A FTIR spectroscopy evidence of the interactions between wheat germ agglutinin and *N*-acetylglucosamine residues

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Abstract Wheat germ agglutinin (WGA), a lectin binding a *N*-acetyl-D-neuraminic acid (NeuNAc) and/or *N*-acetyl-D-glucosamine (GlcNAc) group, was studied by Fourier transform infrared (FTIR) spectroscopy. Deconvolution of the FTIR spectrum of WGA alone indicated the presence of few α-helices and β-sheets, in contrast to many other lectins. These results agree with previous WGA crystal data. The WGA conformational changes, induced by GlcNAc-bearing liposomes or GlcNAc oligomers, were studied by infrared differential spectroscopy. The GlcNAc binding to WGA resulted in a decrease of turns and α-helices and a concomitant appearance of β-sheets, inducing more or less peptidic N-H deuteration.

Key words: Fourier transform infrared spectroscopy; Hydrogen bond; Lectin interaction; *N*-acetyl-D-glucosamine; Neoglycolipid

1. Introduction

Lectins are proteins involved in many cell recognition processes on account of their property to bind carbohydrates [1–5]. In vitro investigations were realized to model the molecular mechanism of recognition between a glycolipid sugar and a lectin at the membrane surfaces. In this regard, biomimetic systems such as liposomes containing natural or synthetic glycolipids were prepared and incubated with a specific lectin [6–10]. In the case of *N*-acetyl-D-glucosamine (GlcNAc)-bearing neoglycolipids (NG), differing by their alkyl-chains and by the presence or not of a spacer, a minimal alkyl-chain length for the anchor and the presence of a spacer were found to be necessary to observe a NG sugar recognition by a specific lectin, the wheat germ agglutinin (WGA) [11].

WGA is a dimeric protein, containing two protomers of 171 amino acid residues each [12]. Each protomer is an assembly of four structurally homologous and spatially distinct domains. Each domain contains amino acid residues folded in an irregular fashion with very little folded structure but with four disulfide bonds in its interior. WGA has two sugar binding sites per protomer (four per dimer) located in the interface between the protomers. The primary binding sites can be occupied by GlcNAc as well as by terminal *N*-acetyl-D-neuraminic acid (NeuNAc), while the secondary binding sites can recognize only GlcNAc. The binding of different oligosaccharides to WGA was studied by X-ray crystallography, by NMR or by chemically induced dynamic nuclear polarization [13–19] but most of the results were obtained with NeuNAc residues.

The purpose of this work was to obtain information on the GlcNAc-induced conformational changes of WGA by using different GlcNAc-bearing compounds and Fourier transform infrared (FTIR) spectroscopy. This technique was chosen because the correspondence between the position of the amide band maxima in the infrared (IR) spectrum and the protein conformations was well-studied [20–24]. The conformation of the lectin alone was studied by deconvolution of its FTIR spectrum and the knowledge of the lectin crystal structure was helpful for the interpretation of the FTIR band assignments. When the lectin was incubated with liposomes containing GlcNAc-bearing NG [11], the resulting WGA conformational changes were determined by IR differential spectroscopy. The results were compared with those obtained by incubating WGA with GlcNAc oligomers, known to bind this lectin [25–28].

2. Materials and methods

2.1. Materials

Dimyristoylphosphatidylcholine (DMPC), $(GlcNAc)_2$ and $(GlcNAc)_3$ were supplied by Sigma, ^2H_2O by Merck and WGA by Boehringer. NGs (Fig. 1) were prepared as previously reported [11].

2.2. Preparation of FTIR samples

Multilayer liposomes were obtained by (a) hydrating a dried film of NG and DMPC (in a 15:100 mol ratio) with a ^2H_2O buffer containing Tris-HCl 10 mM at pH 7.5 (^2H_2O buffer) at a final lipid concentration of 10 mg/ml and (b) vigorous shaking for 5 min at 25°C. The 20 μl FTIR samples were 5 μM WGA solution in ^2H_2O buffer either alone or with liposomes in a GlcNAc/WGA mol ratio of 4:1.

2.3. FTIR spectroscopic measurements

IR data were acquired with a Nicolet 510M FTIR spectrometer equipped with a DTGS detector, using a temperature-controlled flow-through (Harrick, Ossining, NY, USA). The spectrometer was continuously purged with dry filtered air (Balston regenerating desiccant drier). Spectra were recorded at 25°C with 128 interferograms and Fourier-transformed. The resolution was 4 cm^{-1}. 20 μl samples were filled in a 50 μm pathlength CaF_2 cell. The ^2H_2O buffer spectrum was substracted to the sample spectrum taken under the same conditions. Each spectrum is representative of at least three independent measurements. The final spectrum was fitted by the mean of a curve fitting program computing the Lorentzian band shape components in the amide I and II regions as previously described [29,30]. After decomposition, the percentage of each component band was calculated as 100 (A_x/A_t), where A_x is a component band area and A_t is the amide band area. IR difference spectra were obtained by using the intensity of the characteristic Tyr band at 1516 cm^{-1} as a reference for the WGA concentration.

