FOR THE RECORD

Competing protein:protein interactions are proposed to control the biological switch of the *E. coli* biotin repressor

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(RECEIVED August 9, 2001; FINAL REVISION October 1, 2001; ACCEPTED October 1, 2001)

Abstract

A model is suggested for the complex between the biotin repressor of *Escherichia coli*, BirA, and BCCP, the biotin carboxyl carrier protein to which BirA transfers biotin. The model is consistent with prior physical and biochemical studies. Measurement of transfer rates for variants of BirA with single-site mutations in the proposed BirA:BCCP interface region also provides support. The unique feature of the proposed interaction between BirA and BCCP is that it uses the same β -sheet region on the surface of BirA that the protein uses for homodimerization into a form competent to bind DNA. The resulting mutually exclusive protein:protein interfaces explain the novel feature of the BirA regulatory system, namely, that transcription of the genes involved in biotin synthesis is not determined by the level of biotin, per se, but by the level of unmodified BCCP. The model also provides a role for the C-terminal domain of BirA that is structurally similar to an SH3 domain.

Keywords: Biotin; acetyl-CoA carboxylase; SH3 domain; biotin repressor; biotin carboxyl carrier protein

The biotin repressor of *Escherichia coli*, BirA, is both an enzyme and a sequence-specific DNA-binding protein. As an enzyme, it adenylates biotin and transfers it to a unique lysine residue on the biotin carboxyl carrier protein (BCCP) subunit of acetyl-CoA carboxylase. As a DNA-binding protein it binds to the biotin operator and represses expression of genes that encode the biotin biosynthetic enzymes (Croan 1989). Apo-BirA is monomeric, but when complexed to the small-molecule corepressor bio-5'-AMP, two holoBirA (BirA · bio-5'-AMP) monomers bind cooperatively to the biotin operator (Abbott and Beckett 1993; Eisenstein and Beckett 1999). This same cooperative process of dimeriza-

tion and DNA binding is induced, although less strongly, by binding of biotin alone (Prakash and Eisenberg 1979; Weaver et al. 2001).

The crystal structure of monomeric apo-BirA (Wilson et al. 1992) showed it to consist of three domains, an N-terminal helix–turn–helix DNA-binding domain, a central biotin-binding domain, and a C-terminal domain that bears structural (although not sequence) similarity to SH3 domains (Noble et al. 1993), but for which no function was immediately apparent.

The purpose of the present communication is to point out that the same β -sheet that participates in homodimerization of BirA is indicated by model-building to also be poised to bind the BCCP. The model implies that binding of holo-BirA to BCCP, on the one hand, and, on the other, the binding of BirA to itself in the homodimer, are mutually exclusive. The model provides a structural rationale for the partitioning between competing physiological functions of BirA as well as a role for the C-terminal domain of BirA.

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Article and publication are at <http://www.proteinscience.org/cgi/doi/10.1101/ps.32701>.

The structure of the BCCP domain of acetyl-coenzyme A from *E. coli* has been determined by both crystallography (Athappilly and Hendrickson 1995) and NMR spectroscopy (Yao et al. 1997; Roberts et al. 1999). The target lysine to which the biotin is transferred from bio-5'-AMP is located in a highly conserved Met-Lys-Met sequence motif at a surface hairpin turn (residues 121–123; Fig. 1). BirA · bio-5'-AMP is the functional complex in biotin transfer to BCCP, and results of protein footprinting studies indicate that the structure of the bio-5'-AMP-bound enzyme differs from that of the biotin-bound protein (Streaker and Beckett 1999). However, the structural changes that occur simultaneously with biotin binding constitute a subset of those that occur upon binding of the intermediate. Therefore, the BirA · biotin complex serves as a reasonable starting point for modeling the heterologous protein–protein interaction. If the model of BCCP is aligned with that of biotin-bound BirA such that Lys122 is brought close to the biotin, the β -sheet strand Lys122–Glu128 of BCCP can be readily aligned so that it forms a parallel β -sheet interaction with strand Val189–Lys194 of BirA. There are also additional interactions between residues 88–90 plus Pro145 of BCCP and the region of BirA that includes residues 280–281 and 310–312 (Fig. 1). The parallel β -sheet interaction between BirA and BCCP has similarities to, but also distinct differences from, the interaction at the interface of the homodimer of BirA. In the homodimer, strand Val189–Lys194 from the first BirA subunit forms an antiparallel β -sheet interaction with the same strand in the second subunit (see Fig. 3 of Weaver et al. 2001). In the proposed heterodimer, the same strand of BirA participates, but the β -sheet interaction between BirA and BCCP is parallel rather than antiparallel.

