ORIGINAL PAPERS

Adv Clin Exp Med 2006, **15**, 2, 253–258
ISSN 1230-025X

Wojciech Janusz Baranowski

UV-VIS Spectra of Intestinal Mucins with Change in Solution Concentration and pH

Zmiany widm UV-VIS mucyn przewodu pokarmowego w zależności od stężenia i wartości pH w roztworze

Professional College of Łódź Educational Corporation, Trace Elements Laboratory, Poland

Abstract

Background. The composition and physicochemical properties of mucus are interesting since they are able to influence digestion.
Objectives. Determining whether chemical properties of mucin change under the influence of change in their concentrations and pH values in water solutions.
Material and Methods. Solution of mucin type II crude from porcine stomach (Sigma-Aldrich); in: water; 0.5 M KOH; 0.1 M HCl; diluted with: water; water or 0.5 KOH; water or 0.1 M HCl, respectively. The influence of dilution and pH-value change on the chemical properties of mucin solutions were studied with the UV-VIS spectrophotometry method.
Results. All solutions studied showed characteristic minimum wave absorption at 370 nm and maximum at 376 nm in the VIS spectrum. The mucin solution with 0.5 M KOH showed the band of maximum absorption centred at 238 nm in the UV spectrum. Dilution with water or lowering the pH value of the solution widened and shifted that band toward shorter waves and revealed additional absorption maxima. The mucin solution with 0.1 M HCl had a band with maximum wave absorption at 213 nm. Dilution of this solution with water caused the formation of additional band with a maximum at 202 nm. The changes in the course of the absorption curve produced by dilution are reversible – the curve returns to its original form after restoring the original mucin concentration of the solution.
Conclusions. The changes described result from structural transformations characteristic of mucin. Mucin concentration and the pH value of the gastric environment determines its chemical activity. Gastric mucus turns into liquid during acidification and dilution, and becomes thick during alkalization and increase in its concentration. It seems that gastroprotection and the caustic properties of gastric juices arise from the known properties of gastric mucin and its peculiar location. The environment of the mucous membrane surface and the environment of the digestive tract lumen are separated by mucus. The mucous membrane surface is covered with mucus of high cohesion and chemical passiveness, which depends on continuous mucin excretion (high mucin concentration) and high HCO_3^- concentration. The mucus of the gastric lumen side is diluted and acidified, which turns it into a liquid that can enter into chemical reactions with food (**Adv Clin Med 2006, 15, 2, 253–258**).

Key words: mucus, mucin, stomach, gastroprotection, UV-VIS spectra.

Streszczenie

Wprowadzenie. Skład i właściwości fizykochemiczne śluzu budzą zainteresowanie, ponieważ mogą mieć wpływ na przebieg trawienia.
Cel pracy. Ustalenie, czy właściwości chemiczne mucyn zmieniają się pod wpływem zmian ich stężenia i wartości pH w wodnych roztworach.
Materiał i metody. Roztwór *mucin type II crude from porcine stomach* firmy Sigma-Aldrich w: wodzie; 0,5 M KOH; 0,1 M HCl rozcieńczano odpowiednio: wodą; wodą albo 0,5 KOH; wodą albo 0,1 M HCl. Metodą spektrofotometrii UV-VIS badano wpływ rozcieńczania i zmian wartości pH roztworów mucyn na ich właściwości chemiczne.
Wyniki. Wszystkie badane roztwory wykazują w zakresie VIS charakterystyczne minimum absorpcji fali 370 nm i maksimum – 376 nm. W zakresie UV roztwór mucyn w 0,5 M KOH ma pasmo o maksimum absorpcji fali 238 nm. Rozcieńczanie wodą albo zmniejszanie wartości pH roztworu poszerza i przesuwa to pasmo w stronę krótszych fal oraz wykształca dodatkowe maksima absorpcji. Roztwór mucyn w 0,1 M HCl ma pasmo o maksimum absorpcji fali 213 nm. Rozcieńczanie tego roztworu wodą powoduje wykształcanie się dodatkowego pasma o maksimum –

