JAP&T
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Journal of Agroalimentary Processes and Technologies 2009, *15* (4), 599-610

Journal of Agroalimentary Processes and Technologies

Physiologically active natural glycoproteins

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Abstract

In this paper, the authors aim at making up a bibliographical synthesis of the main research directions in the field of some glycoproteins (mucins) of major biological importance. The authors achieve a classification, present structures and conformational competences, as well as some possibilities of isolation, purification, and characterisation. They underline the physiological importance of mucins and possible sources of mucins.

Keywords: glycoproteins, carbohydrates, mucoproteides, mucins, conjugated proteins

1. Introducing the problem

An important feature of ***modern food processing*** is ***modelling***, respective ***fractioning natural raw materials*** and using the products thus obtained as ingredients to obtain ***"formulated foods"***. Literature suggests insistently that ***invertebrate*** protein fractioning should follow the model supplied by milk processing in protein fractioning by using combined, undenatured methods based particularly on separations with semi-permeable membranes and enzymatic processes, that allow the development of an impressive range of products destined to specific applications in the food industry.

Genetic manipulation of some water species is accepted with reservation as far as near future is concerned, and that allows the selection of genetic and physiological competences specific to some species and their transfer to other species. Another model in focus nowadays among academics and that has known some success is ***cloning the production capability of some antifreeze proteins*** (***„antifreeze proteins"***) [1,2,3]. These proteins achieve the inhibition of ice crystal formation and control the size of crystals formed, an extremely important effect for the maintenance of frozen food product quality.

The ability of biosynthesising these proteins is characteristic to ***freshwater*** species and they are trying to transfer the genes responsible for this synthesis in species widely spread that do not have normally this ability. Genetic manipulation are naturally still under intense study through the prism of side effects that are potentially hazardous and possibly responsible.. There has been an increase of the trend to accept complex food (***"formulated foods")***. From this point of view, accepting surimi-based products has known a spectacular evolution that practically opens the way to accepting other protein derivatives (***glycoproteins***).

Methodically, the attempt to use as many as possible water resources and, therefore, supply consumers with a high amount of protein concentrates or hydrolytes from vertebrates respective invertebrates, both marine and terrestrial, have practically failed because they have tried to change feeding routines of the populations.

Though the population needs more proteins, experience confirmed that they need to be supplied to consumers within accepted ***organoleptic limits*** accepted by traditional feeding routines. Thus, at present, it is well known that if they aim at converting fish (in general and invertebrates) into sources of protein, the possibility that consumers accept it increases only if these proteins are used as ***ingredients with***

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functional value in feeding systems known to the consumers.

Functional protein preparations that will be produced, with real chances of tolerance, from under-used water species include, among others: ***surimi, glyco-proteins***, ***thermal stable proteins*** with ***low viscosity*** respective ***protein hydrolisates***.

2. Present state of knowledge

Literature points out the fact that in milk, saliva, tears, seminal fluids, *mucins*, and neutrophils there is a *glycoprotein* called ***lactoferrin (LF)*** with outstanding ***antimicrobial activities***, structural-functional competences since it can coordinate Fe^{3+} cations together with two HCO_3^- anions[4-7]. Physiological activity of LF depends on the conformation of the glycoprotein and on environmental parameters.

Its bacteriostatic effect can be optimised by attaching to the surfaces of microbe cells in tissues specific to both Gram positive and Gram negative bacteria, when starts to turn "***the cell membrane porous"*** [8-20]. Isolating ***LF*** represents a chain of very sensitive operations since it affects the conformation of the glycoprotein [***pH***; Ca^{2+}, PO_4^{3-} and Fe^{3+} ion excess; mass ratio of the buffered citrate/bicarbonate medium; etc.].

Activated lactoferrin (***ALF***) is processed by immobilising ***LF*** on carrageenan (***E-407***) solubilised in a strictly citrate/bicarbonate buffered solution containing ***NaCl***. ***ALF*** as an antiseptic favours the blocking of the existing microbiota attachment to biosurfaces; its detachment; inhibition of microbiota proliferation by coordinating Fe^{3+} necessary to bioenergetic processes in the microbial cell. ***ALF*** also has ***antiviral activity***. Acknowledged by the ***USA legislation*** as an ***antiseptic*** (***1%*** solution of ***ALF*** in deionised water) for bovine and poultry carcasses, it is of interest in accessing (incorporating) in mass food and packaging materials (including membranes for meat products) in the food product mass. They accepted initially that ***glycosilated proteins*** are restricted exclusively to eukaryotes, but later they also noted them in prokaryotes [21-24]. The carbohydrate content of glycoproteins is variable from less than ***1%*** (collagens), to over ***99%*** (glycogen). Acting on the cell, cytoplasm, organitelor subcellular, cellular and extracellular fluids. Blood serum is a rich source of glycoproteins (over ***100*** proteins, most glycosylated).

Serum albumin is one of the rare exceptions in this respect although a genetic variant which is glycosylated has been discovered [25].

A striking feature of almost all glycoproteins is the ***polymorphism associated with glycans*** (microheterogeneity). This type of diversity derives from the role of individual molecules of a given glycoprotein glicanilor various oligosaccharides in relation to the same protein. In ***1962*** were first observed ***α1-acid glycoprotein*** from human serum by electrophoresis [26]. The two major forms of protein glycosylation found in eukaryotes are ***N***-linked and ***O***-linked, designated as such by virtue of their glycosidic linkage to the corresponding amino acid. The most common mucin involves the grafting of an ***N-acetylgalactosamine*** residue (***GalNAc***) to the hydroxyl group of either serine or threonine to form the ***Tn antigen (Tn Ag)*** [27,28].

