

EXPLORING THE DOMAIN STRUCTURE AND DNA BINDING REGIONS OF β PROTEIN FROM BACTERIOPHAGE λ BY MASS SPECTROMETRY

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Introduction: β protein from bacteriophage λ promotes a single-strand annealing reaction that is central to red-mediated recombination at dsDNA breaks and chromosomal ends. Previous studies have shown that β protein binds most tightly to an intermediate of annealing that contains two complimentary oligonucleotides. Here we have characterized the domain structure of β protein using limited proteolysis, lysine modification agent, and mass spectrometry.

Method: Purified β protein underwent limited proteolysis by trypsin, chymotrysin and subtilisin. The reaction was stopped by adding 5% acetic acid and the mixtures were injected to LC-ESI-MS (C4 column) directly to identify the fragments. In a separate study, β protein was incubated with N-hydroxysuccinimidobiotin (NHS-biotin) under physiological relevant condition. The biotinylated protein was subjected to complete trypsin digestion and LC-ESI-MS (C18 column) was used to identify the different biotin patterns of DNA binding and control β protein.

Results: In the absence of DNA, residues 1-134 form a stable N-terminal “core” domain that is protease-resistant. When protein is complexed with two sequentially added complementary 33-mer oligonucleotides, residues 135-230 form a “central” domain that becomes protease-resistant only upon DNA-binding. The C-terminal residues 231-261 of protein are protease sensitive, and presumably unstructured, in both the presence and absence of DNA. We probed the DNA-binding regions of protein further using a technique combining biotinylation of lysine residues and mass spectrometry. Several lysine residues in the N-terminal core and central domains of protein were protected from biotinylation in the DNA-complex, whereas none of the lysine residues in the C-terminal portion were protected. The results lead to a model for the domain structure and DNA-binding of protein in which a stable N-terminal core and a more flexible central domain come together to bind DNA, whereas a C-terminal tail remains disordered.

Supported by NIH Grant GM 067947.