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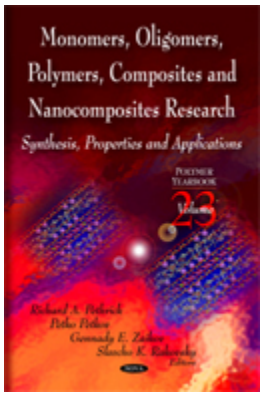

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Chapter 10

NEOGLYCOPROTEINS OF MANNAN: PREPARATION, CHARACTERIZATION, PROPERTIES, AND APPLICATIONS

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ABSTRACT

The present overview is summarizing the results of neoglycoproteins produced from mannan, isolated from *Saccharomyces cerevisiae*, and proteins as bovine serum albumin, human serum albumin and the enzyme penicillin G acylase. The reductive amination of oxidized mannan was used to obtain a set of neoglycoconjugates with different molar mass and content of saccharide. The influence of these characteristics on the specific interaction of mannan-proteins with lectin Concanavalin A and on the stability of mannan-enzyme was investigated. Since the mannan affinity to Concanavalin A was not significantly changed after mannan conjugation with proteins, the procedures applied are proper for the biospecific immobilization of mannan-protein conjugates. In comparison with unmodified penicillin G acylase, the stability of mannan-penicillin G acylase was higher at examined conditions (in acidic and basic conditions, at higher temperature as well as in the presence of organic solvents). The original antioxidative, antimutagenic, and immunogenic activities of *Saccharomyces cerevisiae* mannan were increased after its conjugation with proteins.

1. INTRODUCTION

Glycoproteins are common products of post-translation of proteins in all biological organisms. The presence of carbohydrate units in naturally occurring glycoproteins modifies their physicochemical and biological properties. The glycosylation of proteins plays a critical role on their expression and folding, increases their thermal and proteolytic stability and modulates their interaction with other biomolecules [1-3]. Carbohydrate components contained in natural glycoproteins usually have complex and non-homogeneous structures. The modification of proteins with saccharides in order to obtain new functional glycoconjugates named "neoglycoproteins" has been known for about three decades. Neoglycoproteins are compounds mimicking natural glycoproteins. The advantage of neoglyconjugates prepared from proteins is that they contain carbohydrates of known structures and assured purity. The modification of proteins with saccharides has several advantages, such as better protein solubility and availability of different types of reactive side chains (amino-, carboxyl-, phenol-, and thiol-). The attachment of saccharides to proteins may change their stability and improve their emulsifying and antimicrobial properties [4]. Neoglycoproteins can be also prepared from bioactive proteins as enzymes, immunoglobulins, or growth factors. The chemical modification with water-soluble polysaccharides is a proper strategy for enzyme stabilization [5-7]. Modified enzymes, i.e., neoglycoenzymes, are powerful tools in cytochemistry and in biochemical detection of lectins in solid-phase assays [8]. Neoglycoproteins containing saccharides with specific affinity to endogenous membrane lectins can be utilized as carriers for selective delivery of drugs to cell membranes [9]. The conjugation of polysaccharides with immunological, antioxidative, and antimutagenic activity to a protein carrier can extend these activities [10-12]. In natural glycoproteins, the oligosaccharides, are attached to proteins mainly via *N*-glycosidic bonds to asparagine or via *O*-glycosidic ones to hydroxylated amino acids, such as serine and threonine [13]. The most frequently used functional group for the modification of proteins is the amino group of lysine. Primary amino groups of proteins can be conjugated with saccharides by reductive amination, amidination, or acylation. Reductive amination of carbonyl groups of saccharides or polysaccharides with proteins is a simple reaction for the preparation of neoglycoproteins. A new carbonyl group can be created by oxidizing the reducing end of oligo- or polysaccharides with mild oxidizing agents, as e.g., periodate. By this way, the newly generated aldehydic groups can then react with amino groups of the protein by using a suitable water-soluble carbodiimide or other coupling agents [4].

The goal of this overview is to summarize the optimization of preparation of mannan dialdehydes and their conjugation with proteins – bovine serum albumin (BSA) and penicillin G acylase (PGA). The properties of the obtained neoglycoproteins were studied from the point of view of interaction with Concanavalin A (Con A) lectin, the stability of the formed mannan-PGA and of its application in biotechnology. The immunogenicity, antioxidative, and antimutagenic activity of HSA- and PGA-mannans were also investigated.

