N-Linked glycosylation of *Drosophila* rhodopsin occurs exclusively in the amino-terminal domain and functions in rhodopsin maturation

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Abstract Immature *Drosophila* rhodopsin is *N*-glycosylated, but undergoes complete deglycosylation during the process of protein maturation. In order to elucidate the site of glycosylation and its role in rhodopsin synthesis, we investigated the in vitro and in vivo synthesis of rhodopsin whose putative *N*-glycosylation sites (Asn-20 and Asn-196) were replaced by isoleucine. The results demonstrated that immature rhodopsin binds a single oligosaccharide chain exclusively at Asn-20 in the N-terminal extracellular domain. Furthermore, the results gave the first evidence directly indicating that deletion of the oligosaccharide chain markedly impedes rhodopsin maturation.

Key words: Rhodopsin; *ninaE*; Glycosylation; Photoreceptor cell; Protein maturation; *Drosophila melanogaster*

1. Introduction

N-Linked glycosylation is often observed in the membrane and secretory proteins. Extensive studies on the roles of glycosylation have been carried out, and elucidated its roles as the recognition determinants for cell-protein and cell-cell interaction [1]. In addition to these extracellular functions, another role of glycosylation in intracellular events has recently been noted. For instance, correct folding and subcellular distribution require glycosylation in several kinds of proteins [2,3]. The visual pigment rhodopsin is a light-sensitive membrane protein included in the superfamily of G-protein-coupled membrane receptors. In vertebrates, rhodopsin undergoes *N*-glycosylation [4] in addition to some other post-translational modifications. Microscopic analysis of the frog retina revealed that tunicamycin, a drug that blocks *N*-glycosylation, inhibits disk morphogenesis and the incorporation of the newly synthesized opsin into rod photoreceptor outer segment (ROS) [5–7]. While these data suggest that *N*-glycosylation of rhodopsin is required for rhodopsin localization to ROS, other studies using tunicamycin on bovine rhodopsin contrarily suggest that glycosylation is not essential for its proper localization [8,9]. These results thus indicate that the role of rhodopsin *N*-glycosylation is still not clear.

In contrast to vertebrate rhodopsin, fly rhodopsin is not glycosylated in its mature form [10,11]. However, it was found that glycosylated opsin (an apoprotein of rhodopsin) is accumulated when flies were raised in the carotenoid-deprived condition [12,13]. Carotenoid is a precursor of the rhodopsin chromophore, 3-hydroxyretinal, and the glycosylated opsin in the carotenoid-deprived fly is an immature intermediate of rhodopsin before binding the chromophore. The intermediate stays in the rough endoplasmic reticulum (rER) presumably due to improper folding of the peptide. When the deprived fly is provided with chromophore, the intermediate binds the chromophore, takes the correct conformation, and is exported from the rER. The oligosaccharide chain of the intermediate is trimmed during the transport from rER toward the rhabdomeric membrane of photoreceptor cells, and completely removed when it reaches the rhabdomeric membrane as a mature rhodopsin. These characteristics of fly rhodopsin suggest that the oligosaccharide chain functions in the pathway of rhodopsin synthesis and transport.

Drosophila rhodopsin has two asparagine residues (Asn-20 and Asn-196) possibly provided for *N*-glycosylation [14,15]. In a previous study, O'Tousa constructed a transgenic fly, Δ*Asn20*, in which Asn-20 of rhodopsin was replaced by isoleucine (N20I) to eliminate one of two possible glycosylation sites [16]. In this transgenic fly, the amount of mature rhodopsin is markedly reduced, while the opsin mRNA level is equal to that in the wild-type control. A trace amount of N20I rhodopsin, however, appears to function normally to initiate the phototransduction cascade. Although these results suggest that the substitution of Asn-20 to Ile affects the synthesis or degradation of rhodopsin, there is no direct evidence that Asn-20 is actually glycosylated in immature rhodopsin, and whether Asn-196 also undergoes glycosylation or not. Furthermore, it still remains obscure how and in which step of rhodopsin metabolism the glycosylation functions. In order to answer these questions, we here analyze the protein synthesis and maturation in two kinds of rhodopsin mutants, N20I and N196I, using both the cell-free translation system and transgenic flies.

