POLYANIONIC CHARACTER OF PLASMA MEMBRANE SIALOGLYCOPROTEINS

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1. Introduction

Glycoproteins have been implicated in a variety of membrane functions [1]. The oligosaccharide chains of these macromolecules which generally contain terminal sialic acid residues are located on the outer surface of the cell membrane [2]. However, the role of sialic acid residues is not fully understood. Their influence on the isoelectric point is evident and shows that many glycoproteins carry a high negative molecular charge. Consequently they have a very low isoelectric pH which can be markedly raised by the removal of sialic acid residues with neuraminidase [3].

These observations suggest that such accumulation of negative charges might provide specific local physicochemical properties. That is, we assumed that the accumulation of sialic acid residues on a glycoprotein molecule might determine a polyanionic microenvironment and consequently a number of physicochemical properties and implications might be explained in terms of well-known but scarcely exploited polyelectrolyte theory [4–7].

This theory could be helpful in the understanding of the specific behaviour or/and the role of glycoconjugates, specifically for membrane glycoproteins.

An approach towards a solution of this problem is the study of modification of the protease activity by the cell coat glycoprotein microenvironment since endogeneous and exogeneous protease have been implicated in the cell growth control [8]. In order to test this role, we have investigated, in vitro, the kinetic behaviour of a hydrolytic enzyme (trypsin used as a probe) in non-covalent electrostatic interaction with a sialoglycopeptide fraction isolated from major hepatoma cell surfaces.

2. Materials and methods

2.1. *Materials*

Trypsin from bovine pancreas (type III), neuraminidase (*Vibrio cholerae*), and soybean trypsin inhibitor were obtained from Sigma. Pronase came from Calbiochem and benzoyl-L-arginine ethyl ester hydrochloride (BzArgOEt) (highest quality grade) was from Fluka. TPCK-trypsin was purchased from Worthington.

Sialoglycopeptide fraction was prepared according to [9] from glycoproteins released by trypsin from Zajdela hepatoma ascites cells. This fraction shows a single band in SDS–polyacrylamide gel with app. M_r 70 000. The sugar analysis of this fraction by gas–liquid chromatography [10] revealed the presence of 30% galactose, 10% glucose, 5% mannose, 25% *N*-acetylglucosamine, 15% *N*-acetylgalactosamine and 15% sialic acid. The total carbohydrates represented up to 85% of the entire glycopeptide fraction.

2.2. *Removal of sialic acid*

2.2.1. Enzymatic procedure

Sialoglycopeptide fraction was incubated with neuraminidase at an enzyme/substrate ratio of 1:25, at 37°C for 48 h in 0.02 M sodium acetate buffer (pH 5.0) and then dialysed against water. Sialic acid (70%) was released from glycopeptide as determined by the thiobarbituric acid method [11].

2.2.2. Acid hydrolysis

Sialoglycopeptide fraction was incubated in 0.1 M H_2SO_4 at 80°C for 1 h and neutralized by barium hydroxide.

2.3. *Tryptic activity*

Tryptic activity was determined using 4 buffer systems (Merck), sodium acetate (pH 4.0–5.5), sodium

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cacodylate (pH 6.0–7.0), Tris–HCl (pH 7.5–9.0) and glycine–NaOH (pH 9.5–10.0). The final concentration of buffers was 10 mM, and the ionic strength was adjusted with molar NaCl. Trypsin stock solution was maintained in 10 mM HCl at 4°C. Substrate stock solution was prepared daily.

The hydrolysis of BrArgOEt by trypsin was followed spectrophotometrically at 255 nm and at 20°C on a Beckman Acta III spectrophotometer [12,13]. The reaction was initiated by the addition of 20 μl of the enzyme stock solution (10 μM) to 2 ml of buffer containing the substrate (0.5 mM) in the presence or absence of sialoglycopeptide fraction. Saturating substrate concentrations were used (app. K_m = 4 μM) at pH 9.0 [14]. The rate of the reaction was calculated from the slope of the initial linear part of the kinetic curves ($k_{cat} = k_3$) [15].

3. Results and discussion

The pH activity profiles of the tryptic hydrolysis of BzArgOEt obtained under various conditions have been plotted (fig.1). It can be seen that at low ionic strength (10 mM), the pK_{Es} of the reaction in the absence of glycopeptide, is 5.95 which is in agreement with the value quoted in [16].

In the presence of sialoglycopeptide (0.85 μg/ml, 27 sialic acid residues/mol 70 000 M_r glycopeptide fraction) and at the same ionic strength (10 mM), the pH activity profile of the reaction is shifted 1.5 units towards the alkaline region raising the pK_{Es} to 7.50. However, when the ionic strength is increased in presence of the same amount of sialoglycopeptide, the

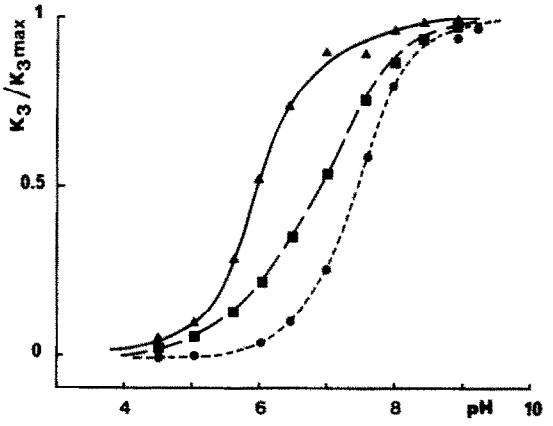

Fig.1. pH dependence of k_3/k_{3max} for the tryptic hydrolysis of BzArgOEt, ionic strength 10 mM, in buffer (▲), in the presence of sialoglycopeptide (●), in the presence of neuraminidase-treated glycopeptide (■). Glycopeptide was 0.01 μM.

