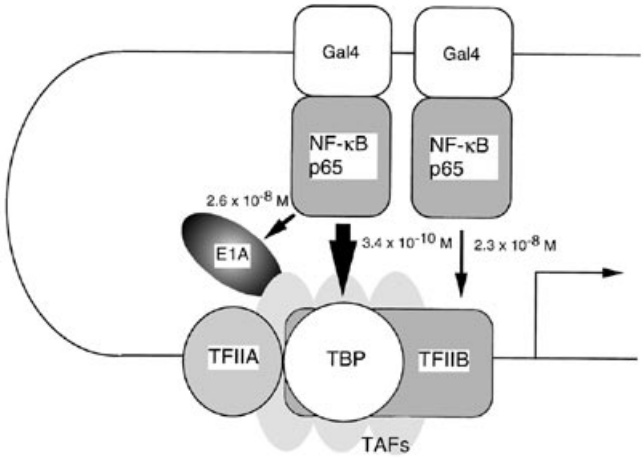

Figure 6. Model for the simultaneous association of NF-κB p65 TADs with the co-activating E1A 13S protein and components of the basal transcription apparatus. The calculated K_d values are given next to the arrows, which symbolize the different binding affinities. The transcription start site is indicated.

by limiting amounts of this basal transcription factor in the cell nucleus. Alternatively, TBP overexpression could compete for negative transcriptional regulators such as NC2/DR1 or DR1/p19, which directly contact TBP. Both models can explain the stimulatory effect of TBP co-expression, which was also seen in transient transfection experiments on the RAR-β2 promoter activated by E1A (30). The inability of TFIIB to further enhance transcription might be due to saturated amounts of this protein or to more indirect effects. It is known that TBP, TFIIB and E1A 13S interact with numerous cellular regulatory proteins, including TAFs and co-activators. Some of these proteins, such as human $TAF_{II}250$ and *Drosophila* $TAF_{II}110$ are bound by both E1A 13S and TBP (31). It is quite possible that these associated regulatory proteins contribute to the observed transcriptional effects. Potential mechanisms could involve squelching, post-translational modifications or conformatorial changes within the PIC. On the

other hand, it is well documented that TBP is crucial for the functioning of acidic TADs (32,33) and the dual role of E1A 13S in NF-κB activation is known in some detail (10). The physiological relevance of interaction between TFIIB and NF-κB p65 is evident from yeast one-hybrid experiments in intact cells (20).

It has been suggested that simultaneous contact of TADs with several components of the basal transcription apparatus is the underlying mechanism for transcription activation and synergistic effects, as seen in functional and biochemical experiments (34). In support of this idea, structural studies on the TBP–TFIIB TATA element ternary complex revealed the presence of exposed surfaces from both proteins that are accessible for simultaneous interaction with TADs (35,36), as schematically displayed in Figure 6. The high affinity of the NF-κB p65 TAD for TBP suggests that this interaction is of especial structural relevance to formation of the PIC. The physiological importance of this interaction is highlighted by the finding that co-transfection of TBP resulted in stimulation of p65-dependent transcription in monkey COS cells. The concept that interactions between activators and the general transcription machinery are important *in vivo* was supported by an analysis of a yeast strain mutated in GAL11P, a protein associated with the RNA polymerase II complex. The point-mutated GAL11P protein allowed contact with the DNA binding domain of GAL4, a region that is normally not involved in transactivation. This interaction led to rescue of the defunct GAL4 protein and resulted in transcription activation (37). Further studies showed that covalent or non-covalent fusion of TBP to a promoter-bound protein lacking a TAD could bypass the need for an activation domain (32,33). The strong affinity of TBP for a TAD might possibbly also account for the finding that the TAD of the VP16 protein could adopt a stable secondary structure exclusively upon contacting TBP, but not upon contacting TFIIB (38). It is tempting to speculate that the high affinity of TBP for the TAD of NF-κB p65 significantly contributes to the driving force required to target this transcription factor to its promoter, while the intervening DNA stretch is looped out. In this scenario the weaker binding TFIIB and E1A 13S proteins would contribute to this driving force to a significantly lesser extent. They would rather be important for non-structural functions such as transcription initiation site selection and stimulation of already ongoing transcription.

ACKNOWLEDGEMENTS

We thank C.Kaltschmidt for helpful discussions, Jan Preiß for his engagement in this project as a summer student and Patricia Müller for critically reading the manuscript. This work was funded by a EC-supported Biomed-2 grant awarded to M.L.S. and P.A.B. The BIAcore™ biosensor was financed by SFB 388.

