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Role of the Carbohydrate Moiety and of α-L-Fucose in the Stabilization and the Dynamics of the *Lens culinaris* Agglutinin–Glycoprotein Complex. A Fluorescence Study

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Interaction between the fluorescent *Lens culinaris* agglutinin–fluorescein complex (LCA-FITC) and two glycoproteins, lactotransferrin (LTF) and serotransferrin (STF), was studied. The two glycoproteins have the same glycan structures, with one difference: the lactotransferrin glycans contain a fucose residue α-1,6-linked to the *N*-acetylglucosamine residue involved in the *N*-glycosylamine linkage. Fluorescence intensity quenching of the LCA–FITC complex shows that affinity between LCA and lactotransferrin is 50 times higher than that between LCA and serotransferrin, the fucose playing a major role in this high affinity (K_a is equal to 9.66 and 0.188 μM^{-1} for the LCA–LTF complex and LCA–STF complex, respectively). Time-resolved anisotropy decay indicates that the rotational correlation time of LCA (20 ns) does not change to a large extent whether the glycoproteins are bound to LCA or not. This suggests that there is no extended physical contact between LCA and the glycoproteins. The interaction between LCA and the glycoproteins occurs likely only via the carbohydrate chains, the STF and the LTF rotating almost-freely in the vicinity of LCA, with the glycans as an anchor.

KEY WORDS: *Lens culinaris* agglutinin–glycoprotein complex; carbohydrate moiety; α-L-fucose; lactotransferrin; serotransferrin; fluorescein; fluorescence intensity quenching; time-resolved anisotropy decay; protein dynamics.

INTRODUCTION

Lectins are proteins that play an important role in immunology and hematology and they are used as specific probes for membrane glycoprotein structures. They recognize and bind specific carbohydrate structures.[1,2] The binding constant of the specific free sugar with a lectin may be several orders of magnitude lower than that of a glycoconjugate containing this sugar.[3]

Lectins can be regarded as model systems for studying the molecular basis of protein–carbohydrate interactions since they are involved in cell–cell recognition events (for a review, see Ref. 4). Although lectins display extensive variations in chemical and physical properties, a structure–function relationship has been demonstrated among related lectins from leguminous plant seeds.[5]

Composed of two α and two β chains (MW = 5710 and 20,572 Da, respectively), the lentil lectin *Lens culinaris* agglutinin (LCA) is a $\alpha_2\ \beta_2$ tetramer with a molecular weight of 52,570 Da.[6] LCA is specific primarily for α-mannopyranosyl residues.[7] The lectin recognizes α-mannopyranosyl end groups or those substituted at the 0–2 position.[8] Additional requirements for strong binding involve the presence of an L-fucose residue α-1,6-linked to a *N*-acetylglucosamine (GlcNAc-1), which is linked to the protein via a *N*-glycosamine linkage.[9,10]

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Up to now, experiments related to the interaction between LCA and glycoproteins have been performed with isolated glycoprotein fragments. Therefore, the association constants between LCA and the glycoproteins were not yet determined.

Serotransferrins from blood plasma and lactotransferrins from mammalian milk are among the glycoproteins that bind to the LCA. Besides their role in iron transport and in the inhibition of the growth of microorganisms, human sero- and lactotransferrins share the following common properties: (i) their molecular mass is about 76 kDa;[11–15] (ii) they are constituted of a single polypeptide chain of 679 and 691 amino acid residues for sero- and lactotransferrin, respectively, organized in two lobes originating from a gene duplication; (iii) each lobe reversibly binds one Fe^{3+} ion; (iv) the protein moiety presents a high degree of homology (about 62%);[16] and (v) they are glycosylated (6.4%, by weight).[17]

The two lobes correspond to the N-terminal and C-terminal halves of the molecules and are tightly associated by noncovalent interactions.[18] Also, both are joined by a connecting short peptide of 12 and 11 amino acids in sero- and lactotransferrin, respectively. The three-dimensional pictures of the two proteins are perfectly superimposable, with very few differences.[19]

Human serotransferrin contains two glycans of the *N*-acetyllactosaminic type, located in the C-terminal lobe of the polypeptide chain. The two glycosylation sites (Asn-413 and -611) may be occupied by bi-, tri-, and tetraantennary glycans.[20,21]

Glycans of human lactotransferrins are located in both the N- and the C-domains, at three glycosylation sites (Asn-137, -478, and -624).[22,23] (For more details on the glycosylation of lacto- and serotransferrin and the spatial conformation of the glycans, see Refs. 24 and 25.)