The model for the BirA–BCCP interaction is supported by prior structural and biochemical studies. In particular, Reche et al. (2000) showed using heteronuclear NMR spectroscopy that the chemical shifts associated with two regions of the BCCP sequence (residues 119–128 and 87–90) are perturbed in the presence of BirA. These correspond precisely to the two regions of BCCP that are implicated in the model as having the most extensive contacts with BirA. (Pro145 of BCCP is also implicated but, lacking an amide proton, would not be detected in the NMR spectrum.) Reche et al. (1998) have also shown it to be critical that the acceptor lysine in BCCP be at position 122. This is consistent with the model because a lysine in either of the adjacent positions could not accept biotin without large structural rearrangements (Fig. 1). The model is also consistent with the results of mutagenesis studies of BCCP in which residues Glu119 (close to Lys122) and Glu147 (close to Pro145) were shown to be critical for the biotin transfer reaction (Chapman-Smith et al. 1999). Finally, mutation of BCCP residue 126, which is located in the proposed interface in the model, from glutamine to glutamic acid has been shown to have a small but measurable effect on biotinylation (Reche and Perham 1999). BirA must also be able to distinguish BCCP from the structurally similar lipoyl domains of 2-oxo acid dehydrogenase complexes (Perham 1991; Athappilly and Hendrickson 1995). The corresponding target lysine of the lipoyl domain is found in a conserved sequence motif Asp-Lys-Ala. If this tripeptide is imported into BCCP, replacing Met-Lys-Met, the mutant BCCP is not biotinylated in vivo (Reche et al. 1998). Likewise, if the methionine at position 121 is replaced by lysine the mutant BCCP is biotinylated at the correct position but

Fig. 1. Stereo drawing showing the proposed interaction between holo-BirA and BCCP. The figure includes the backbone of the central and C-terminal domains of BirA (residues 65–315, open bonds) and the backbone of the C-terminal part of BCCP (residues 77–155, solid bonds) as seen in the crystal structure (Athappilly and Hendrickson 1995). The bound biotin is shown in a ball-and-stick representation, and the essential lysine to which it is transferred on BCCP is residue 122. The coordinates of BirA are from the crystal structure of the dimer obtained in the presence of biotin (Weaver et al. 2001; PDB Code 1HXD). The coordinates of BCCP are from Athappilly and Hendrickson (1995), PDB Code 1BIA.

Fig. 2. Proposed complex between BCCP and the ATP-bound biotin carboxylase subunit of acetyl CoA carboxylase. The backbone of BCCP (Athappilly and Hendrickson 1995; PDB code 1BIA) is drawn solid with a model-built biotin ligated to Lys122. The backbone of the biotin carboxylase (Thoden et al. 2000; PDB code 1DV2) is drawn with open bonds with the A and C domains at the bottom (labeled A, C) and the B domain, which rotates $\sim 45^\circ$ on binding ATP, at the top (labeled CARBOX DOMAIN B). The bound ATP is drawn with solid bonds. In the crystal structure of ATP-bound biotin carboxylase the two residues at the C terminus (448–449) are not seen and are presumably disordered. In the model the C-terminal residues that are seen are located close to the presumed binding site of BCCP. Therefore, it would not be surprising if there were structural rearrangements in this region and elsewhere to allow somewhat more intimate interactions between BCCP and the carboxylase than suggested by the figure.

at a lower rate (Reche et al. 1998). (Substitution of Met121 in the yeast enzyme also reduces the rate [Polyak et al. 2001].) Either an aspartic acid or a lysine at position 121 would require a very unfavorable burial of charge in the proposed interface.