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Abbreviations: DMPC, dimyristoylphosphatidylcholine; ^2H_2O buffer, 10 mM Tris-HCl buffer in ^2H_2O pH 7.5; FTIR, Fourier transform infrared; GlcNAc, *N*-acetyl-D-glucosamine; NeuNAc, *N*-acetyl-D-neuraminic acid; NG, neoglycolipid; WGA, wheat germ agglutinin

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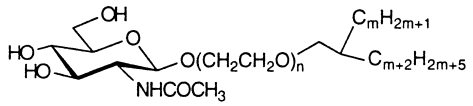

Fig. 1. Chemical structures of NG 24:0 ($n=0$, $m=10$), 24:3 ($n=3$, $m=10$), 28:0 ($n=0$, $m=12$), 28:3 ($n=3$, $m=12$), 32:0 ($n=0$, $m=14$) and 32:3 ($n=3$, $m=14$).

3. Results

3.1. FTIR spectrum of WGA

Amide I bands, at 1700–1610 cm^{-1}, are principally C=O stretching vibrations with some N-H bending and C-H stretching vibrations while amide II bands, at 1575–1480 cm^{-1}, are principally N-H bending vibrations with some C-N stretching vibrations. The amide I and II band decomposition of the ^2H_2O buffer-subtracted FTIR spectrum of WGA alone is shown in Fig. 2. The remaining intense amide II band at 1543 cm^{-1} pointed out an incomplete NH/N^{2}H exchange. This incomplete deuteration corresponds to N-H residues buried in the hydrophobic regions of the protein since the amide II band intensity was not modified by increasing the incubation time in the ^2H_2O buffer. Deconvolution of the amide I band gives five component bands at 1686, 1670, 1657, 1645 and 1623 cm^{-1}, representing 2, 20, 17, 53 and 8%, respectively, of the original band. The 1670 cm^{-1} band corresponds to β-turns while the 1623 cm^{-1} band associated with the 1686 cm^{-1} band corresponds to β-sheets [20–24]. The 1657 cm^{-1} band in ^2H_2O is usually produced by α-helices but it cannot be excluded to originate from turns or 3_{10}-helices [25,31]. The 1645 cm^{-1} band in ^2H_2O corresponds to unordered structures. Besides, it is well known that some amino acid side chain groups absorb in amide I and II regions [22]. The 1516 cm^{-1} band, corresponding to Tyr residues, was easily recognized while the bands at 1585 and 1567 cm^{-1} might correspond to Asp and Glu COO^- residues.

3.2. Influence of liposome-incorporated neoglycolipids on the FTIR spectrum of WGA

When liposomes containing the different NGs were added to WGA (Fig. 3), all the ^2H_2O buffer-subtracted FTIR spectra show the ester band of DMPC C=O groups at 1729 cm^{-1} and no important changes in the amide I region. Only the

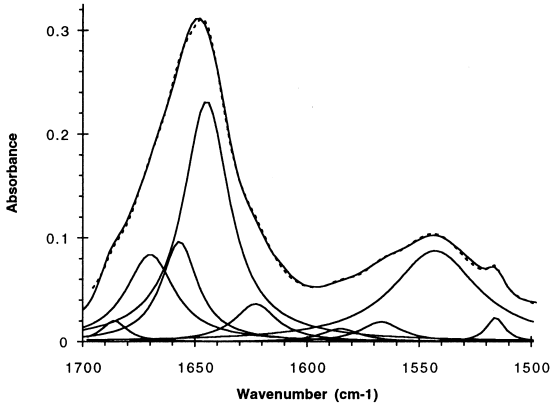

Fig. 2. ^2H_2O buffer-subtracted FTIR spectrum of WGA alone, best-fitted individual component bands of the amide I and II regions (—) and their resulting summation (—). The WGA concentration was 5 μM.

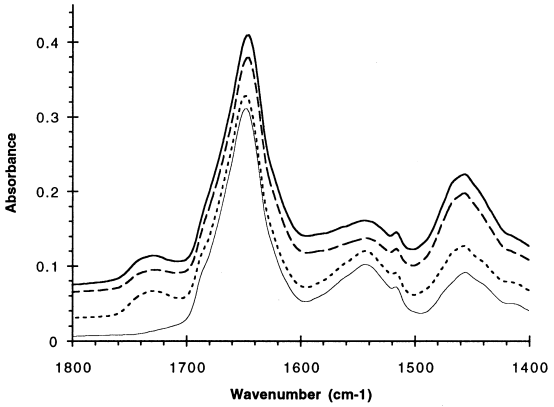

Fig. 3. ^2H_2O buffer-subtracted FTIR spectra of WGA interacting with liposome-incorporated NG 24:3 (—), 28:3 (——), 32:3 (—) and FTIR spectrum of WGA alone (—). In all the cases, the GlcNAc/WGA mol ratio was 4:1.

