Purification and Partial Biochemical Characterization of Glycoproteins in a Champenois Chardonnay Wine

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Seven proteins have been isolated from a champenois Chardonnay still wine by concanavalin A affinity chromatography. The proteins of 24/25, 30 and 60/64 kDa are then purified by preparative isoelectric focusing (pH gradient 2.5−5) and by preparative SDS−PAGE. The 30 kDa protein presents a low hydrophobicity (780 cal/amino acid residue), a homogeneous molecular weight, and an isoelectric point close to 2.5. It also has the characteristic of being retained by *Lens culinaris* agglutinin (LCA). Proteins of 24/25 and 60/64 kDa present heterogeneous MW, a p*I* close to 3.9, and a hydrophobicity 30% superior to that of the 30 kDa molecule. Moreover, these two proteins are not retained by LCA. The three analyzed proteins are not susceptible to *O*-glycosidase activities. In return, the 24/25 kDa protein undergoes a 3100 Da variation after treatment with the peptide-*N*-glycanase F: it is a true *N*-glycosyl protein. The comparison of the must and the corresponding wine proteic fractions isolated by concanavalin A shows that the two heterogeneous MW molecules (24/25 and 60/64 kDa) originate from the grape berry. In addition, they suffer no modification during the alcoholic fermentation.

Keywords: *Wine; glycoproteins; affinity chromatography; foam stabilizer; must; hydrophobicity*

INTRODUCTION

A champagne wine's foaming properties largely depend on its protein concentration (Maujean et al., 1990; Brissonnet and Maujean, 1991; Malvy et al., 1994). Not all proteins participate identically in the foam (Brissonnet and Maujean, 1993). As a matter of fact, polypeptide foamability is governed by three interrelated properties: its isoelectric point and solubility (Bastiaens et al., 1990; Le Meste et al., 1990), its hydrophobicity (Slack and Bamforth, 1983; Townsend and Nakai, 1983; Yokoi et al., 1989), and its flexibility (Graham and Philips, 1979; Damodaran, 1988). According to another source (Roberts, 1977, 1975) glycoproteins are more able to stabilize foam than nonglycosylated proteins, because hydrophilic glycans reside in the liquid region between the bubbles. When the film becomes thinner, the viscosity increases and retards liquid drainage, but the polypeptide moiety equally acts on the liquid surface tension, an essential property for foam formation and stabilization. A better comprehension of the foam depends on knowledge of protein biochemical characteristics. As a result, studying champagne wine glycoproteins is very interesting.

The presence of arabinogalactan protein (AGP) in must (Saulnier and Brillouet, 1989) and of yeast mannoproteins in wine (Waters et al., 1994a) is clearly demonstrated. In both cases, these studies concern real glycoproteins, named proteoglycans by convention. The protein moiety represents less than 10% of the molecule, and the molecular weights are, respectively, 165 000 and 420 000. With regard to protein of less than 70 000, protein−polysaccharides have been put in evidence for the first time in a wine by Hsu and Heatherbell (1987). The proteins are separated by lithium dodecyl sulfate−polyacrylamide gel electrophoresis (LDS−PAGE) and revealed with the method of Clegg (1982) after blotting. Using the same technology, Tusseau and Van Laer (1993) also noted the presence of protein−polysaccharide complexes in a champenois still wine. In a Sauvignon must, Paetzold et al. (1990) separated by chromatofocalization seven proteic fractions, all of them containing sugars and amino acids. Yokotstuka et al. (1991) observed by the periodic acid−Schiff method (PAS) (Zaccharius et al., 1969) after SDS−PAGE that all of the proteins of a Koshu must and the majority of the corresponding wine proteins contain sugars. In a Muscat of Alexandria, Waters et al. (1993) also show the existence of four molecules which with the PAS staining gave a fuchsia coloration. However, for all of these proteins having molecular weights of less than 70 000, the covalent link between the glycan and the polypeptidic moiety has never been clearly evidenced. The term glycoprotein is therefore employed by these authors without a true demonstration. The interest of this study is consequently to demonstrate the existence of potential real glycoproteins in wines and to characterize them biochemically.

