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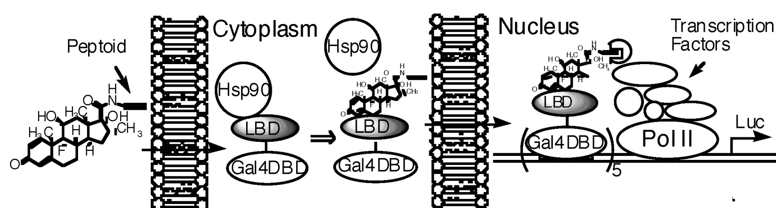

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A Potent Transactivation Domain Mimic with Activity in Living Cells

Bo Liu, Prasanna G. Alluri, Peng Yu, and Thomas Kodadek*

Departments of Internal Medicine and Molecular Biology and Center for Biomedical Inventions, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390

Received March 9, 2005; E-mail: Thomas.Kodadek@utsouthwestern.edu

There is considerable interest in the development of synthetic molecules that are capable of stimulating the expression of specific genes.[1] Essentially, all efforts in this area have focused on making chimeric molecules that link a sequence-specific DNA-binding molecule with a compound capable of recruiting the transcriptional machinery,[2] thus reconstituting a basic function of native activators.[3−14] This strategy has been made feasible by the extensive work done on synthetic, sequence-specific DNA-binding molecules, such as hairpin polyamides[15,16] and peptide nucleic acids (PNAs).[17,18] The development of cell-permeable holoenzyme-binding molecules, which would represent the synthetic equivalent of an activation domain (AD) of a native activator, is less advanced. Two general strategies have been explored. One is to employ molecules that resemble native ADs or, more specifically, are peptide fragments of native ADs.[3−5,7,8,13] The other is to design[19] or screen for[6,9,10] molecules that bind coactivators or other binding partners of native ADs. We report here an example of the latter approach and show that a cell-permeable peptoid, isolated in a screen against the KIX domain of the coactivator CBP (CREB-binding protein), functions as a potent AD equivalent in living mammalian cells.

A combinatorial library of ≈ 100 000 hexameric peptoids[20,21] was screened for binding to the KIX domain of the mammalian coactivator CBP,[22] a target of several native ADs. Three peptoids were identified that bound the protein much better than the other compounds in the library.[23] Two of these were sequenced successfully and are shown in Figure 1 (KIX-binding peptoid KBPo1 and KBPo2). A titration experiment using fluorescein-labeled peptoids revealed that the KDs of the KBPo1− and KBPo2−GST−KIX complexes were similar, 3 and 5 μM, respectively (Figure 2). However, KBPo2 had little affinity for two other proteins, GST and BSA, while KBPo1 bound them almost as well as GST−KIX, showing that it is a relatively nonspecific protein ligand. This may be due to the presence of aromatic, hydrophobic residues at five of the six variable positions in this molecule, whereas KBPo2 is a more polar molecule (Figure 1).

To determine if either peptoid could function as an activation domain equivalent in mammalian cells, we set up a system in which the peptoid could be delivered to a Gal4 DNA-binding domain (DBD) fusion protein via noncovalent interactions. This was done by conjugating these peptoids to dexamethasone, a high affinity agonist of the glucocorticoid receptor (GR).[24−26] In the absence of ligands, a Gal4 DBD-GR ligand-binding domain (LBD) fusion protein is trapped in an inactive form in the cytoplasm through interaction with heat shock protein 90 (Hsp90), but dissociates upon steroid binding. We anticipated that since this construct lacks an AD, it would not activate transcription of a Gal4-responsive reporter gene alone. However, if the peptoid acts as an AD, then delivery of these molecules to the Gal4 DBD via dexamethasone-GR LBD interaction should result in the activation of transcription. Note that this approach is a variant of a general strategy employed previously

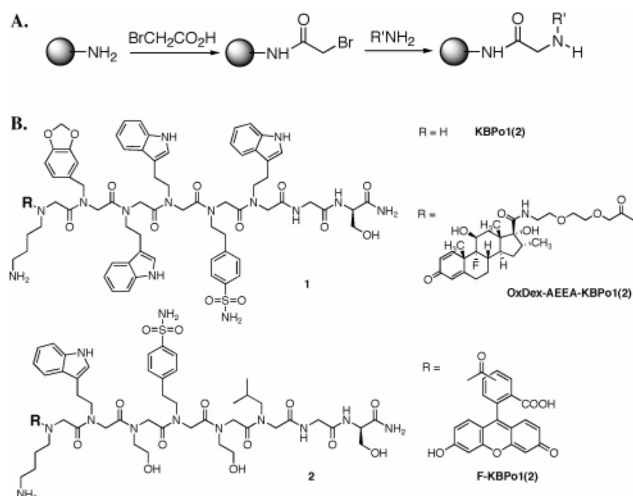

Figure 1. (A) Scheme of peptoid synthesis. (B) Chemical structures of the KIX domain-binding peptoid conjugates employed in this study.