2. Materials and methods

All experiments were carried out on white-eyed (*w*) *Drosophila melanogaster* (Oregon R). Transgenic flies, Δ*Asn20* and Δ*Asn196*, were constructed in J.E. O'Tousa's laboratory. Methods for the construction of Δ*Asn20* have been previously described [16], and were used for the construction of the Δ*Asn196* transgenic mutant, too. Flies were maintained at 25°C on a carotenoid-rich medium (6% yellow corn meal, 5% dry yeast, 3.2% sucrose, and 0.5–1.5% agar) under a 12 h light/12 h dark cycle of fluorescent lighting at an intensity of 50 lux. Carotenoid deprivation was achieved by raising flies from egg to adult on a medium composed of 10% dry yeast, 10% sucrose, 0.02% cholesterol and 1–2% agar. Unless specified, flies used for experiments were kept in constant darkness from third instar larva to adult in order to

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Abbreviations: ROS, rod photoreceptor outer segment; rER, rough endoplasmic reticulum; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PNGaseF, peptide-*N*-glycosidase F; PPIase, peptidylproline *cis-trans*-isomerase

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minimize the light-dependent damage of rhodopsin and photoreceptor structure.

Poly(A)$^+$ RNA was isolated from *Drosophila* heads by an oligo-dT-cellulose column, and reverse-transcribed using a primer of poly-dT sequence added to the end of linearized pUC9 vectors. cDNA encoding opsin was amplified using a polymerase chain reaction (PCR) with a vector primer (M13 Primer RV, Takara) and a synthetic oligonucleotide primer corresponding to the N-terminal sequence of opsin [14,15]. The amplified cDNA fragments consisting of a whole coding sequence and the full length of the 3′ non-coding region were inserted into pBS (Stratagene) vector plasmid. The sequence of the cDNA clone was confirmed by the dideoxynucleotide chain-termination method [17]. cDNA fragments that encode the mutant opsins lacking putative *N*-glycosylation sites were synthesized by PCR using mismatched oligonucleotide primers including the coding sequences for residues 18–28 and 194–199 of opsin (5′-GTGACCTTGTCCAC-CACCGATCCAA(←T)TGGAC-3′ for N20I, 5′-CGAGGTCAG-GA(←T)TACCCTCCG-3′ for N196I), respectively. Each primer contained a single replacement (bold letters) for the residue in parentheses, which causes substitution of Asn-20 or Asn-196 to Ile. The complete cDNAs of N20I and N196I were prepared by replacing the adequate restriction fragments in the wild-type (*Eco*RI-*Tth*111I for N20I, *Eco*T38-*Bst*EII for N196I) with the mutagenized cDNA fragments. The sequences of the mutant cDNAs were confirmed by sequencing as described above.

cDNA encoding wild-type or each mutant rhodopsin was inserted into the transcription vector (pBS) carrying a T7 transcriptional promoter. RNA was transcribed from the linearized pBS-rhodopsin construct and 5′ capped using an mCAP mRNA capping kit (Stratagene) according to the instruction manual. RNA derived from each pBS-rhodopsin construct (0.15 mg) was translated using a rabbit reticulocyte lysate translation kit (DuPont) containing [^{35}S]methionine (293.0–445.8 kBq, 39.9–44.1 TBq/mmol, ICN biomedicals and DuPont). Unless otherwise noted, each reaction mixture (12.5 μl) was supplemented with 1 μl of canine pancreatic microsome suspension (DuPont). Translation reactions were carried out at 20°C for 3.0 h (Fig. 2A), 3.5 h (Fig. 2B) or 1 h (Fig. 3). Radiolabeling was terminated by the addition of cold methionine or putting the mixture on ice. Microsome membranes in the reaction mixture were precipitated by centrifugation at 15 000×*g* for 30 min at 4°C, solubilized in SDS loading solution (2.3% SDS, 5% 2-mercaptoethanol, 5% glycerol, 62.5 mM Tris-HCl [pH6.8]), and subjected to SDS-PAGE. For a control experiment, translation was carried out without microsome membranes. In this case, the reaction mixture was centrifuged after the translation, and the resultant supernatant was mixed with 4×SDS loading solution and subjected to SDS-PAGE.

