N-TERMINAL GROUP OF OVALBUMIN

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Despite many efforts to find the N-terminal group of ovalbumin, no conclusive result has been obtained by the usual N-terminal analysis methods, although a C-terminal proline has been found (Niu and Fraenkel-Conrat, 1955). Thus several possibilities to explain the reason why the N-terminus of ovalbumin has escaped the detection of the terminal group analysis have been suggested. These are a loop peptide structure (Linderstrom-Lang, 1952; Ottesen, 1956; Haruna, 1960) and an N-substituted terminal group. No positive results for the former possibility have been reported thus far. As to the latter structure, two possibilities were considered: the one was N-phosphorylation (Flavin, 1954) and the other was N-substitution with a carbohydrate moiety (Porter, 1950; Anfinsen and Redfield, 1956). The possibility of N-phosphorylation was excluded by the isolation of the two kinds of phosphoserine peptides (Flavin, 1954). In turn a glycopeptide in which the peptidic part linked through aspartic β-carboxyl group with an oligosaccharide consisting of all the constituent glucosamine and mannose (Neuberger, 1938), has been isolated by three different research groups (Cunningham et al, 1957; Johansen et al, 1958; Jevons, 1958). The present author has also isolated glycopeptides from the 10% trichloroacetic acid-

soluble peptic digest of ovalbumin by the aid of molecular sieve of Dowex 50 columns (Narita et al, 1958). The glycopeptides could be retained on an acid form of Dowex 50-X4 column and passed through from a X8 column. The yield of the glycopeptide fraction thus fractionated was 38.4% in carbohydrate content measured by the anthrone reaction and 1.2% in weight. The crude glycopeptides were further fractionated by cellulose powder block zone electrophoresis at pH 5.6. Two main ninhydrin-positive glycopeptides were isolated. Thus the possibility that the N-terminal group might be substituted by carbohydrate was entirely ruled out. Finally the possibility of N-substitution with an acyl group was considered as was found in the protein of tobacco mosaic virus (Narita, 1958) and other proteins.

The performic acid-oxidized ovalbumin (1 g.) was subjected to the digestion with the proteinase produced by Streptomyces griseus (Nomoto et al, 1959) at pH 8.0 for 24 hours. The digest was treated with an acid form of Dowex 50-X2 column (2.0 x 25 cm., 50~100 mesh) and the column was washed with water until the washings became ninhydrin-negative after alkaline hydrolysis (Hirs et al, 1956). The effluent and washings (about 100 ml.) were collected and lyophilized. The acidic peptide fraction, which passed through the column, and which supposedly contain cysteic acid peptides, phosphoserine peptides and N-acyl peptides was further fractionated by the chloride form of Dowex 2-X8 column (1.5 x 23 cm., 200~400 mesh) as is shown in Fig. 1. Homogeneity of each peak was examined by one dimensional paper chromatography using n-butanol-acetic acid-water (4:1:1). No peaks in the figure were homogeneous except peak 3. The results of amino

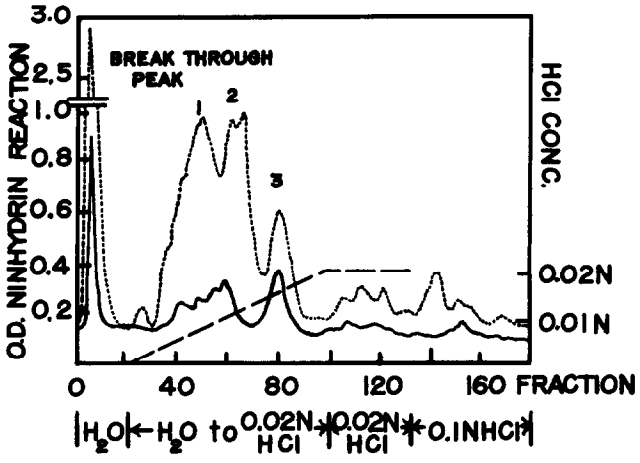

Figure 1. Chromatographic fractionation on a column of Dowex 2-X8 (chloride form, 1.5 x 23 cm.) of the acidic peptide fraction from the enzymatic digest of ovalbumin. Solid and dotted lines show the ninhydrin color values before and after alkaline hydrolysis respectively. One fraction was 5 ml. The break-through peak was large neutral peptide fraction.

acid and N-terminal analyses indicated that peak 3 was Val.$CySO_3H$ (Rf 0.20) which was already isolated by Flavin and Anfinsen (1954). Peak 1 contained several ninhydrin-positive cysteic acid peptides or phosphoserine peptides as well as several other ninhydrin-negative but chlorine-iodide-starch-positive components. The ninhydrin-negative spots were extracted with water from the paper. One of them was predominant (about 1 mg., Rf 0.46) and contained equimolar quantities of serine and glycine. Paper chromatogram of the hydrazinolyzate of the above ninhydrin-negative peptide showed the presence of acetyl hydrazide (Rf 0.65, pyridine-aniline-water, 9:1:4; 0.45, collidine-picoline-water, 5:3:2), serine hydrazide (Rf 0.46; 0.16) and glycine hydrazide (Rf 0.37; 0.11) which were revealed with the ammoniacal silver nitrate reagent. One could not be certain

which was the C-terminal amino acid judging from the above results of hydrazinolysis experiment alone. C-Terminal glycine and serine gave rise to their hydrazides partly by the action of anhydrous hydrazine at 100° for several hours. Therefore the acetyl peptide was incubated with carboxypeptidase A for 24 hours. The paper chromatogram of the incubate showed that serine was liberated from the peptide. The results of the C-terminal analysis by the hydrazinolysis-DNP method (Niu and Fraenkel-Conrat, 1955) gave also serine as the terminal group. Consequently it was decided that the peptide had to be N-acetyl.Gly.Ser. Confirmation of the structure of the present acetyl peptide by synthesis is now in progress. The present results strongly indicate that the N-terminal group of ovalbumin appears to be substituted by acetyl group. Johansen, Marshall and Neuberger (1960) suggested the presence of N-terminal acetylation of the protein from the results of the estimation of acetyl group in ovalbumin and in glycopeptide isolated from it. Recently they (Marshall and Neuberger, 1961) reported the isolation of an acetyl peptide from enzymatic digest of ovalbumin, but the structure was not described.

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