Glycans of human serotransferrin are not fucosylated, while those of human lactotransferrin have an α-1,6-fucose bound to the *N*-acetylglucosamine residue linked to the peptide chain and an α-1,3-fucose bound to the *N*-acetyllactosamine residues. Since strong binding between the transferrins and the LCA requires the presence of the α-1,6-fucose,[9,10] the affinity between the lectin and the lactotransferrin should be more important than that between the lectin and the serotransferrin.

Fluorescence spectroscopy is currently used as a tool to study the interaction between proteins and to determine the stoichiometry and the binding constant of the complex.[26–29]

In this work, we studied the interaction between the fluorescent LCA–FITC complex and lactotransferrin (LTF) from human milk (LTF) and serotransferrin (STF) from human plasma. The interaction between the LCA and the two glycoproteins was performed following fluorescence intensity change of fluorescein bound to LCA. Our results indicate that the association constants (K_a) are 9.66 and 0.188 μM^{-1} for the LCA–LTF complex and LCA–STF complex, respectively. Therefore, the affinity between LCA and lactotransferrin is 50 times higher than that between LCA and serotransferrin, the fucose playing an important role in this difference.

Fluorescence spectroscopy with polarized light allows us to follow the dynamics of proteins.[30–37] In the present work, we studied the dynamics of the LCA–FITC complex in the absence and in the presence of LTF and STF. Fluorescence anisotropy decay indicate that the rotational correlation time (20 ns) of LCA is identical whether or not the glycoproteins are bound to LCA; i.e., there is no tight physical contact between the lectin and the glycoproteins. The interaction between the proteins involves only some amino acids of the lectins and a flexible region of the glycoproteins, the glycan moeity.

MATERIALS AND METHODS

The LCA–FITC complex was purchased from Sigma. Its concentration was determined spectrophotometrically using an extinction coefficient of 132.996 mM^{-1} cm^{-1} at 495 nm. Two molecules of fluorescein are bound to one molecule of LCA.

Lactotransferrin and serotransferrin were isolated and purified as described previously.[38] Their concentrations were obtained spectrophotometrically at 280 nm, with an absorption $E^{1\%}_{1\,cm}$ = 14.3 and 14.0, respectively.[39]

Absorbance data were obtained with a Perkin–Elmer 555 spectrophotometer using 1-cm-pathlength cuvettes.

Fluorescence intensity quenching experiments were performed with a Perkin–Elmer LS-5B spectrofluorometer. Bandwidths used for excitation and emission were 2.5 nm.

The quartz cuvettes had optical pathlengths of 1 and 0.4 cm for the emission and excitation wavelengths, respectively. The observed fluorescence intensities from fluorescein (λ_{ex} = 495 nm, λ_{em} = 515 nm) were corrected for the dilution. Correction for the absorption at the excitation and emission wavelengths was not necessary, since optical densities did not exceed 0.01.

Steady-state anisotropy was measured with the same Perkin–Elmer fluorometer used for fluorescence in-