2.1. Structure

Glycoproteins[1] ***(GP),*** physiologically active biomacromolecular structures, widespread in the animal world, are ***heteroproteins (proteins conjugated)*** structured from a carbohydrate (*poly-saccharide* with fragments of ***N-acetyl hexosamine***, different ***monosaccharides***, and ***uronic acids***), is called ***mucopoly-saccharide*** (***immunopoly-saccharide***) as ***prostetic group*** and ***proteins proper***, predominantly quantitatively (***figure 3***).

They can also be considered glycolised proteins if we accept that protein proper (non-glucidic component) is ***aglycone*** (***figure 9***). Depending on the nature of the linking, in most glycoproteins, there are three glycosidic bridges: (**O**) – glycosides, (**C**) – glycosides and (**N**) glycosides, respectively, the most used being presented in ***figure 4***.

They contain short carbohydrate chains covalently linked to protein, by three types of links, ***N-glycosidically*** via the amide side chain of asparagines, ***O-glycosidically*** via the hydroxyl

[1]IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), *Pure* & *Appl. Chem.*, **1988** Vol. 60, No. 9, pp. 1389-1394.

groups of serine and threonine and the ***C-glycosidic*** attachment to tryptophane. Less common are *O*-glycosidic linkages to hydroxylysine [29], hydroxyproline [30] and tyrosine [31], and the ***C-glycosidic*** attachment to tryptophane [32]. In structure of glycoproteins these oligosaccharide chains are often branched, and they do not contain repeating disaccharides (***figure 1***). They are abundant in secretions of mucus, produced by some cells ***(salivary mucins)*** (***figure 2***).

Tabelul 1. Structural and functional classification of glycoproteins

Nr.	Structural classification	Skills classification	Sources
1	Glycoproteins themselves (under *4%* polysaccharide)	pH	Acidic (plant)
			Neutral (blood plasma, urine, gastrointestinal tract)
		Function	Plastic
			Enzymatic
			Hormonal
			Transfer phase
			Immunology
		Carbohydrates content	"High – mannose"
			"Hybrid"
			"Complex"
2	Mucins over *4%* polysaccharide	Soluble	Blood group A
			Saliva
			Egg α ovomucoid
			Anthrax bacillus
			Gonadotrope hormone
		Insoluble	Egg (β ovomucoid)
		Acidic	Submaxillary secretion

They contain short carbohydrate chains covalently linked to protein, by three types of links, ***N-glycosidically*** via the amide side chain of asparagines, ***O-glycosidically*** via the hydroxyl groups of serine and threonine and the ***C-glycosidic*** attachment to tryptophane. Less common are *O*-glycosidic linkages to hydroxylysine [29], hydroxyproline [30] and tyrosine [31], and the ***C-glycosidic*** attachment to tryptophane [32].

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2.2. *Glycoproteins classification*

Table 1 is the classification, major structural and functional glycoproteins and in ***Table 2*** monosaccharides commonly found in the structure. **2.3 Biosynthesis**

Involves simultaneous grafting of sugars to proteins through a complex process involving two cell organites (***ER endoplasmatic reticulum*** and the ***Golgi apparatus***). Grafting functional groups on proteins is indicated by both co- and post-translation. ***RNA*** codifies protein sequences that penetrate the cytoplasm where they link to ribosomes making up ***RNA-protein complexes*** that represent the basis of protein biosynthesis.

Cytoplasmatic proteins are synthesised without ribosomes, but to form glycoproteins ribosomes are linked to the endoplasmatic reticulum (a long-shaped, expanded ***ER*** organite compared to the other cell organites).

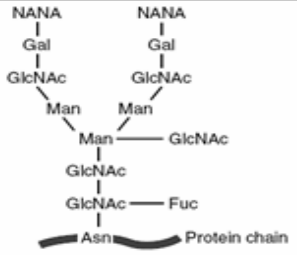
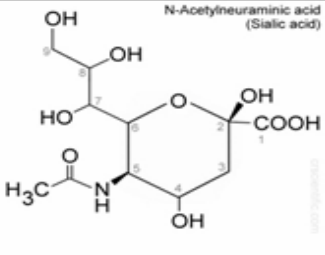


Figure 1. Branched glycoprotein. *NANA*=*N*-acetyl-neuraminic acid; ***Gal***=galactose; ***GlcNAc*** = *N*-acetylglucosamine; ***Man*** = mannose; **Fuc** = fucose.



Sialic acid
(N-acetylneuraminic acid)

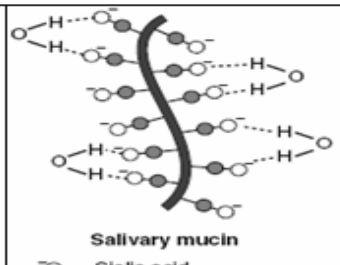


Figure 2. Structure of salivary mucin.