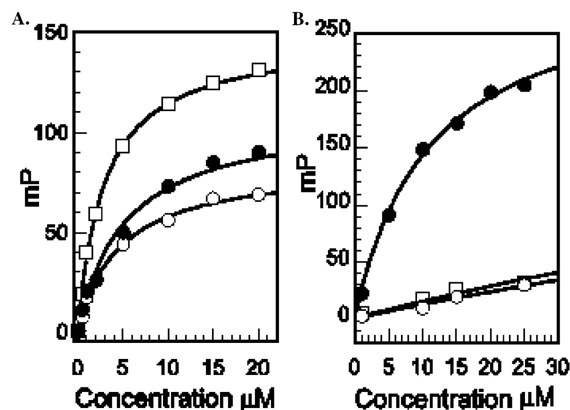

Figure 2. Binding affinities and specificities of the KIX-binding peptoids. (A) Fluorescein-labeled KBPo1 was titrated with the indicated concentrations of GST-KIX (●), GST (○), or BSA (□). Binding was monitored by fluorescence polarization. (B) Same as A, except fluorescein-labeled KBPo2 was employed in the experiment.

by several investigators to tether various small molecules to native DNA-binding proteins.[3,7,27−29]

The glucocorticoid receptor (GR) agonist dexamethasone was oxidized with periodic acid to yield OxDex-COOH,[28] which was then conjugated to KBPo1 and KBPo2 via an ethylene glycol-containing linker AEEA (Figure 1). HeLa cells were transfected with a plasmid encoding a Gal4 DBD (residues 1−147)-GR LBD (residues 499−777) fusion protein, a firefly luciferase gene with five Gal4-binding sites in the promoter, and a *Renella* luciferase gene as a transfection control.

As shown in Figure 3A, addition of OxDex-AEEA-$CONH_2$ did not result in any detectable increase in luc gene expression, as expected. The same result was obtained with OxDex-AEEA-KBPo1

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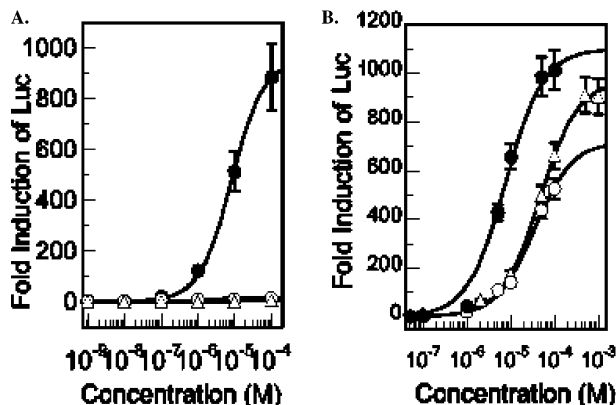

Figure 3. Evaluation of the transcriptional activity and cell permeability of the KIX-binding peptoids in living cells. Dose dependence of the level of Gal4-responsive *luc* gene expression when cells expressing the Gal4DBD-GRLBD fusion protein (A) or Gal4DBD-GRLBD-VP16 (B) were incubated with OxDex-AEEA-KBPo2 (●), OxDex-AEEA-KBPo1 (○), or OxDex-AEEA-$CONH_2$ (△). In the permeability assay (B), the titration curve revealed EC_{50} values of 37 and 8 μM, respectively, for these peptoid conjugates. As a comparison, OxDex-AEEA-$CONH_2$ gave a maximum induction of 950-fold with an EC_{50} value of 48 μM.

even at a concentration of 100 μM. However, when the same experiment was done in cells expressing a Gal4 DBD-GR−LBD-VP16 AD fusion protein, which is a potent activator due to the presence of the VP16 AD, robust luc expression was observed (Figure 3B). This shows that OxDex-AEEA-KBPo1 is cell-permeable and binds to the GR LBD, arguing that its failure to support luc activation in the Figure 3A experiment simply reflects its inability to function as an AD in living cells.

In contrast, titration of Gal4DBD-GRLBD-expressing cells with the other peptoid conjugate, OxDex-AEEA-KBPo2, resulted in a dose-dependent increase in expression of the reporter gene (Figure 3A). At an OxDex-AEEA-KBPo2 concentration of 100 μM, expression of the Gal4-responsive luc reporter gene was induced about 900-fold above the basal level. Half-maximal stimulation was observed at about 10 μM OxDex-AEEA-KBPo2. This experiment did not involve liposome-mediated introduction of the peptoid conjugate into cells or any other artificial manipulations; the steroid−peptoid conjugate is simply cell-permeable. We conclude from this experiment that the peptoid KBPo2 is capable of functioning as a cell-permeable activation domain surrogate in living mammalian cells.

The fact that KBPo2 functions as a potent AD while KBPo1 does not raises at least two important issues. First, it demonstrates that not all coactivator-binding molecules will function as ADs in cells. Second, since KBPo2 is a specific KIX domain ligand, while KBPo1 is far less specific, it is possible that coactivator specificity, in addition to simply coactivator affinity, is an important attribute of AD surrogates (also see ref 30). This is somewhat curious since most native ADs, particularly those of the acidic variety, are notorious for being relatively nonspecific protein interacting domains.[31,32] Of course, a firm conclusion cannot be made on the basis of only two molecules, and more studies of such AD surrogates will be required. However, even the possibility that specificity is critical for in vivo function argues that it will be important to build this into future screens for AD mimics.

To the best of our knowledge, KBPo2 is one of only three synthetic molecules that have been demonstrated to act as an AD surrogate in living cells and the first that is not a peptide modeled after the VP16 AD.[3,13] Efforts to couple KBPo2 to appropriate synthetic DNA-binding molecules and thus create synthetic activators are in progress as are screens directed toward the discovery of even higher potency AD surrogates.

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Supporting Information Available: Detailed experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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