REFERENCES

1 Zawel,L. and Reinberg,D. (1995) *Annu. Rev. Biochem.*, **64**, 533–561.
2 Halle,J.-P. and Meisterernst,M. (1996) *Trends Genet.*, **12**, 161–163.
3 Verrijzer,C.P. and Tjian,R. (1996) *Trends Biochem. Sci.*, **21**, 338–342.
4 Pugh,B.F. (1996) *Curr. Opin. Cell Biol.*, **8**, 303–311.
5 Goodrich,J.A., Cutler,G. and Tjian,R. (1996) *Cell*, **84**, 825–830.
6 Blair,W.S., Fridell,R.A. and Cullen,B.R. (1996) *EMBO J.*, **15**, 1658–1665.
7 Meisterernst,M., Roy,A.L., Lieu,H.M. and Roeder,R.G. (1991) *Cell*, **67**, 557–567.
8 Triezenberg,S.J. (1995) *Curr. Opin. Genet. Dev.*, **5**, 190–196.
9 Akusjärvi,G. (1993) *Trends Microbiol.*, **163**, 163–170.
10 Schmitz,M.L., Indorf,A., Limbourg,F., Staedtler,H., Traenckner,E.B.-M. and Baeuerle,P.A. (1996) *Mol. Cell. Biol.*, **16**, 4052–4063.
11 Baeuerle,P.A. and Henkel,T. (1994) *Annu. Rev. Biochem.*, **12**, 141–179.
12 Baeuerle,P.A. and Baltimore,D. (1988) *Science*, **242**, 540–546.
13 Israel,A. (1995) *Trends Genet.*, **11**, 203–205.
14 Verma,I.M., Stevenson,J.K., Schwarz,E.M., Van Antwerp,D. and Miyamoto,S. (1995) *Genes Dev.*, **9**, 2723–2735.
15 Baldwin,A.S.,Jr (1996) *Annu. Rev. Immunol.*, **14**, 649–683.
16 Schmitz,M.L. and Baeuerle,P.A. (1991) *EMBO J.*, **10**, 3805–3817.
17 Perkins,N.D., Schmid,R.M., Duckett,C.S., Leung,K., Rice,N.R. and Nabel,G.J. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 1529–1533.
18 Blair,W.S., Bogerd,H., Madore,S. and Cullen,B.R. (1994) *Mol. Cell. Biol.*, **14**, 7226–7234.
19 Schmitz,M.L., dos Santos Silva,M.A., Altmann,H., Czisch,M., Holak,T.A. and Baeuerle,P.A. (1994) *J. Biol. Chem.*, **269**, 25613–25620.
20 Schmitz,M.L., Stelzer,G., Altmann,H., Meisterernst,M. and Baeuerle,P.A. (1995) *J. Biol. Chem.*, **270**, 7219–7226.
21 Schmitz,M.L. and Baeuerle,P.A. (1994) *BioTechniques*, **17**, 714–718.
22 Jönsson,U., Fägerstam,L., Ivarsson,B., Johnsson,B., Karlsson,R., Lundh,K., Löfås,S., Persson,B., Roos,H., Rönnberg,I., Sjölander,S., Stenberg,E., Ståhlberg,R., Urbaniczky,C., Östlin,H. and Malmqvist,M. (1991) *BioTechniques*, **11**, 620–627.
23 Karlsson,R. and Ståhlberg,R. (1995) *Anal. Biochem.*, **228**, 274–280.
24 Lopata,M.A., Cleveland,D.W. and Sollner-Webb,B. (1984) *Nucleic Acids Res.*, **12**, 5707–5717.
25 Weigel,R.J., Devoto,S.H. and Nevins,J.R. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 9878–9882.
26 Israel,N., Gougerot,P.M., Aillet,F. and Virelizier,J.L. (1992) *J. Immunol.*, **149**, 3386–3393.
27 Liu,F. and Green,M.R. (1994) *Nature*, **368**, 520–525.
28 Wu,Y., Reece,R.J. and Ptashne,M. (1996) *EMBO J.*, **15**, 3951–3963.
29 Schmitz,M.L. and Baeuerle,P.A. (1995) *Immunobiology*, **193**, 116–127.
30 Keaveney,M., Berkenstam,A., Feigenbutz,M., Vriend,G. and Stunnenberg,H.G. (1993) *Nature*, **365**, 562–565.
31 Geisberg,J.V., Chen,J.-L. and Ricciardi,R.P. (1995) *Mol. Cell. Biol.*, **15**, 6283–6290.
32 Chatterjee,S. and Struhl,K. (1995) *Nature*, **374**, 820–822.
33 Klages,N. and Strubin,M. (1995) *Nature*, **374**, 822–823.
34 Chi,T., Lieberman,P., Ellwood,K. and Carey,M. (1995) *Nature*, **377**, 254–257.
35 Nikolov,D.B., Chen,H., Halay,E.D., Usheva,A.A., Hisatake,K., Lee,D.G., Roeder,R.G. and Burley,S.K. (1995) *Nature*, **377**, 119–128.
36 Bagby,S., Kim,S., Maldonado,E., Tong,K.I., Reinberg,D. and Ikura,M. (1995) *Cell*, **82**, 857–867.
37 Barberis,A., Pearlberg,J., Simkovich,N., Farrell,S., Reinagel,P., Bamdad,C., Sigal,G. and Ptashne,M. (1995) *Cell*, **81**, 359–368.
38 Shen,F., Triezenberg,S.J., Hensley,P., Porter,D. and Knutson,J.R. (1996) *J. Biol. Chem.*, **271**, 4827–4837.