202 nm. Przywrócenie pierwotnego stężenia mucyn w roztworze powoduje wycofanie się wywołanych rozcieńczaniem zmian w przebiegu krzywej absorpcji.
Wnioski. Opisane zmiany widma UV-VIS wynikają z przekształceń strukturalnych mucyn. Aktywność chemiczna żołądkowych mucyn zależy od ich stężenia i wartości pH w środowisku. Zakwaszanie i rozcieńczanie upłynnia śluz żołądkowy, a alkalizacja i zatężanie powoduje jego gęstnienie. Odkryte właściwości żołądkowego śluzu i jego charakterystyczne umiejscowienie odpowiada za gastroprotekcję i trawiące właściwości soku żołądkowego. Śluz rozgranicza środowisko powierzchni błony śluzowej i światła przewodu pokarmowego. Spoistość i chemiczna bierność śluzu na powierzchni błony śluzowej wynika z dużego stężenia mucyn, które są nieustannie wytwarzane i wydzielane na jej powierzchnię oraz ze stopnia wysycenia śluzu jonami HCO_3^-. Po stronie światła żołądka śluz jest rozcieńczany i zakwaszany, co upłynnia go i umożliwia wchodzenie w reakcje chemiczne z pokarmem (**Adv Clin Med 2006, 15, 2, 253–258**).

Słowa kluczowe: śluz, mucyny, żołądek, gastroprotekcja, widma absorpcyjne.

It is well known that the mucous membrane that is build out of the enterocytes forms the wall of the digestive tract. The mucous membrane is covered by a tight layer of mucus produced by enterocytes. Until recently it was assumed that the mucus covering the mucous membrane of the digestive tract plays only a protective role. Such a conception of the role of mucus is however too great a simplification, since the physicochemical properties of digestive-tract mucus change with the age of the enterocyte producing it, with its location and the micro-environment in the lumen of the digestive-tract. In recent years attention has been focussed on the part played by mucus in processes of nutrient absorption from the digestive tract [1–4]. The main ingredients of mucus are mucins which come into close contact with food particles and with them, form the chyme. This chyme, while moving along the digestive tract, is subjected to action of the environmental conditions of its various segments: changes occur in the pH values of this environment, as well as in the composition and concentration of mucus [1].

The purpose of the experiment described was to determine whether the absorption spectra of the mucin studied change under the influence of change in their concentration and pH value in aqueous solutions. To measure this, spectrophotometry was chosen in the 200–500 nm wavelength range, because in that range are located the principle absorption bands of the majority of organic groups.

Material and Methods

The studies were performed on "mucin type II crude from porcine stomach" from the firm Sigma-Aldrich, and used demineralized water of 70 μS conduction, and HCl and KOH of analytic purity, produced by POCH in Gliwice, Poland.

The absorption spectra were registered by a Specord UV-VIS from Carl Zeiss (D – Jena), with use of quartz cuvette of 1 cm-layer thickness with respect to the reference material – water.

To test the influence of dilution on mucin absorption spectra, a starter solution of 50 mg mucin to 100 ml water was prepared. A series of four solutions was used in the study. The first 25 ml flask was filled with 0.8 ml starter solution and water to the full mark. The second flask was filled with 12.5 ml of the solution from the first flask, and water to the full mark. The third flask was filled with 12.5 ml of the solution from the second flask and water to the full mark. In contrast, into the forth flask was poured c. 10 ml of the solution from the third flask, 0.6 ml of the starter solution, and solution from the third flask to the full mark. Thus the series contained in the respective flasks 0.016; 0.008; 0.004 and 0.016 mg mucin in 1 ml of solution. Then the absorption spectra of the specific solutions, with respect to water, were registered (see Fig. 1).

