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Cyclodextrin retinylidene: A biomimetic kinetic trap model for rhodopsin

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Abstract—All *trans* retinal was attached to both the primary face and the secondary face of β-cyclodextrin via a Schiff base linkage, analogous to that in rhodopsin. The new models were evaluated and compared with *n*-butylamine retinylidene Schiff base for their rates of hydrolysis, and factors that influence such rates. Competition studies using adamantane carboxylate demonstrated the kinetic trap theory by diminishing the binding of retinal in the cyclodextrin, thereby augmenting the rate of hydrolysis. NMR experiments indicate that the retinylidene is most probably bound in the form of a dimer.

Retinal, **1**, is the chromophore responsible for triggering the enzymatic cascade leading to vision in vertebrates. In its 11-*cis* geometry, **1b**, it is bound by the protein opsin, covalently linked to Lys296 via a Schiff base generating a retinylidene, which forms the photoactive photoreceptor, rhodopsin.[1–3] Upon absorption of light, the 11-*cis*-retinylidene undergoes an isomerization to the all *trans* retinylidene, which in turn triggers the activation of the G-protein. The active state of rhodopsin is referred to as the meta II or MII (see Fig. 1). In the vertebrate visual cycle, the all *trans* retinylidene is then hydrolyzed and is released out of the chromophore binding pocket. The all *trans* retinal is then shuttled through a series of retinol binding proteins (RBPs) that convert it back to the 11-*cis* geometry, thereby completing the visual cycle (Fig. 1).[3] The hydrolysis of the Schiff base is a crucial step and has not been fully explored mechanistically. Exploring the rate of hydrolysis is pivotal in understanding the abnormalities that affect the visual cycle, such as Stargardt disease.[4] It has been shown that some mutants of rhodopsin result in varying hydrolysis rates for the all *trans* retinal, often leading to adverse visual effects.[5,6] For instance, an accumulation of all *trans* retinal can lead to severe problems including a common form of age-related macular degeneration (AMD), in which two retinal molecules will condense with a phosphatidyl ethanolamine leading to a highly fluorescent pigment known as A2E.[7–10] The role of hydrogen bonding in Schiff base hydrolysis in rhodopsin has been carefully studied via conservative mutations of critical residues near the binding site.[11] We hereby present an alternative approach to understanding hydrolysis via a simple artificial biomimetic model using β-cyclodextrin[12] as the recognition site for retinal, for which it is known to bind in.[13,14]

β-Cyclodextrin (β-CD), **2**, was functionalized from both the primary and the secondary sides using standard techniques[12,15–20] to afford the primary 6-aminocyclodextrin and the secondary 3-aminocyclodextrin (see Fig. 2). The aminocyclodextrins were subsequently reacted with 1 equiv of retinal, **1**, in the presence of 0.9 equiv of acetic acid in DMSO, to generate **3** and **4** in 98% and 93% yields, respectively. The latter Schiff bases were isolated via lyophilization. The Schiff base of retinal with *n*-butylamine, **5**, was also generated and used as a control in the hydrolysis studies.

The new structures **3** and **4** were characterized by UV, ^{1}H NMR,[21] and FAB mass spectrometry. Their UV absorption in DMSO was at a λ_{max} of 370 and 372 nm, with molar absorptivities of 112,800 and 98,400 $mol^{-1}\,cm^{-1}$, respectively. The ^{1}H NMR in DMSO-d_6 revealed a distinctive peak for the aldimine proton (the former aldehyde proton), which was at δ 8.36 and at 8.45 ppm in structures **3** and **4**, respectively. FAB mass spectrometry revealed a mass of 1401.37 for the two structures corresponding to the [M+H] ion.

Keywords: Cyclodextrin; Retinal; Cyclodextrin retinylidene; Schiff base hydrolysis; Kinetic trap; Rhodopsin mimic; Dimer.
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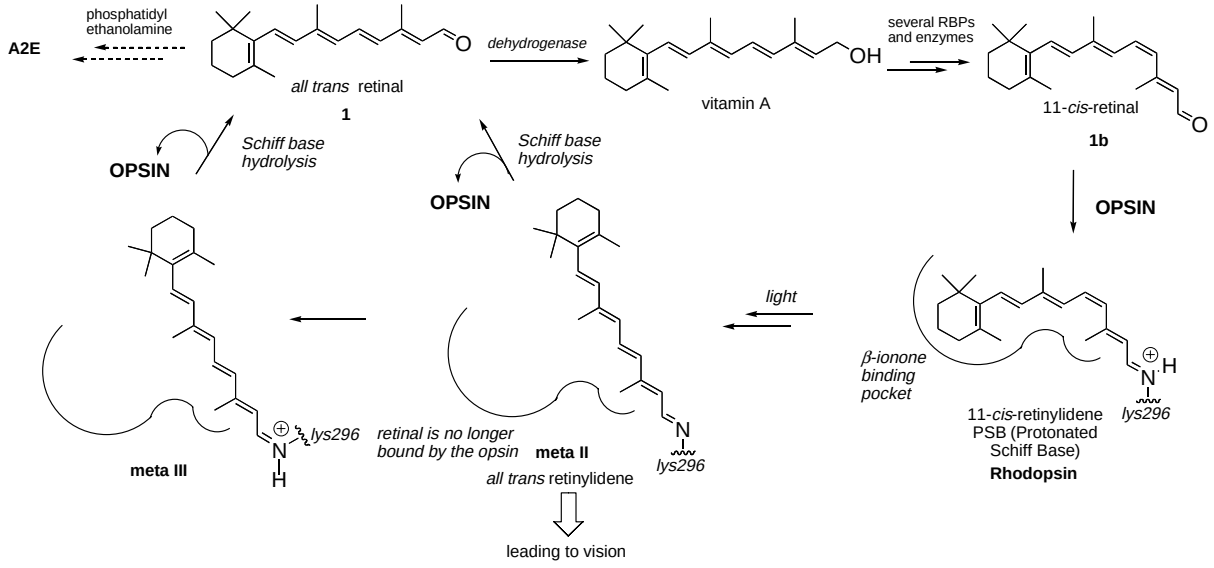