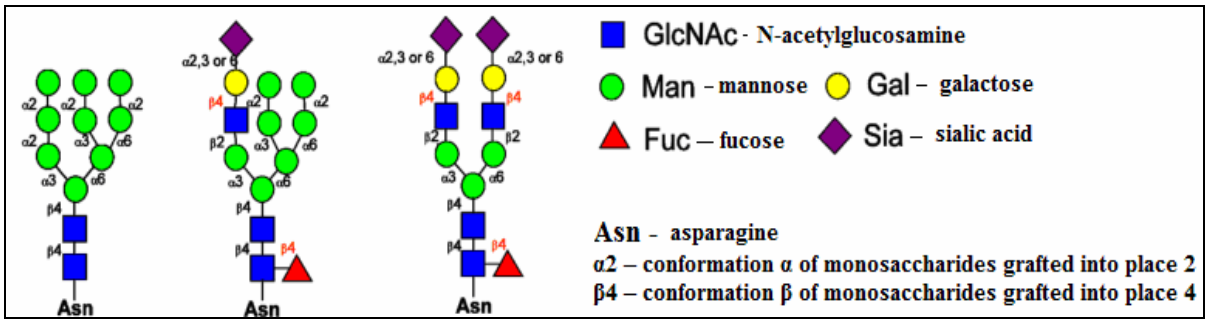
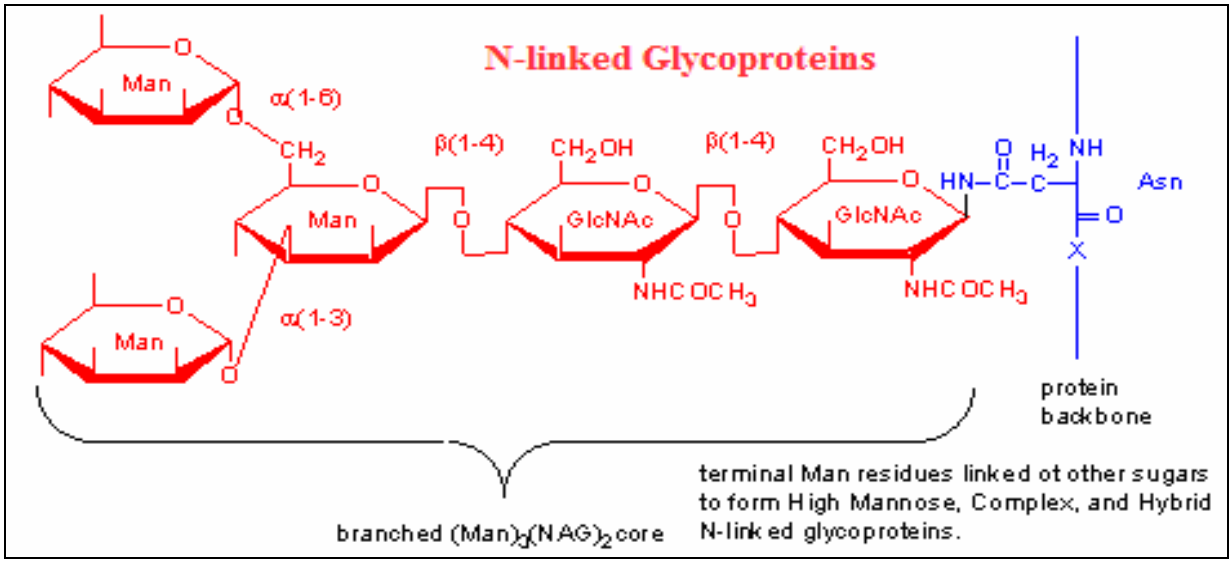


Figura 3. Schematic representation of the architecture of glycoproteins and other structural units [68]



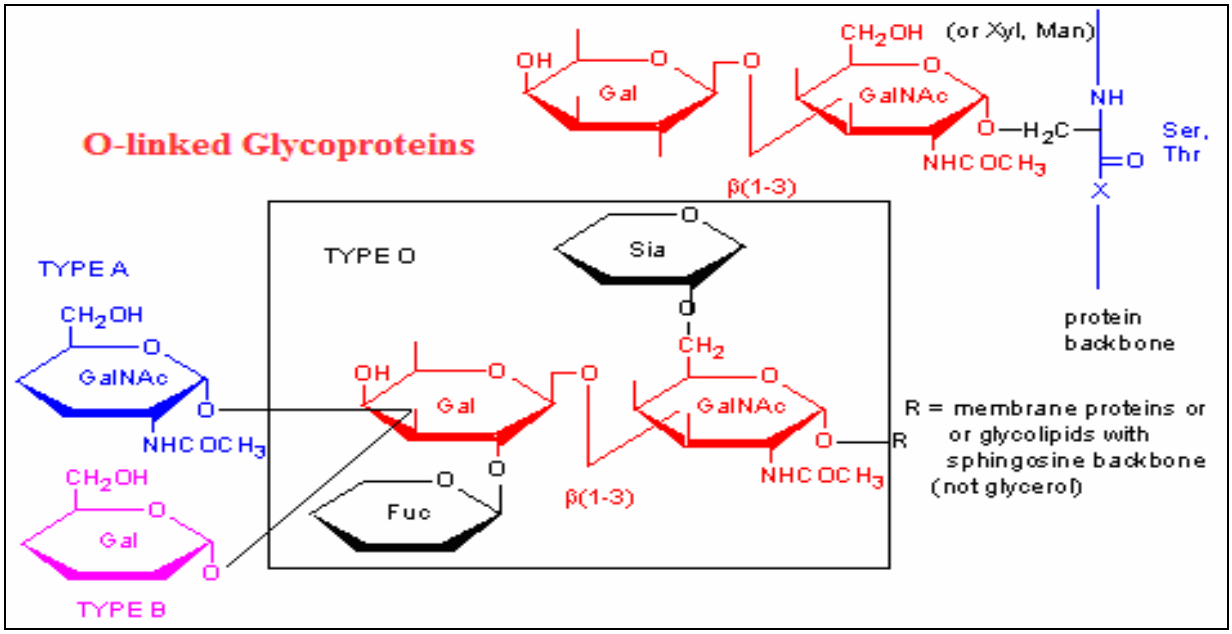
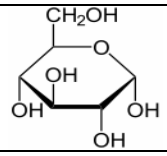
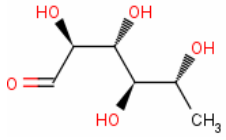
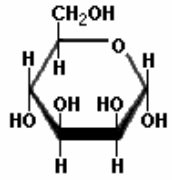
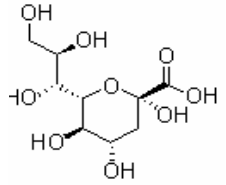
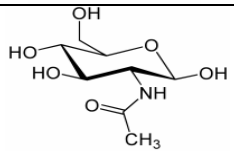
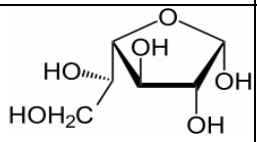
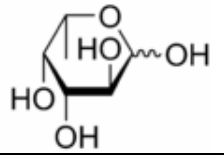
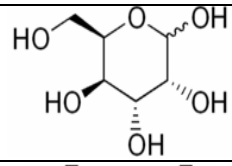
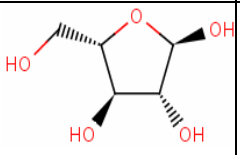
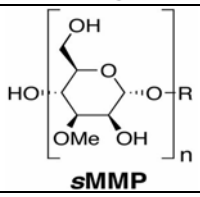
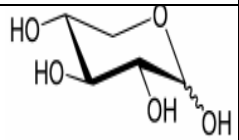
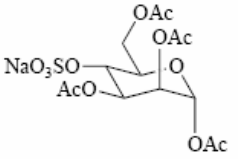
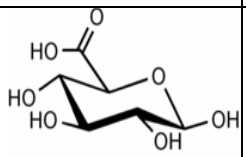
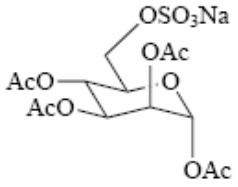
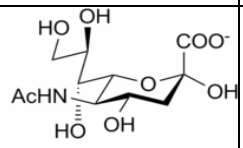
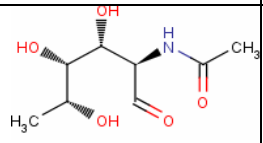