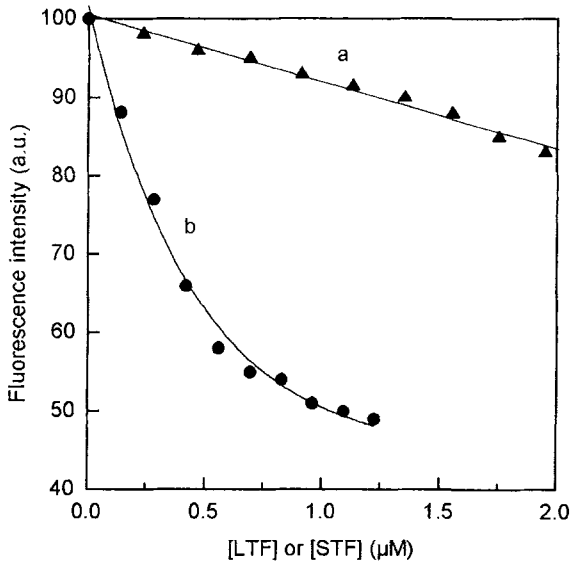

Fig. 1. Fluorescence intensity quenching of fluorescein bound to *Lens culinaris* agglutinin, as a result of serotransferrin–LCA (a) and of lactotransferrin–LCA (b) interactions. [LCA] = 0.7 μ*M*.

tensity quenching. Bandwidths used for excitation and emission were both 5 nm.

Anisotropy values were obtained from parallel and perpendicular intensities after substracting the Raman signal of the buffer.

Fluorescence lifetimes and fluorescence anisotropy decays of fluorescein bound to LCA were obtained by the time-correlated single-photon-counting technique, from the polarized components VV and VH, on the experimental setup installed on the SB_1 window of the Synchrotron Radiation machine super-ACO (Anneau de Collision d'Orsay).[40] The storage ring provides a light pulse with a full width at half-maximum of about 500 ps at a frequency of 8.33 MHz for a double-bunch mode. The excitation wavelength was set at 495 nm and the emission at 515 nm (bandwidth = 10 nm). Cumulation was stopped when 10^5 counts were stored in the peak channel for the total fluorescence intensity decay. The instrument response function was automatically monitored in alternation with the parallel and perpendicular components of the polarized fluorescence decay by measuring the sample-scattering light of the emission wavelength.[41,42] Analysis of the fluorescence intensity decay data as a sum of 150 exponentials was performed by the Maximum Entropy Method (MEM),[43] using the commercially available library of subroutines MEMSYS 5 (MEDC Ltd. UK) as a library of subroutines.[40–46]

All experiments were performed at 20°C in 10 m*M* phosphate, 0.143 *M* NaCl buffer, pH 7.

RESULTS

Binding Parameters of the LCA–LTF and LCA–STF Complexes

Binding of serotransferrin or lactotransferrin to the lectin–fluorescein complex induces a decrease in the fluorescence intensity of the fluorophore (Fig. 1). The decrease is important when lactotransferrin binds to the lectin, while it is not significant in the presence of serotransferrin. The concentration of LTF at saturation was determined by drawing the asymptote to the curve. The stoichiometry of the lectin–lactotransferrin complex is 1:1 (Fig. 1b), while that of the lectin–serotransferrin complex is not reached (Fig. 1a). These results indicate clearly that the affinity of lactotransferrin to the lectin is higher than that of serotransferrin.

Binding of either lactotransferrin or serotransferrin to the lectin does not change the anisotropy value of the LCA–FITC complex (the anisotropy, measured in parallel with the fluorescence intensity, was 0.159 ± 0.003 in the absence and in the presence of the glycoproteins). This result is in good agreement with the finding that the mean fluorescence lifetime $\langle\tau\rangle$ of the fluorescent complex does not change significantly in presence of the glycoproteins ($\langle\tau\rangle$ is 3.27 ± 0.803 and 3.1 ± 0.125 ns in the absence and in the presence of 0.25 μ*M* of lactotransferrin). Thus, a large part of the fluorescence intensity decrease has the nature of static quenching, and the observed mean fluorescence lifetime in the presence of the glycoproteins is the weighted average from the remaining free LCA–FITC complex and from the LCA–FITC–glycoprotein complex.