With the purpose of testing the influence of dilution and pH of the mucin solutions on the absorption spectra on the basis of the starter solution, in measuring flasks of 25 ml capacity were

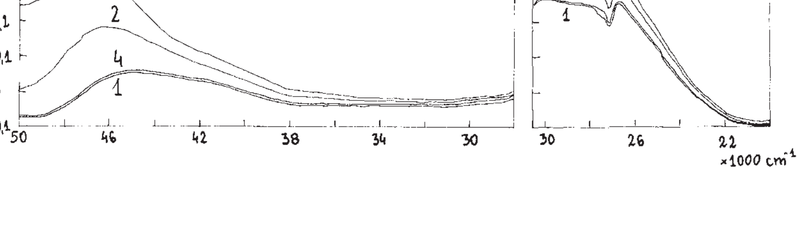

Fig. 1. UV-VIS spectra of aqueous mucin solutions of concentration: 1 – 0.016; 2 – 0.008; 3 – 0.004 and 4 – 0.016 mg/ml with respect to water

Ryc. 1. Widma UV-VIS wodnych roztworów mucyn o stężeniu: 1 – 0,016; 2 – 0,008; 3 – 0,004 i 4 – 0,016 mg/ml względem wody prepared four series of solutions. Into the first flask of each series was measured out 1 ml starter solution; then two of the flasks were filled up to the full-mark with 0.5 M KOH solution, and the other two with 0.1 M HCl solution to the full-mark. The mucin concentration in these flasks was 0.02 mg in 1 ml of solution. Then these solutions were diluted three times in the proportion 1:1. One of the alkaline mucin solutions was diluted with water, and the second, with 0.5 M KOH. With the mucin solutions in acid the procedure was analogous, but in this case the solvent was water and 0.1 M HCl. The absorption spectra of the different solutions with respect to water are presented in Figure 2–5.

Results and Discussion

The mucins studied are hardly soluble in water because, as large molecular compounds – compound proteins, its solutions are colloidal of great turbidity [4]. In previous studies it was seen that at concentrations lower than 500 mg of mucin in 100 ml, registration of absorption spectra is possible in the UV-VIS range [4]. The curve of radiation absorption changes markedly with each change in mucin concentration in solution, as also with each change in solution pH, as illustrated in Figure 1–5. The changes observed result from the intrusion in the internal structure of the mucin – with the chemical transformation new chromophore groups appear which absorb light of another wavelength. Chromophore groups are optically active functional groups (binding sites) which occur in the mucin molecules. The observations described are the confirmation of conclusions drawn from earlier theoretical considerations [1]. Because of the dependencies described and observations carried out, application of the Lambert-Beer law to mucin solutions is not indicated.

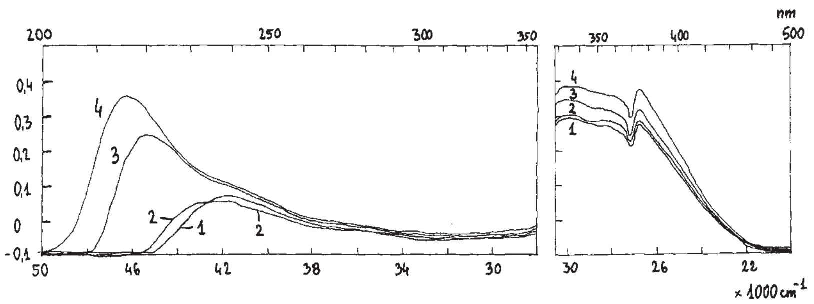

Fig. 2. UV-VIS spectra of basic mucin solutions, varying with concentration (mg/ml) and pH solution value: 1 – 0.02 (pH 13.7); 2 – 0.01 (pH 13.4); 3 – 0.005 (pH 12.5); 4 – 0.0025 (pH 11.9) with respect to water

Ryc. 2. Widma UV-VIS zasadowych roztworów mucyn w zależności od ich stężenia (mg/ml) i wartości pH roztworu: 1 – 0,02 (pH 13,7); 2 – 0,01 (pH 13,4); 3 – 0,005 (pH 12,5); 4 – 0,0025 (pH 11,9) względem wody