Drosophila retinas were prepared from young flies (within 24 h after eclosion). Unless otherwise noted, all procedures were carried out under dim red light according to the method previously described [12]. Briefly, compound eyes were dissected out of the heads and immersed in ice-cold distilled water (100 μl). After 10 min on ice, retinas were separated from corneas and homogenized. The retinal membranes were collected by centrifugation, solubilized in SDS loading solution, and subjected to SDS-PAGE. In vivo radiolabeling of opsin was carried out according to the method described previously [12].

Proteins in each sample were separated by SDS-PAGE [18] with 12.5% polyacrylamide gels. To get autoradiograms, gels were usually stained with Coomassie brilliant blue R-250, treated with radioactivity enhancer solution (EN^{3}HANCE, New England Nuclear), dried in a vacuum drier, and exposed to X-ray film (O-mat AR, Kodak) for 3 days. For the autoradiogram shown in Fig. 2B, the treatment with enhancer solution was omitted, and the dried gel was subjected to exposure against an imaging plate (Fuji Photo Film) for an Image Analyzer BAS2000 (Fuji Photo Film). Immunoblotting was performed as described previously [12] using a monoclonal antibody to the major *Drosophila* rhodopsin [10]. To remove *N*-linked oligosaccharide chains from opsin, the in vitro translation mixture (containing 0.2 μl microsomal membranes) or retinal membrane suspension (containing 12 retinas) was centrifuged to yield the membrane fraction. The fraction was solubilized in 2 μl of SDS loading solution, diluted to 23 μl by adding *n*-octylglucoside (2% final concentration) and peptide *N*-glycosidase F (PNGase F, 2.2 mM final concentration, Boehringer Mannheim) solutions, and incubated at 37°C for 17 h. The