The dissociation constant of the complex was determined by fitting the data to Eq. (1), obtained from the balance of the total fluorescence:

$$\Delta F/F_o = (\Delta F_{max}/F_o)(L_b/P) \quad (1)$$

where ΔF, ΔF_{max}, F_o, L_b, and P are the fluorescence change for a concentration L of the glycoprotein, the maximum fluorescence change at saturation of the protein with the glycoprotein, the fluorescence intensity of LCA–FITC in the absence of glycoprotein, the concentration of bound glycoprotein, and the total concentration of LCA–FITC, respectively. The concentration of bound glycoprotein can be calculated from the root of the quadratic Eq. (2) arising from the definition of the binding constant:

$$L_b = 0.5\ \{(L + P + K_d) + [(L + P + K_d)^2 - 4LP]^{\frac{1}{2}}\} \quad (2)$$

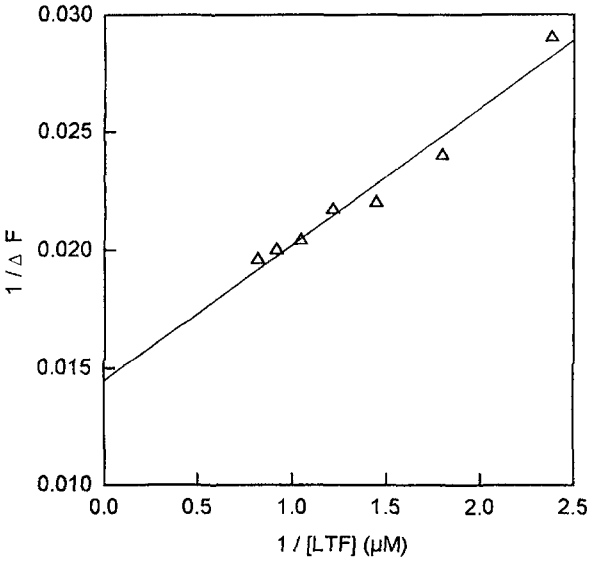

Fig. 2. Double-reciprocal plot of the LCA–FITC–LTF fluorescence intensity variation vs the LTF concentration.

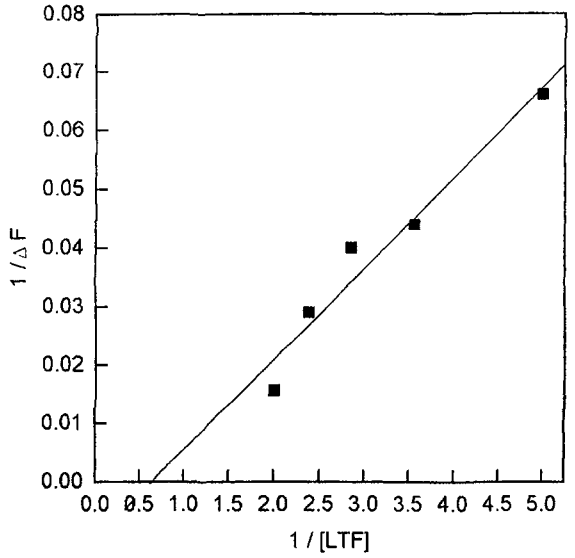

Fig. 3. Double-reciprocal plot of the LCA–FITC–LTF fluorescence intensity variation vs the LTF concentration. The data are obtained from the linear part (low concentrations of LTF) of the graph in Fig. 1b.

Replacing Eq. (2) in Eq. (1) gives Eq. (3):

$$\Delta F/F_o = (\Delta F_{max}/F_o) \times \{P + L + K_d + [(P + L + K_d)^2 - 4PL]^{\frac{1}{2}}\}/2P \quad (3)$$

ΔF_{max} was obtained by plotting $1/\Delta F$ as a function of $1/L$. For the LCA–LTF complex, we found a ΔF_{max} equal to 69.2 (Fig. 2). Since the data for STF binding on LCA does not show a curvature, it is not possible to calculate the ΔF_{max} of the STF–LCA interaction by plotting $1/\Delta F$ as a function of 1/[STF]. In fact, plotting the inverse of the linear region in Fig. 1b. (Fig. 3) does not allow the determination of the ΔF_{max} calculated from the inverse of all the data (Fig. 2). Therefore, we take 69.2 as the value of the maximum fluorescence change for both LCA–LTF and LCA–STF interactions. Equation (3) yields values of K_d equal to 0.1035 and 5.3 μM for the LCA–LTF and LCA–STF complexes, respectively. The equivalent association constants are 9.66 and 0.188 μM^{-1}.

