Preparation and Crystallization of Dynamic NF-κB-IκB Complexes*

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The formation of single, well-diffracting crystals is a requirement for any molecular structure determination by x-ray crystallography. Crystallization of biological macromolecules can represent a significant obstacle when the subject exhibits internal flexibility or indiscriminate self-association. In such cases, the removal of inherently flexible regions and the addition of stabilizing ligands can improve the probability of crystal formation and ordered growth. We have applied these principles in order to form crystals of the Rel homology region of transcription factor NF-κB in complex with its inhibitors IκBα and IκBβ. None of these molecules crystallizes in the absence of a binding partner. Recombinant overexpression of truncated IκBα required selection of the correct start site. NF-κB-IκBα complex crystals formed under relatively stringent conditions. NF-κB-IκBβ complex crystals were formed by analogy to NF-κB-IκBα, although some modifications in purification and complex formation were necessary due to differences between the inhibitors.

Analysis of macromolecules and their complexes by x-ray crystallography requires the preparation of single, well-diffracting crystals. Although current macromolecular crystallization methods have been derived empirically and are generally case-specific in nature, some general guidelines, such as the significance of a pure and homogeneous sample, have emerged. One such principle regards the importance of sample stabilization during the process of crystal formation. Macromolecules exhibiting significant internal relative motion can fail to crystallize alone. Likewise, random self-aggregation poses an impediment to crystal formation. In such cases, either the removal of flexible regions or the addition of ligands can stabilize the dynamic macromolecule and promote crystal formation.

NF-κB is an inducible, dimeric transcription factor involved in coordinating the cellular response to infection and stress (1, 2). In its resting state, NF-κB exists in a stable cytoplasmic complex with a member of the IκB family of transcription factor inhibitor proteins. Bacterial and viral products, inflammatory cytokines, and a host of other activation signals lead to removal of the complex-associated IκB molecule from the NF-κB-IκB complex, rendering the transcription factor constitutively nuclear and leading to enhanced expression of NF-κB-responsive genes (3).

The primary active form of NF-κB in immune cells is a heterodimer composed of p50 and p65 subunits (Fig. 1a). Both subunits contain the approximately 300 amino acid rel homology region (RHR). All of the amino acid residues necessary for subunit dimerization, sequence-specific DNA binding, nuclear localization, and IκB binding are contained within the RHR (4). The crystal structures of several DNA-bound NF-κB homo- and heterodimers indicate that the RHR consists of two immunoglobulin-like domains connected by a short linker (5–9). Although both domains contact DNA, intersubunit dimer-forming contacts are mediated exclusively through the carboxyl-terminal immunoglobulin-like domain, which we consequently refer to as the dimerization domain. Carboxyl-terminal to the dimerization domain lies a non-conserved sequence of 30 amino acids containing a basic type I nuclear localization sequence (NLS). This region, which we denote the NLS polypeptide, does not exhibit an ordered structure in the NF-κB/DNA x-ray crystal structures.

The principal players involved in the inhibition of NF-κB p50/p65 heterodimer transcriptional activity (10) are the IκB proteins, IκBα and IκBβ (Fig. 1b). Although related by primary sequence, IκBα and IκBβ exhibit striking structural and functional differences (11, 12). The primary structure of these transcription factor inhibitor proteins reveals three distinct regions: (i) a centrally located ankyrin repeat-containing domain (ARD) (13); (ii) an acidic carboxyl-terminal PEST-like region (PEST); (iii) an RHR domain that containsOf the three regions, the RHR is the least conserved among NF-κB family members (4).

The inherent flexibility and modular arrangement of the NF-κB RHR and the propensity for IκB proteins to aggregate hinder their crystallization. We proposed that formation of the NF-κB-IκB complex might sufficiently stabilize these factors and improve the probability for formation of crystals. Here, we report the rationale behind the design of targets for co-crystallization of the transcription factor NF-κB with each of two IκB inhibitor proteins. The proposed targets were based on observations derived from the crystal structures of NF-κB-DNA complexes and from characterization of the NF-κB-IκB interaction by a variety of biochemical and biophysical techniques. We present the recombinant expression and purification of the individual protein complex components. We detail complex formation procedures. Finally, we report crystallization conditions and initial crystallographic characterization of NF-κB-IκBα and NF-κB-IκBβ complex co-crystals.