Figura 4. Structure of *N*, ie ***O*** - glycoproteins

Tabelul 2. Representative monozaharide of glycoproteins composition [33-37]

Mono-saccharide „Classical”	**Structures**	**Sources**	**Mono-saccharide „rare”**	**Structures**	**Sources**
Glucose ***Glc***		collagens	6-Deoxy-altrose ***dAlt***		Salmonid fish eggs
Mannose ***Man***		Sweet fruits	3-Deoxy-D-*glycero*-*galacto*-nonulosonic acid ***Kdn***		Salmonid fish eggs
N-Acetyl-glucosamine ***GlcNAc***		Liver epithelial cells and sebaceous glands	Galacto-furanose ***Galf***		Bacteria, trypano-soma, fungi
L- Fucose ***Fuc***		Algae, insects, and on the surface of plants	Gulose ***Gul***		Thyroglobu-lin, mucins in cystic fibrosis
L-Arabino-furanose ***Araf***		Glycolproteins from plants	3-*O*-Methyl-mannose ***Man3Me***		Snail
Xylose ***Xyl***		In proteo-glycans and plant Glycol-proteins	Mannose-4-sulfate ***Man4S***		Ovalbumin
Glucuronic acid ***GlcA***		In proteo-glycans	Mannose-6-sulfate ***Man6S***		Ovalbumin, slime mold
N-Acetyl-neuraminic acid ***NANA***		Mainly in higher verte-brates and inverte-brates	*N*-Acetyl-fucosamine ***FucNAc***		Pili[2] of *Pseudo-monas aeruginosa*

[2] is a hairlike appendage found on the surface of many bacteria. The terms ***pilus*** and ***fimbria*** are often used interchangeably, although some researchers reserve the term ***pilus*** for the sexual appendage required for bacterial conjugation. All pili are primarily composed of ***oligomeric pilin*** proteins.

The new protein chain is transferred to the endoplasmatic reticulum lumen where the basic nucleus of an oligosaccharide is linked to a protein. Attachment and exclusion (***adjusting and processing***) monosaccharides is achieved in the lumen, to a final sugar structural architecture to which a fragment of manose from the structure is attached. This bioprocess is schematically represented in ***figure 5a*** (in which the two pink lobes represent the ribosome structure that attaches to the ***ER*** membrane. The biosynthesised protein is present in the lumen on both sides of the membrane. The ***ER*** lumen also confirms the presence of hydrophilous sugars that favour protein folding [38].

Additional carbohydrate modifications (post-translational) are made as the protein moves from the lumen of the ***ER*** (***probably by a budding process***) to ***Golgi apparatus*** ***(figure 5b)***. Here terminal carbohydrate modification is completed. The ***Golgi*** does not contain molecular chaperons since protein folding is complete when the proteins arrive. Rather they have high concentrations of membrane bound enzymes (***glycosidases, and glycosyltransferases***). The glycan moieties of the folding glycoprotein also lead to binding of the protein to ***lectins***[3] in the ***ER*** (***molecular chaperones***). The most studied of these chaperones are involved in the ***calnexin-calreticulin cycle*** [39], and facilitate correct disulfide bond formation in the protein. After two glucose residues are removed by glucosidase ***I*** and ***II,*** the monoglucosylated protein binds to ***calnexin (CNX)*** and/or ***calreticulin (CRT)***, two homologous ***ER*** lectins specific for monoglucosylated proteins. If a glycoprotein has not been completely folded (formed), this situation is immediately perceived by a glycotransferase which attaches a sugar and later on re-enter the ***calnexin/calreticulin cycle***. In theory, incompletely formed proteins should be removed from the cells. ***ER*** has developed as to be able to remove these imperfect situations because ***ER*** contains protein ***"accompanying" compounds*** and **folding catalyst**.