Results of kinetic measurements of biotin transfer to BCCP support the proposed model of the holoBirA–BCCP complex. Several single-site mutants located in surface loops of the central domain of BirA are defective in repression in vivo and in DNA binding in vitro (Barker and Campbell 1981a,b; Buoncrisiani et al. 1986; Kwon et al. 2000). The low affinities of these proteins for DNA have been shown to be caused by weak dimerization (Kwon et al. 2000). Furthermore, in the BirA dimer structure these loops are all located in the monomer–monomer interface (Weaver et al. 2001). The initial rates of biotin transfer catalyzed by these dimerization mutants have been measured (Table 1). These kinetic measurements were performed in the k_{cat}/K_m regime of the initial rate versus substrate concentration curve. Therefore, the results do not distinguish between defects in BCCP binding and defects in catalysis. The measurements were, moreover, all performed under conditions in which the enzyme, wild-type or mutant BirA, is saturated with bio-5'-AMP. In all but one case, the rates measured for the mutants were found to differ significantly from that measured for the wild-type enzyme. The exception is D197Y. In the BirA:BirA dimer the side chain of Asp197 forms a hydrogen bond across the interface to the main chain of residue 119. It is not surprising, therefore, that

substitution of tyrosine weakens dimerization. In the proposed BirA:BCCP heterodimer, however, the side chain of Asp197 points away from BCCP toward bulk solvent (Fig. 1). In this context the substitution of tyrosine would be expected to have a small effect on the rate of transfer of biotin, as is observed (Table 1). Therefore, defects in BirA homodimerization generally correlate with defects in the transfer of biotin from BirA to BCCP, although there can be exceptions.

As noted above, the C-terminal domain of BirA is structurally similar to an SH3 domain (Noble et al. 1993). SH3 domains are small β -sandwich modules that present a pep-

Table 1. Initial rates of biotin transfer from bio-5'-AMP to BCCP87^a

BirA variant	Rate (s ⁻¹)
Wild type	0.37 $\pm$ 0.01
R118G	0.61 $\pm$ 0.02
R119W	0.121 $\pm$ 0.004
A147 Δ	0.033 $\pm$ 0.005
D197Y	0.441 $\pm$ 0.006

^a BCCP87 is a truncated form of BCCP that contains the C-terminal 87 residues (70–156, cf. Fig. 1) (Nenortas and Beckett 1996). Its kinetic properties are essentially the same as those of full-length BCCP. Initial rates of biotin transfer were measured by stopped-flow fluorescence as described in Nenortas and Beckett (1996) in buffer containing 10 mM Tris HCl, pH 7.5, 200 mM KCl, 2.5 mM MgCl₂ at 20°C. In all measurements, the final concentration of enzyme-bio-5'-AMP was 0.9 μ M and of BCCP87 was 20 μ M.

tide-binding site composed of a nonpolar patch flanked by loops known as RT-Src and n-Src (Schindler et al. 1999; Xu et al. 1999). These modules are found in many proteins in signaling networks, and bind to proline-rich peptides (Pawson 1995). The C-terminal domain of BirA has a nonpolar patch and a loop (residues 294–297) analogous to n-Src, although the region related to RT-Src (residues 280–283) is greatly reduced (Fig. 4 in Noble et al. 1993). The model interaction between BirA and BCCP presents Val88, Gly89, and Pro145 of BCCP to the nonpolar patch of the C-terminal domain of BirA analogous to that of the SH3 modules. Therefore, the C-terminal domain appears to make a modest favorable contribution to the binding energy of BCCP and possibly to its correct orientation on BirA. This proposed role of the C-terminal domain of BirA in biotin transfer, which is essential for viability, provides an explanation for the inability to isolate mutants in this region using genetic screens (Barker and Campbell 1981a,b).

The interfaces formed between the two BirA monomers in the homodimer (Weaver et al. 2001) and in the BirA · BCCP heterodimer model are formed by hydrogen-bonding of β -strands from each protein to create an extended β -sheet. Such interfaces have been seen in a variety of other contexts, for example, in the interaction of a filamentous bacteriophage with its coreceptor on the surface of *E. coli*. In this structure a domain of the minor phage coat protein, gene 3 protein (g3p), binds to the C-terminal domain of the TolA coreceptor via an antiparallel β -sheet interaction to form an extended β -sheet (Lubkowsky et al. 1999). Like the interaction between BirA and BCCP, the TolA:g3p interaction is weak (Holliger and Reichmann 1997). However, in contrast to the phage coat protein:coreceptor interaction, which is simply noncovalent binding, formation of the BirA:BCCP complex is followed by chemical transfer of biotin to the acceptor protein substrate.

