SHORT COMMUNICATION

A Portable Allosteric Mechanism

Urszula Gryczynski, Robert Schleif*
Biology Department, Johns Hopkins University, Baltimore, Maryland

ABSTRACT **The allosteric mechanism by which the gene expression regulatory protein AraC regulates its DNA-binding activity is shown to be portable by grafting it to β-galactosidase, generating an arabinose-regulated β-galactosidase. A portion of the α-peptide sequence that complements the activity of α-acceptor β-galactosidase was inserted into a nonessential region of the regulatory peptidyl arm of AraC protein. Arabinose, which regulates the position of the arm in AraC protein now regulates the availability of the α-peptide to α-acceptor β-galactosidase, thereby modulating its activity in response to arabinose. Proteins 2004;57:9–11.**

Key words: light switch; binary switch; regulation; AraC; peptidyl arm

INTRODUCTION

Expression of the L-arabinose operon in *Escherichia coli* is regulated by the AraC gene product. In the absence of arabinose, AraC protein represses transcription, which initiates at the *ara* p_{BAD} promoter, and in the presence of arabinose, AraC stimulates the initiation of transcription from the promoter. AraC protein utilizes a particularly simple mechanism of allosteric regulation, a binary switch, in which 18-residue N-terminal peptidyl arms communicate the arabinose binding status from the arabinose binding/dimerization domains to the DNA-binding domains (Fig. 1).[1,2] In the absence of arabinose, the protein's arms hold the DNA-binding domains in a configuration that favors DNA looping, in which AraC binds to two well-separated DNA sites. The presence of the loop both interferes with the access of RNA polymerase to the *ara* promoter and helps hold AraC protein in a conformation in which it does not activate transcription. In the presence of arabinose, the arms reposition over the arabinose molecules, releasing the DNA-binding domains, which in turn reorient to bind two adjacent DNA sites. When bound to these sites, AraC protein activates transcription from the adjacent *ara* promoter.

Is the AraC binary switch mechanism general and can it be ported to another protein? To address this question, a controlling peptide with inhibitory or stimulatory activity on a protein could be inserted into a nonessential region of the AraC arm. The addition of arabinose to this construct should reposition the arm and change the accessibility of the controlling peptide to the protein, thereby altering its activity. We chose to test β-galactosidase because certain sequences (α-peptides) are known to restore activity to truncated and inactive β-galactosidase (α-acceptor[3]) and because colonies can readily be screened for active enzyme with a chromogenic β-galactosidase substrate.

MATERIALS AND METHODS

QuickChange mutagenesis (Stratagene) was used successively to insert DNA coding for 8, 8, 12 and 8 residues of the α-peptide sequence, to introduce the UAG mutation for codon 202 of AraC on plasmid pWR03,[4] to adjust the sizes of α-peptide sequences, to randomize codons 13–15 of the AraC arm and to randomize codons 27–29 of the α-peptide sequence.

Arabinose response was measured by growing strain DH5α overnight in yeast extract-tryptone medium[5] containing 100 μg/mL ampicillin, diluting 1/100 into the same medium containing 0.2 m*M* isopropyl-thio-β-D-galactoside (IPTG), growing 5 h to an OD_{450} value of 4, and assaying β-galactosidase[5] in reactions containing the indicated concentrations of arabinose. Reproducibility of β-galactosidase measurements was better than 2%. The magnitude of the arabinose response varied between 15 and 30% from day-to-day due to physiological differences.

To screen β-galactosidase activities, colonies were spotted on YT plates. After being left overnight, they were overlaid with 3 mL 0.8% agar containing 800 μg/mL 5-bromo-4-chloro-3-indolyl-β-D-galactoside and 100 m*M* Na-azide and scored 4 h later.

RESULTS AND DISCUSSION

Starting with a full-length AraC expression vector, we inserted α-peptide sequences of various lengths between residues 5 and 6 of the AraC arm and terminated protein synthesis after the arabinose-binding domain with a nonsense mutation[6] (Fig. 2). A construct termed αgal-AraC containing a 36-residue α-peptide sequence exhibited regulation, albeit quite modest, by arabinose when co-expressed in

*Correspondence to: R. Schleif, Biology Department, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218. E-mail: rfschleif@jhu.edu.

