

LETTERS TO THE EDITORS

Tryptic Digestion of Native and Chemically Modified α -Lactalbumin

The specific catalytic action of trypsin in the hydrolysis of arginyl and lysyl bonds has been repeatedly demonstrated and recently reviewed (1). The present note confirms this specificity with data on the tryptic hydrolysis of native and chemically modified α -lactalbumin.

In the experiments reported the following preparations were used: Native α -lactalbumin was crystallized 3 times (2) and supplied by Dr. W. G. Gordon of this laboratory; *N*-acetyl- α -lactalbumin was prepared according to the method of Fraenkel-Conrat *et al.* (3), which is specific for the acetylation of *N*-terminal and ϵ -amino groups. This preparation contained 12.7 acetyl groups determined by the method of Clark (4) in agreement with the 12 lysine residues and single terminal amino groups in this protein (2). No free amino groups could be detected by the Van Slyke procedure. Guanidinated α -lactalbumin was prepared according to the procedure of Wilcox and Chervenka (5). It contained 12.9 guanido groups, determined by the Sakaguchi (6) method in agreement with 1 arginine originally present in the protein plus 12 homoarginine residues formed by guanidination of lysine. The resulting product gave only traces of amino nitrogen values by the Van Slyke procedure, due to the slight reactivity of the guanido groups. The preparation of the dinitrofluorobenzene derivative of the protein was similar to that used by Sanger (7) except that 40% isopropyl alcohol was used as solvent, which permitted the reaction to proceed without precipitation of protein. The resulting product contained no free amino groups as measured by the Van Slyke method.

Tryptic digestion of the protein was carried out at a 100:1 ratio of substrate to enzyme at 30°C. and the enzyme contained about 0.1% chymotrypsin contamination. The increase in α -amino groups resulting from hydrolysis was measured by the Van Slyke procedure with aliquots containing 40 mg. of protein.

The results obtained and presented in Table I show that native α -lactalbumin was hydrolyzed to the extent of about 13 peptide bonds per mole of protein, in accordance with the sum of the arginine and lysine residues (1 + 12 moles). Acetylation or guanidination of the 12 ϵ -amino groups of the lysine residues resulted in a corresponding reduction in trypsin susceptibility, leading to the cleavage of only 1.5 peptide bonds per mole of protein. This value is in fairly good agreement with the presence of 1 mole of arginine in the protein molecule (2), not affected by the chemical modification. A similar result was obtained with DNP- α -lactalbumin, in agreement with earlier observations of Redfield and Anfinsen (8) on ribonuclease. The slightly higher hydrolytic values observed both for the native and for the modified protein are probably due to the slight contamination of the trypsin with chymotrypsin. The conclusion appears justified that peptide

TABLE I
Tryptic Digestion of Native and Chemically Modified α -Lactalbumin

Substrate	Number of peptide bonds hydrolyzed per mole of protein ^a			
	2 hr.	4 hr.	8 hr.	24 hr.
α -Lactalbumin	8.9	10.8	12.9	13.5
<i>N</i> -acetyl- α -lactalbumin	0.9	1.2	1.2	1.5
Guanidinate- α -lactalbumin	0.9	1.1	1.3	1.5
DNP- α -lactalbumin	0.6	0.9	1.3	1.4

^a Calculation based on molecular weight of 15,500.

bonds adjacent to *N*-acetyllysine and homoarginine are resistant to the action of trypsin. The results also reaffirm the established specificity of trypsin.

It is of additional interest to point out that trypsin is able to distinguish between arginyl and homoarginyl bonds. A similar example of enzymic specificity involving these compounds has been observed by Greenstein (9), who showed that homoarginine was completely inert to arginase action, while arginine was highly susceptible.

This finding is in contrast to a recent report of Lindley (10), in which it was observed that trypsin catalyzed not only the hydrolysis of lysyl bonds but also bonds involving *S*-(β -aminoethyl)cysteine, a compound in which sulfur has replaced a methylene group of lysine.

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