2. Preparation of Mannan Dialdehydes and their Conjugation with Proteins

2.1. Mannan Oxidation

The mild periodate oxidation of *Saccharomyces cerevisiae* mannan was used to prepare dialdehydes suitable for synthesis of a new type of neoglycoproteins [14]. The oxidation reaction results in cleavage of the diol bonds and the production of reactive aldehyde groups. According to the amount of 50 mM sodium periodate solution used, oxidized mannans with various aldehyde group content and molar mass (M) were obtained (Table 1). The higher concentrations of periodate (100 mM and 150 mM) caused an extensive degradation of polysaccharide chains. The mean molar masses of the original as well as oxidized mannans (after their reduction with $NaBH_4$) were determined by the HPSEC method using HEMA BIO 100 column. The contents of aldehyde groups in samples were determined by the Park-Johnson method [15]. The modified mannans with sufficient content of reactive aldehydes were obtained at the molar ratios $n(NaIO_4)/n$(mannose) = 0.135–0.270. A relatively slight decrease of the polysaccharide M value (about 10 %), modified in such a way, was observed. The content of carbonyl groups in mannan was significantly reduced by using a higher molar excess of periodate. This effect is connected with a more extensive degradation of molar mass of the sample as well as with a lower solubility of the oxidized mannans prepared (cf. Table 1; ManO4 and ManO5) (14).

The aim of preparation of mannan dialdehydes is to obtain reactive polysaccharides available for synthesis of a new type of glycoproteins with an affinity to lectin Concanavalin A. The influence of structure changes (caused by periodate oxidation) of modified polysaccharides on their interaction with Con A was investigated by precipitation experiment [14]. Oxidized samples were reduced with $NaBH_4$ before precipitation to eliminate the reaction between aldehyde groups of polysaccharides and amino groups of Con A.

Table 1. Products of periodate oxidation of mannan

Sample	$n(NaIO)_4/n$(monosacch.)	Content of aldehyde groups n(aldehyde)/n(polysacch.)	M (Da)
Mannan	–	1	55 000
ManO1	0.054	11	54 500
ManO2	0.135	20	51 200
ManO3	0.270	28	49 500
ManO4	0.540	22*	46 100
ManO5	0.710	13*	39 600

* Determined in the sample soluble part.

2.2. Conjugation of Oxidized Mannans with BSA

The oxidized mannans with the best affinity to Con A [14] were used to prepare BSA conjugates by reductive amination; various mannan/BSA ratio (4:1, 1:1, 2:3, and 2:5) were applied. The reaction was performed in the presence of $NaCNBH_3$ at pH 7 during 24 hours at room temperature. The remaining free aldehyde groups were reduced at pH 9.5 with $NaBH_4$, the product was adjusted to neutral pH and dialyzed against H_2O to remove un-reacted mannans [16,17] The M_w and M_w/M_n values of the neoglyconjugates with various content of mannan, were characterized (cf. Table 2. A similar procedure was used to prepare mannan-neoglycoproteins from PGA (cf. Table 3), but in this case, the conjugation reaction was performed at 4 °C [18-20].

3. Characterization of Neoglycoproteins Obtained

The prepared lyophilizates of mannan-BSA and mannan-PGA neoglycoconjugates were characterized to determine the M values as well as the content of saccharides [21] and proteins [22]. The molar mass parameters of the prepared neoglycoconjugates were estimated by HPSEC on the tandem columns HEMA BIO 1000 + HEMA BIO 100 (using UV detection and pullulan and dextran calibrants) [16,18] as well as on the Shodex OH Pak column using a LS detector [17,23]. The characteristics of neoglycoconjugates prepared from three mild oxidized mannans at the mannan/protein weight ratio = 1:1 are summarized in Tables 2 and 3. Data of the original mannan, BSA and PGA are also presented there.

Two activated mannans with a low content of dialdehyde groups ManO1 and ManO2 were used to prepare four different conjugates with PGA applying various mannan/PGA weight ratios. Two distinct fractions were detected in all conjugates prepared using SEC fractionation on Sephacryl S-300 HR column. Every neoglycoconjugate contained a minor amount of the high-molar-mass fraction (fr.1) with apparent M above 10^3 kDa (characterized by HPSEC chromatography using HEMA-BIO 1000 column) and a greater amount of the low-molar-mass fraction (fr.2) with M within the range of 286-395 kDa determined by SEC-MALS-SCV system [23].

