Expression in Escherichia coli of N- and C-terminally Deleted Human Holocarboxylase Synthetase

INFLUENCE OF THE N-TERMINUS ON BIOTINYLATION AND IDENTIFICATION OF A MINIMUM FUNCTIONAL PROTEIN

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Biotin functions as a covalently bound cofactor of biotin-independent carboxylases. Biotin attachment is catalyzed by biotin protein ligases, called holocarboxylase synthetase in mammals and BirA in prokaryotes. These enzymes show a high degree of sequence similarity in their biotinyltransfer domains but differ markedly in the length and sequence of their N terminus. BirA is also the repressor of the biotin operon, and its DNA attachment site is located in its N terminus. The function of the eukaryotic N terminus is unknown. Holocarboxylase synthetase with N- and C-terminal deletions were evaluated for the ability to catalyze biotinylation after expression in Escherichia coli using bacterial and human acceptor substrates. We showed that the minimum functional protein is comprised of the last 349 of the 726-residue protein, which includes the biotinyltransfer domain. Significantly, enzyme containing intermediate length, N-terminal deletions interfered with biotin transfer and interaction with different peptide acceptor substrates. We propose that the N terminus of holocarboxylase synthetase contributes to biotinylation through N- and C-terminal interactions and may affect acceptor substrate recognition. Our findings provide a rationale for the biotin responsiveness of patients with point mutations in the N-terminal sequence of holocarboxylase synthetase. Such mutant enzyme may respond to biotin-mediated stabilization of the substrate-bound complex.

Biotin is a water-soluble molecule essential for all forms of life. From bacteria to animals and plants, biotin is used as a cofactor in various carboxylation and decarboxylation reactions (reviewed in Refs. 1–3). In Escherichia coli, a single enzyme, acetyl-CoA carboxylase, requires biotin as a cofactor. However in mammals, four enzymes, acetyl-CoA carboxylase, methylcrotonyl-CoA carboxylase, propionyl-CoA carboxylase, and pyruvate carboxylase, utilize biotin. In such species, biotin is covalently linked to the ε-amino group of a lysine residue, which is part of the conserved (A/V)MKM sequence (3). The attachment of biotin to the lysine residue is enzymatically catalyzed by biotin protein ligase (BPL). In E. coli, BirA adds biotin to the biotin carboxyl carrier protein (BCCP) of acetyl-CoA carboxylase, whereas in mammals, plants, and yeast, holocarboxylase synthetase (HCS) adds biotin to the respective domain of each biotin-dependent enzyme. The BPL attaches the biotin to the apocarboxylase in an ATP-dependent process that occurs in the following two half-reactions.

ATP + Biotin → Biotinyl-5'-AMP + PP, 

REACTION 1

Biotinyl-5'-AMP + Apocarboxylase → Holocarboxylase + AMP 

REACTION 2

To attach biotin to its substrate, the BPL recognizes a 60–90-amino acid domain containing the acceptor lysine residue (3). The biotin binding site is often part of a larger subunit of the carboxylase enzyme. E. coli BCCP is a 156-amino acid polypeptide of which only the last 82 are required for biotin attachment by the BirA protein (4, 5). The 1.3 S biotin acceptor subunit of Propionibacterium shermanii transcarboxylase is a 123-amino acid peptide of which the last 75 have been shown to be sufficient to be biotinylated (6). Human pyruvate carboxylase, a homotrimer of a 1180-amino acid polypeptide, has its biotin acceptor sequence confined to the last 86 residues of the protein (referred to as p-86, Ref. 7). Human propionyl-CoA carboxylase, a heteropolymer of α- and β-subunits, contains its biotin binding region in the last 67 of the 703-amino acid α-subunit (referred to as p-67, Ref. 8). All of these sequences have been expressed independently of their larger polypeptide and are readily biotinylated in vitro. BCCP and the 1.3 S subunit have been characterized structurally and have been shown to share a similar three-dimensional structure, which is related to the lipoyl acid binding domains of dehydrogenase multienzyme complexes (9–12).