MATERIALS AND METHODS

Must. The Chardonnay must comes from sound grape berries harvested in September 1993. No treatment was done before alcoholic fermentation. The natural clarifying with visible flocculation does not occur during static settling of the must (24 h at 14 °C). A fraction of this must is then centrifuged (5 min at 3000*g*) filtered through a Sartopure GF membrane (Sartorius) and then through a 0.45 μm membrane (HA Millipore).

Wine. The settled Chardonnay must is racked and chaptalized with sucrose (35 g/L). The alcoholic fermentation is done by *Saccharomyces bayanus* at 18 °C. After malolactic fermentation, the wine is filtered through diatoms and then through a 0.45 μm membrane (HA Millipore). The wine

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protein concentration is 7.1 mg/L, determined by the direct Bradford (1976) method using a bovine serum albumin (fraction V powder, Sigma) standard curve. The blank contains the same alcohol concentration (11% v/v) as the studied wine (Marchal et al., 1996). The pH of this wine is 3.04 (HEITO PSD11).

Protein Isolation. The must (4 L) and the wine (4 L) are concentrated (10 times) and then four times dialyzed with distilled water (0.4 L concentrate plus 3.6 L water). A hydrophilic polysulfone membrane of 10 000 molecular weight cutoff was used. The crossflow filtration module is connected to the Hi-Flow system (pumping system plus glass tank). Ultrafiltration is done at 4 °C with injection of nitrogen to avoid must and wine oxidation. The ultrafiltrate flow is 40 mL/min. For the concentration step, the must is laced with distilled water (v/v) to reduce viscosity and to avoid complexation between proteins and polyphenolic compounds. The dialyzed retentates are freeze-dried (Serail CS 5L) and conserved at −20 °C.

Affinity Chromatography with Immobilized Lectins. *(a) Concanavalin A (Con A).* The must and the wine proteins containing glucose or mannose are isolated with Con A−Sepharose (Pharmacia) (column 10 × 100 mm). These fractions are respectively noted M-Con A^+ and W-Con A^+. The chromatography conditions are as follows: fixation buffer, 0.1 M sodium acetate + 0.15 M NaCl adjusted to pH 5; elution buffer, 0.1 M sodium acetate + 0.15 M NaCl + 0.1 M methyl α-D-mannopyranoside (Sigma) adjusted to pH 5; sample volume, 1 mL; fixation buffer, 10 column volumes; flow rate, 0.5 mL/min. The A_{280} is registered continuously with an UV detector (Uvicord SII 2238 LKB). The isolated fractions are concentrated (eight times) and then desalted in three steps (2 mL concentrate + 13 mL distilled water) with membranes of 10 000 molecular weight cutoff (Centriprep 10, Amicon).

(b) Lens Culinaris Agglutinin (LCA). The W-Con A^+ fraction is passed through a LCA−Sepharose (Sigma) column (7 × 50 mm). The chromatography conditions are the same as for Con A: sample volume, 0.4 mL; fixation buffer, 10 column volumes. The proteic fraction isolated by LCA is desalted with Centriprep 10 as previously.

Preparative Isoelectric Focusing. The proteins of W-Con A^+ are separated by liquid IEF using the Rotofor apparatus (Bio-Rad). The pH gradient is established between 2.5 and 5 with 0.8 mL ampholines (Pharmalytes 2.5−5, Pharmacia). The anode tank is filled with 0.1 M NaOH and the cathode tank with 0.1 M H_3PO_4. The power in the focalization chamber is kept at 12 W during the entire migration time. After 30 min of stable tension, the 20 partitions are drained by aspiration. The pH of each fraction is measured (HEITO PSD 11) (Table 2).

Analytical SDS−PAGE. Discontinuous SDS−PAGE was performed according to the method of Laemmli (1970) using slab gels (0.75 mm thick). For the stacking gels $T = 5\%$ and $C = 2.7\%$, and for the separating gels $T = 15\%$ and $C = 2.7\%$. A Mini-Protean (Bio-Rad) is used to run the gel at a constant voltage setting of 150 V until the bromophenol blue tracker dye reaches the bottom of the gel (usually 65 min at room temperature). After electrophoresis, the separated proteins are fixed in a 50% methanol solution (50 min) and stained according to the procedure of Wray et al. (1981). However, the silver nitrate (Sigma) concentration is 35% lower. Standard proteins from 14 000 to 94 000 are used as molecular weight markers (LMW Pharmacia). These proteins are treated like sample proteins and loaded in the wells for each analysis. The molecular weights (MW) of unknown molecules are calculated from the linear regression equation of log MW vs mobility. The presence of sugars is put in evidence by the periodic acid−Schiff method (PAS) (Riebe and Thorn, 1991).