A key feature of the proposed interaction of holo-BirA with BCCP is that it uses the same surface of holo-BirA that is required for dimerization. Therefore, the binding of BCCP to BirA precludes BirA dimerization and operator binding. This explains the novel feature of the biotin regulatory system, namely, that the rate of biotin operon transcription in the cell is not determined by the level of biotin per se, but by the supply of the biotin acceptor protein (Cronan 1989). Unmodified BCCP binds available molecules of holo-BirA, competes with dimerization and operator binding, and allows transcription of genes involved in biotin synthesis to remain constitutive or unrepresed.

Following its transfer from BirA to BCCP, biotin is subsequently carboxylated by biotin carboxylase in the presence of bicarbonate, ATP, and Mg^{2+} . The structure of the *E. coli* biotin carboxylase subunit of acetyl CoA carboxylase, with and without ATP, has been determined (Waldrop et al. 1994; Thoden et al. 2000). Because BCCP appears to complex with BirA via a β -sheet interaction it would not be

surprising if it were to interact with the carboxylase in a similar fashion. Indeed, it is straightforward to construct such a model. In the proposed complex (Fig. 2) a parallel β -sheet interaction occurs between residues 122–126 of BCCP and residues 168–172 of ATP-bound biotin carboxylase. In the model the ureido ring of the covalently bound biotin can readily be placed close to the γ -phosphate of the ATP, consistent with the envisaged mechanism of carboxylation (Thoden et al. 2000).

The coordinates for both models have been deposited in the PDB (BirA-BCCP, 1K67; BCCP-carbox., 1K69).

Acknowledgments

We are most grateful to Hazel Holden for helpful discussions on her structural studies of the biotin carboxylase. This work was supported in part by NIH grants GM46511 to D.B. and GM20066 to B.W.M.

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References

- Abbott, J. and Beckett, F. 1993. Cooperative binding of the repressor of biotin biosynthesis to the biotin operator. *Biochemistry* **32**: 9649–9656.
- Athappilly, F.K. and Hendrickson, W.A. 1995. Structure of the biotinyl domain of acetyl-coenzyme A carboxylase determined by MAD phasing. *Structure* **3**: 1407–1419.
- Barker, D.F. and Campbell, A.M. 1981a. The *birA* gene of *Escherichia coli* encodes a biotin holoenzyme synthetase. *J. Mol. Biol.* **146**: 451–467.
- . 1981b. Genetic and biochemical characterization of the *birA* gene and its product: Evidence for a direct role of biotin holoenzyme synthetase in repression of the biotin operon. *J. Mol. Biol.* **146**: 469–492.
- Buoncristiani, M.R., Howard, P.K., and Otsuka, A.J. 1986. DNA-binding and enzymatic domains of the bifunctional biotin operon repressor (BirA) of *Escherichia coli*. *Gene* **44**: 255–261.
- Chapman-Smith, A., Morris, T.W., Wallace, J.C., and Cronan, J.E., Jr. 1999. Molecular recognition in a post-translational modification of exceptional specificity. Mutants of the biotinylated domain of acetyl-CoA carboxylase defective in recognition by biotin protein ligase. *J. Biol. Chem.* **274**: 1449–1457.
- Cronan, J.E., Jr. 1989. The *E. coli* bio operon: Transcriptional repression by an essential protein modification enzyme. *Cell* **58**: 427–429.
- Eisenstein, E. and Beckett, D. 1999. Dimerization of the *Escherichia coli* biotin repressor: Corepressor function in protein assembly. *Biochemistry* **38**: 13077–13084.
- Holliger, P. and Reichmann, L. 1997. A conserved infection pathway for filamentous bacteriophages is suggested by the structure of the membrane penetration domain of the minor coat protein g3p from phage fd. *Structure* **5**: 265–275.
- Kwon, K., Streaker, E.D., Ruparelia, S., and Beckett, D. 2000. Multiple disordered loops function in corepressor-induced dimerization of the biotin repressor. *J. Mol. Biol.* **304**: 821–833.
- Lubkowsky, J., Hennecke, F., Pluckthun, A., and Wlodawer, A. 1999. Filamentous phage infection: Crystal structure of g3p in complex with its coreceptor, the C-terminal domain of TolA. *Fold. Des.* **7**: 711–722.
- Nenortas, E. and Beckett, D. 1996. Purification and characterization of intact and truncated forms of the *Escherichia coli* biotin carboxyl carrier protein subunit of acetyl CoA carboxylase. *J. Biol. Chem.* **271**: 7559–7567.
- Noble, M.E.M., Musacchio, A., Saraste, M., Courtneidge, S.A., and Wierenga, R. 1993. Crystal structure of the SH3 domain in human Fyn; comparison of the three-dimensional structures of SH3 domains in tyrosine kinases and spectrin. *EMBO J.* **12**: 2617–2624.
- Pawson, T. 1995. Protein modules and signalling networks. *Nature* **373**: 573–580.
- Perham, R.N. 1991. Domains, motifs, and linkers in 2-oxo acid dehydrogenase multienzyme complexes: A paradigm in the design of a multifunctional protein. *Biochemistry* **30**: 8501–8512.