BPLs share a common catalytic domain (see Fig. 1), which was initially determined from the crystal structure of E. coli BirA complexed with biocytin (biotinyl-lysine, Ref. 13). It spans about 130 of the 321 amino acids of BirA. This sequence also shows a high level of identity with sequences in the C-terminal half of the human, yeast, and Arabidopsis enzymes (3). In E. coli BirA, the N terminus contains a DNA binding domain
involved in transcription repression of the biotin operon (14–16). In contrast, the N terminus of the 726-amino acid human HCS is unrelated to the bacterial sequence and has no known role in transcriptional regulation (17). The yeast BPL shares some resemblance to the human enzyme, encoding a polypeptide of 690 amino acids (18, 19). A cDNA for the Arabidopsis thaliana BPL has recently been cloned and found to encode a protein of 367 amino acids (20, 21). This BPL is similar to the bacterial enzyme except for a chloroplast-targeting sequence at its N terminus.

Years ago, it was shown that BPLs were interchangeable between organisms (6, 22–25). More recently, this has been applied to the expressed biotin attachment domains as substrates, where *E. coli* BirA has been shown to successfully biotinylate BCCP87 (*E. coli*), p-75 (*P. shermanii*), p-86 (yeast), and p-67 (human) among the above carboxylases (6–8, 26). This made it possible to clone BPLs by functional complementation between species, and this has been accomplished for the human, *Bacillus subtilis*, *A. thaliana*, and yeast BPLs using a temperature sensitive *E. coli* BirA mutant as host (17–20).

Cloning of human HCS allowed the first functional dissection of an eukaryotic BPL through mutation analysis of patients suffering from an inherited deficiency of HCS activity. This deficiency results in depressed activity of all four biotin-dependent carboxylases, hence the term early onset "multiple carboxylase deficiency" to describe the disease (27–30). Most patients with HCS deficiency can be successfully treated with pharmacologic doses of biotin (27, 31, 32), and several point mutations have been localized to the region of homology between *BirA* and HCS (see Fig. 1; Refs. 33, 34). HCS containing these mutations has been shown to be "bixinot responsive" in expression experiments and has been shown to be defective in biotin binding (35–37). Several other point mutations (R183P, L216R, L237P, V333E, and V363D) form a cluster outside the region of homology and, as such, appear to define a distinct region important for HCS activity (35–39). This suggests that some residues in the N-terminal domain of HCS might participate in the reaction even though this region has no bacterial counterpart. To test this hypothesis, we randomly generated N-terminal deletions and tested their effect on HCS activity toward either the human (p-67) or bacterial peptide substrates (BCCP or BCCP-87). Also, two C-terminal deletions were engineered to test the importance of the C-terminal domain.

**EXPERIMENTAL PROCEDURES**

**Materials and Reagents—** *E. coli* strain birA104 and purified apoMCCP87 were the kind gifts of A. Campbell (Stanford University) and D. Beckett (University of Maryland at College Park), respectively. p-67 was expressed and purified using the Fichia pastoris expression system from Invitrogen. The exo-size deletion kit was from New England BioLabs, [3H]biotin (53 mCi/mmol) from DuPont Chemicals, IPTG from Life Technologies, Inc., the Wizard miniprep kit from Promega, and the Qiafilter Midiprep kit from Qiagen. The High Fidelity long PCR kit was used for subcloning underlined). The PCR product was digested with SacI/SmaI and inserted into pQE32. For HCS ending at G655, the primers were: primer I and GGAGGAAGCTTGTGTTGCCCCTTTT. The PCR product was digested with SacI/SmaI for subcloning. Each construct was generated twice from two independent PCR reactions. Plasmid constructs were verified by sequencing the insert boundaries. The various constructs were purified using the Qiafilter Midi prep kit, and 50–100 mg of plasmid was used to transform competent *E. coli* birA104 that had already been transfected with plasmid pEPF4 (Qiagen), encoding the lacI repressor. Three colonies were picked for each construct and tested independently for reproducibility of results.

**Measurement of HCS Activity by Complementation of birA104—** *E. coli* birA104 carrying each construct was streaked onto LBagar plates containing 50 mM biotin, 2 mM IPTG, and 100 mg/ml ampicillin for incubation at the restrictive temperature of 42 °C or on plates containing 50 mg/ml biotin and 100 mg/ml ampicillin and for incubation at the permissive temperature of 30 °C. In these conditions, growth of *E. coli* at 42 °C is directly correlated with the level of residual HCS activity.