Preparative SDS−PAGE. For each of the two fractions obtained by preparative IEF, the proteins are separated by preparative SDS−PAGE according to the method of Laemmli (1970). For the stacking gels $T = 5\%$ and $C = 2.7\%$. Con A−p$I_{2.5}$ proteins are separated with a $T = 13.5\%$ and $C = 2.7\%$ resolving gel, and Con A−p$I_{3.9}$ proteins are separated with a $T = 9\%$ and $C = 2.7\%$ resolving gel. The Prep-Cell apparatus (Bio-Rad) is used to run the gel at a constant power setting of 12 W during all migrations. When the bromophenol blue tracker dye reaches the bottom of the gel, proteins are collected with a peristaltic pump (flow rate, 0.5 mL/min). Each tube (3 mL) is then analyzed by analytical SDS−PAGE. The fractions containing the same protein are pooled, concentrated, and desalted with distilled water by ultrafiltration through 10 000 MWCO membranes (Centriprep 10 Amicon).

Table 1. Amino Acid Separation with the Pico-Tag Column (Gradient Eluent Conditions)

time (min)	eluent A %	eluent B %
0	100	0
8	60	40
17	0	100
19	0	100
20	100	0
38	100	0

Enzymatic Treatments. The 24/25 kDa purified protein [20 μg equivalent BSA (Bradford, 1976) in a 50 mM Tris-HCl, pH 8.5 buffer] is treated with 1 unit of peptide-*N*-glycosidase F for 24 h at 30 °C. The enzyme is purified from *Flavobacterium meningosepticum* (Boehringer Mannheim). The five purified proteins (70 μL) are also treated with *O*-glycosidases (20 μL) produced by *Bifidobacterium bifidum* (20 000 Psi French press supernatant). The proteins are contaminated by SDS because of the purification scheme, but enzymatic activities are preserved owing to Nonidet P40 (8% v/v).

Amino Acid Composition. Acid hydrolysis and free amino acid derivation are carried out with a Pico-Tag station (Waters) following the manufacturer's instructions. Hydrolysis changes glutamine and asparagine into glutamic and aspartic acids, respectively. Moreover, the tryptophan is destroyed. The separation of derivated amino acid is undertaken on an HPLC system (pump, Spectra-Physics 8100; UV detector, Knauer at 269 nm; integrator, Spectra-Physics 4290). The column is a Pico-Tag (Waters) 3.9 × 150 mm column. Gradient eluent conditions are outlined in Table 1. Solution composition: (**A**) 19 g of $CH_3COONa{\cdot}3H_2O$ + 1 L of H_2O Milli-Q, adjust to pH 6.4 with acetic acid, filter, take 940 mL + 60 mL of acetonitrile; (**B**) 600 mL of acetonitrile + 400 mL of H_2O Milli-Q; degassed for 20 min with helium; flow rate, 1 mL/min. Each amino acid concentration is calculated (70 μL) with regard to standard amino acids (Standard H, Pierce) using the external calibration method.

Hydrophobicity. The proteic fractions and the purified protein hydrophobicities are calculated from amino acid composition according to the method of Bigelow (1967). Each amino acid percentage is multiplied by its hydrophobicity coefficient, determined by Tanford (1962).

RESULTS AND DISCUSSION

Evidence of the Existence of Protein−Polysaccharide Complexes. PAS staining reveals the presence of numerous proteins associated with sugars (Figure 1). An intensely colored area appears in the upper part of the separating gel. For the must, this area is due to arabinogalactan proteins (Saulnier and Brillouet, 1989). In the wine, the deep spot could be attributable to arabinogalactan proteins originating from the juice (Waters et al., 1994b; Saulnier et al., 1992) and to yeast mannoproteins released during alcoholic fermentation. This observation was carried out with a Carginan noir wine, using the same technique (Waters et al., 1993). The biochemical characteristics of these macromolecules have been extensively studied. In return, the numerous proteins with MW less than 70 000 have never been studied. In plants, all soluble vacuolar and parietal glycoproteins are, except AGP, N-glycosylated (Fournet et al., 1987; Kimura et al., 1988; Takahashi et al., 1990). Elucidated glycan structures have all been complex or oligomannosidic types. For this reason, Con A is chosen for the isolation of the potential wine glycoproteins.

