doi:10.1016/j.jmb.2006.07.039 J. Mol. Biol. (2006) 363, 98–113

JMB

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Methyl Substituents at the 11 or 12 Position of Retinal Profoundly and Differentially Affect Photochemistry and Signalling Activity of Rhodopsin

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The C-11=C-12 double bond of the retinylidene chromophore of rhodopsin holds a central position in its light-induced photoisomerization and hence the photosensory function of this visual pigment. To probe the local environment of the HC-11=C-12H element we have prepared the 11-methyl and 12-methyl derivatives of 11-Z retinal and incorporated these into opsin to generate the rhodopsin analogs 11-methyl and 12-methyl rhodopsin. These analog pigments form with much slower kinetics and lower efficiency than the native pigment. The initial photochemistry and the signaling activity of the analog pigments were investigated by UV-vis and FTIR spectroscopy, and by a G protein activation assay. Our data indicate that the ultrafast formation of the first photointermediate is strongly perturbed by the presence of an 11-methyl substituent, but much less by a 12-methyl substituent. These results support the current concept of the mechanism of the primary photoisomerization event in rhodopsin. An important stronghold of this concept is an out-of-plane movement of the C-12H element, which is facilitated by torsion as well as extended positive charge delocalization into the C-10–C-13 segment of the chromophore. We argue that this mechanism is maintained principally with a methyl substituent at C-12.

In addition, we show that both an 11-methyl and a 12-methyl substitutent perturb the photointermediate cascade and finally yield a low-activity state of the receptor. The 11-methyl pigment retains about 30% of the G protein activation rate of native rhodopsin, while the 12-methyl chromophore behaves like an inverse agonist up to at least 20 °C, trapping the protein in a perturbed Meta-I-like conformation.

We conclude that the isomerization region of the chromophore and the spatial structure of the binding site are finely tuned, in order to achieve a high photosensory potential with an efficient pathway to a high-activity state.

Keywords: rhodopsin analog pigment; retinal synthesis; quantum yield; photoactivation; fourier-transform infrared spectroscopy

Introduction

Rhodopsin is the G protein-coupled photoreceptor protein in the retina of vertebrates that initiates the visual transduction cascade in dim light vision.[1,2] Rhodopsin contains a protonated 11-Z retinylidene Schiff base in its active site, which functions as an inverse agonist. The protein binds this ligand in the 12-s-*trans* conformation and the non-bonding interactions between the C-13 methyl group and the C-10 hydrogen atom give rise to a

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Abbreviations used: Meta, metarhodopsin; Batho, bathorhodopsin; FTIR, Fourier transform infrared spectroscopy; HOOP, hydrogen-out-of-plane.

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non-planar structure.[3–5] The resulting torsions in the chromophore configure the polyene tail for fast photoisomerization around the C-11=C-12 double bond.[3,6,7] Within 1 ps following light absorption, the primary photoproduct bathorhodopsin (Batho) is formed, which contains a highly strained protonated all-E retinylidene Schiff base in the active site.[8–11] Subsequent thermal steps lead within milliseconds to the signaling intermediate metarhodopsin-II (Meta-II), which contains a relaxed unprotonated all-E retinylidene Schiff base in the active site that acts as a full agonist.[1,2,12] Studies on rhodopsin analogs without the 9-methyl or 13-methyl group (C-19, C-20) have established that these methyl groups contribute significantly to the propagation of the photochemical cascade leading to the signaling state of the protein.[13–22] For example, the absence of the 13-methyl group leads to a significantly lower quantum yield of $\Phi = 0.47$ compared to $\Phi = 0.65$ for native rhodopsin, while upon light absorption by 9-desmethyl rhodopsin, the active state 9-desmethyl Meta-II is generated with much lower efficiency.

