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Method of reduction of the volume of proteolytic databases and the structural interpretation of irregularities on the example of analysis of proteolysis kinetics of beta casein by trypsin mutants

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Kinetic information from the time courses of the major peptide components in the enzymatic hydrolysates of food proteins was transformed to the set of kinetic constants. This procedure allows decrease the number of points from 5-10 (points of kinetic curve) to 1 point (kinetic constant). Taking into consideration the common regularities of proteolysis kinetics allows substantially simplify and reduce volume of proteolysis databases.

Time courses of the major peptide components in the enzymatic hydrolysates of food proteins represent valuable information on protease specificity and proteolytic availability of peptide sequences possessing bioactivity (Vorob'ev and Goncharova, 1998). The objective of the study was to apply kinetic methods of modern enzymology and computer modelling to be able to reduce database without loss of biochemical information. We have transformed information from the time courses of the major compounds to the set of kinetic constants. This procedure allows decrease the number of points from 5-10 (points of kinetic curve) to 1 point (kinetic constant).

Kinetic constants for tryptic hydrolysis of peptide bonds containing Arg and Lys residues in β-casein were determined by kinetic analysis of 17 major peptide components as revealed by HPLC (Vorobev et al., 2000). Arg/Lys bond cleavage preference and averaged rate constants over several Arg-X and Lys-X bonds were used for the analysis of wild-type trypsin, K188H, K188F, K188Y, K188W, and K188D/D189K mutants (Chobert et al., 1998). Considerable preference for the cleavage of Arg over Lys containing peptide bonds was demonstrated for all trypsins with engineered S2 site except for K188H and K188F (TABLE 1).

Comparison of individual kinetic constants for various bonds showed that during the hydrolysis by wild-type trypsin the probabilities of splitting depend on secondary specificity and masking of peptide bonds. The improvement of prediction of hydrolysis rates performed by the used computer program was achieved after considering the presence of hydrophobic neighbourhoods of Lys48-Ile49 and Arg202-Gly203 bonds.

Clusters of hydrophobic amino acid residues in these segments of polypeptide chain cause hydrophobic interaction to be responsible for the steric obstacles shielding peptide bonds against enzymatic attack. Taking into consideration the common regularities of proteolysis kinetics allows substantially simplify and reduce volume of proteolysis databases.

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TABLE 1. Arg/Lys preference for tryptic hydrolysis of nitroanilide and peptide bonds in ß-casein.

Enzyme	pH	Substrate	
		tetrapeptide p-nitroanilide[a]	peptides from ß-casein[b]
		k_{cat}/K_M(Arg) / k_{cat}/K_M(Lys)	k_{Arg}(Arg25-Ile26) / k_{Lys}(max)
Wild – type trypsin	7	22	7.4
	8	7.0	8.0
	9	4.2	5.1
	10	4.3	3.7
K188H	7	-	1.3
	8	8.7	1.5
	9	-	1.4
	10	-	3.8
K188F	7	8.7	1.1
	8	8.2	1.2
	9	6.3	1.2
	10	6.0	0.7
K188Y	7	10	7.3
	8	5.2	14
	9	7.7	11
	10	8.8	10
K188W	7	8.8	4.3
	8	10	13
	9	15	11
	10	-	-
K188D/D189K	7	-	12
	8	22.5	13
	9	-	11
	10	-	6.6

[a] Substrate: Suc-Ala-Ala-Pro-X-pNA (X=Arg, Lys).
[b] k_{Lys}(max) means rate constant for hydrolysis of Lys28-Lys29, Lys32-Phe33, Lys99-Glu100, Lys105-His106, Lys113-Tyr114, Lys169-Val170, Lys176-Ala177.

Literature:

Chobert J.-M., Briand, L., Haertlé, T, 1998, J. Food Biochem. 22, 529.

Vorobev, M.M., Goncharova, I.A., 1998, Nahrung, 42, 61.

Vorobev, M.M., Dalgalarrondo, M., Chobert, J.-M., Haertlé, T., 2000, Biopolymers, 54, 355.