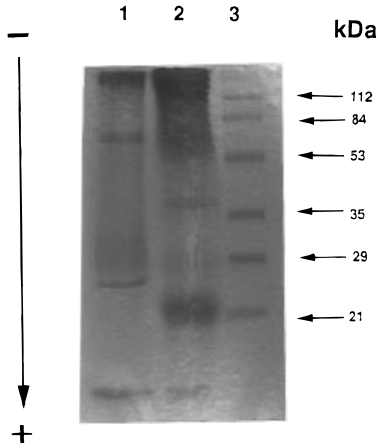

Figure 1. SDS−PAGE analysis of a Chardonnay must and the corresponding wine total proteins isolated by a 10 kDa ultrafiltration: lane 1, must total proteins; lane 2, wine total proteins; lane 3, Bio-Rad low MW prestained markers. Relative molecular weights ($\times 10^{-3}$) of protein standards are given at the right side of the gel.

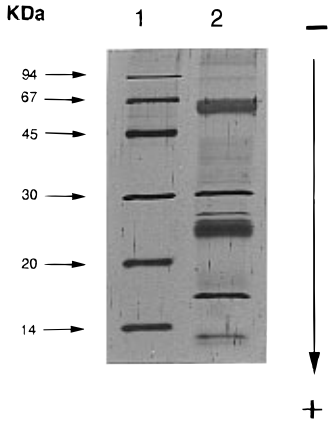

Figure 2. SDS−PAGE analysis of the wine proteic fraction isolated by affinity chromatography as described under Materials and Methods: lane 1, MW markers; lane 2, W-Con A^+. Relative molecular weights ($\times 10^{-3}$) of protein standards are given at the left side of the gel.

Table 2. pH of the Proteic Fractions Isolated by Preparative Isoelectric Focusing

fraction	pH	fraction	pH
1	2.53	11	4.03
2	2.95	12	4.21
3	3.16	13	4.26
4	3.35	14	4.37
5	3.51	15	4.45
6	3.59	16	4.67
7	3.66	17	5.04
8	3.75	18	5.39
9	3.86	19	5.56
10	3.97	20	5.91

The protein purification scheme includes three steps: affinity chromatography with Con A, preparative isoelectric focusing, and preparative SDS−PAGE.

Wine Proteins Isolated by Con A. The proteic fraction isolated by Con A chromatography (W-Con A^+) contains seven major proteins with MW of, respectively, 14 000, 17 000, 24 000/25 000, 26 000, 27 000, 30 000, and 60 000/64 000 (Figure 2). Numerous minor proteins appear with MW between 32 000 and 44 000. Finally, two proteins are weakly stained with MW more than 70 000.

Preparative Isoelectric Focusing. In a second phase, the proteins of W-Con A^+ are subjected to preparative IEF with a pH gradient between 2.5 and 5. The pH of the collected fractions is reported in Table 2. For the proteins isolated by Con A, the value $\Delta(pH_{wine} - pI_{protein})$ is between 0.6 and 0.8 unit. This biochemical characteristic is then favorable to the expression of a great foaming capacity (Bastiaens et al., 1990; Yokoi et al., 1989). The proteins are sharply divided into two groups (Figure 3). Fractions 1 and 2 contain four proteins with homogeneous MW (respectively, 14 000, 17 000, 27 000, and 30 000), and one protein weakly stained with more diffuse MW (25 000). Their isoelectric point is close to 2.5. These fractions are combined and noted as Con A−p$I_{2.5}$. These proteins are then negatively charged in the studied wine. Fractions 5−11 contain two heterogeneous MW proteins (24 000/25 000 and 60 000/64 000) and numerous minor proteins with MW between 32 000 and 44 000. Fractions 7−11 are combined and labeled Con A−p$I_{3.9}$. In contrast to the previous one, the pI = 3.9 proteins are positively charged in this wine.