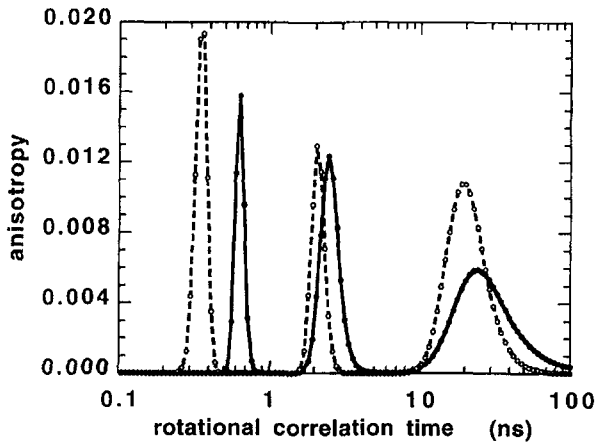

Fig. 4. Correlation–time profile of the FITC–LCA complex (open circles) and of the FITC–LCA–LTF complex (filled circles) at 20°C obtained by the Maximum Entropy Method. λ_{ex}, 495 nm; λ_{em}, 515 nm.

Rotational Correlation Time of LCA

The rotational correlation time (Φ_P) of a hydrated sphere is obtained from Eq. (4):[47,48]

$$\Phi_P(T) = \eta(T)3.8 \times 10^{-4} M \quad (4)$$

where M is the protein molecular weight (=52,570 Da) and η is the viscosity of the medium. At 20°C, the Φ_P value of LCA is 20 ns. In the presence of the LTF–LCA complex and if the two proteins rotate as one resulting spherical entity, M and Φ_P would be 128,570 Da (52,570 + 76,000 Da) and 49 ns, respectively.

The fluorescence anisotropy decay of the LCA–FITC complex shows three rotational correlation times: 0.36, 2.0, and 18.8 ns (Fig. 4). The value of χ^2 is 1.00. The shorter correlation time (360 ps) describes the motion of the fluorescein. This value does not exceed that measured for a completely mobile probe (0.1 ns).[49,50] The correlation time of 2 ns can be attributed to the

segmental motion of the binding site of the fluorescein. The value of 18 ns corresponds to the rotational correlation time of LCA. This value is close to that (20 ns) calculated theoretically for a globular protein [Eq. (4)].

In the presence of lactotransferrin, the fluorescence anisotropy decays with three rotational correlation times: 0.63, 2.50, and 24 ns (Fig. 4). The value of χ^2 is 1.030. The presence of the short rotational correlation time (0.63 ns) indicates that binding of LTF to LCA does not hinder the motion of the fluorescent probe. The value of 24 ns corresponds to the rotational correlation time of LCA and not to that (49 ns) of the LCA–LTF complex.

DISCUSSION

The affinity of LCA to LTF is more important than that observed for STF as shown in Fig. 1 and by the values of K_a. Thus, as established previously by either inhibition of agglutination or by interaction of complex oligosaccharides with the immobilized lectin,[9,10] the α(1 → 6) fucose present in the glycans of LTF plays an essential role in this high affinity.

The fact that no changes were observed in the fluorescence anisotropy of FITC with the glycoprotein concentrations indicates that the physical contact between the protein parts is very weak, even nonexistent; otherwise, the anisotropy would increase with the size of the complex.[51]

In the present study, fluorescence anisotropy decay experiments show three rotations: (i) local rotation of the fluorescein, (ii) rotation of a larger segment of the protein, and (iii) rotation of the whole molecule.

In theory, a fast local motion of the fluorophore would make measurements of the global rotation of the protein difficult, especially if the fluorescence lifetime is short (in our case, $\langle\tau\rangle$ = 3.2 ns) and if the rotational correlation time of the protein is very large compared to the fluorescence lifetime.[52,53] However, the difference between the two times was not really defined. Some examples follow.