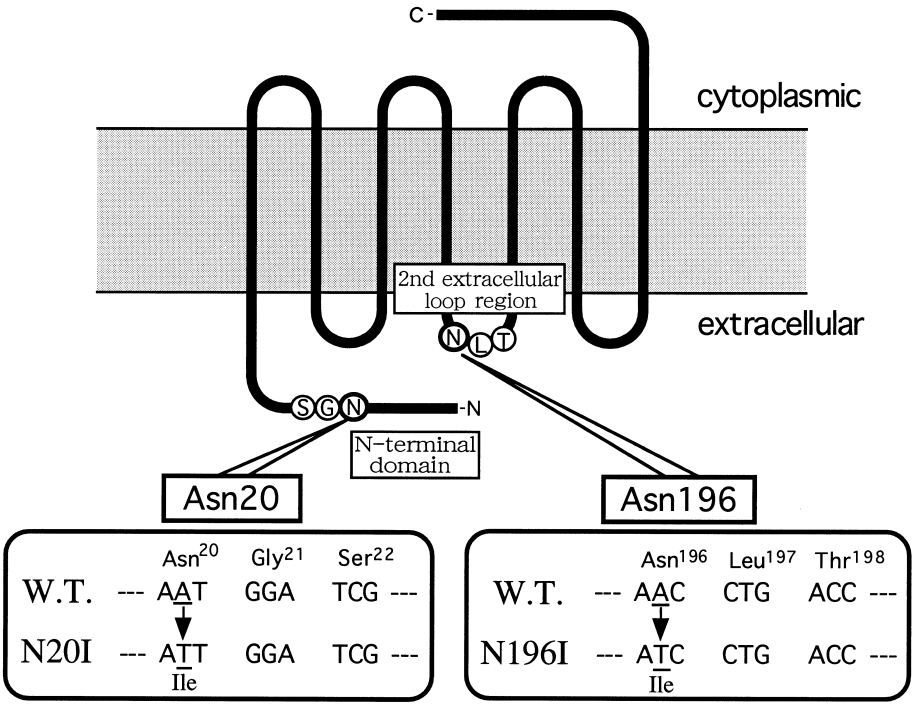

Fig. 1. Schematic model of major *Drosophila* rhodopsin showing two putative *N*-glycosylation sites and the mutations used for their substitutions. *N*-Linked oligosaccharide chain can be attached to the Asn residue in the sequence Asn-X-Ser/Thr (where X is any amino acid). The major *Drosophila* rhodopsin contains two possible glycosylation sites, Asn-20 and Asn-196, in the N-terminal and the second extracellular loop regions, respectively. Lower panels show the partial peptide and DNA sequences of the wild-type and two mutant proteins, N20I and N196I. A single nucleotide was changed in each of the mutant DNAs to replace one of the asparagine residues to isoleucine. Details are described in Section 2.

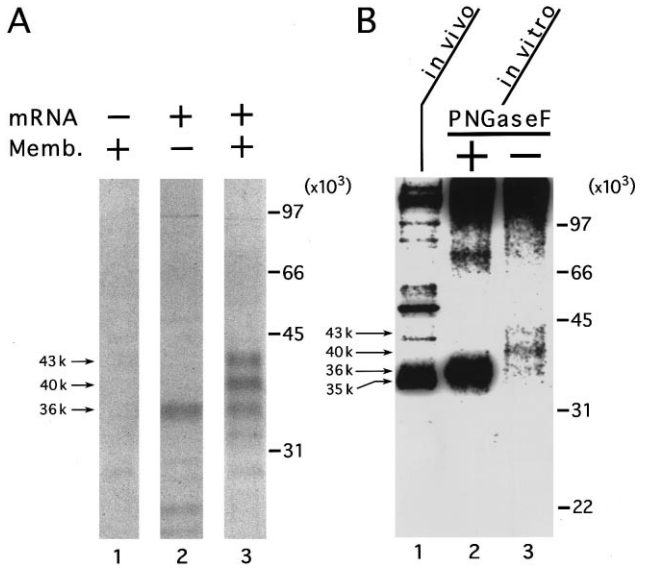

Fig. 2. In vitro translation of wild-type opsin in the cell-free translation system. A: mRNA of wild-type opsin was translated in the reticulocyte lysate translation system supplemented with microsome membranes. Lane 1, control reaction without mRNA showing that no labeled products are detectable. Lane 2, another control reaction without microsome membranes. Only a non-glycosylated core peptide (36 kDa) is detectable. Lane 3, translation products in the presence of both mRNA and microsome membranes. Two kinds of opsin peptides (40 kDa and 43 kDa) can be identified in addition to the 36 kDa core peptide. B: PNGaseF digestion of in vitro translation products. In the absence of PNGaseF, three different bands of opsin peptide were detected at 36, 40 and 43 kDa (lane 3). By digesting with PNGaseF, the apparent molecular weights of the two larger opsin peptides (40 kDa and 43 kDa) shift to 36 kDa (lane 2). Mature rhodopsin synthesized in vivo (35 kDa) was also electrophoresed in lane 1. Note that the core peptide of opsin (36 kDa) is still larger than mature rhodopsin (35 kDa). The positions and molecular weights of protein size markers are indicated on the right.

solution was then mixed with 7 μl of 4×SDS loading solution for SDS-PAGE.