Table 2. Molecular characteristics of mannan-BSA conjugates

Sample	Content of sacch. [%]	M_w [kDa]	Polydispersity M_w/M_n
BSA	0	66.7	-
Mannan	100	53.0	1.3
ManO1-BSA	25	176.2	2.8
ManO2-BSA	28	246.3	3.2
ManO3-BSA	30	169.1	3.6

Table 3. Molecular characteristics of mannan-PGA conjugates

Sample	Content of sacch. [%]	M_w [kDa]
PGA	0	80.0
Mannan	100	55.0
ManO1-PGA	22	260.0
ManO2-PGA	41	310.0
ManO3-PGA	40	240.0

Table 4. Characteristics of mannan-PGA neoglycoproteins

Sample	Conjugation ratio ManO/PGA(w/w)	Proportion of fractions (%, w/w)	Saccharide content (%, w/w)	M_w (kDa)	M_w/M_n
PGA	–	–	–	78.4	–
ManO1-PGAa-fr.1	1:1	28	51±3	$>10^3$	–
ManO1-PGAa-fr.2	1:1	72	47±5	332	2.1
ManO1-PGAb-fr.1	1:3	31	53±4	$>10^3$	–
ManO1-PGAb-fr.2	1:3	69	42±4	283	1.5
ManO2-PGAa-fr.1	3:1	46	67±3	$>10^3$	–
ManO2-PGAa-fr.2	3:1	54	61±5	395	1.8
ManO2-PGAb-fr.1	1:3	38	47±3	$>10^3$	–
ManO2-PGAb-fr.2	1:3	62	43±6	286	1.8

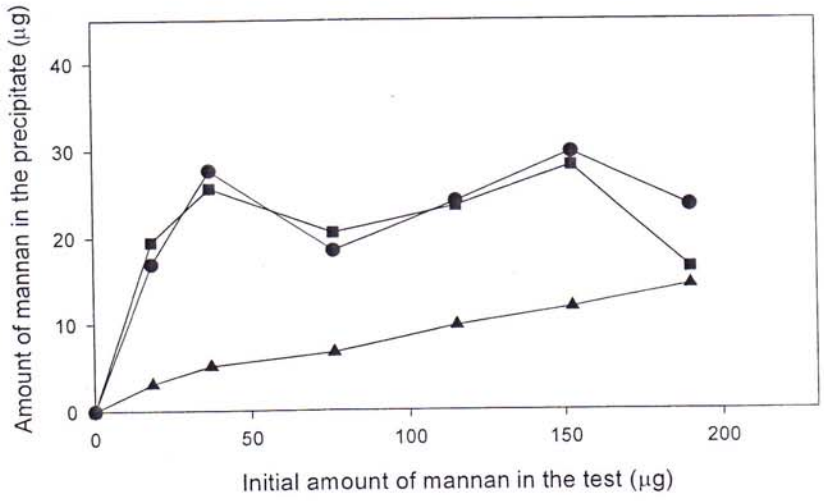

Figure 1. Precipitation of mannans with Con A. -●- original mannan, -■- ManO2, -▲- ManO5 (Ref. 14).

Data in Table 4 show that the amount of the high-molar-mass fractions is dependent upon the degree of mannan oxidation as well as on the conjugation ratio. All high-molar-mass fractions contained a higher saccharide content than the fractions with low-molar-mass prepared from the same sample of oxidized mannan. The mannan content in preparations is dependent on the mannan/PGA conjugation ratio; this is more noticeable in the case of the ManO2 sample, where two significantly distinct ratios were used. The molar-mass distribution of the main fractions (fractions 2) was broad, with two or three shoulders [23]. The presence of the shoulders was more obvious in the samples prepared at higher mannan/PGA ratios (1:1 and 3:1) compared with those prepared at lower ratio of 1:3. The polydispersity index M_w/M_n of the fractions 2 ranged from 1.5 to 2.1, and their conformations estimated were very similar and relatively compact.