Received 15 May 2004; Accepted 2 June 2004

Published online 12 July 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/prot.20233

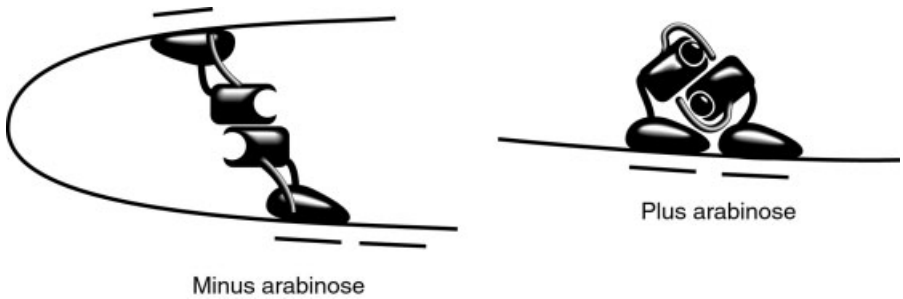

Fig. 1. Schematic of the binary switch mechanism of intact AraC protein. In the absence of arabinose, the N-terminal peptidyl arms from the arabinose binding/dimerization domains bind to the DNA-binding domains and the protein binds to the two DNA sites shown, forming a DNA loop. In the presence of arabinose, the arms bind over the arabinose, freeing the DNA-binding domains, which then bind to the adjacent DNA sites.

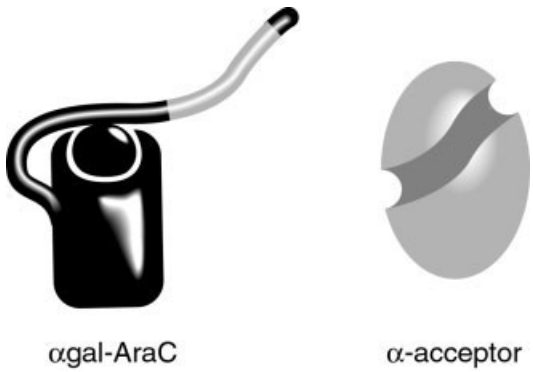

Fig. 2. Regulating the altered availability of α-peptide to α-acceptor β-galactosidase. Residues 7–42 of β-galactosidase were inserted between residues 5 and 6 of AraC, and translation was terminated beyond residue 201 with a nonsense mutation. The α-acceptor was β-galactosidase with residues 11–41 deleted. DNA constructions used oligonucleotide-directed QuickChange (Stratagene) mutagenesis.

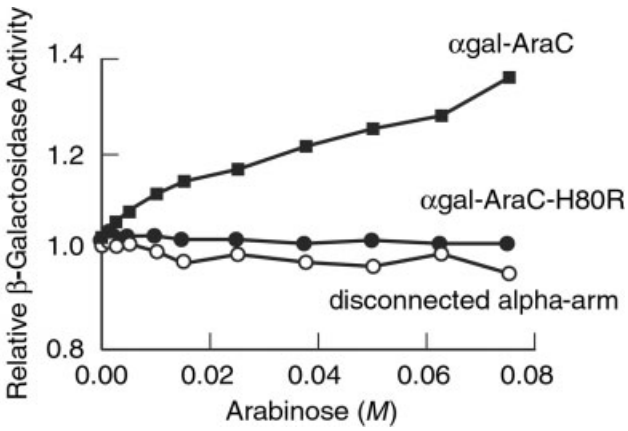

Fig. 3. Regulation of β-galactosidase by arabinose. DH5α cells containing plasmids coding for the αgal-AraC constructs were grown overnight in a broth medium containing 100 μg/mL ampicillin, diluted 1/100 into the same medium containing 0.2 m*M* IPTG, grown 5 h to an OD_{450} of 4 and assayed for β-galactosidase[5] in reactions containing the indicated concentrations of arabinose: (■) native-sequence αgal-AraC, (●) α-AraC containing the His80-to-Arg mutation, (○) αgal-AraC containing the Phe15-to-stop mutation.

a strain with α-acceptor (Fig. 3). The arabinose response represented a ≈30% enhancement. This response clearly derives from αgal-AraC because introducing the His80-to-Arg mutation, which prevents the protein from binding arabinose,[7] eliminates the regulation effect. Furthermore, both α-peptide alone and the αgal-AraC construct truncated near the end of the N-terminal arm possess complementing activity but lack regulation by arabinose. Finally, the regulation is specific; glucose does not stimulate.

To test whether the system behaves as expected, we used oligonucleotide-directed mutagenesis to randomize codons in the AraC arm region and in the α-peptide region. Sequencing showed that more than 90% of the candiate colonies were changed from the starting sequence. Candidates were spotted on plates, grown and then screened for β-galactosidase activity, as described in the Methods section. As anticipated, most mutations in the AraC arm portion of αgal-AraC retained β-galactosidase complementing activity (94% of 272 mutants), while most mutations in the α-peptide region eliminated β-galactosidase activity (82% of 277 mutants). Many of the mutations introduced at residues 6 through 15 of AraC, which bind over the arabinose, retain complementing activity but leave αgal-AraC with no detectable arabinose response. Amongst these are the substitutions of Asp or Ser for Leu9 or Glu for Leu10. These same mutations also prevent the arms from binding to the DNA-binding domains.[7] Thus, the side chains of residues 9 and 10 appear to make functionally important contacts in two different structural contexts.