3. Results

The major *Drosophila* rhodopsin encoded by the *ninaE* gene has two possible glycosylation sites within the N-terminal and second extracellular loop regions, respectively (Fig. 1) [14,15]. To determine the actual numbers and sites of *N*-glycosylation in the immature rhodopsin, we first carried out in vitro synthesis of opsin using a cell-free translation system. In order to enable protein integration into the membrane and *N*-glycosylation of the peptide, we added canine microsomal membranes to the translation system of rabbit reticulocyte lysate. In the absence of microsomal membranes, a single kind of polypeptide having an apparent molecular weight of 36 kDa was synthesized from the wild-type opsin mRNA (Fig. 2A, lane 2). On the other hand, no translated product was detected in the control experiment without opsin mRNA (Fig. 2A, lane 1). Because the rabbit reticulocyte lysate used here does not contain a membrane fraction, the products do not undergo post-translational modifications that require rER or Golgi membranes. Therefore, the above results indicate that the 36 kDa peptide is a core peptide of opsin without *N*-glycosylation. We next examined the opsin synthesis in the presence of microsomal membranes. Two kinds of polypeptides, 40 kDa and 43 kDa, were synthesized in addition to the 36 kDa core peptide (Fig. 2A, lane 3). To examine whether the 40 kDa and 43 kDa peptides are *N*-glycosylated or not, these products were digested with peptide-*N*-glycosidase F (PNGaseF), which cleaves the linkage between asparagine and every type of *N*-linked oligosaccharide chain (Fig. 2B). By the treatment with PNGaseF, two upper bands of opsin (40 kDa and 43 kDa) completely disappeared, and the 36 kDa core opsin increased instead (Fig. 2B, lane 2). This result indicates that the 40 kDa and 43 kDa peptides are both *N*-glycosylated products of the 36 kDa core opsin. *Drosophila* opsin encoded by the *ninaE* gene has two possible sites for *N*-glycosylation (Fig. 1). It is therefore most likely that the 40 kDa and 43 kDa peptides bind one and two oligosaccharide chains, respectively. In Fig. 2B, mature rhodopsin (lane 1) synthesized in vivo was also electrophoresed with cell-free translated products. It should be noted that the 36 kDa core opsin is slightly larger than mature rhodopsin (35 kDa). This suggests that, during maturation in vivo, rhodopsin would undergo additional modification reducing the apparent molecular weight from 36 kDa to 35 kDa.

Using the same system as above, we next synthesized two mutant opsins, N20I and N196I, whose Asn-20 and Asn-196 were replaced by Ile. As shown in Fig. 3 (lanes 2 and 3), 36 kDa and 40 kDa peptides were synthesized in both mutant opsins, while the 43 kDa peptide was not detected in either case. Because each mutant opsin carries a single *N*-glycosylation site, the result indicates that the 40 kDa peptide binds a single oligosaccharide chain at Asn-196 (N20I) or Asn-20 (N196I). The apparent molecular weight of the 40 kDa peptide is very similar to that of immature opsin accumulated in rER of carotenoid-deprived flies [12]. Because the initial struc-

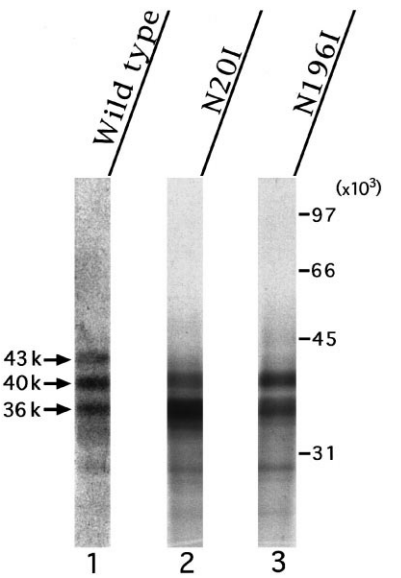

Fig. 3. In vitro translation and *N*-glycosylation of mutant opsins N20I and N196I. mRNA of wild-type (lane 1), N20I (lane 2) and N196I (lane 3) opsins was translated in the reticulocyte lysate translation system supplemented with microsomal membranes. The products derived from the mutant mRNAs lack the largest peptide (43 kDa), while 36 kDa and 40 kDa peptides can be synthesized from each mutant mRNA. Note that *N*-glycosylated opsin (40 kDa) in the N196I mutant (lane 3) is more abundant than that in N20I (lane 2). The numbers and small bars on the right indicate the positions and molecular weights of protein size markers.