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1 The abbreviations used are: RHR, rel homology region; NLS, nuclear localization signal; ARD, ankyrin repeat-containing domain; PEST, proline-, glutamic acid-, serine-, and threonine-rich region; PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol; MES, 2-(4-morpholino)ethanesulfonic acid; PEG, polyethylene glycol; NTA, nitrilotriacetic acid.
EXPERIMENTAL PROCEDURES

Preparation of IκB Protein Expression Plasmids—A fragment of the human cDNA encoding amino acid residues 70 to 302 of IκBα was prepared by the polymerase chain reaction with primers that introduced NdeI and BamHI enzyme restriction sites. This DNA construct was subsequently ligated into the corresponding sites of the T7 promoter driven plasmid vector pET11b (Novagen). In a similar manner, human IκBβ gene constructs encompassing amino acid residues 70–302, 71–302, and 67–302 and murine IκBα gene constructs encoding amino acids 50–325 and 50–331 were also ligated into NdeI and BamHI restriction sites of the pET11a vector.

Introduction of Phospho-mimetic Glutamate Mutations in IκBβ—
IκBβ bearing five glutamic acid mutations in the PEST (E5-IκBβ) expression plasmids were prepared by a two-step polymerase chain reaction strategy using internal primers with the following sequences: E5 primer 1, 5'-CTT AGC CCT TGC -3'; E5 primer 2, 5'-GTT GTC ACT GTC -3'. In a similar manner, human IκBα gene constructs encompassing amino acid residues 70–302, 71–302, and 67–302 and murine IκBα gene constructs encoding amino acids 50–325 and 50–331 were also ligated into NdeI and BamHI restriction sites of the pET11a vector.

Expression and Purification of Untagged Recombinant IκBα—Purified plasmid DNA was used to transform Escherichia coli strain BL21[DE3] (Stratagene). Transformed bacterial cells were cultured in LB media with 200 mg/ml ampicillin and were grown at 37 °C until A600 of approximately 0.1. At this point the cultures were removed from the shaker and placed on stir plates, induced with 0.1 mM isopropylthiogalactopyranoside, and left for 16 h at approximately 22 °C with vigorous stirring to ensure sufficient aeration. Cells were pelleted by centrifugation at 6000 rpm for 15 min and resuspended in 50 ml of lysis buffer per 1 liter cell pellet. Lysis buffer consisted of 25 mM Bis-Tris-HCl (pH 6.0), 0.5 mM DTT, 50 mM NaCl, 0.5 mM EDTA, and 50% glycerol. Cell lysis occurred on sonication on crushed ice. Insoluble cell material was clarified by centrifugation at 12,000 rpm for 40 min. The crude cell lysate was passed over a Q-Sepharose column (Amersham Pharmacia Biotech) that had been equilibrated with lysis buffer. The column was washed with 10 column volumes of lysis buffer and then eluted at a linear salt gradient of 225 mM NaCl to 700 mM NaCl over 20 column volumes. Peak fractions were pooled and dialyzed against phosphate buffer containing 20 mM KH₂PO₄ (pH 7.5), 0.5 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 10 mM β-mercaptoethanol. Cell lysis occurred on sonication on crushed ice. Insoluble cell material was clarified by centrifugation at 12,000 rpm for 40 min. The crude cell lysate was passed over a Q-Sepharose column (Amersham Pharmacia Biotech) that had been equilibrated with lysis buffer. The column was washed with 10 column volumes of lysis buffer and then eluted at a linear salt gradient of 225 mM NaCl to 700 mM NaCl over 20 column volumes. Peak fractions were pooled and dialyzed against phosphate buffer containing 20 mM KH₂PO₄ (pH 7.5), 0.5 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 10 mM β-mercaptoethanol. The dialyzed peak fractions were next loaded on a hydroxyapatite Bio-Gel (Bio-Rad) column that had been equilibrated in phosphate buffer. Washing the column with 20 column volumes of phosphate buffer recovered a significant fraction of the loaded IκBα at >80% purity, as judged by Coomassie-stained SDS-PAGE and ultraviolet absorbance spectrophotometry. The phosphate wash fraction was then concentrated to 5 mg/ml in Amicon concentrators containing a 10,000 molecular weight cut-off membrane. The concentrated protein was finally loaded on 10-ml samples on a Superdex 75 (Amersham Pharmacia Biotech) gel-filtration column and run isocratically in 25 mM Tris-HCl (pH 7.5), 50 mM NaCl, 2 mM dithiothreitol (DTT).