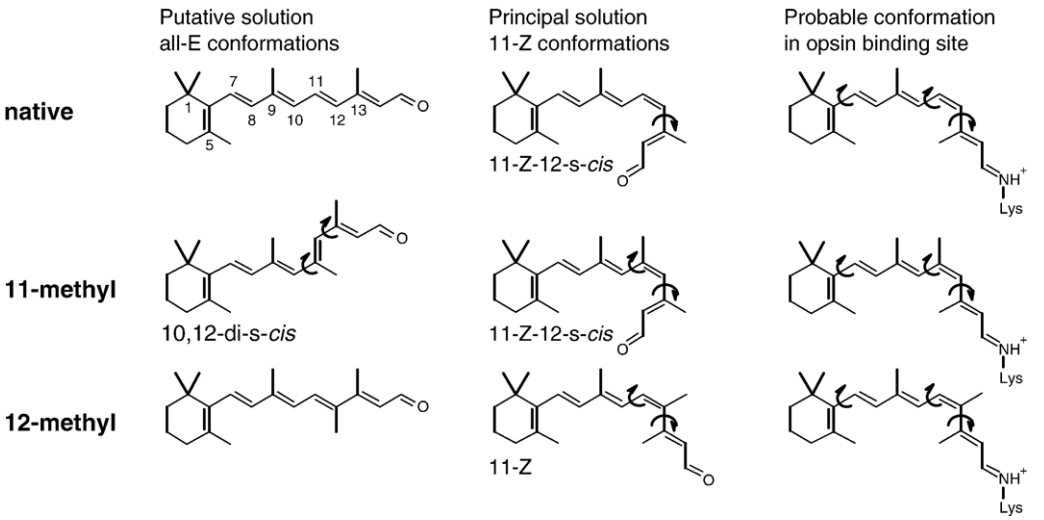
Chemical modification of the isomerization region of the 11-Z retinylidene moiety (C-10••C-13) can help to provide insight into the structural basis of the high rate and efficiency of the photochemical reaction.[23,24] Earlier studies have pursued the effect of an additional methyl group at the 10 position.[4,23,25] For instance, it was observed that 10-methyl rhodopsin has a lower quantum yield ($\Phi = 0.55$) than the native system ($\Phi = 0.65$), and that thermal conversion of the first photoproduct (Batho) to the next intermediate is shifted to a higher temperature. An additive effect is observed upon simultaneous removal of the 13-methyl group yielding 10-methyl-13-desmethyl rhodopsin, which shows a further reduction in quantum yield to $\Phi = 0.35$.[13]

In this study, the effect of methyl substitution at the C-11 and C-12 position of retinal (Scheme 1) is investigated. In earlier work, the corresponding rhodopsin analog pigments were generated in low yield and only the value of λ_{max} was reported.[26,27] It was claimed very recently that a methyl group at position C-12 would downshift the pK_a of the Meta-I ↔ Meta-II equilibrium, which should result in strongly reduced signalling activity of 12-methyl rhodopsin at physiological pH.[22]

We have improved the efficiency of the chemical synthesis of 11-methyl and 12-methyl retinal significantly, as well as the production of the corresponding rhodopsin analogs. The photochemical and signalling activity of the 11-methyl and 12-methyl rhodopsin analogs have been studied by means of UV-vis and Fourier transform infrared (FTIR) spectroscopy and a G protein binding assay. The aim of this study is to resolve and understand the details of the ligand–receptor communication and photoactivation in the native pigment. The results indicate mixed effects of methyl group insertion at positions C-11 or C-12 on: (i) protein–ligand interaction as well as at two stages in the photosequence; (ii) the primary photoisomerization step; and (iii) the signaling activity of the receptor at the final stage.

Results and Discussion

The use of modified retinal derivatives allows us to investigate ligand–protein interactions, the photoisomerization process and, finally, the effect of substituents on the mechanisms leading to the signaling activity of the visual receptor rhodopsin. First, the efficiency and rate of binding, and the spectral properties of the retinal analog in the binding site provide information about ligand–



Scheme 1. Principal solution conformations of the 11-Z retinal derivatives used in this study determined by NOE NMR spectroscopy (middle column). For comparison, the putative all-E solution structures are shown in the left-hand column, and the probable conformation in the opsin binding site is shown in the right-hand column. The arrows indicate bond torsion that occurs due to intramolecular steric repulsion.