**Measurement of HCS Activity in Vitro—** Each construct was tested for its ability to biotinylate the bacterial substrate BCCP or the human substrate fragment, p-67 (8). The *E. coli* extract was the source of unbiotinylated BCCP. *E. coli* birA104 harboring each construct was grown overnight at 30 °C in LB medium containing 5 mM biotin and 100 mg/ml ampicillin. They were diluted 1:100 in fresh LB with 5 mM biotin and 100 mg/ml ampicillin and grown at 30 °C until they reached an *A* \(_{\text{595}} \) of 0.4–0.5. HCS expression was induced by adding 2 mM IPTG and incubating the cells at 30 °C for 2 h. The cells were centrifuged and washed twice with phosphate-buffered saline. They were resuspended in resuspension buffer (50 mM potassium phosphate, pH 6.0, 1 mM dithiothreitol, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride) and lysed by freeze-thaw in a methanol-dry ice water bath followed by sonication. Protein concentrations were determined using the Bio-Rad assay kit. For the HCS assay, 150 mg of protein lysate was mixed with 25 mg of 2x biotinylation assay buffer (100 mM reduced glutathione, 600 mM Tris-HCl, pH 7.5, 50 mM MgCl2, 2 μl of 100 mM ATP, 2 μl of [3H]biotin (0.1 μCi), and 5 μg p-67 in a final volume of 50 μl. Lysates were incubated in duplicate at 42 °C for 1–2 h. After termination of the reaction by the addition of 50 μl of SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 0.71 mM β-mercaptoethanol, 3% SDS, 0.01% bromphenol blue), the lysates were denatured 5 min at 100 °C and loaded onto a 12.5% SDS-polyacrylamide gel and migrated at 30 mA for 3 h. The gels were stained with a silver staining reagent for 3 h. The gels were then destained and subjected to western blotting overnight at 34 °V. The membranes were exposed to a Fujifilm BAS 2000 Bioimaging analyzer phosphorimaging plate to quantify the amount of [3H]biotin incorporated into p-67 and/or BCCP. The membranes were subsequently incubated with the MRGS-His antibody according to the supplier's instructions for detection of the polyHis/HCS fusion protein.

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2 E. Campeau and R. Gravel, manuscript in preparation.
3 L. Del Rio and R. Gravel, unpublished results.
RESULTS

Functional Analysis of poly(His)/HCS—HCS clone BL-04, starting at Met-58 relative to the longest cDNA clone isolated by Leon Del Rio et al. (17), was subcloned into the E. coli expression vector pQE30 to generate pQE30/HCS4 as described under “Experimental Procedures.” The HCS protein produced by this system is fused at its N terminus with a polyhistidine tag. This poly(His) HCS construct was tested for its ability to complement the temperature-sensitive E. coli mutant birA104. Transformation of birA104 with pQE30/HCS04 and incubation at the restrictive temperature of 42 °C results in normal growth comparable with that obtained for HCS fused to the lacZ gene in Bluescript or HCS expressed from plasmid pSE936 used in previous experiments (Ref. 35 and results not shown). No growth is obtained for birA104 grown alone at 42 °C. Western transfer of bacterial cell lysates onto nitrocellulose membranes and detection of the poly(His) fusion proteins using the MRGS-His antibody gives a protein of the expected size (see Figs. 2–5, Control or M58 lanes).

Effects of C-terminal Deletions on HCS Activity—We designed oligonucleotides encoding restriction endonuclease sites to subclone C-terminal truncated versions of HCS after amplification by long PCR. Two C-terminal deletions of HCS were generated: a 31-amino acid deletion ending at Leu-695 and a 71-amino acid deletion ending at Gly-655 (Fig. 1). Each deletion was tested for its ability to complement E. coli birA104 at the restrictive temperature of 42 °C, as well as for its ability to biotinylate BCCP and p-67 in extract assays. Both C-terminal deletions fail to complement birA104 at 42 °C and do not display any biotinylating activity toward p-67 or BCCP in vitro despite production of a protein of the expected size by Western blot (Fig. 2). In contrast, full-length HCS clones (from Met-58) generated similarly by long PCR support growth of birA104 at 42 °C, show biotinylation of both substrates in vitro and produce a protein of the expected size (Fig. 2).