Expression and Purification of Untagged Recombinant IκBβ—E. coli strain BL21(DE3) LyS5 (Stratagene) that was transformed with expression plasmids bearing deletion variants of murine IκBβ, and E5-IκBβ was treated in an identical manner as the bacterial cells expressing IκBα through the Q-Sepharose chromatography step. At this peak fraction was pooled and purified over a Superdex 200 size exclusion column.

Expression and Purification of Histidine-tagged IκBα—Bacteria expressing the IκBα (70–302) amino-terminal hexa-histidine fusion were treated identically to the untagged IκBα-expressing cells. The histidine-tagged protein was affinity-purified on a NTA-agarose column (Qiagen) by following standard procedures. Specifically, EDTA and β-mercaptoethanol were removed from the lysis buffer, and the protein was eluted in steps of 50, 250, and 600 mM imidazole. Protein of sufficient purity for size exclusion chromatography was obtained in the 250 mM imidazole-eluted fraction. Removal of the histidine tag was accomplished by incubating 1 ml of 0.1 mM histidine-tagged IκBα (70–302) with 15 μg (50 units) of thrombin (Sigma) at room temperature for 30 min. The reaction was then quenched by the addition of 10 mM phenylmethylsulfonyl fluoride, and the protein was re-purified either on NTA-agarose or by anion exchange chromatography on a Mono-Q column (Amersham Pharmacia Biotech).

Expression and Purification of NF-κB Homo- and Heterodimers—
Bacterial expression plasmids containing gene constructs for both the NF-κB p50 and p65 subunits under the same promoter were introduced into BL21(DE3) cells and expressed in a manner similar to IκBα. Growth and harvesting of the NF-κB heterodimer expressing E. coli was identical to that of the IκB recombinant bacterial cells except that the NF-κB lysis buffer contained 25 mM Tris-HCl (pH 7.5) rather than Bis-Tris. Nucleic acids present in the clarified soluble NF-κB bacterial lysate were precipitated from solution by the slow addition of 10% streptomycin sulfate solution to a final concentration of 1%. Nuclease acid precipitation was allowed to proceed for 20 min at 4 °C and a second round of centrifugation clarified the lysate. Crude soluble NF-κB heterodimer was then loaded onto an SP-Sepharose column (Amersham Pharmacia Biotech) pre-equilibrated with lysis buffer. The column was washed with 1 ml of protein (7 mg/ml) in 25 mM MES buffer and then further purified by size exclusion chromatography on a Superdex 75 column in 25 mM Tris-HCl (pH 7.5), 50 mM NaCl, and 1 mM DTT. The same protocol was employed for expression and purification of p65 homodimers with the lone exception that 25 mM MES buffer at pH 6.5 was employed in the place of Tris-HCl for ion exchange chromatography.

NF-κB/IκBα Complex Formation and Crystallization—The two purified proteins were combined with an approximately 1.2 times molar excess of IκBα to NF-κB and concentrated in a centri-prep30 (Amicon) concentrator to 20 mg/ml. A second round of size exclusion chromatography was then performed on the complex to remove the excess IκBα from the complex. Peak fractions were pooled and concentrated in Centricron 30 (Amicon) concentrators to a final concentration of 30–40 mg/ml. Small (300 μg) aliquots of the purified complex were flash-frozen in liquid nitrogen and stored at −80 °C. Crystals containing the NF-κB/IκBα complex were grown by the hanging drop vapor diffusion method. Drops containing 4 μl of protein (7 mg/ml) in 25 mM MES buffer (pH 6.35), 5.5% polyethylene glycol (PEG) 8000, and 2.5 mM DTT were equilibrated against 1 ml of reservoir solution containing 50 mM MES (pH 6.35), 10% PEG 8000, and 5 mM DTT at 23–24 °C. Rod-like crystals (0.5 × 0.1 × 0.05 mm) formed in 48–72 h amid heavy precipitate.

