Characterization of the long-term-terminal repeat single-strand tail-binding site of Moloney-MuLV integrase by crosslinking

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Processing of viral DNA by retroviral integrase leaves a dinucleotide single-strand overhang in the unprocessed strand. Previous studies have stressed the importance of the 5? single-stranded (ss) tail in the integration process. To characterize the ss-tail binding site on M-MuLV integrase, we carried out crosslinking studies utilizing a disintegration substrate that mimics the covalent intermediate formed during integration. This substrate carried reactive groups at the 5? ss tail. A bromoacetyl derivative with a side chain of 6 Å was crosslinked to the mutant IN 106-404, which lacks the N-terminal domain, yielding a crosslinked complex of 50 kDa. Treatment of IN 106-404 with N-ethylmaleimide (NEM) prevented crosslinking, suggesting that Cys209 was involved in the reaction. The reactivity of Cys209 was confirmed by crosslinking of a more specific derivative carrying maleimide groups that spans 8Å approximately. In contrast, WT IN was not reactive, suggesting that the N-terminal domain