^2H_2O buffer-substracted FTIR spectra of WGA in the presence of liposomes with NG 28:3 or 32:3 show a decrease of the amide II band and an increase of the amide II' band in the 1500–1400 cm^{-1} region. This pointed out a NH/N^{2}H exchange increase, induced by the structural rearrangements of WGA occurring during the lectin-ligand interaction. In order to verify that the lectin has no aspecific affinity for DMPC, the FTIR spectrum of WGA incubated with liposomes containing only DMPC was compared with the spectrum of the lectin alone. No difference was detected in the amide I region of the spectra (data not shown), proving that there is no interaction between WGA and liposomes which do not bear GlcNAc residues.

3.3. Interactions between liposome-incorporated neoglycolipids and WGA studied by IR difference spectroscopy

The WGA conformational changes induced by the addition of mixed liposomes could be seen on the IR difference spectra, obtained by substracting the FTIR spectrum of WGA alone to the FTIR spectrum of WGA in the presence of liposomes containing a NG. Each IR difference spectrum was compared to the FTIR spectrum of the corresponding mixed liposomes without any incubation with WGA (Fig. 4A). On the one hand, the IR difference spectra of WGA and liposomes with NG 28:3 or 32:3 were quite different to the FTIR spectra of the corresponding liposomes alone proving the WGA recognition. Table 1 gives the tentative assignments of the bands in the 1700–1400 cm^{-1} region. On the other hand, the IR difference spectrum of WGA and NG 24:3 liposomes was very similar to the FTIR spectrum of NG 24:3 liposomes alone. Similar results were obtained with NG 24:0, 28:0 or 32:0 liposomes, indicating the absence of interaction between WGA and the sugar residue of NG 24:3, 24:0, 28:0 or 32:0.

3.4. Interactions between WGA and GlcNAc oligomers

The significance of the results obtained with WGA and liposome-incorporated NG was studied by analyzing the interactions between WGA and GlcNAc oligomers. When WGA was interacting with $(GlcNAc)_2$ and $(GlcNAc)_3$, the IR difference spectra (Fig. 4B) give positive and negative bands located at similar wavenumbers to those observed with NG (Fig. 4A). There were only differences in the relative intensity of the various bands. Table 1 gives the tentative

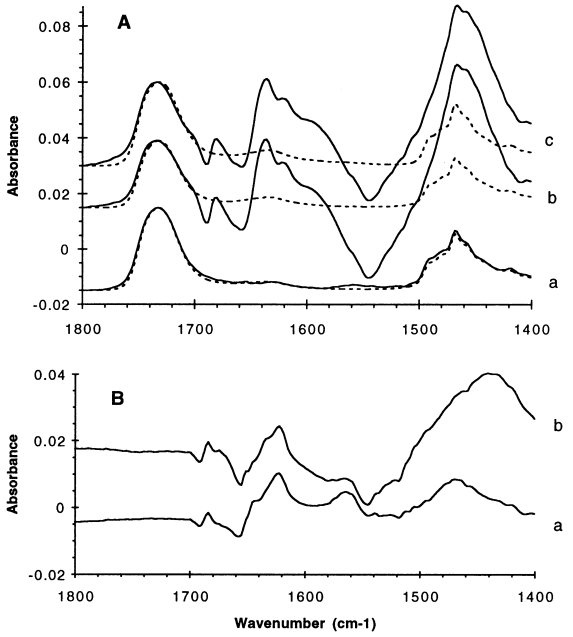

Fig. 4. (A) FTIR spectra of liposome-incorporated NG (—) and IR difference spectra obtained by substracting the FTIR spectrum of WGA alone to the FTIR spectrum of WGA interacting with liposome-incorporated NG (—). Curves a, b and c correspond to NG 24:3, 28:3 and 32:3, respectively. (B) IR difference spectra obtained by substracting the FTIR spectrum of WGA alone to the FTIR spectrum of WGA interacting with $(GlcNAc)_2$ (curve a) and $(GlcNAc)_3$ (curve b). In all the cases, the GlcNAc/WGA mol ratio was 4:1.

assignments of the 1700–1400 cm^{-1} bands. The spectral changes observed when WGA interacts with GlcNAc oligomers are less intense than those of WGA with NG liposomes (Fig. 4), indicating that the conformational changes are more important when WGA was interacting with NG 28:3 or 32:3 than with GlcNAc oligomers. Moreover, when the WGA concentration varied with the same amount of ligand, there was no change in the position of the various bands in the IR difference spectra but only the band intensities were modified (data not shown). This confirms that the different bands arise from conformational changes of WGA.