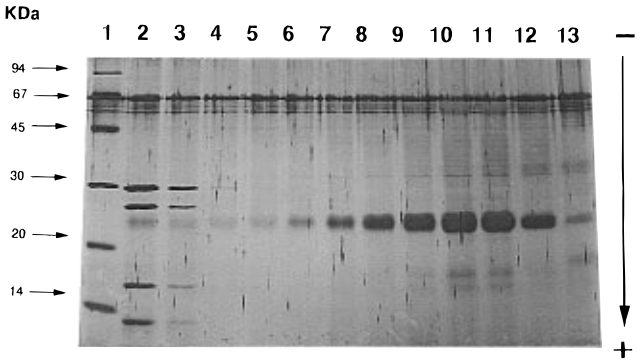

Figure 3. SDS−PAGE analysis of proteins fractionated by preparative isoelectrofocusing with the Rotofor apparatus: lane 1, MW markers; lanes 2−13, fractions 1−12 of the Rotofor. The gradient of pH is established between 2.5 and 5.9. Relative molecular weights ($\times 10^{-3}$) of protein standards are given at the left side of the gel.

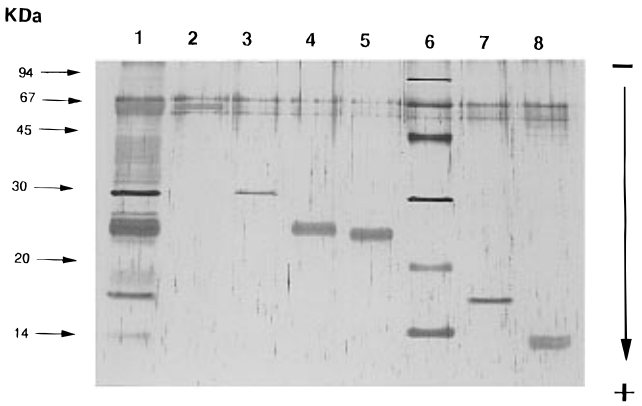

Figure 4. SDS−PAGE analysis of purified wine proteins obtained by affinity chromatography, preparative IEF, and preparative SDS−PAGE (see Materials and Methods for details): lane 1, proteic fraction isolated by Con A; lane 2, 62 kDa protein (it comes from only one fraction obtained by preparative SDS−PAGE); lane 3, 30 kDa protein; lanes 4 and 5, 24 and 25 kDa proteins showing the heterogeneity of MW; lane 6, molecular weight markers; lane 7, 17 kDa protein; lane 8, 14 kDa protein. Relative molecular weights ($\times 10^{-3}$) of protein standards are given at the left side of the gel.

Preparative SDS−PAGE. The Con A−p$I_{3.9}$ and Con A−p$I_{2.5}$ proteins are separated by the Prep-Cell system (Bio-Rad). Five purified proteins are collected (Figure 4): 14 000, 17 000, and 30 000 MW proteins come from Con A−p$I_{2.5}$; 24 000/25 000 and 60 000/

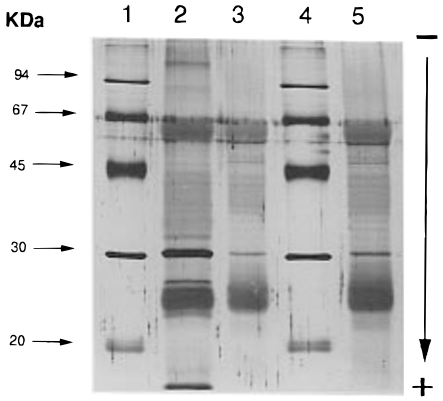

Figure 5. SDS−PAGE analysis of the must and the corresponding wine proteic fractions isolated by Con A chromatography as described under Materials and Methods: lane 1, MW markers; lane 2, W-Con A^+; lanes 3 and 5, M-Con A^+ (quantities of deposits are single and double, respectively); lane 4, MW markers. Relative molecular weights ($\times 10^{-3}$) of protein standards are given at the left side of the gel.

64 000 MW proteins come from Con A−p$I_{3.9}$. Lanes 4 and 5 show that this protein has a heterogeneous MW. This could be a microheterogeneity of the glycosylation, frequently observed among plant glycoproteins (Takahashi et al., 1986; Sturm, 1991). The MW 24 000/25 000, 30 000, and 60 000/64 000 proteins are in sufficient quantities to approach biochemical characteristics, important for foaming capacity.