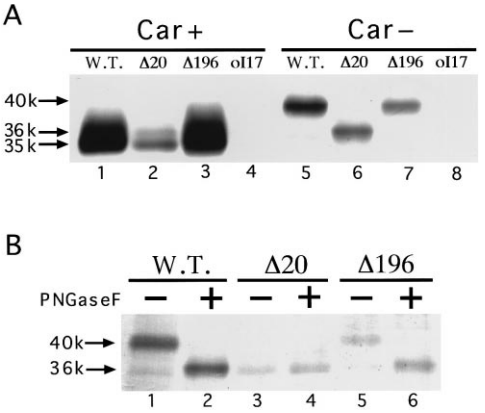

Fig. 4. Immunoblot analysis of rhodopsin maturation in the Δ*Asn20* and Δ*Asn196* transgenic mutants. A: Wild-type (lanes 1, 5), Δ*Asn20* (lanes 2, 6), Δ*Asn196* (lanes 3, 7) and *ninaE*^{oI17} (lanes 4, 8) flies were raised under carotenoid-replete (Car+, lanes 1–4) or carotenoid-deprived (Car−, lanes 5–8) conditions. In the Car− condition, 40 kDa immature opsin is accumulated in the wild-type and Δ*Asn196* flies, whereas 36 kDa immature opsin is synthesized in the Δ*Asn20* flies. Note that the amount of immature opsin in Δ*Asn20* is similar to that in Δ*Asn196*. In the Car+ condition, the wild-type, Δ*Asn20* and Δ*Asn196* flies yield 35 kDa mature rhodopsin. In Δ*Asn20*, however, the amount of mature rhodopsin is greatly reduced, and 36 kDa immature opsin coexists with mature rhodopsin. *ninaE*^{oI17} is a null mutant of major *Drosophila* rhodopsin, showing no opsin peptide in either Car+ or Car− condition. Extract from one eye (lane 1) or five eyes (lanes 2–8) is loaded in each lane. B: *N*-Glycosylation of immature opsin in the wild-type (lanes 1, 2), Δ*Asn20* (lanes 3, 4) and Δ*Asn196* (lanes 5, 6) flies. Retinal extracts from carotenoid-deprived flies were incubated with (lanes 2, 4, 6) or without (lanes 1, 3, 5) PNGaseF. The enzyme reduces the size of the 40 kDa immature opsin to 36 kDa (wild-type and Δ*Asn196*), but is ineffective against 36 kDa immature opsin (Δ*Asn20*).

ture of the oligosaccharide chain and its processing in rER is common in all eukaryotes examined [19], it is most likely that the 40 kDa immature opsin synthesized in vivo also has a single oligosaccharide chain similar to that binding to opsin synthesized in the cell-free system. In addition, Fig. 3 shows that the amount of the 40 kDa peptide of N20I opsin is less than that of N196I opsin. This result suggests that the Asn-196 residue of N20I opsin is less easily glycosylated than Asn-20 of N196I opsin.

