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Synthesis of a β-Estradiol-Biotin Chimera that Potently Heterodimerizes Estrogen Receptor and Streptavidin Proteins in a Yeast Three-Hybrid System

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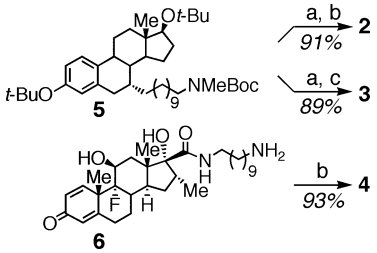
Small molecules that dimerize proteins in living cells provide important tools for probing diverse biological processes.[1] Chemical inducers of protein dimerization (CIDs) have been used to control intracellular signal transduction pathways, protein subcellular localization, and gene expression.[2] This approach has also been used to identify protein targets of small molecule natural products.[3] Pioneering work by Liu linked the steroid dexamethasone to the natural product FK506 to identify the protein target FKBP by screening this chimeric compound against a genetically encoded library of proteins in a yeast three-hybrid system.[3] This system employed an engineered glucocorticoid receptor (GR) protein as a DNA-bound platform to display dexamethasone-tethered FK506 to target proteins that activate gene expression upon binding.

Although screening natural products against protein targets with yeast three-hybrid systems is a potentially elegant alternative to traditional affinity chromatography methods, dexamethasone derivatives are limited in this regard by the relatively low activity of glucocorticoids in recombinant yeast.[4] This low activity relates in part to the observation that yeast-expressed GR proteins bind dexamethasone with $>10^3$-fold lower affinity than GR proteins expressed in mammalian cells.[5] In contrast, steroidal estrogens are highly active in yeast systems,[6] and 7-α-substituted estradiol derivatives such as the antiestrogen ICI 182,780 (**1**) bind tightly to both the estrogen receptor α (ER-α, $K_d \approx 1.0$ nM) and the estrogen receptor β (ER-β, $K_d \approx 3.6$ nM) isoforms.[7] Furthermore, high-resolution X-ray crystal structures of these proteins bound to cognate ligands are available for design of CIDs.[8] To investigate these potential advantages for the analysis of natural products in yeast-based systems, we employed the previously reported protected 7-α-substituted β-estradiol derivative **5**[6b] to synthesize the chimeric 7-α-substituted β-estradiol derivatives **2** and **3** linked to the natural product biotin (Scheme 1). Other biotinylated β-estradiol derivatives have also been reported in the literature.[9] Biotin was chosen because molecular recognition by the bacterial streptavidin (SA) protein has been extensively characterized.[10] Moreover, interactions between biotin and streptavidin have not been previously investigated in a yeast three-hybrid system, and biotin provides a simple model of more complex natural products. The dexamethasone-biotin derivative **4** was prepared from **6**[3] (Scheme 1) to directly compare yeast three-hybrid systems on the basis of GR-dexamethasone and ER-estradiol molecular recognition in vivo.

The availability of X-ray crystal structures of the ligand binding domain (LBD) of ER-β (PDB code 1HJ1)[8] bound to the antiestrogen ICI 164,384 (structurally similar to **1**) and tetrameric streptavidin (PDB code 1SWR)[11] bound to biotin enabled construction of a simple molecular model of a ternary protein−ligand complex (Figure 1, see Supporting Information for details). Actual protein−ligand interactions formed in vivo will be much more complex; estrogen binding to ER monomers promotes ER homodimerization, and tetrameric SA binds four biotin ligands. Modeling suggested that the longer linker of ligand **2** as compared with ligand **3** would be necessary to effectively bridge both binding sites and heterodimerize these proteins.

Scheme 1 [a]



[a] (a) HCl (aq.)/dioxane (1:9). (b) D-Biotinamidocaproate NHS ester, DIEA, CH_2Cl_2/MeOH or THF. (c) D-Biotin NHS ester, DIEA, CH_2Cl_2/MeOH.

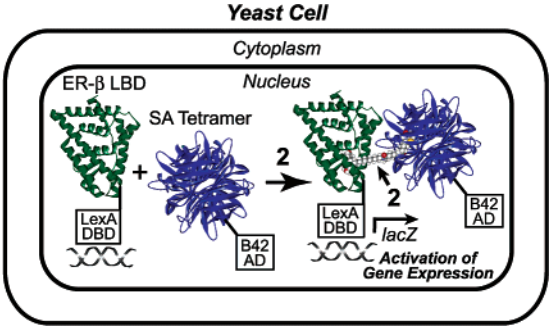
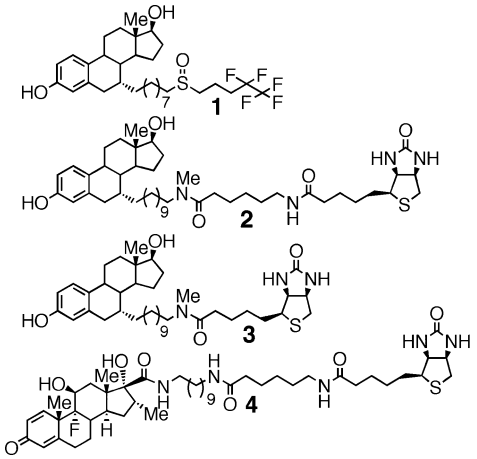