Ability of N-terminal-deleted HCS Constructs to Complement birA104—N-terminal deletions were generated by exonuclease treatment and subcloned into reading frame variants of the expression vector so as to permit expression of the truncated HCS. In this manner, eight N-terminal deletions were obtained with HCS starting at Ala-80, Leu-165, Leu-166, Ala-235, Thr-266, Pro-338, Lys-378, and Pro-569 (Fig. 1). Two additional constructs, generating HCS starting at Arg-290 and Leu-460, took advantage of restriction endonuclease sites present in the HCS cDNA. Each construct was tested for its ability to complement E. coli birA104 at the restrictive temperature (Fig. 3A). HCS cDNAs starting at Ala-80, Leu-165, or Leu-166 complement birA104 at 42 °C, showing growth at levels only slightly less than obtained for Met-58. In contrast, HCS starting at Ala-235 fails completely to complement the E. coli mutant, providing an initial border to the minimum functional enzyme. However, further deletion to Thr-266 shows partial growth and still further deletion of N-terminal sequences to
Arg-290, Pro-338 or Lys-378 restores growth to levels obtained for constructs starting at Met-58 to Leu-166. Finally, removal of additional sequences to Leu-460 or Pro-569 completely abolishes growth of birA104. These data indicate that the complementing deletion constructs generate truncated enzymes capable of biotinylating BCCP at a level sufficient to support growth of the E. coli mutant.

In Vitro Assay of N-terminal-deleted Constructs—Each of the N-terminal-deleted constructs was assayed in extracts for biotinylation of BCCP and p-67, the former derived from the broken cell extract and the latter added directly into the assay (Fig. 3B). Most of the in vitro assay results are in accord with those obtained by the complementation growth of birA104. Several HCS constructs that are able to complement birA104 (HCS starting at Met-58, Arg-290, Pro-338, and Lys-378) are able to biotinylate BCCP in vitro, whereas constructs unable to complement birA104 (HCS starting at Ala-235, Leu-460, Pro-569) fail to show BCCP biotinylation. One construct, HCS starting at Thr-266, which complements birA104 weakly, also shows weak biotinylation of BCCP. Some constructs are discordant between the complementation growth tests and the in vitro assays: HCS starting at Ala-80 is able to complement birA104 but does not incorporate any labeled biotin into BCCP in the in vitro assay. However, this proved to be because the BCCP was already fully biotinylated in culture, thereby preventing any further biotinylation in the extract assay. When the same log phase cell extract was mixed with an equivalent source of unlabeled BCCP, transfer of biotin was observed (result not shown). Western detection of the fusion protein also shows that the Ala-80 clone produced a stable protein and activity in the in vitro assay were purified using Ni²⁺-NTA agarose to further examine their substrate specificity. These included HCS starting at Met-58, Ala-80, Arg-290, Pro-338, and Lys-378 (Fig. 4). This procedure clearly shows enrichment of the derivative HCS by Coomassie staining and Western blot, although the presence of contamination proteins or proteolytic products prevented direct quantification of the isolated enzymes. Each sample was tested for biotinylation of p-67, BCCP87, or an equimolar mixture of both substrates (Figs. 1, 5). HCS starting at Met-58 and Ala-80 show biotinylation of both BCCP87 and p-67 with a similar level of biotinylation of the mixture. HCS starting at Arg-290 and Pro-338 fail to biotinylate BCCP87 but show weak biotinylation of p-67, whereas HCS starting at Lys-378 shows a much stronger biotinylation of p-67 and weak biotinylation of BCCP87. These results for the purified Arg-290 and Pro-338 protein contrast with those obtained with the crude cell lysates in which biotinylation of full-length BCCP was favored over p-67. The BirA enzyme from a host expressing the wild-type sequence was tested for its ability to biotinylate each substrate in this assay and, as shown in Fig. 5 (top), both BCCP87 and p-67 are biotinylated at similar levels. Detection of the HCS fusion proteins by Western blotting shows that similar amounts of enzyme were added in each set of assays (Fig. 5, bottom).

**DISCUSSION**

In this study, we showed that deletion of the first 377 amino acids of the 726 amino acid human HCS yields a protein that retains biotinylating activity, both in vivo and in vitro. This 349-amino acid segment contains the biotinylination domain, residues Gly-466 to Val-674, that is homologous in all BPLs. This region contains the sites shown to make contact with biotin in the co-crystallized E. coli BirA-biocytin (biotinyl-lysine) complex (13). As well, it includes a sequence, GK/RGXXG, initially thought to be an ATP binding site in BirA but shown recently to be involved in birA biotin/biotin-AMP binding (42). It also includes a short segment (P/D)DGN(S/T)FD (residues 709–715) that is also found in yeast, A. thaliana, and Caenorhabditis elegans BPL, which may explain our finding of an absolute requirement for the C terminus by the human enzyme.