W-Con A^+ Protein Origin. The must and the wine proteic fractions retained by Con A are compared using the SDS−PAGE method (Figure 5). The 60/64 kDa molecule exists without ambiguity in the two samples. It originates from the grape berries and does not undergo modification during alcoholic fermentation. The same seems to be true for the 24/25 kDa molecule. A 30 kDa protein is weakly stained in the Chardonnay must, even if the loaded quantity in the well increases (lane 5). The ratio of intensity in the must to intensity in the wine does not plead for a plant origin, but it is impossible, with this technique, to give a definitive answer. The 14, 17, 20, and 27 kDa proteins seem to be absent in the must or are in quantities too small to be clearly identified. Finally, numerous minor proteins between 32 and 44 kDa and a band at >90 kDa are present in wine but are not detected in must. These glycoproteins are probably released by yeasts during alcoholic fermentation.

Enzymatic Treatment. The 24/25, 30, and 60/64 kDa proteins, which apparently come from grape berries, are insensitive to *O*-glycosidases produced from *B. bifidum*. These results are in harmony with the literature, which demonstrates that, except for AGP, there are no soluble *O*-glycosylproteins in plants. In return, the 24/25 kDa molecule presents, after treatment with peptide-*N*-glycanase F, a molecular mass decrease of about 3100 Da (Figure 6). The absence of lower MW bands indicates there has been no proteolytic degradation. Consequently, the MW decrease effectively results from a deglycosylation. It is more than likely that it concerns a plant *N*-glycosylprotein. Because of this endoglycosidase specificity, the proximal *N*-acetylglucosamine will not be α1-3-fucosylated. An oligosaccharide MW is between 1000 and 2000. Consequently, this protein could bear two and even three glycans.

Affinity for LCA. This lectin fixes biantenned glycans containing α-D-glucose and/or α-D-mannose and

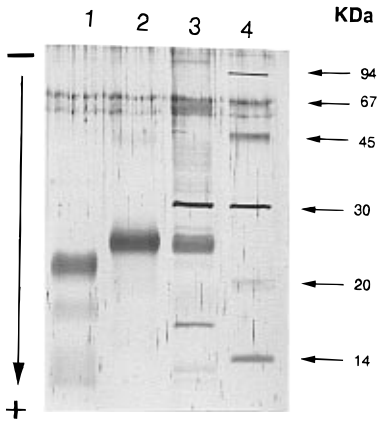

Figure 6. Susceptibility of 24/25 kDa wine purified protein to peptide-*N*-glycanase F from *F. meningosepticum* as shown by analysis with a 15% polyacrylamide gel electrophoresis in denaturating conditions: lane 1, protein incubated 24 h at 30 °C with the *N*-glycosidase; lane 2, untreated protein; lane 3, wine proteins isolated by Con A; lane 4, MW markers. Relative molecular weights ($\times 10^{-3}$) of protein standards are given at the right side of the gel.

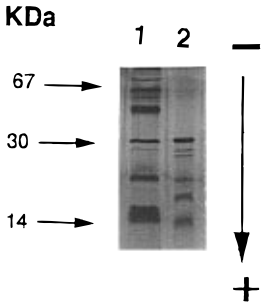

Figure 7. SDS−PAGE analysis of the wine proteic fraction isolated by LCA chromatography: lane 1, MW markers; lane 2, retained proteins by LCA after being passed through a Con A column. Relative molecular weights ($\times 10^{-3}$) of protein standards are given at the left side of the gel.

especially fucose linked on the proximal *N*-acetylglucosamine (Lee et al., 1990). The higher affinity corresponds to glycans containing *N*-acetylglucosamine in nonreducing terminal position. Moreover, galactose and fucose of lateral chains reduce interactions between the lectin and the oligosaccharide. LCA does not adsorb all of the proteins adsorbed by Con A, and vice versa, because of steric differences. Among the seven major proteins in W-Con A^+, five proteins are complexed by LCA (Figure 7). The MW are, respectively, 14 000, 17 000, 26 000, 27 000, and 30 000. A minor protein (19 kDa) is weakly stained but is absent in W-Con A^+ (Figure 2), perhaps because of the difference in protein content. This step provides valuable indications on the structure of the oligosaccharides borne by these proteins. The glycans successively hung by Con A and LCA can only correspond to four structures (Lee et al., 1990) (Figure 8). In the wine, proteins exclusively originate from yeast and plant. The B and D structures contain, respectively, an intercalary β1-4 *N*-acetylglucosamine and neuraminic acids. They must therefore be eliminated. The A and C glycans solely can be envisaged.