Figure 1. Schematic of the ER-SA yeast three-hybrid assay showing a hypothetical model of the ternary complex. Addition of ligand **2** heterodimerizes the DNA-bound LexA-ER fusion protein and the SA-B42 fusion protein to activate expression of a *lacZ* reporter gene.



To analyze ligand-mediated protein heterodimerization in vivo, yeast were engineered to express ER-β LBD and SA fusion proteins as shown in Figure 1. In this novel yeast three-hybrid system, the DNA binding domain (DBD) of the bacterial LexA protein[12] was fused to the N-terminus of the steroid receptor to anchor this protein

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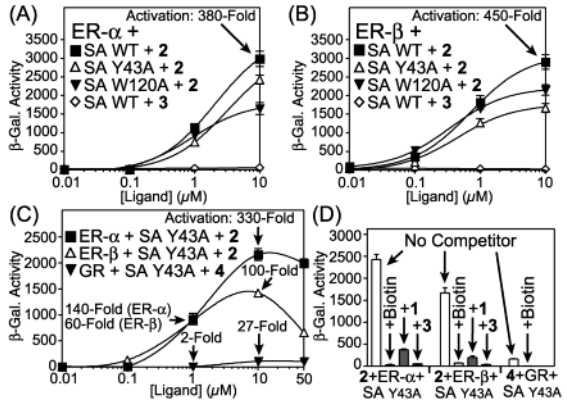

Figure 2. Dose−response curves and competition experiments. Panels A−C: Fold activation = observed β-Gal. activity/β-Gal. activity without ligand. Panel D: [Ligand] = 10 μM. [Biotin] = 100 μM. [**1**] and [**3**] = 50 μM.

on DNA sites that control expression of a *lacZ* (β-galactosidase) reporter gene. The bacterial B42 activation domain (AD)[12] was fused to the SA C-terminus to activate gene expression upon small molecule-mediated heterodimerization with the ER-β-LexA fusion protein. Analogous three-hybrid assays were constructed by substituting the ER-β LBD with the ER-α LBD and the GR LBD.

Functional SA has been expressed in bacteria fused to an N-terminal T7 peptide tag to facilitate protein folding.[13] We employed this approach to express functional SA proteins in yeast. However, expression of wild-type (WT) SA fused to the B42 AD resulted in a substantial reduction in the rate of yeast cell growth (data shown in the Supporting Information), presumably due to the high affinity ($K_d \approx 100$ fM)[11] of SA for endogenous biotin, which is an essential vitamin. In an attempt to attenuate this toxicity, site directed mutagenesis was employed to generate the known lower-affinity SA mutants: SA Y43A ($K_d \approx 100$ pM)[10a] and SA W120A ($K_d \approx 100$ nM).[11] As expected, yeast expressing these mutant proteins exhibited substantially enhanced rates of cellular growth (data shown in the Supporting Information).

Addition of ligands **2** and **3** to yeast three-hybrid systems and analysis of ligand-mediated gene expression provided the dose−response curves shown in Figure 2 (panels A and B). Ligand **2** potently activated gene expression in yeast expressing either the ER-α (~380-fold activation at 10 μM, panel A) or the ER-β LBD (~450-fold at 10 μM, panel B) as compared with levels of gene expression in the absence of ligand. Surprisingly, the lower affinity mutant SA Y43A and SA W120A proteins were nearly as effective as SA WT in mediating this dose−response (Figure 2), revealing that moderate-affinity interactions can be detected with this approach. Analysis of the toxic SA WT protein in this three-hybrid system was possible because expression of this protein was controlled by the galactose-inducible Gal1 promoter.[12] As predicted from molecular modeling, ligand **3** did not significantly activate gene expression in ER-SA three-hybrid assays.