4. Interaction of Mannan-proteins with Concanavalin A

Mannan is a polysaccharide with a strong affinity to lectin Concanavalin A and, therefore, is important for glycoproteins synthesis for their prospective use in lectinology. It was found that this polymer carrying multiple specific mannosyl residues exhibiting several times higher affinity toward Con A than methyl α-D-mannopyranoside (α-MMP), where the later is considered to be the most interacting monosaccharide [24]. The mannan from *Saccharomyces cerevisiae* showed a good precipitation with Con A at pH 7 and in the presence of a low concentration of Ca^{2+} and Mn^{2+}. It is caused by highly branched polysaccharide with α-D-(1→2)-mannopyranosyl units on the terminal ends, highly specific for affinity to Con A. The conservation of mannan structure at mild oxidation condition was confirmed by interaction study with Con A (Fig. 1). Some small changes of amount of saccharides in the precipitates were observed in precipitation profiles of mild oxidized mannan ManO2 (Table 1). However, the precipitation profile of highly modified mannan ManO5 appears to be different from those observed with unmodified mannan [14].

The interaction of mannan-protein conjugates with Con A was studied using three different methods: *1)* quantitative precipitation; *2)* sorption method; and *3)* surface plasmon resonance (SPR). The experiments were performed at pH 7 (or 7.4 in SPR method) and in the presence of a low concentration of Ca^{2+} and Mn^{2+}:

1. The measured parameter in the quantitative precipitation method is the amount of precipitate formed from soluble neoglycoprotein and Con A at equilibrium. The biospecificity of this interaction was proved by inhibition with α-MMP.

The precipitation profiles of mannan-BSA conjugates (shown in Table 2) with Con A are presented in Fig. 2. The similar aggregation courses of original mannan and its conjugates with BSA were obtained [17]. By precipitation of mannan-BSA conjugates with Con A aggregates are created with higher content of mannan than in the case of original mannan. It might be caused by partial association of mannan-BSA.

The precipitation profiles of selected Man-PGA fractions 2 (see Table 4) with Con A are shown in Figure 3.

The courses of the precipitation of individual low-molar-mass fractions were similar exhibiting the maximum. The highest molar ratio Man-PGA/Con A (0.58) was determined at the apex for the sample ManO2-PGAa-fr.2 (two molecules Con A/one molecule of the conjugate in the aggregate). The continual decrease of enzyme activity dependent on the

amount of the conjugates in precipitation tests was found out. The retained activity of PGA in the precipitates was about 50% for the majority of low-molar-mass fractions at the precipitation maximum.

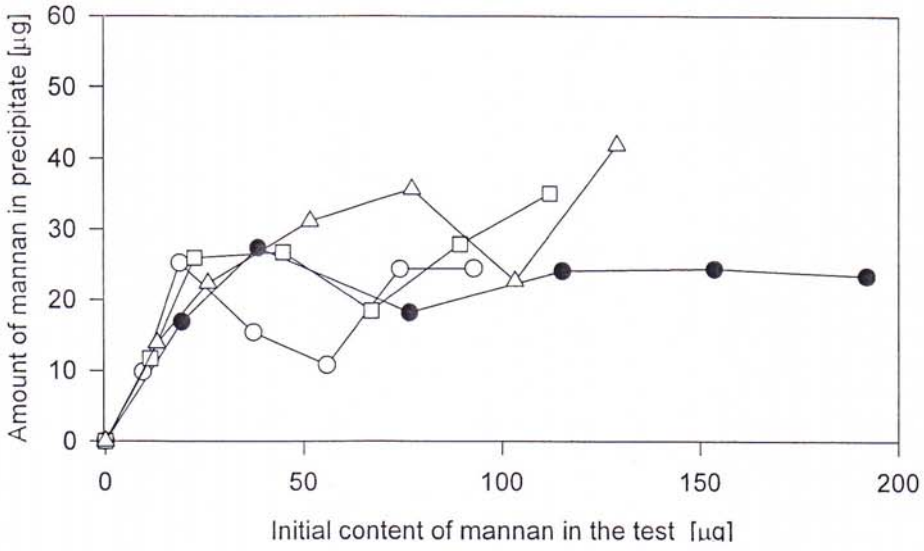

Figure 2. Precipitation of mannan and mannan-BSA conjugates with Con A. -●- original mannan, -○- ManO1-BSA, -□- ManO2-BSA, -△- ManO3-BSA (Ref. 17).