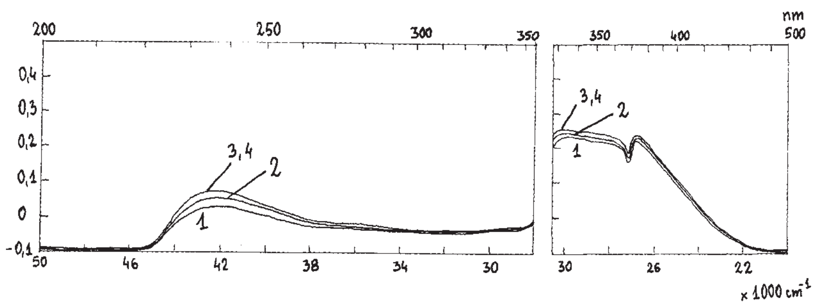

Fig 3. UV-VIS spectra of mucin solutions of concentration: 1 – 0.02; 2 – 0.01; 3 – 0.005; 4 – 0.0025 mg/ml in 0.5 M KOH with respect to water

Ryc. 3. Widma UV-VIS roztworów mucyn o stężeniu: 1 – 0,02; 2 – 0,01; 3 – 0,005; 4 – 0,0025 mg/ml w 0,5 M KOH względem wody

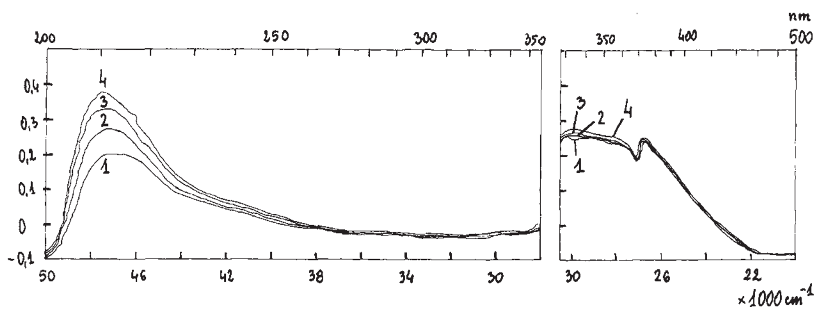

Fig. 4. UV-VIS spectra of mucin solutions of concentration: 1 – 0.02; 2 – 0.01; 3 – 0.005; 4 – 0.0025 mg/ml w 0.1 M HCl with respect to water

Ryc. 4. Widma UV-VIS roztworów mucyn o stężeniu: 1 – 0,02; 2 – 0,01; 3 – 0,005; 4 – 0,0025 mg/ml w 0,1 M HCl względem wody

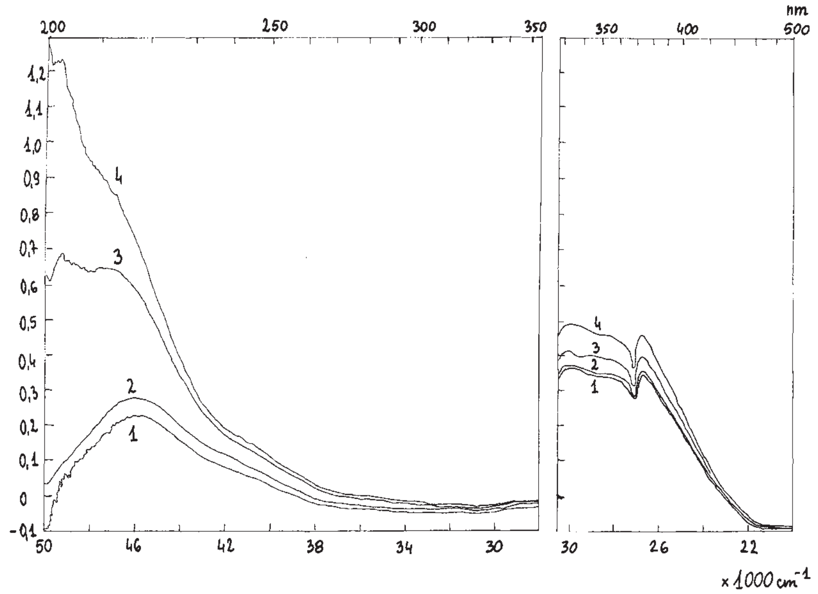

Fig. 5. UV-VIS spectra of acid mucin solutions, varying with concentration (mg/ml) and pH solution value: 1 – 0.02 (pH 1.0); 2 – 0.01 (pH 1.3); 3 – 0.005 (pH 1.6); 4 – 0.0025 (pH 1.9) with respect to water

