Interaction between Arrestin1 and Enolase1 in Rod Photoreceptor Cells

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Abstract

Purpose. This research aimed to determine the effect arrestin1 has on the catalytic activity of enolase1 and to identify the interacting domains between arrestin1 and enolase1 to provide a structural understanding for this interaction. Understanding the interaction between arrestin1 and enolase1 will provide a foundation to further study the link between phototransduction and glycolysis in the rod photoreceptor cells of the retina.

Methods. The effect arrestin1 has on the catalytic activity of enolase1 was determined by measuring the consumption of NADH as 2-phosphoglycerate is converted to lactic acid. Also, by labeling 24 site-directed cysteine mutants in loops of arrestin1 with monobromobimane, fluorescence emission was measured to determine conformational changes in arrestin1 induced by enolase1 binding.

Results. Arrestin1 was shown to slow the enzymatic activity of enolase1 by as much as 25%. Fluorimetric scanning of all arrestin loops showed an increase in fluorescence emission on loops XIV-XV, XVI-XVII, and XVII-XIX which indicates a decreased aqueous exposure of the bimane fluorophore from either burying of the bimane into arrestin or from shielding provided by the enolase. A decrease in emission occurred on loop I-II at residue 18 indicating an increased aqueous exposure of the fluorophore.

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**Conclusions:** The catalytic activity assay proved that the interaction of arrestin1 with enolase1 affects enolase1 catalysis. Due to arrestin1 slowing down the activity, light-driven translocation may regulate the metabolic activity of the photoreceptors. Fluorimetric scanning indicates that multiple loops of arrestin have significant conformational mobility. The interaction with enolase induces conformational changes in several of these loops.

**Introduction**

In the mammalian retina, rod and cone photoreceptor cells capture photons by opsin-based visual pigments to initiate the phototransduction cascade. The arrestin family of molecules modulates the activity of the visual pigment by quenching and halting phototransduction until the vitamin-A chromophore is released and rhodopsin is regenerated. The arrestin molecule is quite dynamic in that it moves between the inner and outer segments of the photoreceptors. In the dark, arrestin resides in the inner segments and the perinuclear regions of the photoreceptors, and translocates to the outer segments during light adaptation.

The method of arrestin translocation has been investigated by many laboratories, and one hypothesis involves the use of the cell’s cytoskeletal elements and a visual pigment called rhodopsin. It was thought arrestin binds to activated rhodopsin in the outer segments in the light and binds to microtubules in the inner segments of the photoreceptor cells in the dark, with arrestin diffusing through the connecting cilium.

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3 These sections have already been published in the Investigative Ophthalmology and Visual Sciences Journal. Title: “Interaction of Arrestin with Enolase1 in Photoreceptors”. Author: Smith et al.
In vitro, the evidence supporting the microtubule hypothesis is strong, but many other studies show this is in fact not what is occurring in the photoreceptors. If the diffusion of arrestin occurs through cytoskeletal elements, then the distribution of arrestin binding to microtubules in the dark-adapted inner segments would be expected to be more linear, conforming to the distribution of the microtubules. However, many studies show that arrestin is distributed uniformly throughout the cytoplasm of the inner segments indicating another possible method of arrestin translocation.\textsuperscript{5,12,13,14}

Our lab has recently shown arrestin1 and enolase1 are co-localized in the inner segments of the photoreceptors during the dark-adapted state. This project explores the effect arrestin1 has on the catalytic activity of enolase1 and the interacting domains on arrestin1 with enolase1 induced binding.

**Methods**

**Enolase Catalytic Activity\textsuperscript{3}**

The enzymatic activity of enolase1 was measured by monitoring the loss of absorbance at 340 nm, as NADH is oxidized when 2-phosphoglycerate is converted to lactic acid. The rate of enolase activity was determined from a linear fit of the decline in absorbance at 340 nm for data collected at 1-minute intervals over 1 to 10 minutes.