(a) The rotational correlation time of the γ_G-immunoglobulins was measured using 1-dimethylaminonaphthalene-5-sulfonyl (DNS) as a probe ($\langle\tau\rangle$ = 9.5 ns), by steady-state anisotropy as a function of sucrose (Φ_P measured = 86 ns) [54] and by fluorescence anisotropy decay (Φ_P measured = 97 ns).[55]

(b) Lakowicz *et al.* measured a rotational correlation time of 44.6 ns at 5°C for human serum albumin, following fluorescence anisotropy quenching with oxygen of Trp residues ($\langle\tau\rangle$ = 5.4 ns).[56]

(c) Vincent *et al.* measured rotational correlation times of 63.4 and 46.5 ns at 10 and 20°C, respectively, by fluorescence anisotropy decay of the Trp residues ($\langle\tau\rangle$ = 1.76 and 1.56 ns at 10 and 20°C, respectively).[41]

Thus, one can measure a rotational correlation time that is 10 to 30 times higher than the fluorescence lifetime. In our case, as the fluorescein lifetime is 3.2 ns, it is, in principle, possible to measure a rotational correlation time of 50 ns (≃16 times the fluorescence lifetime).

The long correlation time (18.8 ns) measured from the anisotropy decay (Fig. 4) is close to that (20 ns) calculated theoretically for LCA alone or to that (18.6 ns) measured with the TNS as a probe.[57] When LTF or STF is bound to LCA, the long correlation time (24 ns) is still in the same range as the theoretical value (20 ns). If the lectin–glycoprotein complex rotates as one entity, the rotational correlation time would be expected to be 49 ns. As this is not the case, our results suggest the absence of any strong physical interaction between the protein part of the glycoprotein and LCA. Thus, the possibility of a strong interaction between the glycans of LTF and STF with some amino acids of LCA has been established. In fact, X-ray diffraction studies have indicated that Tyr β 100 and Trp β 128 make Van der Waals contact with α-L-fucose.[6] These interactions increase the affinity between LCA and the glycoproteins.

X-ray diffraction studies on the complex *Lathyrus ochrus* isolectin II–glycosyl fragment of human lactotransferrin have indicated that the oligosaccharide adopts an extended conformation, suggesting that the peptide part of the glycopeptide has no influence on the binding.[58,59] Thus, as we did not observe any significant increase in the rotational correlation time of LCA or in the anisotropy of the fluorescein in the presence of the glycoproteins, we may conclude that there is no rigid contact between the protein parts. This may be the result of the extended conformation of the carbohydrate moiety of LTF and STF.

X-ray diffraction and fluorescence studies performed on LCA have indicated that the carbohydrate binding site is flexible.[6,57] The carbohydrates in LTF and STF are highly flexible.[60] This flexibility is maintained when the glycoproteins are bound to the LCA. Otherwise, the carbohydrates will not conserve their extended conformation and we observe a more compact structure between LCA and the glycoproteins; i.e., we observe an increase in the rotational correlation time of the LCA–glycoprotein complex. Thus, if the α(1 → 6) fucose increases the affinity of the glycoproteins to LCA, it does

not hinder the global motion of the carbohydrate and does not modify their spatial conformation.

From the results obtained in our work, we suggest that the LTF rotates freely in the vicinity of LCA, with the carbohydrates as an anchor.

