C-TERMINAL RESIDUES OF OVOMUCOID AND OVALBUMIN

by

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The C-terminal residue of ovomucoid has been identified as phenylalanine by PÉNASSE, JUTISZ, FROMAGEOT AND FRAENKEL-CONRAT[1], who reduced the protein with $LiAlH_4$, hydrolyzed the reduction product, and identified phenylalaninol in the hydrolyzate. By means of carboxypeptidase STEINBERG[2] has shown that alanine is a C-terminal residue of ovalbumin, with several amino acids as other possible C-terminal residues. We wish to report here the results of our experiments in determining the C-terminal moieties of ovomucoid and ovalbumin by means of the thiohydantoin method[3,4,5].

A mixture of 140–190 mg of protein in 3.0–4.0 ml of a solution of 90% acetic anhydride in acetic acid was shaken to dissolve as much of the protein as possible, 35 mg of NH_4CNS was added, and the mixture was heated for 90 min in a steam-bath. The orange solution was shaken with 8 ml of petrol ether, which was then pipetted off and discarded. After six more extractions with petrol ether, 0.2 *N* $Ba(OH)_2$ was added until the pH of the solution was *ca.* 12. At this point all of the solid matter had dissolved. After two hours the solution was acidified to pH 6.5 by addition of 0.25 *N* H_2SO_4. The solution was centrifuged, decanted from the $BaSO_4$, and extracted seven times with 10 ml portions of ethyl acetate. When the united ethyl acetate extracts had been concentrated nearly to dryness, the remainder was diluted with 15 ml of water, and then extracted five times with 10 ml portions of ethyl acetate. The united extracts were dried over anhydrous $MgSO_4$, concentrated to a volume of 1 ml, and transferred to a small combustion tube. The solution was evaporated to dryness in a stream of nitrogen. After addition of 0.2 ml of 48% HBr to the residue, the tube was sealed and then heated at 150° for six hours. The contents of the tube were diluted with water, filtered, and distilled to dryness *in vacuo*. Addition of water and distillation to dryness were repeated alternately three more times. The residue was stored in a vacuum desiccator with KOH overnight, dissolved in 0.3 ml of water, and chromatographed on paper.

When used in the above method a sample of ovomucoid[6] yielded a single spot of phenylalanine. This spot was obtained in several experiments, and its identity was demonstrated by comparison with authentic phenylalanine in several different solvents: R_F 0.59 in butanol-acetic acid; R_F 0.87 in 80% phenol; R_F 0.66 in butanol-formic acid[7] (Whatman No. 4 paper). This result confirms that of PÉNASSE *et al.*[1] completely.

Similarly, a sample of twice recrystallized ovalbumin yielded a single spot, identified as alanine: R_F 0.42 in butanol-formic acid; R_F 0.61 in 80% phenol. STEINBERG[2] has concluded that alanine, at least, is a C-terminal residue in ovalbumin on the basis of its appearance first during incubation of the protein with carboxypeptidase; subsequently five other amino acids appeared. Whether these were other C-terminal residues or were adjacent to a singly terminal residue of alanine remained in doubt. Since in our experiments alanine was obtained as the only spot, they support the hypothesis that alanine is the only C-terminal residue in ovalbumin.

The application of our modification of the thiohydantoin degradation to the determination of C-terminal residues in small peptides will be reported in a forthcoming paper.

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