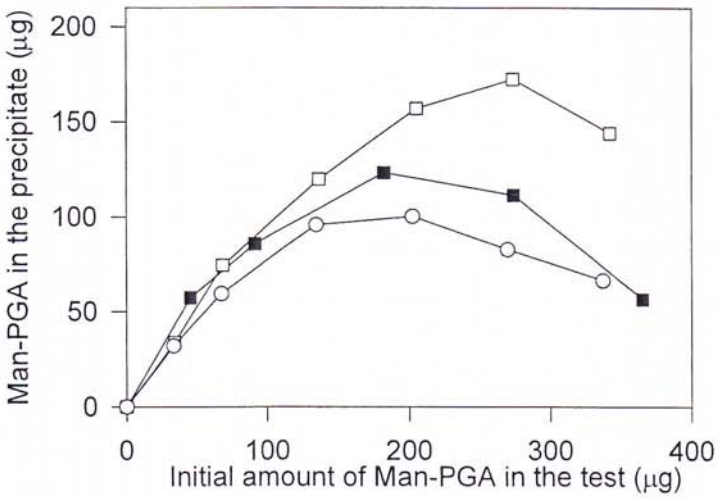

Figure 3. Precipitation of mannan-PGA conjugates (fr.2) with Con A. -○- ManO1-PGAb-fr.2, -■- ManO2-PGAa-fr.2, -□- ManO2-PGAb-fr.2 (Ref. 23).

Table 5. Interaction of mannan and its BSA conjugates with Con A determined as Langmuir parameters for adsorption on Con A-bead cellulose sorbent and as dissociation constants by SPR

Sorbate	Q_m (μmol sorbate/g wet sorbent)	K_D (M)	R[a]	K_D (SPR) (M)
Mannan	3.16×10^{-3}	5.2×10^{-8}	0.941	–
ManO1-BSA	8.68×10^{-3}	5.67×10^{-7}	0.962	5.32×10^{-7}
ManO2-BSA	3.44×10^{-3}	8.98×10^{-8}	0.945	3.25×10^{-7}
ManO3-BSA	5.71×10^{-3}	2.81×10^{-7}	0.721	5.02×10^{-7}

[a] Correlation coefficient.

The results obtained by sorption experiments confirmed that the affinity of mannan molecules in the conjugates with BSA to Con A was retained. ManO2-BSA conjugate showed the highest affinity to immobilized Con A among the three tested conjugates.

2. The sorption experiments were performed on Con A-bead cellulose MT-50 (Con A-TBC MT-50) sorbent with a small diameter of pores, to eliminate the sorption of the conjugates into the pores. Therefore, Langmuir isotherm curves and Scatchard plots for the sorption of the conjugates Man-BSA only on the surface of cellulose beads were performed. The values of K_D and Q_m estimated from Scatchard plot and expressed for the mannan-BSA conjugates are listed in Table 5 [17].

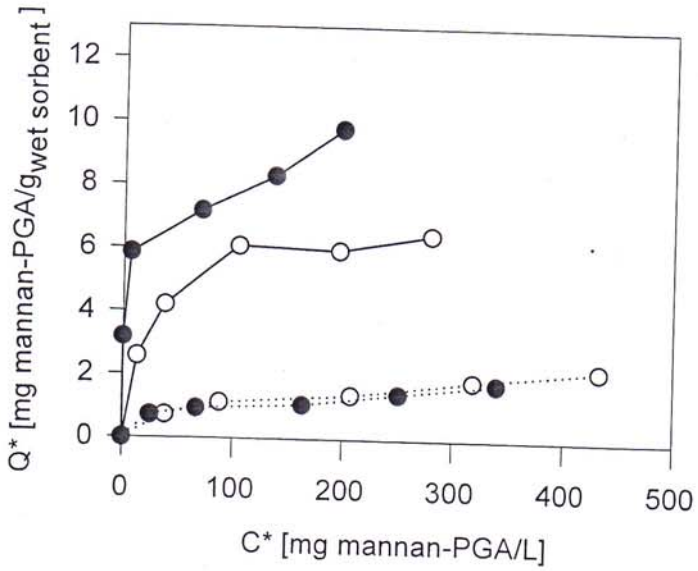

Figure 4. Affinity sorption of ManO1-PGAa-fr.1 (○) and ManO1-PGAa-fr.2 (●) on Con A-bead cellulose with different porosities. Dotted lines: BC MT 50, full lines: BC MT 100 (Ref. 23).

