MODIFICATION OF PEPSIN FRAGMENTS WITH DNP-HEXAMETHYLENEDIAMINE

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The modification of porcine pepsin by mono-DNP-hexamethylenediamine (DNPHMDA) in the presence of the water-soluble 1-cyclohexyl-3-(2- morpholinoethyl)carbodiimide p-toluenesulfonate (CMECDI) at pH 5.5 and 20°C leads to the inclusion of one DNPHMDA residue in the molecule of the enzyme. The stoichiometry of the reaction is determined by the binding of the DNPHMDA by the hydrophobic zone on the surface of the pepsin, followed by the reaction of the amine with one of the three carboxy groups concentrated in this zone, and activated by the carbodiimide [1]. We have found that the large peptide fragments B-1 and B-2 formed in the cleavage of pepsin by cyanogen bromide at the methionine residues [2] can undergo modification under similar conditions. The treatment of B-1 with DNPHMDA and CMECDI (molar ratios 1:6:20) at pH 5.9 and 20°C led to the inclusion of 0.6 mole of the DNP-amine per mole of peptide.

From a thermolysin hydrolyzate of the modified peptide

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Asp-	Val-	Pro-	Thr-	Ser-	Ser-	Gly-	Glu-	Leu-	Try-	Ile-	Leu-	Gly-	Asp-	Val-	Phe-	Ile-	Arg-	Gln-	Tyr-	Tyr-	Thr-

23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Val-	Phe-	Asp-	Arg-	Ala-	Asn-	Asn-	Lys-	Val-	Gly-	Leu-	Ala-	Pro-	Val-	Ala

we have isolated colored peptides containing the DNPHMDA group and corresponding to sequences 5-8, 1-11, and 1-16.

It may be assumed that the addition of DNPHMDA to the carboxy groups of aspartic and glutamic acids in B-1, as in the modification of pepsin, prevents the binding of the DNP-amine by the hydrophobic part of the peptide fragment. A prerequisite for such a sequence of reactions is the capacity for maintaining spatial organization, which is unusual for a comparatively short fragment, as is shown by the well-defined tendency of the fragment B-1 to form aggregates that persist even in 8 M urea solution, and its resistance to the action of a number of proteases.

The characteristic short-wave shift in the fluorescence spectrum of the tryptophan residue [3] in B-1 (λ 334 nm; for free tryptophan in aqueous solution 350 nm, and for tryptophan in pepsin λ 338 nm) shows the hydrophobic environment of the tryptophan residue in B-1 and also the existence of at least elements of a spatial structure.

The modification of B-1 differs from the modification of the native pepsin in which the reaction affects the C-terminal alanine residue, the carboxy group of an aspartic acid residue not included in B-1, and glutamic acid probably belonging to sequence 6-8 of B-1. The absence of substitution at the C-terminal alanine in B-1 confirms the specificity of the reaction.

The modification of the large N-terminal pepsin fragment B-2 which includes about 175 amino-acid residues [2] leads to the inclusion of 1.2 DNPHMDA residues per molecule of peptide. No DNPHMDA is included in the B-4 and B-5 fragments, which serves as one more indication of the specific nature of the reaction which is apparently characteristic of carboxy groups located close to hydrophobic sections of the molecule.

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