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RESULTS

Rational Design of NF-κB1sXα Complexes

Crystal structures of NF-κB RHR homo- and heterodimers complexed to DNA illustrate that the RHR contains two domains that are connected by a short linker. The NF-κB RHR fails to crystallize in the absence of DNA. Removal of the amino-terminal domain from the RHR of p65 and p50, however, resulted in a NF-κB dimers containing only their dimerization domains and NLSs. These proteins readily crystalize in multiple forms (16). These observations suggest that in the absence of DNA, the two domains and NLS of the NF-κB RHR exhibit significant relative motion.

Concurrent with early attempts at NF-κB1sXα co-crystallization, fluorescence polarization competition assays and surface plasmon resonance spectroscopy studies aimed at measuring binding affinities for native and deletion mutant NF-κB1sXα complexes were performed. To summarize the results of this study, which appear separately (17), native NF-κB1sXα binding requires the ARD and part of the PEST of IkBα and the entire RHR of both p50 and p65 NF-κB subunits, with the notable exception being that removal of the p50 amino-terminal domain does not affect binding affinity. Coupling this knowledge with our prior hypothesis concerning the dynamic nature of unbound NF-κB amino-terminal domains provided our crystallization targets. Specifically, we supposed that an IkBα construct encompassing the ARD and PEST (roughly amino acid residues 70 to 302) in complex with NF-κB containing the entire RHR of p65 (amino acid residues 19 to 304) and the dimerization domain and nuclear localization sequence only of p50 (amino acids 245 to 363) might sufficiently stabilize the inherent flexibility in the p65 RHR and yield to crystallization.

Expression of IkBα—Initially, an IkBα construct encoding amino acid residues Leu-70 to Glu-302 was overexpressed and purified as an amino-terminal hexa-histidine fusion protein possessing a thrombin protease cleavage site. Attempts at crystallization of this protein construct, both independently and in complex with NF-κB, did not meet with success. Removal of the amino-terminal hexa-histidine tag with thrombin proved problematic. IkBα bears cryptic thrombin cleavage sites and continued to degrade in crystallization drops even after repurification (data not shown).

Efforts to express the same IkBα construct as an untagged protein proved unsuccessful. The protein does not overexpress well in E. coli. We supposed that the failure to express the untagged protein might be due to the presence of a bad codon in the second position (18, 19) and so prepared a second construct encompassing amino acid residues Thr-71 to Glu-302. This second construct also failed to result in any appreciable protein expression.

Comparison of the amino acid sequences at the start of the IkBα ARD and the corresponding portion of 53BP2, a p53 binding protein of unknown function, suggested a possible explanation. The x-ray crystal structure of 53BP2 bound to the DNA binding domain of p53 (20) reveals that the 53BP2 amino acid residues homologous to IkBα Leu-70 and Thr-71 form a short β strand that starts a hairpin turn. Supposing that this super-secondary structural motif might somehow complicate protein expression at the translational level (21), a new construct encoding amino acid residues Lys-67 to Glu-302 of IkBα was prepared and ligated into a protein expression vector. This protein fragment overexpresses in E. coli under standard conditions (Fig. 2).

NF-κB1sXα Purification and Complex Formation—The untagged recombinant IkBα (67–302) protein was purified by two ion exchange chromatography steps (Fig. 3, a and b). Although solubility and long term storage of IkBα alone is a problem, recombinant IkBα exhibits an entirely different character in complex with NF-κB. Purification of NF-κB, therefore, always accompanied the IkBα purifications.

Purification of recombinant NF-κB centers on the preference for p50/p65 heterodimer formation over formation of either the p50 or p65 homodimers. The p50(245–363) subunit overexpresses in E. coli to a much higher degree than does p65(19–304) so that effectively no p65(19–304) homodimer is present. Purification of the heterodimer from excess p50(245–363) homodimers occurs by cation exchange chromatography (Fig. 3c).

Formation of stoichiometric NF-κB1sXα complexes was accomplished by size exclusion chromatography (Fig. 4a). After running both the complex components separately, a mixture of NF-κB with excess IkBα allows for resolution of the purified 1:1 NF-κB1sXα complex from excess IkBα (Fig. 4b). Purified NF-κB1sXα complex can be concentrated to at least 40 mg/ml and does not denature with one freeze-thaw cycle.