Stress (thermal shock) enhances their activity. As final defence mechanisms, incomplete proteins are degraded by the cytoplasmatic proteasic complex. Certain forms can avoid degradation by accumulating and can generate ***Alzheimer's*** and ***Parkinson*** diseases [40].

2.4. Functions

Since sugars and proteins as such are involved in very many biological functions, their association in biomolecules form ***"structural architectures"*** with different biological functions. Thus, the active physiological importance of the glycoproteins can be grouped into:

* ***structural*** when glycoproteins are found throughout matrices. They act as receptors on cell surfaces that bring other cells and proteins (collagen) together giving strength and support to a matrix [41]. ***Glycoproteins and proteoglycan*** molecules are involved into the structure of cartilage. In nerve tissue glycoproteins appear to be associated with synaptosomes, axons, and microsomes. In plants, glycoproteins have roles in cell wall formation, tissue differentiation and embryogenesis [46]. Glycoproteins of conjunctive tissue include: ***hyaluronic acid, chondroitin sulphate and keratan sulphate (figure 10)*** [47-50]***;***

****protection-***High molecular weight polymers (***mucins***) are found on internal epithelial surfaces. They form a highly viscous gel that protects epithelium against pathogenic factors (*chemical, physical, and microbial*). Examples of mucin sites are the human *digestive tract, urinary tract, and respiratory tracts.* Recently it was discovered that mucins may be responsible for aiding in metastasis of transformed cancer cells. Lacrimal glands produce a glycoprotein which protects the corneal epithelium from desiccation and foreign particles. Mucins are also found on the outer body surfaces of fish to protect and lubrication the skin [51].

****mutual linking*** of the cells and of the latter with substrata is a bioprocess that is the basis of tissue forming in the body. ***N-CAM glycoprotein*** plays a role in the attachment and recovery of the nervous cells and is also identified in muscular cells where they from myoneural junctions;

****hormonal*** **(**the ***HCG glycoprotein – gonadotropin crorionic***, present in human pregnancy and erythropoietin, regulate erythrocyte genesis);

[3] Lectin - a class of proteins or glycoproteins with agglutinating properties compared to other cells and that can graft reversibly sugars without changing their structure.

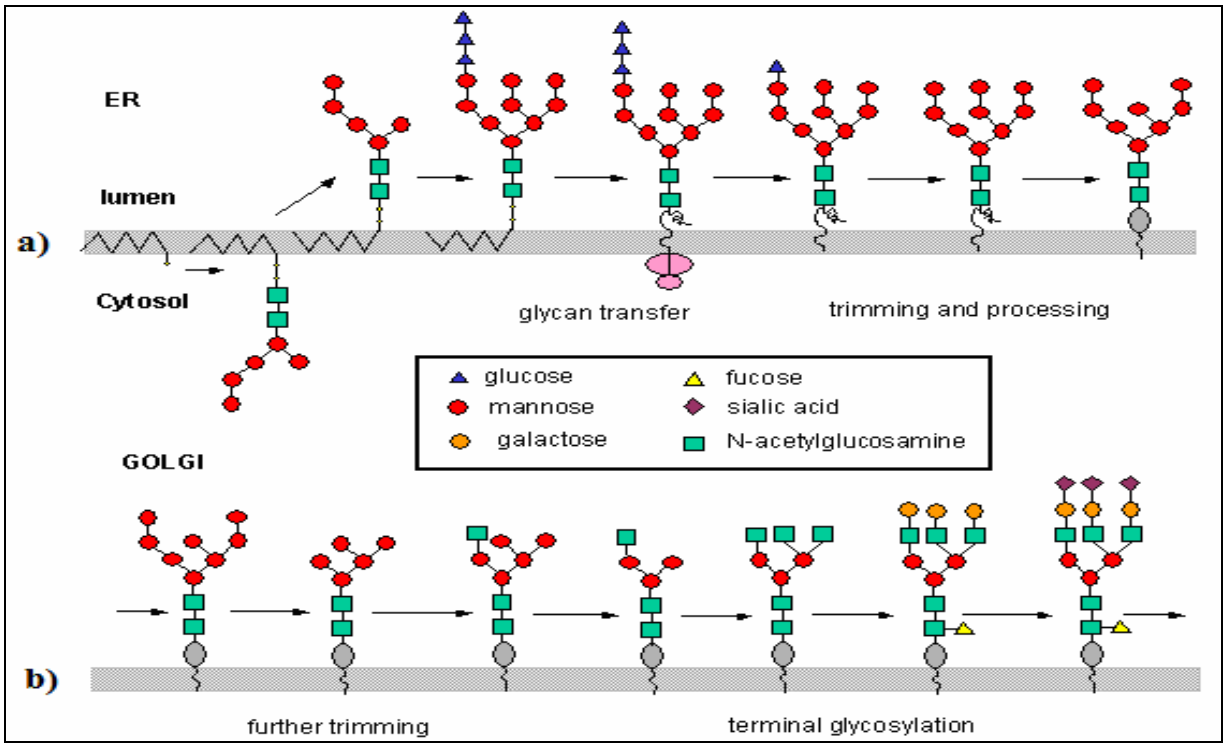


Figure 5. The general scheme of biosynthesis of glycoproteins: a) endoplasmic reticulum (***ER***); b) ***Golgi apparatus*** [38, 39, 40]