Amino Acid Composition. In total Chardonnay still wine proteins, six amino acids reach values of 9% or more of total amino acids. These amino acids are alanine (Ala), aspartic acid and aspragine (Asx), glycine (Gly), proline (Pro), serine (Ser), and threonine (Thr) (Table 3). Except Pro, these amino acids are also preponderant in the protein of a Koshu wine (Yokotsuka

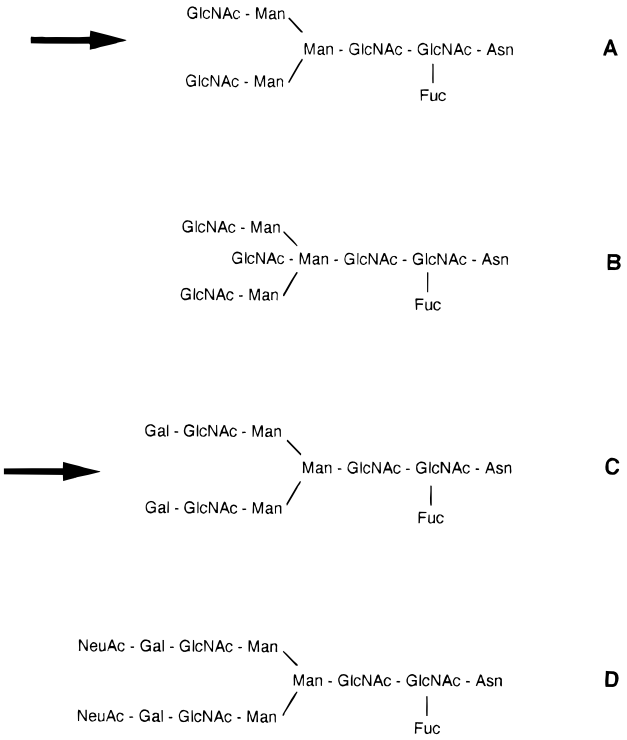

Figure 8. Structures of *N*-glycans recognized by Con A and LCA (Lee et al., 1990).

Table 3. Amino Acid Compositions of the Three Purified Proteins, the Proteic Fraction Isolated by Con A, and the Total Wine Proteins[a]

amino acid	wine proteins	W-Con A^+	24/25 kDa protein	60/64 kDa protein	30 kDa protein
Asx	9.3	8.8	13.7	14.9	12.0
Glx	6.7	7.9	7.6	12.1	7.2
Hyp	3.2	0.1	0.0	0.0	0.0
Ser	13.8	27.7	9.8	8.8	14.0
Gly	10.3	5.9	6.0	6.0	6.0
His	1.0	0.9	1.4	1.2	8.0
Arg	0.7	0.3	4.0	2.6	2.0
Thr	11.2	11.2	8.3	7.2	7.7
Ala	11.8	14.8	10.4	6.7	7.1
Pro	9.0	4.8	5.9	7.1	4.7
Tyr	0.4	0.1	3.2	2.9	1.8
Val	5.8	6.3	7.2	7.1	5.5
Met	1.1	0.7	1.6	2.5	1.0
Cys	0.6	0.2	0.6	0.2	1.7
Ile	3.6	2.8	6.1	5.5	6.5
Leu	5.7	3.6	7.5	8.2	6.0
Phe	3.8	1.7	4.2	3.3	3.3
Lys	1.9	2.0	2.7	3.8	5.6

[a] Results are expressed in number of residues for 100 amino acids.

et al., 1991). It is interesting to note that the two above-mentioned studies, on wines of different grape variety, vintage, and region, give relatively comparable results. Hydroxyprolin (Hyp) reaches 3.2% of total amino acids. This amino acid is a characteristic of plant-soluble AGP, and it generally reaches 25% of the total amino acids. The grape berry's AGP could therefore represent about 13% (3.2% × 4) of the total wine's proteins. A large proportion of Ala, Ser, and Thr comes from parietal yeast mannoproteins released during the alcoholic fermentation (Leroy, 1986; Freyssinet, 1988; Waters et al., 1993). The concentration in Ala and Ser is higher in the W-Con A^+ fraction than in wine for the same reason we described above since yeast mannoproteins are hung by Con A (Table 2). The absence of hydroxyproline was foreseeable since sugars of AGP (arabinose and galactose) are not recognized by Con A.

