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.

JACQUELINE LABAT and K. SCHMID

Department of Biochemistry,
Boston University School of Medicine,
Boston University Medical Center,
Boston (Massachusetts 02118, USA), 10 February 1969.

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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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The jejunum of Greek Tortoise (*Testudo hermanni*) was isolated according to BAILLIEN and SCHOFFENIELS[9] and put between 2 lucite chambers of the same volume. The apparent tissue area was 1.33 cm². The perfusion fluids were perfused with O_2 and the temperature was of 24 ± 2 °C. The perfusion fluid, tortoise Ringer, was always the same on both sides and its composition was the following: NaCl 110 m*M*, KCl 2 m*M*, $CaCl_2$ 2.5 m*M*, Na_2HPO_4 3.7 m*M*, NaH_2PO_4 1.7 m*M*, glucose 5.5 m*M*.

During the experiment, the transepithelial electrical potential was continuously held at zero with an electrical short-circuitation device (VESCOVINI and MARRO[10]).

In a first set of experiments, we measured the mucosa-serosa (in-flux) and serosa-mucosa (out-flux) fluxes of $^{36}Cl^-$ in 2 adjacent tracts of the same intestine. The fluxes were determined as follows: at the beginning of the experiment Ringer solution labelled with ^{36}Cl (about 0.1 μc/ml) was put at one side of the epithelium and unlabelled Ringer at the other. The labelled sides were opposite in the 2 tracts. After a 1-h period, required to obtain steady fluxes, every half an hour a small quantity of fluid from the unlabelled side was collected.

A second set of experiments was run as before, but with the addition, after the control periods, of 2,4-dinitrophenol (DNP) ($10^{-4}M$ or $5 \times 10^{-4}M$) to both perfusion fluids. Then the experiment went on over a 1-h period.

The radioactivity readings were performed with a Tri-Carb scintillation spectrometer (3000 series).

As can be seen from Table I, the in- and out-fluxes of ^{36}Cl detected in adjacent tracts of jejunum differ in that the serosa-mucosa flux prevails over the opposite one. The flux ratio is lower than unity and the difference is statistically significant. It can be seen from Table II that DNP lowers significantly both mucosa-serosa and serosa-mucosa fluxes.

The results of Table I allow us to agree with GILLES-BAILLIEN and SCHOFFENIELS[8] conclusion that across tortoise small intestine a net transport of Cl^- from the serosa to the mucosa is detectable. This transport occurs in the absence of electrochemical gradients and according to ROSENBERG[11] criteria must be considered an active transport. In order to check this statement, we have studied the effect of a metabolic inhibitor such as DNP on the transepithelial fluxes of Cl^-. The results summarized in Table II confirm this statement, as the serosa-mucosa flux of Cl^- is partially inhibited after DNP treatment. It is interesting to note that also the mucosa-serosa flux is lowered by DNP. This observation is consistent with the presence of an active Cl^- transport towards the serosal side. It seems reasonable to exclude a direct influence of DNP on Cl^- permeability. In fact it is well known that this substance increases the permeability of artificial lipid membranes (BIELAWSKY et al.[12]), while in this tissue DNP causes a reduction in Cl^- fluxes. Besides, we have observed that the permeability of a substance which crosses the intestinal epithelium passively, such as thiourea, is not affected by DNP (LIPPE et al.[13]).

At first sight the existence of 2 active fluxes in different directions across the same tissue seems rather strange. However, different types of cells of opposite functional polarity are present in the intestinal epithelium (TRIER[14]). Therefore Cl^- active transport presumably occurs in both directions across different types of cells.

Furthermore, the hypothesis that the 2 active transports are different in their mechanism cannot be excluded; while the serosa-mucosa flux could be due to the presence of a Cl^- pump, the Cl^- transport from mucosa to serosa could be dependent on the glucose pump according to the hypothesis advanced by FORDTRAN et al.[15].

Table I. Mucosa-serosa (Φ_i) and serosa-mucosa (Φ_o) fluxes of $^{36}Cl^-$ in adjacent tracts of the Greek Tortoise jejunum

Φ_i μ equiv. cm^{-2} h^{-1} mean value	Φ_o μ equiv. cm^{-2} h^{-1} mean value	$\frac{\Phi_i}{\Phi_o}$ mean value ± S.E.
1.99	2.90	0.68 ± 0.02

Numbers of experiments = 10.

Table II. $^{36}Cl^-$ fluxes across the jejunum of Greek tortoise

	Φ_1 μ equiv. cm^{-2} h^{-1} mean value	Φ_2 μ equiv. cm^{-2} h^{-1} mean value	$\frac{\Phi_2 - \Phi_1}{\Phi_1} 100$ mean value ± S.E.
Serosa-mucosa fluxes	3.21	1.47	− 55.7 ± 4.5 (6)
DNP $10^{-4}M$			
Mucosa-serosa fluxes	4.09	2.31	− 37.7 ± 10.5 (5)
DNP $10^{-4}M$			
Mucosa-serosa fluxes	3.12	1.36	− 59.5 ± 6.3 (4)
DNP $5 \times 10^{-4}M$			

Φ_1, control; Φ_2, DNP treatment. The figures in parentheses represent the number of experiments.

Riassunto. Nel digiuno di *Testudo hermanni* in assenza di gradienti elettrochimici esiste un trasporto netto di Cl^- dal mezzo serosale a quello mucosale. Il trattamento con DNP riduce significativamente il flusso sierosa-mucosa di Cl^-, per cui si conferma l'esistenza di un trasporto attivo di questo ione. Anche il flusso mucosa-sierosa di Cl^- è inibito dal DNP, per cui è probabile che anche questo flusso sia dovuto in parte a un trasporto attivo.

S. HÉNIN, A. BIANCHI and C. LIPPE

Istituto di Fisiologia Generale dell'Università, 20133 Milano (Italy), 24 February 1969.

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