Crystallization of the NF-κB1sXα Complex—Analysis by commercially available sparse matrix screen played an invaluable role in profiling NF-κB1sXα complexes against standard crystallization parameters. However, finer screening was required to grow the first NF-κB1sXα complexes. Tiny, multiple needle-like crystals were produced by the hanging drop vapor diffusion method with PEG 8000 precipitant buffered to pH 6.3. Optimization of these crystals proved quite
difficult as they show extreme sensitivity to starting protein concentration, ionic strength, pH, precipitant concentration, and temperature. The NF-κB-IκBα crystals grow to maximum size in only 2 to 3 days amid heavy precipitate. Since neither IκBα nor NF-κB crystallizes on its own, we suspected that crystallization of the complex and precipitation of the individual complex components might be competing processes within the crystallization drop. We endeavored, consequently, to permit the complex the maximum time possible at or near its ideal crystallization condition during our 72-h window.

Raising the starting precipitant concentration in the drop relative to the final concentration in the reservoir well and allowing the crystals to grow at room temperature finally permitted the complex the maximum time possible at or near its ideal crystallization condition during our 72-h window.

**The Design of Relevant NF-κB-IκB Complexes for Co-crystallization**

One surprise to emerge from the NF-κB-IκB complex crystal structures was the involvement of the NF-κB p65 subunit amino acids 305 to 319 in contacting IκBα. These residues correspond to a nonhomologous region within the NF-κB RHR carboxyl-terminal to the NLS (Fig. 1). To incorporate analogous interactions into the complex between NF-κB and IκBα, a new p65 protein was prepared spanning amino acids 19–325. A second observation from the NF-κB-IκBα crystal structures centered on the paucity of direct interactions between the amino-terminal immunoglobulin-like domain of p65 and IκBα. A p65 protein construct containing only the dimerization domain and NLS polypeptide (amino acids 191–325) was prepared. p65(191–325) binds to IκBα with affinity comparable with the full p65 RHR.

Murine IκBα containing the ARD and PEST (amino acids 50–325) was prepared by analogy to the IκBα (67–302) protein construct that had co-crystallized with NF-κB. Complexes of IκBβ(50–325) and NF-κB failed to purify as a single peak by size exclusion chromatography, however, leading to the evaluation of new IκBβ protein constructs. Fluorescence polarization competition assays revealed that a slightly longer IκBβ protein construct (amino acids 50–331) exhibits NF-κB binding properties similar to the full-length protein. Furthermore, constitutive phosphorylation of the IκBβ PEST has been reported as necessary for full IκBβ activity (24). To address this issue, we tested the inhibitory properties of an IκBβ(50–331) protein construct containing phospho-mimetic glutamic acid residues in place of the native side chains that undergo constitutive phosphorylation by protein kinase CK2 (casein kinase II), namely, Ser-312, Ser-314, Ser-315, Ser-316, and Ser-318. The resulting IκBβ molecule, which we denote E5-IκBβ(50–331), was in fact a slightly better inhibitor of NF-κB DNA binding.
To address all of the structural and functional information available, we employed a combinatorial approach to the problem of NF-κB-IκB δ crystallization. In all, six different NF-κB homo- and heterodimers were complexed with IκBα(50–331) and E5-IκBβ(50–331) for crystallization trials (Table I).

**NF-κB-IκB δ Complex Purification**—Both NF-κB and IκB δ were purified to homogeneity. We were surprised to note that, in contrast with monomeric IκB α, the recombinant IκB δ protein purifies as an oligomer. ² Due to the oligomeric nature of the IκB δ, NF-κB-IκB δ complexes were prepared with excess NF-κB. Stoichiometric NF-κB-IκB δ complexes were then resolved from excess NF-κB by size exclusion chromatography (Fig. 6).

**NF-κB-IκB δ Crystallization**—Like NF-κB-IκB α, NF-κB-IκB δ complexes failed to crystallize in standard sparse matrix screening conditions. A total of six different IκB δ-NF-κB p50/ p65 heterodimer complexes were screened extensively without success (Table I). Attempts at crystallization of four different IκB δ-p65 homodimer complexes were made. Room temperature trials of p65(191–325)-IκB δ(50–331) and p65(191–325)-E5-IκB δ complexes lead to the formation of p65 subunit crystals. SDS-PAGE analyses of these crystallization drops revealed that the IκB δ components had completely degraded and that the p65(191–325) component only was present in the drops. Incubation of these same complexes at 4°C abated the IκB δ proteolysis problems. At this temperature crystals of E5-IκB δ(50–331) in complex with either p65(191–325) or p65(191–321) formed (Fig. 7, a and b). Diffraction data taken on our home source x-ray generator indicate that the E5-IκB δ(50–331)p65(191–325) crystals belong to the space group P1 with unit cell parameters a = 46.40 Å, b = 48.92 Å, c = 59.47 Å, α = 95.17°, β = 91.80°, and γ = 105.45°. The crystals contain 1 complex per asymmetric unit and are 43% solvent by volume.