Ryc. 5. Widma UV-VIS kwaśnych roztworów mucyn w zależności od ich stężenia (mg/ml) i wartości pH roztworu: 1 – 0,02 (pH 1,0); 2 – 0,01 (pH 1,3); 3 – 0,005 (pH 1,6); 4 – 0,0025 (pH 1,9) względem wody

On the basis of Figure 1 it can be maintained that, with a reduction in mucin concentration, the wide band of maximum wave absorption lying above 220 nm shifts in the direction of shorter waves, with a simultaneous increase in absorption and a pronounced bending point at c. 230 nm wavelength. The change in the curve under the influence of dilution of the mucin solution could indicate their hydrolysis, i.e. the fragmentation of the macromolecule. The increase in mucin concentration in the solution causes the reversal of the changes observed and the return of the curve to its original course. This phenomenon should be explainable by the reversibility of processes occurring in the solution. The disappearance of the short-wave band indicates that they originate from chromophore groups occurring in the mucin molecule, and not in the products of its continuous degradation. In given conditions of the mucin solution concentration these groups become chemically active and can react with other substances. The dependency discovered has far-reaching significance, since it explains the physiological mechanism of gastroprotection.

From Figure 2 results that in a basic environment a pronounced band of 238 nm max. wave absorption occurs which together with an increase in dilution with water and, decrease in solution pH, shifts in the direction of shorter waves. On these curves clearly forms a bending point at c. 230 nm wavelength, also seen in Figure 1. Certain of the absorption curves registered cut through each other in a way typical of isosbestic points, which indicates the co-existence of various spatial forms of mucin.

In aqueous KOH solution and with an insignificant increase in pH, small absorption increases are observed without a visible shift of the max. wave absorption at 238 nm, as seen in Figure 3. This fact indicates that in spite of significant lowering of mucin concentration, in the solution is present a constant concentration of certain chromophore groups displaying wave absorption at 238 nm. This phenomenon indicates the weak chemical reactivity of stomach mucus in conditions of increased pH. It seems that precisely this chemical property of stomach mucus promotes incipient infection with *Helicobacter pylori*. It is well known that this bacteria lives in stomach mucus. The ammonia produced by it prevents hydrolysis of stomach-mucus mucin, which protects it from the destructive action of digestive juices and makes invasion possible. Regardless of the above remark it seems that stomach-mucus mucins (glycoproteins) are nutritious for *Helicobacter pylori*, and ammonia is the main product of the metabolism of the protein portion of mucin in the metabolic processes of that microorganism: ever more frequent are current reports of the nutritious effect on *Helicobacter pylori* of substances freed by the stomach epithelium [5].

In Figure 4 is seen the band of max. wave absorption at 213 nm, which, with the increase in dilution, shifts insignificantly in the direction of shorter waves. Besides that, formation of a bending point at c. 230 nm wavelength is noticeable, similarly as in Figure 1 and 2. The existence of this point indicates the occurrence in the acidic solutions of optically active chromophore groups, which also occur in the alkaline solutions.

Figure 5 presents very interesting changes in the course of the UV-VIS radiation absorption curve for solutions of various mucin concentrations in aqueous HCl solutions. With dilution in water, the broad absorption band in the region of 210–250 nm waves widens visibly in the direction of shorter waves, and becomes divided. At the first dilution occurs one band of max. wave absorption at 220 nm, which is only insignificantly shifted towards shorter waves with respect to the absorption of the initial test. With subsequent dilutions one more band forms with max. wave absorption at 202 nm. On all the curves are observed also a bending point at a wavelength of c. 230 nm. Additional absorption bands appearing with progressive solution dilution point to the emergence of still other chromophore mucin groups.

