Neuraminidase-Resistant Sialyl Residues of α_1-Acid Glycoprotein

The sialyl residues of plasma glycoproteins, the terminal groups of the carbohydrate units of these macromolecules, are thought to be readily cleaved by neuraminidase. In a recent study on α_1-acid glycoprotein (orosomucoid), a well characterized globulin of normal plasma[1], the cleavage of these residues was investigated over the pH range from 1–6, and it was found that a small but significant percentage of the sialic acid was split off at a rate very much lower than that of the rest[2].

The present study was initiated to further investigate the linkage between the 'acid-resistant' sialyl residues and the carbohydrate moiety. For this purpose, α_1-acid glycoprotein was hydrolyzed at pH 2.2 and 80 °C for 1 h[2] effecting cleavage of the readily hydrolyzable sialic acid. Incubation of the resulting modified glycoprotein with highly purified neuraminidase (free of other hydrolases and peptidases), led to hydrolysis of only a small fraction of the remaining sialyl residues as compared with the extensive cleavage of this sugar from the native glycoprotein carried out in a simultaneously performed control experiment.

Additional evidence in support of this observation was obtained as follows: An aliquot of the modified α_1-acid glycoprotein was digested with pronase (1% w/w) at pH 8.5 and 37 °C for 8 h and the resulting digest fractionated by gel filtration through a Sephadex G-25 column to remove amino acids and small peptides and then through a Sephadex G-100 column to remove minute amounts of undigested glycoprotein. The presence of this undigested glycoprotein in the modified glycoprotein probably explains the relatively high rate of cleavage of sialyl residues from the latter preparation as the sialic acid content of the former was found to be relatively high. The isolated glycopeptide mixture contained approximately 5% sialic acid and was incubated with the mentioned neuraminidase. The sialyl residues were liberated at a rate very much lower than that of the parent protein (Table).

This observation confirms the above described finding and suggests that in α_1-acid glycoprotein 2 types of linkages exist between these residues and the carbohydrate moiety of this protein: one of these bonds is cleaved readily, while the other is split very slowly[3].

The relative rate of cleavage of the sialyl residues by neuraminidase from native human plasma α_1-acid glycoprotein (Native α_1-AG), from this protein after acid hydrolysis at pH 2.2 (pH 2-α_1-AG) and from the glycopeptide mixture (Glycopeptide) isolated from a pronase digest of the modified α_1-acid glycoprotein[a].

Incubation (min)	Glycopeptide (% of NANA cleaved)	pH 2-α_1-AG	Native α_1-AG
5	3	6	31
22	11	21	53
35	12	30	77

[a] These 3 experiments were carried out simultaneously and each contained the same amount of bound sialic acid.

Zusammenfassung. Untersuchungen über die Bindung der Sialinsäure des α_1-Säure-Glykoproteins (Orosomucoid) als typisches Glukoproteid des menschlichen Plasmas zeigten, dass dieses endständige Monosaccharid auf zwei verschiedene Arten gebunden ist. Ein zwar kleiner, aber signifikanter Anteil von Sialinsäure wird äusserst langsam durch das Enzym abgespalten.

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Active Transport of Cl^- Across the Isolated Intestinal Mucosa of *Testudo hermanni*

The intestinal epithelium is the site for transcellular active transport of many inorganic ions. While the existence of sodium active transport is clearly demonstrated, Cl^- active transport is much debated. Some authors (CURRAN and SOLOMON[1]; CURRAN[2]; CAPRARO et al.[3]) have given good evidence for an active transport from the lumen to the serosa; others (CLARKSON et al.[4], SCHULTZ et al.[5]) were not able to observe the same phenomenon. Even the presence of a Cl^- secretion towards the intestinal lumen has been observed under particular experimental conditions by some authors (TIDBALL[6]; TAYLOR et al.[7]).

Recently, GILLES-BAILLIEN and SCHOFFENIELS[8] have well established the existence of an active Cl^- transport from the serosa to the lumen across the isolated intestine of the Greek Tortoise. In the present work we have studied the effect of a metabolic inhibitor such as DNP, on Cl^- fluxes in both directions across tortoise intestinal mucosa.

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