Table 6. Dissociation constants of Man-PGA conjugate interaction with Con A determined by SPR

Conjugate	K_D [M]
ManO1-PGAa-fr.2	6.07×10^{-7}
ManO1-PGAb-fr.2	4.09×10^{-7}
ManO2-PGAa-fr.2	3.64×10^{-7}
ManO2-PGAb-fr.2	3.94×10^{-7}

Two Con A-bead celluloses with different porosities (MT50 and MT100) with similar content of Con A (≈ 1 mg/1g wet BC) were used in the affinity sorption experiments of low- and high-molar-fractions of ManO1-PGA. The sorption curve patterns were practically the same for both fractions in the case of low-porosity bead cellulose (Fig. 4). The influence of molar mass was proved by the sorption on a higher porosity bead cellulose (MT-100). While the saturation of this sorbent with high-molar-mass fraction was reached at the concentration of 100 $mg.l^{-1}$, the sortion of low-molar-mass fraction was still increasing at 200 $mg.l^{-1}$.

3. Kinetic measurement of the interaction between the neoglycoconjugates and Con A was performed using a biosensor based on surface plasmon resonance. In our study, Con A was immobilized on the SPR chip surface, and the interaction of the neoglycoconjugates present in solution with the lectin bound on the chip was monitored. The calculated dissociation constants K_D of mannan-BSA conjugates with Con A (summarized in Table 5 – last column) are similar to those determined by isothermal adsorption.

The interaction between four low-molar-mass fractions of mannan-PGA neoglycoproteins and Con A determined by SPR method was calculated as K_D (shown in Table 6). The obtained K_D values are very similar, within the range of $(3.64–6.07)\times10^{-7}$ M, that is the scope of biospecific interaction. No influence either of saccharide content in Man-PGA conjugates or their molar mass on values of K_D was observed [23].

5. Stabilization of Penicillin G Acylase with Mannan

The modification of enzymes with polysaccharides is an effective strategy for their stabilization. The improvement of the stability is based on the formation of additional inter- and intra-molecular bridges in the molecule of glycosylated enzyme. The electrostatic and hydrogen interactions between polysaccharides and proteins contribute to the enzyme stabilization [20,25]

The influence of PGA modification with mannan on its stability was investigated. Three aspects of enzyme stabilization were studied: (i) the degree of mannan oxidation; (ii) the saccharide content in the conjugate; and (iii) the average molar mass (M_w) of the conjugates.

The series of Man-PGA conjugates were prepared to vary reaction conditions such as using mannans with different degrees of oxidation and different mannan/PGA ratios. The obtained conjugates had the molar mass varying from 140 to 580 kDa and contained from 18 to 50% (w/w) of saccharide moiety. pH- and thermostability of the conjugates were studied and calculated as half-lives and free activation energies of deactivation [18]. The characteristics and half-lives of investigated neoglycoenzymes are summarized in Table 7.

Table 7. Characteristics and half-lives of mannan-PGA conjugates under various conditions

Sample	Saccharide content (%, w/w)	M_{app}* (kDa)	pH 3, 37 °C $t_{1/2}$ (min)	pH 10, 37 °C $t_{1/2}$ (min)	pH 8, 37 °C $t_{1/2}$ (min)	pH 8, 50 °C $t_{1/2}$ (min)
PGA	–	80	12	23	95	18
MO1-PGA-1	25	520	90	98	210	138
MO1-PGA-2	22	260	36	124	182	78
MO1-PGA-3	18	140	76	71	104	65
MO2-PGA-1	50	580	116	117	229	224
MO2-PGA-2	41	310	40	141	186	108
MO2-PGA-3	33	300	62	72	126	56
MO3-PGA-1	41	490	160	152	334	289
MO3-PGA-2	40	240	52	187	213	136
MO3-PGA-3	28	310	80	122	171	68

* M_{app} determined by HPSEC on HEMA BIO 100 and HEMA BIO 1000 columns system.

The results show that the stability of mannan-PGA conjugates increased in comparison with original PGA and is dependent on the character of mannan derivative attached to the enzyme and on the content of the saccharide in the conjugate. With the exception of condition at pH 10, the stability of PGA is proportional to the content of mannan and molar mass of the conjugate. The effect of MO1 (oxidized mannan having the highest molar mass) on PGA stability was smaller than observed for two other MO2 and MO3 due to active-site "shading" by longer polysaccharide chains. These later modified mannans are composed of shorter chains and contain more aldehyde groups to increase the possibility of multipoint attachment of single mannan molecule to PGA, and the presumption of active-centre "shading" becomes restricted. The results are in accordance with those obtained for PGA conjugates with dextran [26,27].