This initial result did not suggest a requirement for the N-terminal domain of HCS. However, intermediate deletions, leaving about two-thirds of the protein intact, interfered with biotinylating activity, suggesting that some level of interaction occurs between these two halves of the protein. In sum, a short deletion of 79 amino acids (that is, starting at Ala-80) resulted
in a fully active enzyme. Additional deletions to generate proteins beginning at Leu-165 or Leu-166 also complemented the biotin auxotrophy of birA104 in vitro although they did not show any activity in vitro. These proteins were Western blot-negative, suggesting that the protein was unstable and rapidly degraded in cell homogenates. Significantly, further deletions to Ala-235 or Thr-266 were inactive or only partially active in vivo and in vitro, whereas removing still more residues to Arg-290, Pro-338, or Lys-378, restored biotinylating activity. Deletions to Leu-460 or Pro-569, which are within the biotinylating domain, were without any activity. These latter proteins, from Ala-235 onward except Pro-569, were all Western blot-positive indicating that stable proteins are generated from these constructs. We conclude that, in addition to the 349-amino acid active site domain, additional sequences, delimited by residues Leu-166 to Arg-290, interact with the biotinylating domain. Disruption of this N-terminal domain interferes with the biotin transfer reaction. We speculate that partial removal of Leu-166–Arg-290 inappropriately blocks the active site, whereas removing the domain altogether restores basal activity. Consistent with these findings, Narisawa and co-workers (37) reported recently that a truncated HCS initiating at Met-234 was inactive when expressed in human fibroblasts, whereas proteins initiating at Met-1, Met-58, and Ile-117 (with Met substitution) were active.

The yeast and human BPLs are of similar length (690 and 726 residues, respectively) and share weak sequence similarity in the N-terminal half of the protein. Unlike our findings, Polyak et al. (43) have shown an absolute dependence of yeast BPL on inclusion of the N-terminal domain for biotinylating activity, demonstrated using N-terminal deletions and sensitivity of the enzyme to protease cleavage. Significantly, these authors identified a protease sensitive site in yeast BPL, which they propose acts as a hinge region that brings together the two halves of the protein upon biotin binding. This is similar to our conjecture about human HCS. The difference compared with our results may lie in instability of the truncated products of yeast BPL or perhaps that biotin binding, in the absence of the N terminus, is too weak to catalyze significant product formation. The linker region identified in the yeast BPL spans residues Lys-240–Asn-260, which corresponds approximately to Lys-312–Glu-336 (with a four-residue gap included). This region is conveniently located between the biotinylation domain (Gly-466–Val-674) and the Leu-166–Arg-290 N-terminal domain that we identified in the human enzyme. The location of this putative linker region provides an explanation for the results obtained for the human HSC deletion constructs. The shortest HCS constructs that retain biotinylating activity, Arg-290, Pro-338, and Lys-378, all have their N terminus in the sequence surrounding the linker region. Thus, removal of the N-terminal domain up to the linker region results in a stable, functional protein. Indeed, the construct showing the highest level of protein by Western blot is Lys-378, which has its N terminus beyond the carboxyl end of the putative linker region.

A significant property of the truncated human HCS was its differing behavior toward the BCCP, BCCP87, and p-67 acceptor substrates. The complementation assay tested the ability of the constructs to biotinylate BCCP at levels sufficient to permit growth of birA104, and these results were substantiated in the crude extract assays, which measured the biotinylation of remaining BCCP and the coexpressed p-67 substrate. Whereas the longest HCS, starting at Met-58 or Ala-80, were active toward both p-67 and BCCP, Arg-290 and Pro-338 showed a clear bias toward BCCP, with p-67 biotinylation essentially undetectable in the assay. The activity toward both substrates was restored in Lys-378. In the kinetically more interpretable assays using purified enzyme to assay equimolar mixtures of BCCP87 and p-67 (Fig. 5), Met-58 and Ala-80 were similarly active toward the two substrates. In contrast, Arg-290 and Pro-338 showed weak but significant activity toward p-67 but only trace activity toward BCCP87. Similarly, purified Lys-378 also showed a greater affinity for p-67 compared with BCCP87. The lower overall activity of Arg-290 and Pro-338 compared with Lys-378 is explained by the Western blot, which shows less protein in the assay than found for Lys-378. The disparity between the biotinylation of BCCP and BCCP87 by the significantly truncated HCS versus the near full-length HCS is more difficult to explain. It is possible that the N terminus of HCS participates in the interaction with the acceptor substrate, so that any difference in the interaction with BCCP and BCCP87 might become pronounced in the more extensively truncated HCS. However, this conjecture contrasts with the absence of any discernible difference in the rate of biotin transfer to the two substrates by BirA (44). These results, implicating N-terminal interaction with the acceptor substrates, call for dissection of the two half-reactions governing biotin transfer to apocarboxylase substrates.