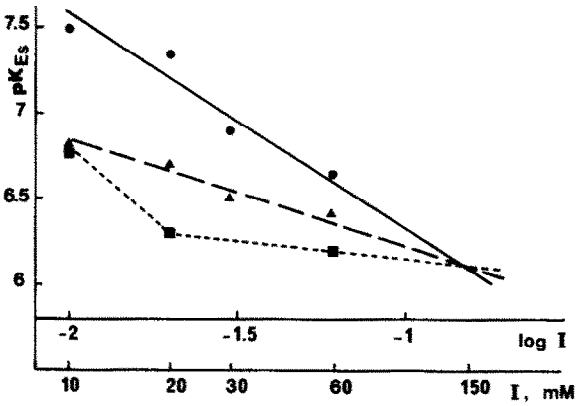

Fig.2. pK_{Es} of the tryptic hydrolysis of BzArgOEt as a function of the logarithm of ionic strength (in NaCl), in the presence of sialoglycopeptide (●), in the presence of partially desialylated glycopeptide with neuraminidase (▲), in the presence of acid treated glycopeptide (■). Glycopeptide was 0.01 μM.

observed values for pK_{Es} are shifted towards 6.60 and 6.10 for 60 mM and 150 mM ionic strength in NaCl, respectively (fig.2). This indicates that at high ionic strength the sialoglycopeptide effect is weaker and that the pK_{Es} tends to coincide with values obtained in the absence of sialoglycopeptide.

It may be mentioned here that the tryptic activity profile without glycopeptide, in present experiments, is almost independent of ionic strength in the range used above (10–150 mM).

When the pK_{Es} of the tryptic reaction in presence of the sialoglycopeptide was plotted as a function of the logarithm of ionic strength a linear representation was obtained (fig.2).

These observations which are the first to be reported for cell membrane sialoglycopeptide indicate that the tryptic activity is markedly influenced by the interacting glycopeptide. These effects can be explained in terms of the polyelectrolyte theory [4,17,18]. The influence of cell membrane polyanion, i.e., sialoglycopeptide on the pK_{Es} of the tryptic reaction can be compared with that observed for different other polyanions [4,16,19].

When the glycopeptide is partially desialylated with neuraminidase, the pK_{Es} is 6.8 at ionic strength 10 mM (fig.1,2). A linear representation of the pK_{Es} as a function of the logarithm of ionic strength is also obtained and the straight line intercepts the line obtained with the non-treated glycopeptide at a value of 6.10 for ionic strength about 150 mM (fig.2).

With the acid-treated glycopeptide free of sialic

acid residues, such linear representation is recordable for ionic strength ≥20 mM (fig.2). However, at 10 mM ionic strength the pK_{Es} (6.8) cannot be plotted on the straight line. This apparently abnormal behaviour could be explained by the very weak ionic strength (10 mM) inducing a hypotonic medium.

However, these results show that the total elimination of sialic acid by acid hydrolysis does not restore pK_{Es} to the value obtained without glycopeptide (5.95) indicating that a little polyelectrolytic influence is found even with desialylated glycopeptide. This remaining polyelectrolyte characteristics are probably due to the presence of other charged groups (Asp, Glu) on the polypeptide moiety. These groups appear to be quite sensitive to ionic strength since only 20 mM NaCl restores a nearly 'normal' pK_{Es}-value.

Thus, in spite of such residual polyanionic environment, it is clear that most of the polyelectrolyte character on glycopeptides is due to the presence of sialic acid residues.

Thus the sialoglycoconjugates can act as inhibitor of the hydrolytic activity of trypsin, while changes in ionic strength are able to restore and regulate this activity depending on the pH. These glycoconjugates are acidic and therefore strongly interact through electrostatic interactions, at low ionic strength, with an enzyme of strongly basic character. Further comprehensive polyelectrolytic studies of glycoproteins are required before generalizing the results reported here.

This present paper could be of major interest for the interpretation of the hypothesis claiming that endogeneous proteases of the cell plasma membrane could be implicated in malignant cell transformation [8,20–23]. This hypothesis is founded upon observation that normal cells treated with trypsin acquire the properties of transformed cells. The sialoglycoproteins which produce a polyelectrolytic type microenvironment, can modify the pK_{Es} of proteases and thus could modulate the activity of endogeneous or exogeneous proteases on the cells.

This hypothesis, founded upon the present results obtained with an acellular system, could be explored with normal and homologous transformed cells with respect to their different sialic acid contents.

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