Discussion

In the work reported here, we found that the light switch mechanism utilized by AraC can be fused to β-galactosidase, producing an enzyme whose activity is regulated, albeit weakly, by the presence of arabinose. In addition to demonstrating the feasibility of fusing the regulation mechanism to other proteins, it was possible to use the resulting β-galactosidase activity to examine the roles of residues in the regulatory arm of AraC.

The binary light switch mechanism utilized by AraC protein[1,2] can easily be understood, and minimal re-engineering appears to be required to fuse this mechanism onto a target protein. Slightly more complicated than the mechanism used by AraC is that used by the actin regulatory switch, N-WASP. Here, autoinhibitory domains can be inactivated by ligands, and the components of this

system have been used to construct synthetic switches.[8] A different simple mechanism for allosteric control or allosteric conformational change is fusing two domains so that only one at a time can properly fold or so that the folding or conformational changes in one assists folding or generates conformational changes in the other. This too, has been successfully used in engineering regulated proteins.[9–11] Another simple mechanism available for the design of regulated systems is the control of oligomerization via phosphorylation.[12] In many instances, of course, nature uses far more complicated schemes for allosteric regulation, such as that used in hemoglobin.

The example presented here utilizes separate polypeptide chains for the regulating module, αgal-AraC, and the regulated module, α-acceptor β-galactosidase. Fusing the two would eliminate the expected concentration-dependent component of the system but would require rebalancing the interactions between the arm and each module.

These results demonstrate the feasibility of transferring the binary switch allosteric mechanism of AraC to other proteins and suggest possible generalizations. Protein design or biochemical selections like phage display could be used to identify peptides that bind to a specific protein in a ligand-dependant fashion as well as peptides that modulate other proteins' activity. Combining the two may allow regulation of the activity of a wide variety of proteins by many different ligands, thereby adding to the collection of useful approaches for the design and redesign of proteins.[13–16]

ACKNOWLEDGMENTS

This work was supported by NIH GM18277. In addition, we thank Michael Rodgers, Jed Weldon, Blake Hill, George Rose and Doug Barrick for comments.

REFERENCES

1. Saviola B, Seabold R, Schleif R. Arm-domain interactions in AraC. J Mol Biol. 1997;278:539–548.
2. Seabold R, Schleif R. Apo-AraC actively seeks to loop. J Mol Biol 1998;278:529–538.
3. Ullman A, Perrin D, Jacob F, Monod J. Identification, by in vitro complementation and purification, of a peptide fraction of *Escherichia coli* β-galactosidase. J Mol Biol 1965;12:918–923.
4. Reed W, Schleif R. Hemiplegic mutations in AraC protein. J Mol Biol 1999;294:417–425.
5. Schleif R, Wensink P. Practical methods in molecular biology. New York: Springer-Verlag 1981.
6. Brunelle A. Mutations and biochemistry of AraC Protein. PhD thesis. Brandeis University, 1988.
7. Ross J, Gryczynski U, Schleif R. Mutational analysis of residue roles in AraC function. J Mol Biol 2003;328:85–93.
8. Dueber J, Yeh B, Chak K, Lim W. Reprogramming control of an allosteric signaling switch through modular recombination. Science 2003;310:1904–1908.
9. Tucker C, Fields S. A yeast sensor of ligand binding. Nature Biotech 2001;19:1042–1046.
10. Radley T, Markowska A,Bettinger B, Ha J, Loh S. Allosteric switching by mutually exclusive folding of protein domains. J Mol Biol 2003;332:529–536.
11. Guntas G, Ostermeier M. Creation of an allosteric enzyme by domain insertion. J Mol Biol 2004;336:263–273.
12. DeGrado W, Signarvic R. *De novo* design of a molecular switch: phosphorylation-dependent association of designed peptides. J Mol Biol 2003;334:1–12.
13. Looger L., Dwyer M., Smith J. Hellinga H. Computational design of receptor and sensor proteins with novel functions. Nature 2003;423:185–190.
14. Dahiyat B, Mayo S. De novo protein design: fully automated sequence selection. Science 1997;278:82–87.
15. Bryson J, Betz S, Lu H, Suich J, Zhou X, O'Neil T, DeGrado W. Protein design: A hierarchic approach. Science 1995;270:935–941.
16. Kuhlman B, Dantas G, Ireton G, Varani G, Stoddard B, Baker D. Design of a novel globular protein fold with atomic-level accuracy. Science 2003;302:1364–1368.