Table 4. Hydrophobicity of the Three Purified Proteins, the Proteic Fraction Isolated by Con A, and the Total Wine Proteins[a]

	hydrophobicity $H\varphi_{av}$
total wine proteins	940
W-Con A^+	656
24/25 kDa protein	1051
60/64 kDa protein	1026
30 kDa protein	784

[a] Results are expressed in cal/amino acid residue.

The two purified proteins of 24/25 and 60/64 kDa have the same amino acid concentrations except for Ala, Glx, and, in a lesser proportion, Pro (Table 3). The 30 kDa protein is distinguishable from the previous by several amino acids the contents of which are largely different: Ser, Pro, Val, Leu, Lys (25−30%), Tyr and Met (60%), and His and Cys (>100%). In yeast mannoproteins and in W-Con A^+ proteins, Ala and Ser percentages are comparable (Leroy, 1986; Freyssinet, 1988; Waters et al., 1993). These results indicate that yeast mannoproteins represent a very important part of W-Con A^+ proteins. Acid hydrolysis changes glutamine and asparagine into glutamic and aspartic acids. Then, the amino acid composition cannot explain the difference of p*I* between the 24/25 and the 30 kDa proteins.

Hydrophobicity. W-Con A^+ proteins are less hydrophobic than the wine proteins considered on the whole (Table 4). This difference can partially be explained by the high proportion of serine, the hydrophobicity coefficients of which is naught. It can also be explained with regard to the smaller concentrations of Pro, Ile, Leu, and Hyp. Among the three studied proteins, the 30 kDa one is by far the least hydrophobic (784 cal/amino acid residue). Its value is even lower than the wine protein average. In return, the two 24/25 and 60/64 kDa molecules have the greater values (1051 and 1026 cal/residue, respectively) owing to apolar amino acids, even if globally unimportant. The difference between these two proteins and the 30 kDa protein is 25%.

A polypeptidic chain is adsorbed at the gas−liquid interface by its hydrophobic segments and undergoes conformational changes favoring the orientation of new hydrophobic segments toward the interface. In theory, the two 24/25 and 60/64 kDa proteins should then have good surface properties and actively participate in the formation of foam, after the use of champagne process (Maujean et al., 1990), owing to the high hydrophobicity of their proteic moiety.

Conclusion. The Chardonnay still wine studied contains numerous proteins that positively react with the PAS staining. Some low molecular weight proteins are therefore isolated by Con A affinity chromatography. These proteins are divided into two groups by preparative isoelectric focusing using a 2.5−5 pH gradient. One fraction contains four major proteins (homogeneous MW: 14 000, 17 000, 27 000, and 30 000) having p*I* close to 2.5. The other group contains two major proteins of 24 000/25 000 and 60/64 000 having heterogeneous MWs. Their p*I* is close to 3.9. The Con A-p$I_{3.9}$ and the Con A-p$I_{2.5}$ proteins are fractioned by preparative SDS−PAGE. Five proteins are purified with this three-step scheme and three of them analyzed (sufficient quantities). The 24/25 kDa protein is sensitive to the peptide-*N*-glycanase activity. It is consequently a *N*-glycosylprotein. The two heterogeneous proteins (24/25 and 60/

64 kDa) present a hydrophobicity largely superior to the wine's protein average. The 30 kDa molecule is of the opposite case. Nevertheless, the proteic fraction isolated by Con A has a low hydrophobicity because of the presence of serine originating from yeast mannoproteins.

This study demonstrates the presence of true plant glycosylproteins in champagne wine. The 24/25 kDa protein is at the present time the best characterized. In theory, its p*I* of close to the wine pH, its high hydrophobicity, and its glycosylated nature confer excellent surface properties. Experiments are underway to verify this hypothesis.

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