- Polyak, S.W., Chapman-Smith, A., Mulhern, T.D., Cronan, J.E., Jr., and Wallace, J.C. 2001. Mutational analysis of protein substrate presentation in the post-translational attachment of biotin to biotin domains. *J. Biol. Chem.* **276**: 3037–3045.
- Prakash, O. and Eisenberg, M.A. 1979. Biotinyl 5'-adenylate: Corepressor role in the regulation of the biotin genes of *Escherichia coli* K-12. *Proc. Natl. Acad. Sci.* **76**: 5592–5595.
- Reche, P. and Perham, R.N. 1999. Structure and selectivity in post-translational modification: Attaching the biotinyl-lysine and lipoyl-lysine swinging arms in multifunctional enzymes. *EMBO J.* **18**: 2673–2682.
- Reche, P., Li, Y.L., Fuller, C., Eichhorn, K., and Perham, R.N. 1998. Selectivity of post-translational modification in biotinylated proteins: The carboxy carrier protein of the acetyl-CoA carboxylase of *Escherichia coli*. *Biochem. J.* **329**: 589–596.
- Reche, P.A., Howard, M.J., Broadhurst, R.W., and Perham, R.N. 2000. Heteronuclear NMR studies of specificity of the post-translational modification of biotinyl domains by biotinyl protein ligase. *FEBS Lett.* **479**: 93–98.
- Roberts, E.L., Shu, N., Howard, M.J., Broadhurst, R.W., Chapman-Smith, A., Wallace, J.C., Morris, T., Cronan, J.E., and Perham, R.N. 1999. Solution structures of apo and holo biotinyl domains from acetyl coenzyme A carboxylase of *Escherichia coli* determined by triple-resonance nuclear magnetic resonance spectroscopy. *Biochemistry* **38**: 5045–5053.
- Schindler, T., Sicheri, F., Pico, A., Gazit, A., Levitzki, A., and Kuriyan, J. 1999. Crystal structure of Hck in complex with a Src family-selective tyrosine kinase inhibitor. *Mol. Cell* **3**: 639–648.
- Streaker, E.D. and Beckett, D. 1999. Ligand-linked structural changes in the *Escherichia coli* biotin repressor: The significance of surface loops for binding and allostery. *J. Mol. Biol.* **292**: 619–632.
- Thoden, J.B., Blanchard, C.Z., Holden, H.M., and Waldrop, G.L. 2000. Movement of the biotin carboxylase B-domain as a result of ATP binding. *J. Biol. Chem.* **275**: 16183–16190.
- Waldrop, G.L., Rayment, I., and Holden, H.M. 1994. Three-dimensional structure of the biotin carboxylase subunit of acetyl-CoA carboxylase. *Biochemistry* **33**: 10249–10256.
- Weaver, L.H., Kwon, K., Beckett, D., and Matthews, B.W. 2001. Corepressor-induced organization and assembly of the biotin repressor: A model for allosteric activation of a transcriptional regulator. *Proc. Natl. Acad. Sci.* **98**: 6045–6050.
- Wilson, K.P., Shewchuk, L.M., Brennan, R.G., Otsuka, A.J., and Matthews, B.W. 1992. *Escherichia coli* biotin holoenzyme synthetase/bio repressor crystal structure delineates the biotin- and DNA-binding domains. *Proc. Natl. Acad. Sci.* **89**: 9257–9261.
- Xu, W., Doshi, A., Lei, M., Eck, M.J., and Harrison, S.C. 1999. Crystal structures of c-Src reveal features of its autoinhibitory mechanism. *Mol. Cell* **3**: 629–638.
- Yao, X., Wei, D., Soden, C. Jr., Summers, M.F., and Beckett, D. 1997. Structure of the carboxy-terminal fragment of the apo-biotin carboxyl carrier subunit of *Escherichia coli* acetyl-CoA carboxylase. *Biochemistry* **36**: 15089–15100.