THE N-TERMINAL RESIDUE IN ACID GLYCOPROTEIN*

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Since the introduction of the fluorodinitrobenzene (FDNB)-method for the determination of free amino groups by SANGER[1,2], the N-terminal amino acids of a large variety of proteins have been identified[3,4]. In ovomucoid the amino acid with a free α-amino group could be measured satisfactorily in spite of the high carbohydrate content[5].

This communication summarizes the results obtained by applying the FDNB-method to acid glycoprotein[6], a protein isolated from normal human plasma and also distinguished by its large moiety of hexose and hexosamine.

The dinitrophenyl (DNP)-derivative of the native and of the denatured[6] form of acid glycoprotein were hydrolyzed as indicated by PORTER AND SANGER[2]. In addition the hydrolysis was performed with a sulfonated ion exchange resin on the hydrogen cycle[7]. For identification of the liberated DNP-compounds, the original method of SANGER as well as paper partition chromatography were used[8]. The silica gel prepared for this purpose exhibited the reported properties[1] as judged by the R_F-values of DNP-glycine and ε-DNP-lysine as well as by its capacity to separate DNP-amino acids**.

Hydrolysis of the DNP-derivatives of both the native and denatured acid glycoprotein with HCl as well as with an ion exchange resin yielded the same DNP-compounds, none of which was found identical with any DNP-amino acid, although special care was taken to identify any DNP-fractions which appeared to have R_F-values similar to any DNP-amino acid.

The possibility that a DNP-amino acid could have been destroyed during hydrolysis with HCl under conditions found satisfactory for the determination of the N-terminal amino acid in ovomucoid, was rendered unlikely by the results obtained from the hydrolysate with a sulfonated ion exchange resin. Under the latter conditions, humin formation was prevented by releasing the neutral sugars into the aqueous phase and binding the liberated amino acids and amino sugars to the resin. Since the hydrolysis, both with HCl and with the ion exchange resin, lead to DNP-compounds which were identical with those obtained after hydrolysis of DNP-glucosamine together with native acid glycoprotein, it was concluded that this protein has no N-terminal acid and that the DNP-compounds obtained were artifacts derived from DNP-glucosamine residues of DNP-acid glycoprotein. Due to the instability of DNP-glucosamine it was not possible to quantitate the results. Similar findings have been reported by MEYER AND SCHWARTZ[9].

Furthermore, results obtained by applying EDMAN's phenylisothiocyanate method[10] as modified by FRAENKEL-CONRAT[11,12], confirmed the absence of N-terminal amino acid in acid glycoprotein.

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