Based on the above results, we hypothesized that the 40 kDa intermediate of opsin synthesized in vivo would bind a single oligosaccharide chain at Asn-20. In order to examine this hypothesis, we newly constructed a transgenic fly, Δ*Asn196*, in addition to Δ*Asn20* developed by O'Tousa [16,20]. In a previous study, we demonstrated that the glycosylated intermediate of rhodopsin (40 kDa) is accumulated when wild-type flies are raised in the carotenoid-deprived condition [12]. Therefore, we first investigated rhodopsin synthesis in the carotenoid-deprived condition. As shown in Fig. 4A (Car−), Δ*Asn196* mutant flies accumulated 40 kDa opsin (lane 7) as well as wild-type flies (lane 5). Digestion of the 40 kDa opsin with PNGaseF reduced its apparent molecular weight to 36 kDa (Fig. 4B, lanes 2 and 6), indicating that the 40 kDa opsin is composed of a 36 kDa core peptide and an *N*-linked oligosaccharide chain. This result demonstrates that the rhodopsin intermediate in the Δ*Asn196* mutant can be glycosylated in the same manner as that in the wild-type fly, and that Asn-196 is not essential for the glycosylation of the rhodopsin intermediate. In contrast, the Δ*Asn20* fly synthesized a 36 kDa opsin (Fig. 4A, lane 6), whose apparent molecular weight was not changed by the PNGaseF digestion (Fig. 4B, lane 4). Therefore, it is clear that Asn-20 is indispensable for glycosylation of the rhodopsin intermediate. Based on the above in vitro and in vivo results, we conclude that the 40 kDa intermediate of rhodopsin synthesized in the *Drosophila* photoreceptor cell is a glycoprotein binding a single oligosaccharide chain at Asn-20.

In order to elucidate the metabolic process to which *N*-glycosylation contributes crucially, we next compared the amounts of mature and immature rhodopsins between Δ*Asn20* and Δ*Asn196* mutants. As shown in Fig. 4A, the Δ*Asn20* mutant contains as much immature rhodopsin as the Δ*Asn196* fly (lanes 6 and 7). This result indicates that *N*-glycosylation at Asn-20 is not essential for the synthesis and stabilization of the immature intermediate of rhodopsin. In contrast, the amount of mature rhodopsin synthesized in the carotenoid-replete (Car+) fly was dramatically reduced in the Δ*Asn20* mutant (lane 2) compared with that in Δ*Asn196* (lane 3), although no significant difference in amount of opsin mRNA was found between these mutants (data not shown). These results, therefore, demonstrate that the defect in *N*-glycosylation at Asn-20 greatly inhibits the maturation process from the immature intermediate to the mature form of rhodopsin. Furthermore, it should be noted that, in the Δ*Asn20* mutant, the 36 kDa immature opsin coexists with mature rhodopsin, even when they are raised under carotenoid-replete condition (Fig. 4A, lane 2). Because the immature intermediate (40 kDa) is found neither in the wild-type nor Δ*Asn196* flies under the same condition, this result also indicates that the maturation process is largely blocked by the lack of the oligosaccharide chain at Asn-20. In conclusion, our present results demonstrate that *N*-glycosylation at Asn-20 would contribute to the acceleration of rhodopsin maturation rather than the synthesis and stabilization of immature rhodopsin.

4. Discussion

Recent studies on *N*-glycosylation revealed that the *N*-linked oligosaccharides play important roles in intracellular processes such as protein maturation as well as in extracellular events [21,22]. *Drosophila* rhodopsin is co-translationally glycosylated and processed during its maturation. However, few studies on its glycosylation have been carried out [16,20], because the *N*-linked oligosaccharide is detectable only in the immature molecules of rhodopsin, whose amount is not sufficient for biochemical analysis. In the present study, we first determined the actual glycosylation site by constructing mutant opsins lacking possible glycosylation sites and expressing them both in vitro and in vivo. The results demonstrated that *Drosophila* rhodopsin is glycosylated exclusively at Asn-20 in the extracellular N-terminal region. Binding of carbohydrate in the N-terminal region has also been reported in some other visual pigments [4,23]. In addition, the possible *N*-glycosylation site in the N-terminal region is conserved throughout most members of the G-protein-coupled receptor family. It is therefore very likely that *N*-linked oligosaccharides in the N-terminal region may have a common function in these receptors.

