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Opsin Stability and Folding: The Role of Cys185 and Abnormal Disulfide Bond Formation in the Intradiscal Domain

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The structure in the extracellular, intradiscal domain of rhodopsin surrounding the Cys110–Cys187 disulfide bond has been shown to be important for correct folding of this receptor *in vivo*. Retinitis pigmentosa misfolding mutants of the apoprotein opsin (such as P23H) misfold, as defined by a deficiency in ability to bind 11-*cis* retinal and form rhodopsin. These mutants also possess an abnormal Cys185–Cys187 disulfide bond in the intradiscal domain. Here, by mutating Cys185 to alanine, we eliminate the possibility of forming this abnormal disulfide bond and investigate the effect of combining the C185A mutation with the retinitis pigmentosa mutation P23H. Both the P23H and P23H/C185A double mutant suffer from low expression and poor 11-cis retinal binding. Our data suggest that misfolding events occur that do not have an absolute requirement for abnormal Cys185–Cys187 disulfide bond formation. In the detergent-solubilised, purified state, the C185A mutation allows formation of rhodopsin at wild-type (WT) levels, but has interesting effects on protein stability. C185A rhodopsin is less thermally stable than WT, whereas C185A opsin shows the same ability to regenerate rhodopsin in detergent as WT. Purified C185A and WT opsins, however, have contrasting 11-*cis* retinal binding kinetics. A high proportion of C185A opsin binds 11-*cis* retinal with a slow rate that reflects a denatured state of opsin reverting to a fast-binding, open-pocket conformation. This slower rate is not observed in a stabilising lipid/detergent system, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine/Chaps, in which C185A exhibits WT (fast) retinal binding. We propose that the C185A mutation destabilises the open-pocket conformation of opsin in detergent resulting in an equilibrium between correctly folded and denatured states of the protein. This equilibrium can be driven towards the correctly folded rhodopsin state by the binding of 11-*cis* retinal.

Keywords: membrane protein folding; opsin; 11-cis retinal binding; disulfide bond; stability

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Abbreviations used: BTP, bis-tris-propane; DDM, *n*-dodecyl-β-D-maltoside; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; GPCR, G-protein-coupled receptor; Meta II, metarhodopsin II; ROS, rod outer segment; WT, wild type.

Introduction

Membrane proteins constitute approximately one third of the proteome, but our understanding of this diverse category of proteins at the molecular level is currently based on a small group of representative proteins.[1] Structural study is frequently prohibited by the very challenging process of obtaining sufficient quantities of pure, functionally active and stable protein. A serious problem with purified

membrane proteins is destabilisation and loss of activity that occurs over time, especially in the detergents often needed for crystallisation.[2] Studying the protein conformational changes involved in destabilisation will provide insights into the underlying molecular events and inspire possible ways to preserve active conformations of membrane proteins in solution.

G-protein-coupled receptors (GPCRs) form the largest family of mammalian receptors and represent major targets for drug development. The proteins as a group have proved elusive to structure determination, the exception being the dim-light receptor rhodopsin.[3–5] Rhodopsin is covalently bound to its ligand, 11-*cis* retinal, giving a stable protein. The apoprotein opsin is highly unstable in detergent solution, especially in the absence of any lipids, and previous work has identified denatured states of opsin.[6] Successful attempts to stabilise opsin *in vitro* have focused both on developing stabilising lipid/detergent systems[7] and on mutations that pin the intradiscal domain of the protein together.[8] Research into the intramolecular interactions of the rhodopsin structure that affect folding, function and stability has also greatly improved our understanding of GPCRs, predominately through rational mutation and comparison to disease-associated mutants of opsin.[9–17] Rhodopsin possesses a disulfide bond between Cys110 and Cys187, located within the intradiscal domain (see Fig. 1) that is highly conserved among GPCRs. Formation of this disulfide bond is important for stability of the activated, metarhodopsin II (Meta II) state.[10]