The mannosylated PGA has potential to form very stable insoluble precipitates with Con A, caused by biospecific interaction between the mannan and the lectin Con A (described above). This can be applied as the contribution to the stabilization of PGA, because the stability of Con A-cross-linked mannan-PGA conjugates (precipitates) is higher than that of original mannan-PGA conjugates. This effect was observed for both the low-molar- and high-molar-mass fraction of mannan-PGA conjugates [23].

Derivatization of PGA with mannan and other polysaccharides also influences the enzyme stability in the presence of organic solvents. The conjugation of PGA with dextran and mannan extended its stability significantly at extreme pH 3 and slightly at pH 9 and also showed the increase of the stability at higher temperature (55 °C). The stabilization effect of dextran and mannan in the presence of organic solvents (*n*-butyl alcohol, *n*-propyl alcohol, methyl alcohol and butyl acetate) caused the increase of the deactivation half-time from 1.3-fold to 10.6-fold, dependent on the type of polysaccharide used and the concentration of organic solvents [20].

6. Antimutagenic and Antioxidative Activity of Mannan Neoglyconjugates

The yeast mannan, isolated from *Saccharomyces cerevisiae,* has important characteristics such as good water solubility, available molar mass and relatively good antioxidative and antimutagenic properties. These appear to be promising features for its prospective use as a natural protective agent [28]. The protein occurrence in yeast mannan increases its ability to prevent oxidation and mutagenesis, because of its free radical scavenging activity [12]. The conjugates of the mannan with HSA and PGA were investigated for their antimutagenic and antioxidative activity in comparison with those of mannan alone. HSA was conjugated with the mannan at two different conditions. Samples with a molar mass of 160 kDa containing 25.5% of saccharide moiety and of 240 kDa containing 36% of saccharide moiety, respectively, were obtained. These were studied together with the Man-PGA conjugate (M_w = 153 kDa, 37.2% of saccharides) and the original mannan. By use of the TEAC (trolox equivalent antioxidant capacity) method, it was found that the presence of protein in the conjugates increases its antioxidative activity. The lowest activity was observed for the mannan, followed by the low-molar-mass Man-HSA(1) and the high-molar-mass Man-HSA(2), while the highest one was observed for Man-PGA. From these results, it can be supposed that the antioxidative activity of mannan conjugates depends more on the type of protein used than on the amount of carbohydrate in conjugate [29].

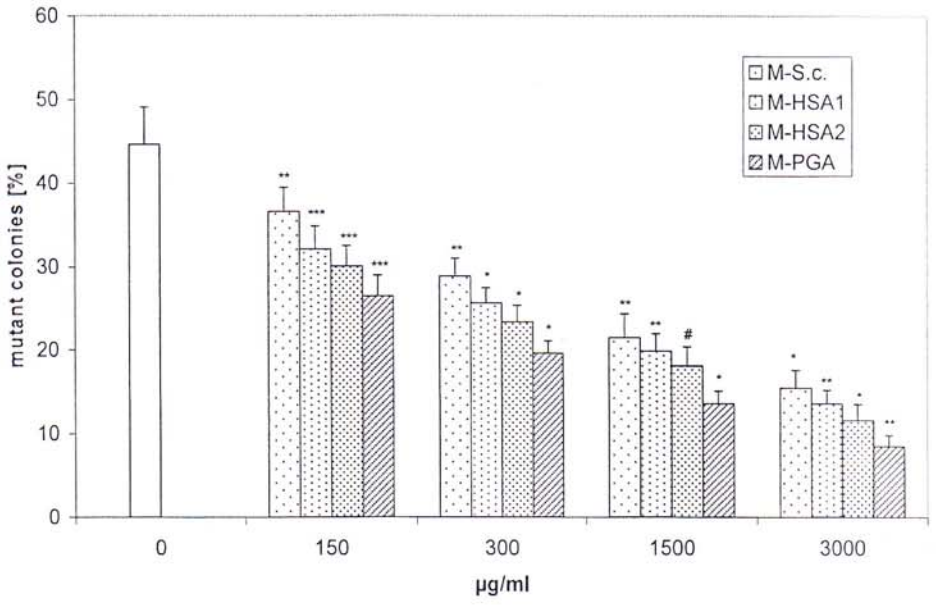

Figure 5. Effect of mannan, Man-HSA (1), Man-HSA (2) and Man-PGA on AO-induced genotoxicity in *E. gracilis* (Ref. 29).