In *Drosophila*, Asn-196 in the second extracellular loop of opsin does not undergo *N*-glycosylation in vivo. Nevertheless, the consensus sequence for *N*-glycosylation within the second

extracellular loop is also found in various kinds of animals [24,25]. It is proposed that the loop region contains important information required for the assembly of the rhodopsin molecule, because any deletions in this region resulted in the formation of misfolded rhodopsin [26]. Therefore, this consensus sequence, together with its flanking region, would not be functioning as a target for *N*-glycosylation, but be essential to construct a correct conformation of rhodopsin. In the present study, the amount of mature rhodopsin in Δ*Asn196* flies was reduced to approximately 20% of that in the wild-type (Fig. 4A, lanes 1 and 3; note that total protein charged in lane 1 is 20% of that in the other lanes). The reduced yield of rhodopsin in the N196I mutant might be attributable to the above reason. When *Drosophila* opsin was translated in vitro, Asn-196 was glycosylated as well as Asn-20. This result suggests that *N*-glycosylation can potentially occur at both sites in rhodopsin. It might be presumed that, in the *Drosophila* photoreceptor cells, co-translational binding of chaperone-like proteins to opsin would block the glycosylation of the second site. NINAA, an eye-specific peptidylproline *cis-trans*-isomerase (PPIase) [27,28], is one of the possible opsin-binding proteins which is specifically distributed in the photoreceptor cells [29,30]. However, it is not likely that NINAA alone contributes to the inhibition of glycosylation, because the 43 kDa rhodopsin bearing two oligosaccharide chains is not detectable in the *ninaA* mutant [12]. Alternatively, elongating peptide halfway through the translation may take a specific conformation that prevents the second site from co-translational glycosylation in vivo.

In previous studies, it was shown that the synthesis of mature rhodopsin is depressed in the presence of tunicamycin [31]. In addition, the amount of mature rhodopsin is dramatically reduced in transgenic flies (Δ*Asn20*) whose opsin contains the amino acid substitution N20I [16,20]. Although these results suggest the important role of *N*-glycosylation in the pathway of rhodopsin synthesis, the critical step requiring the *N*-linked oligosaccharide chain has not been clarified. In this paper, we elucidated that *N*-glycosylation at Asn-20 predominantly functions in the maturation process of rhodopsin (40 kDa → 35 kDa) rather than the synthesis or stabilization of immature opsin (→ 40 kDa). However, it should be noted that the amount of immature rhodopsin in the Δ*Asn20* mutant is significantly lower than that in the wild-type fly, although the mutant contains as much immature rhodopsin as Δ*Asn196*. Therefore, we could not completely exclude the possibility that *N*-glycosylation at Asn-20 may also partly contribute to stabilizing or accelerating the synthesis of immature rhodopsin. Further studies using other amino acid substitutions may clarify this point.

In the wild-type fly, the 40 kDa immature opsin is the earliest intermediate of rhodopsin so far found. The molecule does not bind chromophore yet, possesses an oligosaccharide chain of the high mannose type, and is accumulated in rER [12,32]. The present results thus suggest that the oligosaccharide chain at Asn-20 would be working at one or several steps between the export of the rhodopsin intermediate from rER and the incorporation of mature rhodopsin into the rhabdomeric membrane. A possible way to facilitate rhodopsin maturation is that the oligosaccharide chain enables the interaction between opsin peptides and chaperone-like proteins. Recently, in various kinds of cells lectin-like proteins were found, calnexin [2] and calreticulin [33], which resides in ER and interacts with some glycoproteins. In *Drosophila* photoreceptor cells, it has also been suggested that NINAA (eye-specific PPIase) interacts with rhodopsin in rER and facilitates its biogenesis [30]. Furthermore, it was shown that NINAA and rhodopsin also colocalize to secretory vesicles [32], suggesting that rhodopsin may require NINAA not only in rER but also in its maturation and transport processes. Therefore, one of these proteins may possibly recognize the *N*-linked oligosaccharide chain of rhodopsin to facilitate its maturation. It would be very important to identify proteins that interact with rhodopsin in the maturation process through the oligosaccharide chain.

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