A collection of mutations to the transmembrane and intradiscal domains of rhodopsin result in a neurodegenerative disease of the retina known as retinitis pigmentosa (RP) and affect rhodopsin folding.[16,19] RP is a genetically heterogeneous disease, affecting approximately every 1 in 4000 people worldwide,[20] causing a progressive loss of vision and usually leaving patients blind by middle age. P23H opsin is a commonly occurring RP misfolding mutant[21] that is classed as a partial misfolding mutation. P23H opsin can be purified and separated into 11-*cis* retinal binding (folded) and nonbinding (misfolded) fractions.[16,22] Misfolded P23H opsin has a lower α-helical content than wild-type (WT) opsin,[16] is incapable of binding 11-*cis* retinal, has an immature glycosylation status when expressed *in vivo*,[23] suffers from poor expression yields due to degradation[24] and does not traffic to the plasma membrane. Instead P23H accumulates in aggresome-like bodies within the cell[23] and copurifies with chaperones.[9] Addition of 11-*cis* retinal and other retinal analogues during expression of P23H opsin in cultured cells enhances correct trafficking to the plasma membrane and normal glycosylation,[23] but the rhodopsin is thermally unstable and hydroxylamine sensitive.[25] Most interestingly, WT and correctly folded P23H opsin possess the native Cys110–Cys187 disulfide bond in the intradiscal domain, whereas misfolded P23H opsin

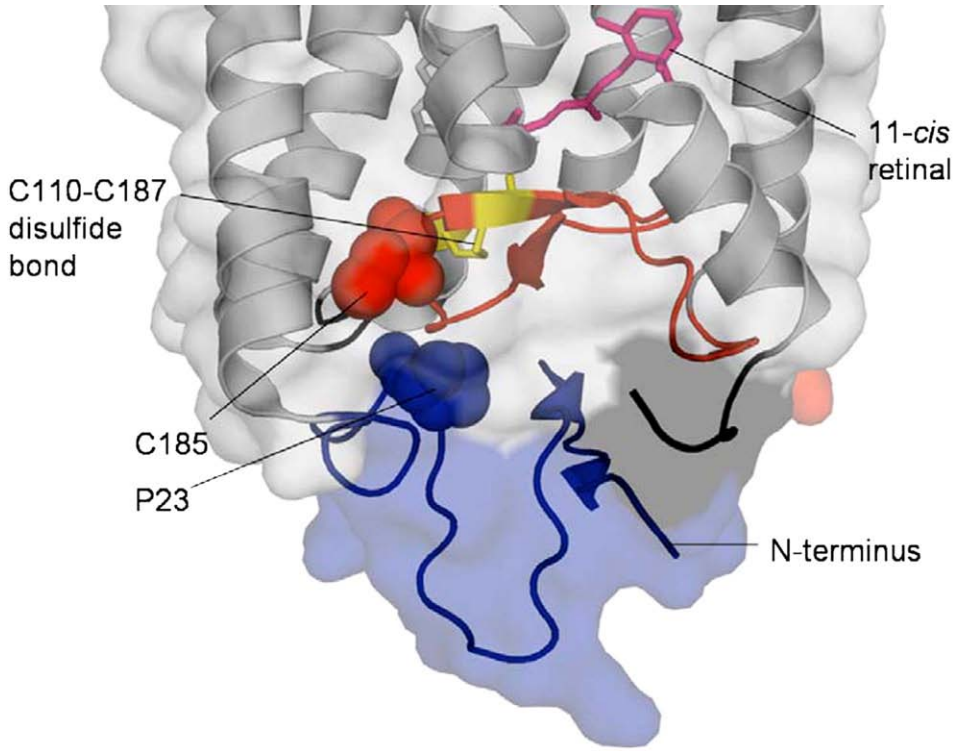

Fig. 1. Structure of the intradiscal domain of rhodopsin. Residues proline 23 (blue) and cysteine 185 (red) are shown as spheres. The N-terminal cap is in blue, the second intradiscal loop is in red and the Cys110–Cys187 disulfide bond is in yellow. 11-*cis* retinal is in pink. Segments of helices VI and VII have been removed for clarity. Rhodopsin coordinates were taken from Protein Data Bank entry 1L9H.[18] Cartoon was generated using PyMol software.