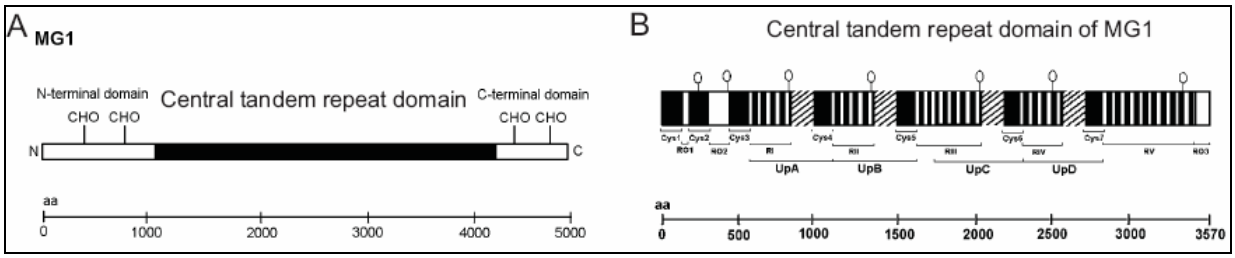


Figure 6. Structure of human sublingual oligomeric mucin ***MG1***, ***A*** – Schematic representation; ***B*** – Structure of the central domain of MG1: Cys (1–7), cysteine-rich subdomains; R01, R02, R03, subdomains with no repeats; UpA, UpB, UpC and UpD, super repeats of 528 amino acids [42, 43].

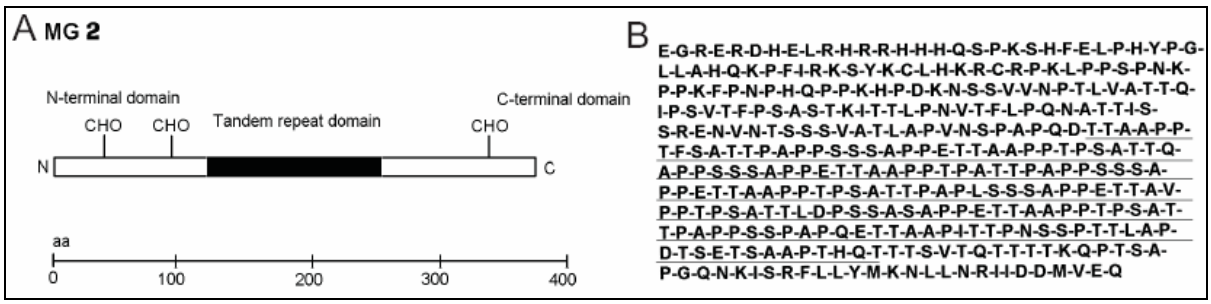


Figure 7. Structure of human salivary monomeric mucin *MG2*. ***A*** – Schematic representation of mucin [42, 43]. ***B*** – Amino-acid sequence of human monomeric mucin MG2 (the six tandem repeat sequences are underlined) [44, 45].

enzymatic **(**glycoproteins playing an enzymatic role ensure three types of action mechanisms: ***oxidoreductase, transferase and hydrolase***);

transfer **[**the conformation of a glycoprotein allows the forming of ***sites (cavities)*** with defined geometrical coordinates capable of ***sequestrating reversibly*** structural units with similar size (vitamins, hormones, organic and/or inorganic cations, etc.) to transfer them intra- or inter-phasically to different biological systems where they release them and comes back to the initial form to start another transfer cycle];

inhibitors [many glycoproteins secreted by blood plasma, protects organs against bacteria and fungi, ***α1 glycoproteins – antichymotripsin,*** that inhibit chymotrypsin];

antimicrobial (antifungal) [Glyco-proteins secreted by different glands protect the organs from bacteria and fungi];

cryoscopic **[*lowering the melting point (and the interval) of fluid (liquid) biological systems.*** Have been identified in fish glycoproteins playing the role of body protectors from the very low temperature of the aquatic environment];

immunological [***The interaction of blood group substances with antibodies is determined by the glycoproteins on erythrocytes***. Adding or removing just one monosaccharide from a blood group structure, the antigenicity and therefore a person's blood type can be altered. Many ***immunoglobulins*** are actually glyco-proteins. ***B*** and ***T*** cells contain surface glycoproteins that attract bacteria to these sites and bind them or can direct phagocytosis].

2.5. Mucins

The apical epithelial surfaces of ammalian respiratory, gastrointestinal, and reproductive tracts are coated by ***mucus, a mixture of water, ions, glycoproteins, proteins, and lipids.*** Mucus provides a protective barrier against pathogens and toxins and contributes to the innate defensive system in mucosal immunology [52]. ***Mucin glycoproteins*** are the major macromolecular constituents of epithelial mucus and have long been implicated in health and disease. Mucins historically are large, highly glycosylated, viscoelastic macromolecules that are difficult to isolate and purify [53]. In recent years increasing attention has been given to a class of carbohydrate-protein complexes, largely because of the important biological activity. In many of these the carbohydrate moiety represents analytically a ***minor portion*** only, though, as in virus inhibitors ***(functional group) (figure 6 and 7)*** [54]. This subclass of complexes, in which protein predominates, make referred to mucoproteins.

