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Toward an antibody peptidase: Mechanistic studies of peptide-bond hydrolysis

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Abstract

To maximize the probability of obtaining an antibody peptidase, an efficient assay for the hydrolysis of peptide bonds was developed. Two methods for detecting the uncatalyzed rate of hydrolysis of a peptide bond, without the use of extreme temperature or pH, are described. The uncatalyzed and carboxypeptidase-catalyzed hydrolysis of hippurylphenylalanine was followed under identical conditions by derivatization of the primary-amine product with naphthalenedicarboxaldehyde (NDA). The half-life of this peptide as well as the rate enhancement and catalytic proficiency of carboxypeptidase A were determined. Progress was made in monitoring the hydrolysis of peptidyl-prolyl bonds by derivatization of the secondary-amine product with 4-(dimethylamino)azobenzene-4$\sp\prime$-sulfonyl (dabsyl) chloride.^ The effect of torsional strain on the rate of peptide-bond hydrolysis was also investigated. Antibodies were elicited against FK520, a hapten that mimics a twisted peptide bond in addition to the transition state for peptide-bond hydrolysis. The six monoclonal antibodies obtained were characterized by competitive ELISA. The binding of FK520 to the antibodies is specifically inhibited by FK506 hut not by rapamycin, indicative that the antibodies do not recognize the α-ketoamide functionality of FK520. Nonetheless, the panel of antibodies characterized may prove useful in future studies of immunusuppression. ^

Subject Area

Biochemistry

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