4. Discussion

The purpose of this work was to study the GlcNAc ligand-induced changes in the WGA conformation by FTIR spectroscopy. In a first step, the protein secondary structure was studied in the absence of ligand. The deconvolution of the amide I region of the FTIR spectrum of WGA showed that about 17% of the amino acid C=O groups are involved in α- or 3_{10}-helices, 8% in β-sheets, 20% in β-turns and 53% in unordered structures. Some errors in these estimations could come from the fact that we did not take account of the absorbances of the 4 Arg, 9 Asn and 10 Gln residues per WGA monomer. Indeed, it is impossible to determine, by FTIR spectroscopy, the real contribution of these amino acids since some of them, located in hydrophobic regions, are more or less accessible to ^2H_2O [32]. PDB data of crystallography studies on WGA with NeuNAc [13] gave 19% of the lectin amino acids involved in α- or 3_{10}-helices, 9% in β-sheets (in good agreement with our FTIR data on WGA with GlcNAc) and 47% in turns. The lower level of turns obtained in the FTIR study can arise from (a) the difference of ligand and/or (b) the overlapping of β-turn hydrogen-bonded C=O [29,30,33] and unordered structures. Furthermore, the low level of β-sheets in WGA is surprising since lectins are generally described as rich β-sheet-containing proteins [34–36].

In a second step, the ligand-induced changes in the protein secondary structure were studied. The results show that only liposome-incorporated NG 32:3 and 28:3 interact with WGA, showing that the presence of a spacer is not sufficient for WGA recognition. A minimal alkyl-chain is also needed. This agrees with previous studies on aggregation between WGA and liposome-incorporated NG [11].

The spectral modifications of WGA interacting with liposome-incorporated NG 28:3 or 32:3 involved about 5% of the C=O residues absorbing in the amide I region, indicating that about 17 amino acids per WGA dimer were affected by the GlcNAc binding. The program HINT was previously used to model the hydrogen bonds involved in the GlcNAc binding to WGA [37]. In this model, none of the lectin amino acids was bound by their peptidic C=O group. This indicates that the lectin changes observed in IR difference spectra did not arise from the formation of hydrogen bonds with some lectin amide I C=O groups but might be due to conformational changes of the protein backbone. These changes might involve turns and α-helices (wide negative bands at 1666–1656 cm^{-1} in IR difference spectra) and β-sheets (positive bands at 1636–1623 and 1681 cm^{-1}). This agrees with the HINT model [37], showing that GlcNAc binds mostly WGA amino acids taking part in turns or α-helices [13].

The conformational changes induced by the interaction of WGA with liposome-incorporated NG 32:3 or 28:3 increase

Table 1
Tentative assignments of the bands in the various IR difference spectra

WGA and NG 28:3 or 32:3 liposomes	WGA and $(GlcNAc)_2$ or $(GlcNAc)_3$	Tentative assignments
1735 (+)		C=O ester DMPC and C=O COOH Asp or Glu
1690 (−)	1691 (−)	β-Sheet
1682 (+)	1684 (+)	β-Sheet
1665 (sh−)		β-Turn, C=O Gln in H_2O
1656 (−)	1656 (−)	α-Helix
1637 (+)	1633 (sh+)	β-Sheet and C=O Gln in ^2H_2O
1621 (+)	1623 (+)	β-Sheet
1596 (+)	1564 (+)	C=O COO^- Asp and/or Glu
1546 (−)	1545 (−)	NH amide II
	1518 (−)	Ring OH Tyr
1460 (+)	1441 (+)	N^2H amide II'

(+) Or (−) means positive or negative bands and (sh) designates a peak shoulder.

the NH deuteration of the protein (negative 1544 cm^{-1} band associated to positive 1466 cm^{-1} band in IR difference spectra). Such a deuteration was observed when concanavalin A binds a sugar ligand [36].

The rates of NH/N^2H exchange and structural changes of WGA were greater in the presence of liposome-incorporated NG 28:3 and 32:3 than in the presence of GlcNAc oligomers. This can be explained by the number of lectin-recognizable residues beared by each kind of ligand [38]. Indeed, WGA forms soluble complexes with entities bearing only one binding site (GlcNAc oligomers) and insoluble complexes with entities bearing more than one binding site (liposomes containing many NG molecules). The formation of such aggregates with liposomes containing NG 32:3 or 28:3 might induce greater conformational changes since two binding sites of the lectin were occupied by ligands. Furthermore, the formation protein aggregates are often associated with the formation of intermolecular β-sheets absorbing at 1628–1610 cm^{-1} [39] and would explain the greater changes observed in the 1630–1610 cm^{-1} region of the IR difference spectra of WGA interacting with liposomes containing NG 32:3 or 28:3.

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