² S. Malek, unpublished data.

**DISCUSSION**

The preparation of single, well-diffracting protein crystals remains the principle impediment to any protein structure analysis by x-ray crystallography. Advances in molecular cloning technologies make it possible for structural biologists to overexpress isolated domains of target proteins. These independent modules often crystallize readily when the native, full-length protein will not. With the soluble protein domain in hand, commercially available sparse matrix screens provide the means for the efficient evaluation of many different crystal growth conditions. Many hundreds of protein crystals have been prepared for x-ray diffraction analysis through the application of such techniques (25).

Crystallography of polypeptides containing two or more independent domains or their complexes is seldom so simple. Size of the multidomain protein or complex alone is not a limitation to its ability of crystallize. Whole viral particles (26, 27), nucleosomes (28), molecular chaperons (29), and ribosomal subunits (30, 31) all readily crystallize. More often it is the physical properties of the subject, such as shape, surface charge distribution, flexibility, and interdomain dynamics that impede its crystallization. In these cases, a knowledge of the biochemistry and biophysical character of the system can be extremely helpful. Such an understanding often suggests logical modifications in the protein complex or multidomain polypeptide,
which render it a better candidate for crystallization.

This principle is illustrated by the crystallographic structure determination of two Src-family protein-tyrosine kinases (32, 33). Despite the fact that many crystal structures of independent protein-tyrosine kinase, SH2, and SH3 domains had each been determined separately, crystallization of the three domains covalently linked as one polypeptide eluded researchers for many years. A kinase engineered with a single phosphorylated tyrosine and the amino-terminal membrane-localization sequence removed eventually crystallized. These modifications apparently promote crystallization by forcing the protein to adopt a closed conformation that minimizes interdomain dynamics. In a similar manner, polysaccharide removal and the addition of stabilizing ligands were required for co-crystallization of the inherently flexible human immunodeficiency virus gp120 envelope protein with a CD4 fragment and monoclonal antibody Fab specific for the gp120 chemokine receptor site (34, 35).

Here, we have demonstrated that crystallization of two NF-κB-IκBα complexes required that we first select the appropriate protein fragments in each protein-protein complex. In the case of NF-κB-IκBα protein complex crystallization, removal of the p50 amino-terminal domain and the IκBα signal response domain proved to be critical for crystal growth. In contrast, only the NF-κB-IκBβ complex containing five glutamates within the carboxyl-terminal PEST region and removal of the signal response domain of IκBβ coupled with removal of the amino-terminal domains of p65 homodimer yielded crystals.

In retrospect, it is interesting to note that the amino-terminal domain of the NF-κB p65 subunit and the PEST of IκBα participate in a dynamic interaction. This hypothesis was inferred by comparison of the NF-κB-IκBα and NF-κB-DNA complex crystal structures and is supported by subsequent oxidative cross-linking studies (36). We suppose that this dynamic and the presence of multiple charged amino acids within this region partially account for the difficulties in obtaining NF-κB-IκBα complex crystals. In the case of NF-κB-IκBα, for example, changes in buffer pH of less than 0.1 unit resulted in significant crystal formation defects.

Nevertheless, we conclude that the most important parameter in crystallizing these protein-protein complexes was the selection of appropriate protein fragments in which high affinity interactions were maximized and non-interacting, flexible portions were removed or stabilized. This point is further corroborated by the fact that although two independent laboratories succeeded in growing the NF-κB-IκBα complex crystals in different space groups and under different crystallization conditions, both crystals contained similarly modified NF-κB and IκBα protein fragments (22, 23). Crystals of two nearly identical NF-κB-IκBβ complexes were obtained only after extensive screening of 10 closely related complexes.

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Fig. 7. NF-κB-IκBα complex co-crystals. a, photograph of E5-IκBα(50–331)p65(191–325) protein complex crystals. b, 18% SDS-PAGE analysis of complex crystals reveals the presence of both complex components. MW, molecular weight.
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