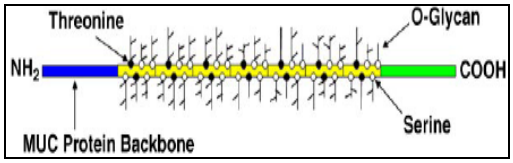


Figure 8. Schematic drawing of a secretory mucin glycoprotein depicting a ***MUC*** protein backbone and its ***O-glycans***

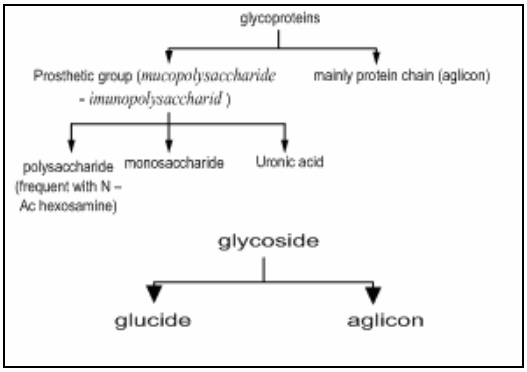
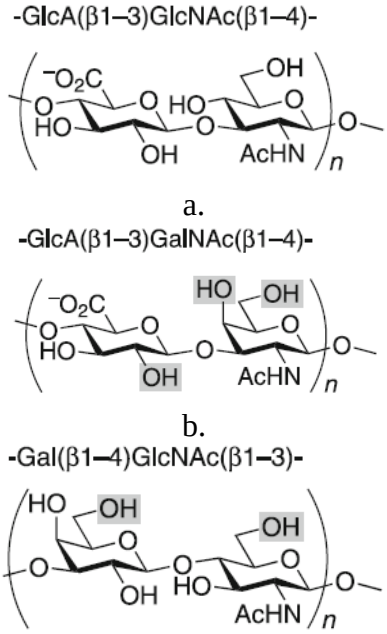


Figure 9. Schematic structure of the glycoprotein



a.

b.

c.

Figure 10. Structure: ***hyaluronic acid (a), chondroitin sulphate (b) and keratan sulphate (c)***

In any quantitative analysis of the carbohydrate moiety of mucoproteins three major difficulties arise:
- the carbohydrate is a mixed polysaccharide;
- pure samples of the material to be analysed are usually available in small amounts only;
- the amino acids liberated on hydrolysis of the mucoprotein are known to interact with sugars, in as far as mucins are heavily ***O-glycosylated*** proteins.

The number of ways in which proteins are modified by carbohydrates has increased, as a result of detailed glycobiological investigations, ***O-linked glycosylation*** has come to mean many things [55], but in the context of mucins it is specifically characterized by an ***α-O-glycosidic linkage*** between ***N-acetylgalactosamine*** and the ***hydroxyl groups of threonine*** and ***serine*** (***figure 8***) [56]. Mucins, for which ***20*** genes have been identified thus far [57], are often divided into two major classes: membrane-associated (***MUC1, MUC3, MUC4, MUC12***) by virtue of a hydrophobic ***C-terminal*** transmembrane domain, and constitutively secreted (***MUC2***), by specialized cells. Mucins found in many mammals, and because of important physiological roles, attempted their isolation and purification from several sources, for example ***snails, jellyfish, the mandibular glands of different mammals, eggs, milk,*** etc.

2.5.1. Block operating systems

Was reported reported these years, in the ***Japanese sea waters***, the excessive presence of some giant jellyfish species (***Nemopilema nomurai***) measuring ***2 m*** in diameter and weighing up to ***200 kg***.
Besides the fact that they have become a serious ecological issue (uncontrolled deaths) they have also resulted in tourism, fish farming discomfort, etc. Though ecologist and professional organisations have suggested that these jellyfish are material carriers of food utilities (***fortifying foods***), they have not developed yet large scale industry for the isolation, purification, and characterisation of the ***jellyfish mucins*****,** except for the „***Qniumucin***" [58] variant (***figure 12***).

Literature [59] reports the possibility of isolating, purifying, and characterising two categories of glycoproteins (***mucoproteins***) from the snail species (***Helix pomatia)*** (***figure 13***).

The first structure was isolated from the mucous secreted by the snail foot and it can be characterised by a low share of the sugar fragment (mainly protein chain).

A second protein isolated from the same superficial biological area is characterised by an increased share of the structures with non-protein nitrogen (***6-8%***), called "***sinistrin***".

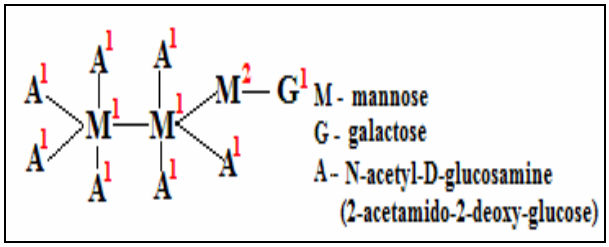


Figure 11. Ovomucoid structure

Mucins (ovomucoids) isolated, purified, and characterised from eggs [60] (***figure 14***) contain about ***25%*** sugars structured from ***D-manose*** (three), ***D-galactose*** (one), and ***2-acetamino-2-deoxi-D-glucose*** (seven) units. Ovalbumins contain ***5%*** sugars [61].

Ovalbumins are present mainly in egg white. To note the presence of a globulin and two proteids [***conalbumin (flavoprotein)*** and ***ovomucoid (mucoid) (figure 11)***] in the studied composition.

The mucoid is responsible for the egg white ***"capacity of threading"***.

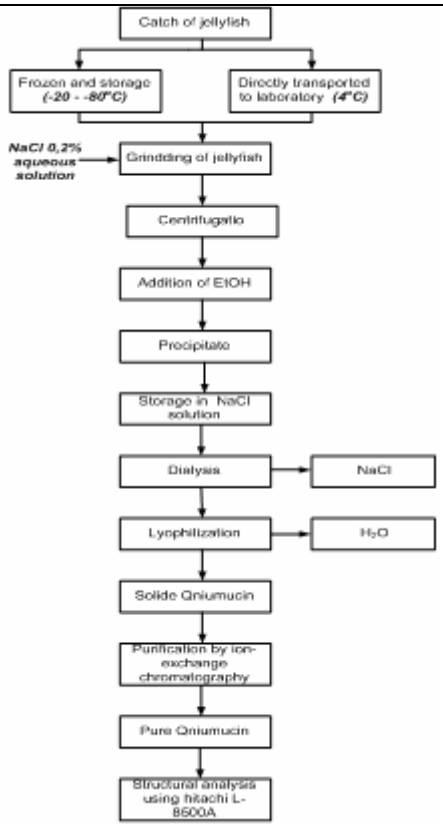


Figura 12. Block operating systems in the processing of the jellyfish mucin (***Nemopilema nomura***i)