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REFERENCES

1. I. E. Liener (1976) *Annu. Rev. Plant Physiol.* **27**, 291–319.
2. N. Sharon (1977) *Sci. Am.* **236**, 108–119.
3. R. Kornfeld and C. Ferris (1975) *J. Biol. Chem.* **250**, 2614–2619.
4. H. Lis and N. Sharon (1986) *Annu. Rev. Biochem.* **53**, 35–67.
5. A. Foriès, R. De Niève, and A. D. Strosberg (1979) *Phys. Véget.* **17**, 597–606.
6. R. Loris, J. Steyaert, D. Maes, J. Lisgarten, R. Pickersgill, and L. Wyns (1993) *Biochemistry* **32**, 8772–8781.
7. S. Toyoshima, T. Osawa, and A. Tonomura (1970). *Biochim. Biophys. Acta* **221**, 514–521.
8. R. Kaifu, T. Osawa, and R. W. Jeanloz (1975) *Carbohydr. Res.* **40**, 111–117.
9. H. Debray, D. Decout, G. Strecker, G. Spik, and J. Montreuil (1981) *Eur. J. Biochem.* **117**, 41–55.
10. K. Kornfeld, M.-L. Reitman, and R. Kornfeld (1981) *J. Biol. Chem.* **256**, 6633–6640.
11. D. Léger, B. Campion, J. P. Decottignies, J. Montreuil, and G. Spik (1989) *Biochem. J.* **257**, 231–238.
12. M. J. Powell and J. E. Ogden (1990) *Nucleic Acids Res.* 4013.
13. A. B. Mason, M. K. Miller, W. D. Funk, D. K. Banfield, J. Savage, R. W. A. Olivier, B. N. Green, R. T. A. MacGillivray, and R. C. Woodworth (1993) *Biochemistry* **32**, 5472–5479.
14. V. Salmon, D. Legrand, B. Georges, M. C. Slomianny, B. Coddeville, and G. Spik (1997) *Protein Expr. Purif.* **9**, 203–210.
15. B. Coddeville, H. Carchon, J. Jaeken, G. Briand, and G. Spik (1998) *Glycoconjugate J.* (in press).
16. J. Mazurier, M. H. Metz-Boutique, J. Jollès, G. Spik, J. Montreuil, and P. Jollès (1983) *Experientia* **39**, 135–141.
17. G. Spik, B. Coddeville, J. Mazurier, Y. Bourne, C. Cambillaut, and J. Montreuil (1994) *Adv. Exp. Med. Biol.* **357**, 21–32.
18. D. Legrand, J. Mazurier, D. Colavizza, J. Montreuil, and G. Spik (1990) *Biochem. J.* **266**, 575–581.
19. E. N. Baker and P. F. Lindley (1992) *J. Inorg. Biochem.* **47**, 147–160.
20. M. W. C. Hatton, L. März, L. R. Berry, M. T. Debanne, and E. Regoeczi (1979) *Biochem. J.* **181**, 633–638.
21. L. März, M. W. C. Hatton, L. R. Berry, and E. Regoeczi (1982) *Can. J. Biochem.* **60**, 624–630.
22. M. H. Metz-Boutique, J. Jollès, J. Mazurier, F. Schoentgen, D. Legrand, G. Spik, J. Montreuil, and P. Jollès (1984) *Eur. J. Biochem.* **145**, 659–676.
23. P. H. C. Van Berkel, H. A. Van Veen, M. E. J. Geerts, H. A. De Boer, and J. H. Nuijens (1996) *Biochem. J.* **319**, 117–122.
24. J. Montreuil (1984) *Biol. Cell.* **51**, 115–132.
25. J. Montreuil, G. Spik, and J. Mazurier (1997) in J. Montreuil, J. F. G. Vliegenthart, and H. Schechter (Eds.), *Glycoproteins, New Comprehensive Biochemistry*, Elsevier, Amsterdam, pp. 203–242.
26. M. A. Thomas, M. Gervais, V. Favaudon, and P. Valat (1983) *Eur. J. Biochem.* **135**, 577–581.
27. J. Albani (1985) *Arch. Biochem. Biophys.* **243**, 292–297.
28. J. Albani (1997) *Photochem. Photobiol.* **66**, 72–75.
29. J. Albani, R. Vos, K. Willaert, and Y. Engelborghs (1995) *Photochem. Photobiol.* **62**, 30–34.
30. J. R. Lakowicz, E. Gratton, H. Cherek, B. P. Maliwall, and G. Lackzko (1984) *J. Biol. Chem.* **259**, 10967–10972.