Figure 1. A simplified outline of the visual cycle.

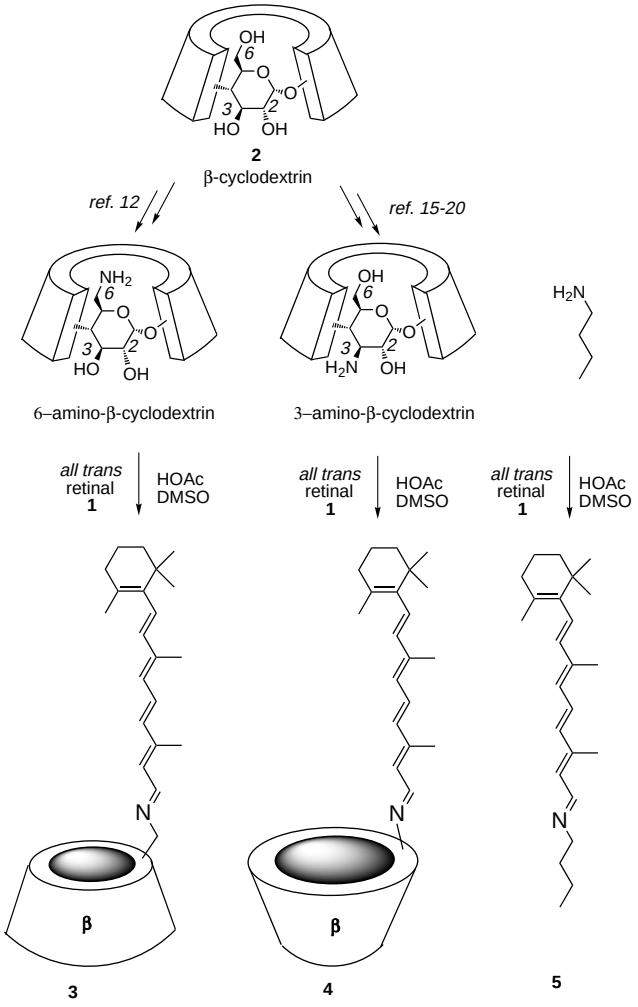

Figure 2. The synthesis of the three retinylidene Schiff bases.

Hydrolysis studies were carried out at 25 °C and in pH 4.0 buffer. Although the hydrolysis of the three Schiff bases was followed by ^{1}H NMR to prove the identity of the products, the rates were measured more precisely by following the decrease in absorption of the protonated Schiff bases at their corresponding λ_{max}, which was 442 nm for **3** and 447 nm for **4**, in pH 4 buffer. Since it is expected that retinal will bind into cyclodextrin[13,14] in water, analogous to retinal's binding into opsin, we sought to evaluate the effect of the binding of the hydrophobic retinoid on the rate of Schiff base hydrolysis. It is established that in rhodopsin, the binding that 11-*cis*-retinal has for opsin ensures its stability in the dark state, such that if hydrolysis of the 11-*cis* conformation occurs, the bound chromophore ligand simply reforms the Schiff base, hence the term kinetic trap.[5] However after isomerization of the 11-*cis*-retinal to the all *trans*, the β-ionone moiety of the all *trans* retinal no longer binds into the opsin binding pocket, thereby exposing the Schiff base to water molecules that will lead to its hydrolysis. Alternatively, the conformational change imposed by the isomerization of retinal could reorient the amino acid residues within the Schiff base binding site in such a manner as to orchestrate a hydrogen bonding network that will catalyze the hydrolysis of the Schiff base.[11] In our model studies, we envision compound **4** forming a dimer in water as depicted in Figure 3. The NMR data presented below support our reasoning behind it. Introduction of a strong hydrophobic β-cyclodextrin ligand, such as adamantane carboxylate which has a binding constant in β-cyclodextrin of 10^{4} M^{-1},[22] will undoubtedly break up the dimer 'releasing' the retinal from the hydrophobic cyclodextrin cavity. It is therefore expected that the rate of hydrolysis will be enhanced in the presence of adamantane carboxylate. In other words, in our model, adamantane carboxylate

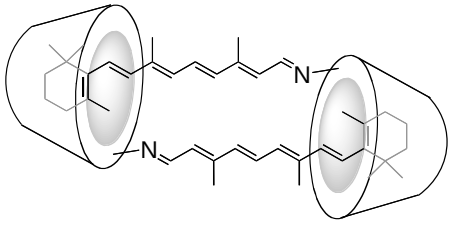

Figure 3. Proposed dimer of **4** formed in aqueous solutions.