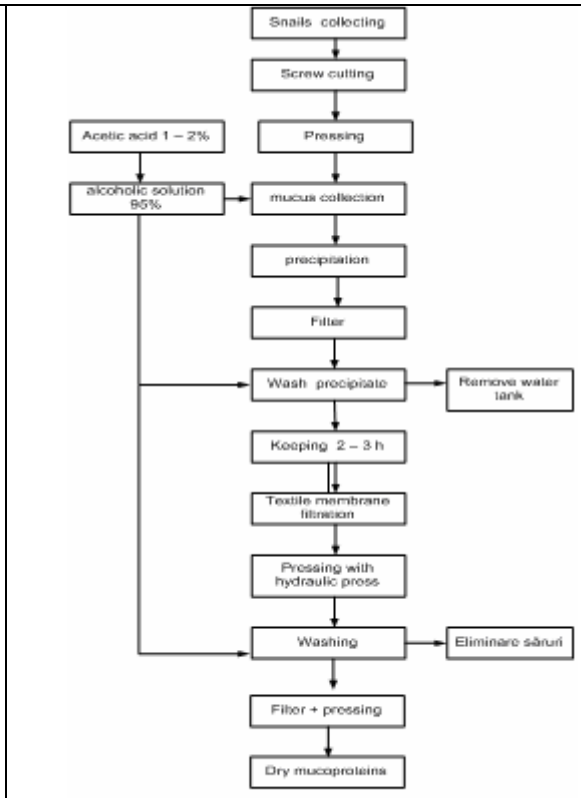


Figura 13. Block operating systems in the processing of the snails mucin (***Helix pomatia***)

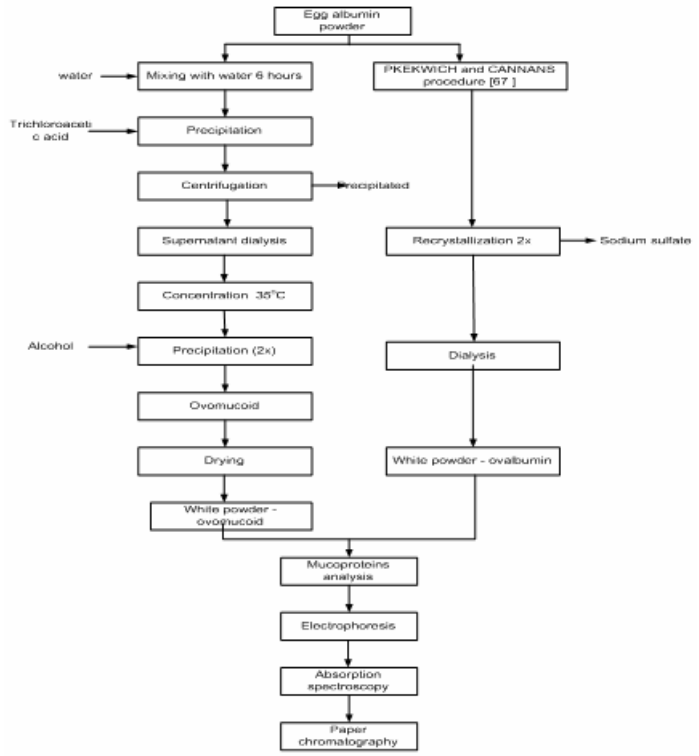


Figura 14 . Block operating systems in the processing of the egg mucoproteinelor

2.5.2. Applications

Snail bodies are covered by a considerable amount of mucus playing the main role of regulating dehydration, facilitating movement, and protecting against mechanical lesions. The idea of antibacterial effects of this secretion was advanced after they noted the low resistance to infections. Microbiological tests carried out with watery extract of mucous from ***giant African snails*** (***Achatina filica Fkrussac***) on Gram-positive and Gram-negative microorganisms have also confirmed these premises.

Mucus is the first barrier with which nutrients and enteric drugs must interact and diffuse through, in order to be absorbed and gain access to the circulatory system and their "***target end organs***". There is great interest in methods to optimize these socalled ***muco-adhesive*** interactions for improved drug delivery. Various molecular interactions have been exploited to ***enhance mucoadhesion***, including, polyelectrolytic interactions (***chitosans, poly-acrylic acid,*** etc.) ***hydrogen bonds (hydrogels)***, [62] and ***disulfide binding (thiomers)*** [63]. Efforts are underway to design pH sensitive drug carriers such as gels which will not release the drug in the acidic environment of the stomach but will do so in the basic environment of the intestine and colon. Mucin can be used as a high molecular weight additive to improve the adherence of artificial tear drops in ***treating dry eye syndrome*** [64]. Efforts to develop nanoparticles for mucosal DNA vaccines [65], artificial saliva and artificial stomach acid [66].

3. Conclusions

Antimicrobial natural glycoproteins are also, due to their diversity, biologically active competences, inter- and pluri-disciplinary application fields, of real interest for the science of food processing in the near future.

Acknowledgment

We wish to thank the ***Banat's University of Agriculture Sciences and Veterinary Medicine Timişoara, Faculty of Food Technology Products***, for a generous helpful of different kinds and for financial support by ***doctoral scholarship programme SOP HRD (Sectoral Opera-tional Programme Human Resources Development) by the Managing Authority of SOP HRD (MA SOP HRD).***

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