31. J. Albani and B. Alpert (1986) *Chem. Phys. Lett.* **131**, 147–152.
32. R. F. Steiner and L. Norris (1987) *Biopolymers* **26**, 1189–1204.
33. J. Albani (1998) *Spectrochimica Acta. Part A.* **54**, 175–183.
34. J. Albani (1994) *J. Biochem.* **116**, 625–630.
35. J. Albani (1996) *Biochim. Biophys. Acta* **129**, 215–220.
36. F. Shen, S. J. Triezenberg, P. Hensley, D. Porter, and J. R. Kunston (1996) *J. Biol. Chem.* **271**, 4819–4826.
37. J. Albani (1997) *Biochim. Biophys. Acta* **1336**, 349–359.
38. D. Legrand, J. Mazurier, J.-P. Aubert, M. H. Loucheux-Lefebvre, J. Montreuil, and G. Spik (1986) *Biochem. J.* **236**, 839–844.
39. M. A. Krysteva, J. Mazurier, and G. Spik (1976) *Biochim. Biophys. Acta* **453**, 484–493.
40. O. P. Kuipers, M. Vincent, J.-C. Brochon, M. H. Verheij, G. H. De Hass, and J. Gallay (1991) *Biochemistry* **30**, 8771–8785.
41. M. Vincent, I. M. Li de la Sierra, M. N. Berberan-Santos, A. Diaz, M. Diaz, G. Padron, and J. Gallay (1992) *Eur. J. Biochem.* **210**, 953–961.
42. M. Vincent, A. M. Deveer, G. H. De Hass, M. H. Verheij, and J. Gallay (1993) *Eur. J. Biochem.* **215**, 531–539.
43. A. K. Livesey and J.-C. Brochon (1987) *Biophys. J.* **52**, 693–706.
44. P. Blandin, F. Mérola, J.-C. Brochon, O. Trémeau, and A. Ménez (1994) *Biochemistry* **33**, 2610–2619.
45. J. Sopkova, J. Gallay, M. Vincent, P. Pancoska, and A. Lewit-Bentley (1994) *Biochemistry* **33**, 4490–4499.
46. E. H. W. Pap, J. J. Ter Horst, A. Van Hoek, and A. J. W. G. Visser (1994) *Biophys. Chem.* **48**, 337–351.
47. A. J. W. G. Visser, N. H. G. Penners, and F. Müller (1983) in H. Sund and C. Veeger (Eds.), *Mobility and Recognition in Cell Biology*, Walter de Gruyter, Berlin, pp. 137–152.
48. R. Leenders, A. Van Hoek, M. Van Iersel, C. Veeger, and A. J. W. G. Visser (1993) *Eur. J. Biochem.* **218**, 977–984.
49. B. J.-M. Thevenin, N. Periasamy, S. B. Shohet, and A. S. Verkman (1994) *Proc. Natl. Acad. Sci. USA* **91**, 1741–1745.
50. J. Oton, E. Bucci, R. F. Steiner, C. Fronticelli, D. Franchi, J. Montemarano, and A. Martinez (1981) *J. Biol. Chem.* **256**, 7248–7256.
51. V. Le Tilly and C. A. Royer (1993) *Biochemistry* **32**, 7753–7758.
52. S. Anderson and G. Weber (1966) *Arch. Biochem. Biophys.* **116**, 207–223.
53. G. Weber (1966) in K. Kustin (Ed.), *Methods in Enzymology. Vol. XVI. Fast Reactions*, Academic Press New York, pp. 380–394.
54. J. K. Weltman and G. M. Edelman (1967) *Biochemistry* **6**, 1437–1447.
55. J.-C. Brochon and P. Wahl (1972) *Eur. J. Biochem.* **25**, 20–32.
56. J. R. Lakowicz, B. P. Maliwal, H. Cherek, and A. Balter (1983) *Biochemistry* **22**, 1741–1752.
57. J. Albani (1996) *J. Fluoresc.* **6**, 199–208.
58. Y. Bourne, J. Mazurier, D. Legrand, P. Rougé, J. Montreuil, G. Spik, and C. Cambillau (1994) *Structure* **2**, 209–219.
59. Y. Bourne, H. Van Tilbeurgh, and C. Cambillau (1993) *Curr. Opin. Struct. Biol.* **3**, 681–686.
60. M. Dauchez, J. Mazurier, J. Montreuil, G. Spik, and G. Vergoten (1992) *Biochimie* **74**, 63–74.