From a comparison of the absorption curve in Figure 1 and 2 results that dilution with water of aqueous mucin solutions leads to a reduction in pH value in those solutions. This effect is similar to the effect appearing during hydrolysis of weakly basic salts with strong acid.

Surprising are however the changes in the spectrum as seen in Figure 5 – dilution of the solution leads to an increase in pH, and in spite of that appear new, short-wave absorption bands. The appearance of new absorption bands points to structural transformations of mucins (including change of conformation) and activation of various functional mucin groups. The mucins studied are stomach mucins, and for that reason it should be assumed that this phenomenon has far-reaching physiological significance in the digestive and nutrient absorption processes, the exact progression of which is still unknown.

It is worth noting that in the wavelength region of 350–460 nm, on all curves appear a min. wave absorption at 370 nm and a max. wave absorption at 376 nm. The form of this section of the absorption curve seems to be characteristic for the mucins studied. The absorption minimum and maximum mentioned is however weakly sensitive to change in concentration of mucin and pH of the solution.

Changes in the course of the absorption curve in the UV-VIS range shows that the mucins studied, influenced by changes in their concentration and pH in aqueous solution, change their chemical properties. In the introduction it was mentioned that intestinal tract mucus has been treated until now as a passive participant in the digestive process, and the reason for its production is only the protection of the intestinal epithelium. The results obtained contradict this assumption, for the stomach-mucus mucins studied change their physicochemical properties under the sole influence of dilution with water. The chemical activity of stomach mucins changes with change in pH of the environment. In a basic environment the mucins studied bind with one another, which makes them inactive with respect to elements composing the chyme. In contrast in an acidic environment these mucins have many active functional groups which can react with foods. For this view speak research results on zymogen of pepsin. It was shown that pepsynogen is a glicoprotein which is stable in a basic or neutral environment, but in an acidic one, is subject to transformation into pepsin, the most important digestive enzyme secreted by the stomach [6–8].

Change in the structure of mucins, expressed in changes in activity of their functional groups, brings changes in their rheological properties, which changes also the rheological properties of the mucus. From the experiments conducted results that acidification and dilution liquefies mucus, while alkalization and concentration cause its thickening. Other authors who have studied duodenum mucus have made similar observations. The only difference is that changes in rheological properties of duodenum mucus, under influence of changes in pH value, occur in the opposite direction [9]. With regard to the above it can be assumed that composition and physicochemical properties of mucus in each segment of the digestive tract are different. This observation is confirmed by earlier reports of biochemical studies [10–13].

Secretion of digestive juices in the digestive tract is a continuous process and occurs also in spite of a lack of stimulation by foods. The mucus layer and the mucins contained in it are continuously diluted and subjected to action of the microenvironment of the given segment of the digestive tract. The rheological properties of mucus are subject to essential changes with the increase in distance from enterocytes: mucus in direct contact with enterocytes is thick and strongly adheres to the cells (unstirred layer), while that furthest away is half-liquid and can mix easily with the chyme [14]. The phenomenon described by those authors can be associated with the effect of dilution and change in pH of the environment, which were observed during performance of the experiments. This phenomenon results from the specific location of the mucus layer. Mucus forms the border between two environments: that of the surface of the mucus membrane, which is relatively constant, and of the environment of the digestive tract lumen, which is changeable. Change in the rheological properties of mucus at the lumen side of the digestive tract is related to the great volume and changeable pH value of digestive juices which dilute it. The cohesiveness of the mucus on the surface of the mucous membrane results from the great concentration of the mucins which are constantly produced and secreted onto its surface, as well as from the degree of its saturation with HCO_3^- ions. Synnerstad et al. report that during secretion of hydrochloric acid the mucous membrane of the stomach secretes considerable amounts of HCO_3^- ions, which saturate the mucus covering it. This hydrocarbon-saturated mucus in their opinion is supposed to protect the mucous membrane from acidic digestive juices [15]. This phenomenon is called gastroprotection.