Yeast three-hybrid systems expressing cognate steroid receptor proteins and SA Y43A were employed to directly compare activation of gene expression by dexamethasone derivative **4** and β-estradiol derivative **2**. Remarkably, ligand **2** was more potent and much more active (ER-β-SA_{Y43A} EC_{50} = 700 nM; 9-fold activation at 100 nM; 60-fold (ER-β) to 140-fold (ER-α) activation at 1 μM) than **4** (GR-SA_{Y43A} EC_{50} = 3.6 μM; 2-fold activation at 1 μM, 27-fold activation at 10 μM). Moreover, the absolute magnitude of the response with **2** was up to 70-fold greater than that with **4** at 1 μM (Figure 2, panel C). At the high concentration of 50 μM, ligand **2** was sufficiently potent to partially competitively inhibit reporter gene expression. Although not commonly observed in three-hybrid systems, this autoinhibition is predicted to occur if all protein binding sites become occupied by excess ligand. Competition experiments confirmed that biotin, **1**, and **3** are antagonists, establishing the specificity of these interactions (Figure 2, panel D).

Estrogen receptors expressed in yeast homodimerize upon addition of 7-α-substituted estradiol derivatives.[6b,14] Thus, substitution of SA-B42 with B42-ER in the three-hybrid assay enables evaluation of the cell permeability of compounds linked to β-estradiol. This analysis of compounds **1**−**3** revealed similar levels of ligand-mediated ER dimerization (data provided in the Supporting Information). This approach provides information regarding compound cellular permeability prior to screening of compounds against libraries of proteins.

These results indicate that 7-α-substituted derivatives of β-estradiol can be employed as highly effective activators of gene expression in living yeast cells. Coupling these compounds to biologically active small molecules may facilitate the identification of cognate protein targets expressed in yeast three-hybrid systems.

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Supporting Information Available: Experimental procedures, additional data and control experiments, and characterization data for new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Lin, H.; Cornish, V. W. *Angew. Chem., Int. Ed.* **2001**, *40*, 871−875.

(2) (a) Spencer, D. M.; Wandless, T. J.; Schreiber, S. L.; Crabtree, G. R. *Science* **1993**, *262*, 1019−1024. (b) Belshaw, P. J.; Ho, S. N.; Crabtree, G. R.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4604−4607. (c) Diver, S. T.; Schreiber, S. L. *J. Am. Chem. Soc.* **1997**, *119*, 5106−5109. (d) Lin, H. N.; Abida, W. M.; Sauer, R. T.; Cornish, V. W. *J. Am. Chem. Soc.* **2000**, *122*, 4247−4248.

(3) Licitra, E. J.; Liu, J. O. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 12817−12821.

(4) Sitcheran, R.; Emter, R.; Kralli, A.; Yamamoto, K. R. *Genetics* **2000**, *156*, 963−972.

(5) Garabedian, M. J.; Yamamoto, K. R. *Mol. Biol. Cell* **1992**, *3*, 1245−1257.

(6) (a) Wooge, C. H.; Nilsson, G. M.; Heierson, A.; McDonnell, D. P.; Katzenellenbogen, B. S. *Mol. Endocrinol.* **1992**, *6*, 861−869. (b) Hussey, S. L.; He, E.; Peterson, B. R. *Org. Lett.* **2002**, *4*, 415−418.

(7) Sun, J.; Meyers, M. J.; Fink, B. E.; Rajendran, R.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Endocrinology* **1999**, *140*, 800−804.

(8) Pike, A. C.; Brzozowski, A. M.; Walton, J.; Hubbard, R. E.; Thorsell, A.; Li, Y.; Gustafsson, J.; Carlquist, M. *Structure* **2001**, *9*, 145−153.

(9) Hauptmann, H.; Paulus, B.; Kaiser, T.; Luppa, P. B. *Bioconjugate Chem.* **2000**, *11*, 537−548.

(10) (a) Perez-Luna, V. H.; O'Brien, M. J.; Opperman, K. A.; Hampton, P. D.; Lopez, G. P.; Klumb, L.; Stayton, P. S. *J. Am. Chem. Soc.* **1999**, *121*, 6469−6478. (b) Sano, T.; Cantor, C. R. *Methods Enzymol.* **2000**, *326*, 305−311.

(11) Freitag, S.; Le Trong, I.; Chilkoti, A.; Klumb, L.; Stayton, P. S.; Stenkamp, R. *J. Mol. Biol.* **1998**, *279*, 211−221.

(12) Gyuris, J.; Golemis, E.; Chertkov, H.; Brent, R. *Cell* **1993**, *75*, 791−803.

(13) Gallizia, A.; de Lalla, C.; Nardone, E.; Santambrogio, P.; Brandazza, A.; Sidoli, A.; Arosio, P. *Protein Expression Purif.* **1998**, *14*, 192−196.

(14) Wang, H.; Peters, G. A.; Zeng, X.; Tang, M.; Ip, W.; Khan, S. A. *J. Biol. Chem.* **1995**, *270*, 23322−23329.

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