

## Processing Site Context Contributes to Determining the Rate of HIV-1 Protease-Mediated Processing of Gag

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The HIV-1 Gag protein must be cleaved by the viral protease at five sites to produce mature HIV-1 particles. Gag processing is dynamic, with cleavage occurring in a particular order. Unique amino acid sequences at each processing site have complicated the identification of determinants controlling cleavage rates, which are likely important determinants of particle maturation. We examined the relationship between cleavage rate, processing site sequence requirements, and processing site context within the MA-CA cleavage site. Using a new gel-based two-substrate proteolysis assay, we directly compared processing of wild type and mutant MA-CA cleavage sites within MA-CA fusion proteins to measure relative specificity constants. The mutant MA-CA sites contained sequences from alternative Gag processing sites and/or isolated the processing site from the normal context by introducing 'spacer' amino acids. Replacing the MA-CA site with the amino acid sequence from the SP1-NC and SP2-p6 sites attenuated the rate of cleavage, despite originating from processing sites that are cleaved at equivalent or faster rates *in situ*. These results are consistent with a role for contextual determinants of processing. However, introducing three 'spacer' glycine residues uniformly decreased processing of wild type and mutant sites by approximately two-fold, suggesting that the contextual determinants may apply at the SP1-NC and SP2-p6 sites. Additionally, we created partial site substitutions to explore sequence requirements for processing at the MA-CA site. Placing the P3P4 or P3'P4' SP2-p6 amino acids into the MA-CA site diminished processing to rates similar to the full SP2p6 exchange. In contrast, substitutions including only the P2-P2' residues improved MA-CA cleavage by about two-fold, and this enhancement was also obtained by changing only the P1 and P1' amino acid of MA-CA. Swapping only the P4-P1 or P1'-P4' regions of SP1-NC exacerbated the loss in MA-CA cleavage efficiency observed in the full site exchange, which suggests the rate of SP1-NC cleavage is to some extent controlled by interactions spanning the scissile bond. Our results support a key role for contextual determinants in controlling processing rate, and highlight the complex interplay of the cleavage site residues in determining the rate of cleavage by the HIV-1 protease.