The antimutagenic activity of mannan, Man-HSA and Man-PGA conjugates was examined in the system involving the genotoxic agents ofloxacin and AO (acridine orange)

with unicellular flagellate *Euglena gracilis*. The mannan and mannan-protein conjugates showed a significant antimutagenic activity against ofloxacin-induced damage of flagallate *E. gracilis*. The effect of mannan-protein conjugates towards the induction of mutants by application of ofloxacin is likely to be based on their antioxidative properties, therefore, the established antimutagenic activity of mannan-protein conjugates increased in the same order (mannan < Man-HSA(1) < Man-HSA(2) < Man-PGA) [29].

In the case of mannan-protein conjugates action towards AO, the antimutagenic activity of the tested conjugates cannot be explained by their antioxidant properties since AO is a frameshift mutagen, acting by virtue of its intercalative DNA-binding ability. The same order of antimutagenic effect of mannan and mannan glycoproteins towards AO as towards ofloxacin was determined [29]

The effect of mannan and neoglyconjugates on AO-induced genotoxicity in *E. gracilis* is shown in Fig. 5.

It can be stated that mannan neoglycoproteins revealed higher antioxidative and antimutagenic activities than the original mannan and may have broad practical application.

7. Immunogenicity of Mannan Neoglycoconjugates

Mannan, a major polysaccharide constituent of yeast cell wall, is a principal surface antigen. The mannan in *Saccharomyces cerevisiae* has species-specific structure and composition, different from other yeast mannans [30]. The conjugation of polysaccharide antigen to a protein carrier changes the immunological response of such a conjugate to a T-cell dependent response [10,11]. The presumption that the conjugation of surface mannan to protein carrier can induce anti-*S.cerevisiae* serum IgG and IgM antibodies in animal model was proved using neoglycoconjugates of mannan with human serum albumin in immunization study [31]. Two Man-HSA conjugates with different saccharide/protein ratios (1.35 and 0.66, respectively) were injected into female mice as host animals. The serum levels of elicited anti-*S.cerevisiae* Man-HSA conjugates were tested by ELISA. The comparison of induction of mannan specific IgG and IgM antibodies with Man-HSA to the whole-cell control and preimmune status evidently shows higher levels of specific IgG antibodies elicited with both Man-HSA conjugates (2.14 fold, 2.8 fold and 2.95 fold after first, second and third injection, respectively). Both conjugates showed similar booster effect. On the other hand, a very slight increase of anti-mannan IgM antibodies was observed. In summary, it can be concluded that the immunogenicity and T-cell dependence of the mannan antigen could be improved by conjugation to a protein carrier [31].

8. Conclusion

The modification of proteins with polysaccharides changes relevantly their properties, which can result in their broader utilization. On the other hand, the original antioxidative, antimutagenic, and immunogenic effects of polysaccharides can be extended in consequence of the conjugation with proteins. These aspects, oriented on the conjugates of *Saccharomyces cerevisiae* mannan with proteins prepared by simple reductive amination of oxidized mannan

with various proteins (BSA, PGA, HSA), were incorporated in this review. The obtained macromolecular conjugates were very compact and quite polydisperse due to the polydispersity of oxidized mannan and various stoichiometric mannan/protein ratios used. It was found that in the mannan-protein conjugate the significant affinity of original mannan to lectin Con A was preserved, which was subsequently evaluated in biospecific neoglycoenzyme immobilization [32]. The advantages of such enzyme immobilization is in a/ the higher remained enzyme activity, b/ the reversibility of such immobilization and c/ the possibility to use the technique of biospecific layering. Modification of the enzyme PGA with the mannan also influenced positively its stability in acidic as well as in basic condition, at higher temperature and also in the presence of low aliphatic alcohols. The mannan from *Saccharomyces cerevisiae* had the antioxidative and antimutagenic activities, which were increased after conjugation with the proteins such as HSA and PGA. The extended immunogenicity caused by creation of higher level of the antibody IgG was observed after conjugation of mannan with HSA.

This review has shown a simple manner of conjugation of two macromolecular compounds (polysaccharide and protein) to produce neoglycoproteins having the potential of utilization in biotechnology, pharmacology and medical research.

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