The changes in mucin properties observed during the experiment, and also described by other authors – the binding of mucins between themselves, or dissolution of the bonds between them under influence of change in pH and their concentration in solution – has considerable significance for the activity of the mucus barrier in the digestive tract. In light of the research results presented, the obvious consequence of a lack of gastroprotection and excessive acidification of the stomach contents, leading to hydrolysis of mucin, is the liquefaction of the entire stomach mucus. The products of the hydrolysis of mucin are, among others, digestive enzymes, which has been proved for pepsygen, which transforms itself into pepsyn [6–8]. These enzymes damage the stomach's mucous membrane which can cause bleeding into the digestive tract lumen.

References

[1] **Baranowski WJ:** Rola śluzu jelitowego we wchłanianiu żelaza. Gastroenterol Pol 1999, 6, 63–65.

[2] **Conrad ME, Umbreit JN, Moore EG:** A role for mucin in the absorption of inorganic iron and other metal cations. Gastroenterology 1991, 100, 129–136.

[3] **Quarterman J:** Metal absorption and the intestinal mucus layer. Digestion 1987, 37, 1–9.

[4] **Baranowski WJ:** Stomach mucins bind iron ions. Mengen- und Spurenelemente 2004, 22, 747–752.

[5] **van Amsterdam K, van der Ende A:** Nutrients released by gastric epithelial cells enhance *Helicobacter pylori* growth. Helicobacter 2004, 9, 614–621.

[6] **Tanaka T, Yada RY:** N-terminal portion acts as an initiator of the inactivation of pepsin at neutral pH. Protein Eng 2001, 14, 669–674.

[7] **Kageyama T:** Pepsinogens, progastricsins and prochymosins: structure, function, evolution and development. Cell Mol Life Sci 2002, 59, 288–306.

[8] **Baudys M, Kostka V:** Covalent structure of chicken pepsinogen. Eur J Biochem 1983, 136, 89–99.

[9] **Kaunitz JD, Akiba Y:** Acid-sensing protective mechanism of duodenum. J Physiol Pharmacol 2003, 54, Suppl. 4, 19–26.

[10] **Nordman H, Davies JR, Herrmann A, Karlsson NG, Hansson GC, Carlstedt I:** Mucus glycoproteins from pig gastric mucosa: identification of different mucin populations from the surface epithelium. Biochem J 1997, 326, 903–910.

[11] **Ohara S, Ishihara K, Hotta K:** Comparative study on mucus glicoproteins in rat stomach and duodenum. Comp Biochem Physiol [B] 1986, 83, 273–275.

[12] **Spee-Brand R, Strous GJ, Kramer MF:** Isolation and partial characterisation of rat gastric mucous glycoprotein. Biochim Biophys Acta 1980, 621, 104–116.

[13] **Stauffer A, Lallemand A, Gaillard D:** Mucin histochemistry of the digestive tract in the human fetus. Gastroenterol Clin Biol 1990, 14, 561–566.

[14] **Atuma C, Strugala V, Allen A, Holm L:** The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. Am J Physiol Gastrointest Liver Physiol 2001, 280, G922–G929.

[15] **Synnerstad I, Johansson M, Nylander O, Holm L:** Intraluminal acid and gastric mucosal integrity: the importance of blood-borne bicarbonate. Am J Physiol Gastrointest Liver Physiol 2001, 280, G121–G129.

Address for correspondence:

Wojciech Janusz Baranowski
Laboratorium Analiz Śladowych Pierwiastków Wyższej Szkoły Zawodowej ŁKO
Prusa 6, m. 12
91-315 Łódź
tel. 0502 063 567
e-mail: kosmetologia@wp.pl

Conflict of interest: None declared

Received: 7.04.2005
Revised: 21.06.2005
Accepted: 19.07.2005

Praca wpłynęła do Redakcji: 7.04.2005 r.
Po recenzji: 21.06.2005 r.
Zaakceptowano do druku: 19.07.2005 r.