Whereas eukaryotic and prokaryotic BPLs share an essentially identical biotinylation activity, they have completely distinct N-terminal sequences. In E. coli, the N terminus contains the biotin operon repressor function of BirA. Its corepressor is biotin-AMP, the product of the first half-reaction in the biotinylation of BCCP. When all of the available apo-BCCP becomes biotinylated, newly synthesized biotin-AMP remains associated with BirA and the BirA-biotin-AMP complex accumulates and binds to the operator sequence of the biotin operon, thereby repressing further transcription of the biotin biosynthetic genes. Beckett and co-workers (45, 46) have shown that biotin and biotin-AMP binding to BirA induces large conformational changes in the complex and Wilson et al. (13) showed that addition of biotin or biotin-AMP to crystallized BirA resulted in destruction of the crystals. These data pointed to the critical involvement of the N-terminal domain in tight binding of biotin and biotin-AMP. Xu and Beckett (46) further showed that deletion of the N terminus dramatically reduced the affinity for biotin and biotin-AMP without reducing the maximal rate of biotin-AMP synthesis. They also showed that the dissociation of the BirA-biotin-AMP complex occurs 100 times faster in the truncated protein than full-length BirA. It is not evident whether the molecular basis for the involvement of the N terminus in eukaryotic BPLs is similar. Because the sequences are unrelated, the parallels between these interactions may be coincidental. Alternatively, perhaps eukaryotic BPLs have evolved a related but biochemically distinct role in gene action in eukaryotic cells. To this end, there are several reports of biotin-mediated regulation of transcription or translation of mammalian genes (47–51).
Our results provide an alternate explanation for the biotin responsiveness of HCS-deficient patients with mutations outside the biotin binding region. For patients with mutations within the biotin binding region, their biotin responsiveness can be explained because at least one allele expresses mutant enzyme with an elevated $K_m$ for biotin (35–37). However, for patients with both alleles mapping outside the biotin binding region, or where the second allele is nonexpressing, their biotin responsiveness has been less easy to explain. One suggestion is based on mass action; plasma biotin levels are so far below the $K_m$ of the enzyme that any increase in biotin levels, afforded by high dose treatment with biotin, would result in an increase in HCS activity as long as there remained any unoccupied enzyme (35–37). Whereas this option remains plausible, our results, in combination with the findings for yeast BPL, provide a rationale for biotin responsiveness that takes into account the effect of biotin on the integrity of the enzyme. Three of these point mutations (R183P, L216R, L237P) are found within the region defined by the N-terminal domain Leu-166–Arg-290, described above (Fig. 1). Two others, V333E and V363D, are in the vicinity of the proposed linker sequence or within the sequence defined by the functional, truncated proteins. In these cases, the biotin responsiveness of patients may be associated with a biotin-mediated stabilization of the substrate-bound complex. Our studies raise a caution for the analysis of HCS activity from patients with multiple carboxylase deficiency where BCCP is used as the substrate (35, 37, 52). The implication for varying impact of the N terminus of HCS in biotin transfer, including in the interaction with the peptide substrate, suggests that natural substrates should be favored for diagnostic use. Apyocarboxylase fragments such as p-67, from human propionyl-CoA carboxylase, or p-86 from pyruvate carboxylase (7) may prove to be more informative of the functional state of mutant human enzyme.

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Fig. 5. Comparison of BCCP87 and p-67 as substrates for purified N-terminal deletion constructs. Top panel, each construct was tested for its ability to biotinylate p-67, BCCP87, and an equimolar mixture of both substrates, detected as described in the legend to Fig. 2. Bottom panel, detection of the fusion protein using the poly(His) antibody. The difference in size of the constructs is not as pronounced as in Figs. 3 and 4 because a higher percentage acrylamide (16%) was used to enable separation of BCCP87 and p-67, along with detection of the HCS protein. The panel for BirA is blank because purified BirA was used for this experiment; there is no reaction with MRGS-His antibody.
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