Enolase1 (purified from *Pichia*) was dialyzed into 15 mM Tris-HCl with 1 mM MgCl\textsubscript{2}, 1 mM CaCl\textsubscript{2}, and 0.1 mM DTT at pH7.4 (TMCD buffer). Enolase1 was added at a final concentration of 77 nM to a reaction mix containing 2 mM 2-phosphoglycerate, 3 U/mL pyruvate kinase, and 3.6 U/mL lactate dehydrogenase in 15 mM Tris-HCl with 25 mM MgSO\textsubscript{4}, 100 mM KCl, 0.2 mM NADH, 2 mM ADP, 0.02% (wt/vol) bovine serum
albumen, and 0.01% (vol/vol) P20 surfactant. Bovine arrestin1 purified from heterologous expression in yeast was added in increasing amounts (0–32 μM). The reaction mix was maintained at 25°C during the 10-minute reaction time.

**Fluorimetric Scanning of Arrestin**

The interacting domains between enolase and arrestin were determined by measuring conformational changes in arrestin induced by enolase binding. Site-specific cysteine substitutions were made in bovine visual arrestin1 by polymerase chain reaction. Mutated cDNA’s were introduced into yeast (*Pichia*) by heterologous recombination. Mutated arrestin was purified following heterologous expression in yeast. Twenty-four site-directed cysteine mutants in the loops of arrestin were labeled with monobromobimane and dialyzed into TMCD. Bovine enolase1 (purified from *Pichia*) was dialyzed into TMCD as well. Labeled arrestin was added at a final concentration of 1 μM to a reaction mixture containing 75 μM enolase, in TMCD buffer with 0.005% P20 surfactant. Also, arrestin was added at a final concentration of 1 μM to a reaction mixture containing 0 μM enolase, TMCD buffer, and 0.005% P20 surfactant. Fluorescence emission was monitored with each arrestin containing reaction mixture with excitation at 380 nm, and monitoring emission at 400 – 600 nm.

The language used to represent the 24 different sites was in the form of amino acid, the labeled residue, and labeled with monobromobimane. For example, E302B: 302 is the residue that was labeled, the E stands for the amino acid at that residue (in this case, E stands for glutamate based on the one-letter amino acid code), and B means the residue was labeled mutated to a cysteine and then labeled successfully with monobromobimane.
Results

Arrestin’s Effect on Enolase Enzymatic Activity³

Enolase is a metabolic enzyme functioning in glycolysis (the conversion of the six-carbon glucose to the three-carbon pyruvate) to turn 2-phosphoglycerate to phosphoenolpyruvate which then gets converted to pyruvate. Pyruvate is then used in further steps to make ATP for the cell to use as energy. In light of our lab previously showing that arrestin1 and enolase1 are co-localized in the inner segments of the photoreceptor cells, we next wanted to investigate if arrestin1 modulates the enzymatic activity of enolase1.

In our assay, 77 nM enolase1 was mixed with 2-phosphoglycerate in the presence or absence of arrestin1. The rate that NADH was consumed was measured by comparing the linear slopes from a plot of NADH consumption over time produced from the absence of arrestin1 and with 32 μM arrestin1. Figure 1 shows enolase1 catalyzes the hydrolysis of 2-phosphoglycerate at a rate of 2.55 mmol/(min*g of enolase). However, with the addition of arrestin1, this activity is significantly slowed as NADH is being consumed at a rate of 1.92 mmol/(min*g). This difference represents a 24.7% reduction of enolase1 activity in the presence of 400-fold excess of arrestin1. The concentrations of arrestin1 and enolase1 used in this assay are much lower than normal physiological conditions in the photoreceptors, with arrestin being present in the rod photoreceptors at about 1 mM and enolase being at about 200 μM.
Enolase Interacting Domains on Arrestin

To further study the interaction between enolase1 and arrestin1, we next used fluorimetric scanning of a bimane fluorophore to determine the interacting domains on arrestin1 when binding with enolase1. By labeling 24 site-directed cysteine mutants in loops of arrestin1 with monobromobimane, all arrestin1 loops were able to be studied for enolase1 induced movement. Figure 2 is a diagram of arrestin1 where the spheres represent the residues labeled with the bimane fluorophore.

Figure 1. Modulation of enolase1 catalytic activity by arrestin. Enolase1 enzymatic activity was monitored by measuring the rate of NADH consumption (loss of OD340nm) as 2-phosphoglycerate is converted to lactate. Enolase1 activity was measured in the presence of increasing concentration of arrestin up to 32 μM.

Figure 2. Structure of arrestin1 showing residues labeled with monobromobimane. The red spheres represent the residues with the largest percent difference when interacting with enolase1. The dark blue spheres represent residue 18 which showed a decrease in fluorescence with enolase1 binding. The other colored spheres represent the labeled residues which did not show much of a change in fluorescence emission when interacting with enolase1.
Figure 3 is a plot of the fluorescence emission vs. excitation wavelength for four different arrestin1 mutants in the absence and presence of enolase1. As the graphs indicate, the fluorescence emission increases for residues 302, 37, and 342 when enolase1 interacts with arrestin1 while it decreases for residue 18. An increase in fluorescence when arrestin1 and enolase1 interact indicates a decreased aqueous exposure of the bimane fluorophore from either burying of the bimane into arrestin1 or from shielding provided by the enolase1. A decrease in fluorescence indicates an increase in aqueous exposure of the bimane. Similar graphs were obtained for all 24 arrestins to determine if that residue plays a significant role in the enolase-arrestin interaction. Figure 4 shows the percent differences between the reaction mixtures containing 75 μM enolase and those that do not contain enolase1 for all 24 labeled arrestins. According to the figure, the residues that showed the most activity when binding with enolase1 were 302, 342, 25, 231, 37, 267, and 18. Some of the differences were as much as 12.69% as seen with E231B, 10.64% as seen with L342B/F197W, and 10.62% as seen with R37B. R18B showed a decrease in fluorescence of 7.9%. This study showed loops I-II, XIV-XV, XVI-XVII, and XVII-XIX changed fluorescence with enolase1 binding which could mean a change in conformational structure.
Figure 3. Fluorescence emission for 24 site-directed cysteine mutant arrestins was monitored in the absence and presence (75 μM) enolase1. (A) Fluorimetric scanning of residues 342 and 18 shows an increase in fluorescence and conformational mobility with enolase1 binding. (B) Fluorimetric scanning of residue 302 shows an increase in fluorescence when interacting with enolase1, but a decrease in fluorescence at residue 18.

Figure 4. The percent difference of the fluorescence emission at 460 – 490 nm was calculated for each labeled arrestin to determine the strength of the change in conformational mobility of the arrestin loops with enolase1 induced binding.
**Discussions**

**Importance of Enolase in Glycolysis**

Enolase is an enzyme that functions in glycolysis to turn 2-phosphoglycerate to phosphoenolpyruvate. There are three isozymes—enolase1 (α-enolase), enolase2 (γ-enolase), and enolase3 (β-enolase)—that function as obligate homo- or heterodimers. Typically, enolase1 is broadly expressed in a variety of tissues, enolase2 is specific to neurons and neuroendocrine tissue, and enolase3 is found nearly exclusively in muscle.\(^3\,^{15}\) Glycolysis is the first set of reactions to convert glucose to adenosine triphosphate (ATP). ATP is the main source of energy for living cells. Our studies show that arrestin modulates the enzymatic activity of enolase, decreasing it by as much as 25%. Giving that the photoreceptors are estimated to require \(10^8\) ATP/sec,\(^3\,^{16}\) this interaction has potentially important implications.

Another main part of the glycolysis set of reactions is to produce NADH. NADH/NADPH is required for the reduction of all-\textit{trans} retinal produced by photoisomerization of 11-\textit{cis} retinal in rhodopsin and subsequent suppression of quantum noise. By arrestin moving away from enolase, essentially more NADH could be made to facilitate the reduction of all-\textit{trans} retinal.\(^3\,^{17}\)

**Interacting domains on Arrestin**

The fluorimetric scanning results provide further evidence of the arrestin-enolase interaction by giving insight to the structural understanding of this interaction. These results show that the interaction between enolase1 and arrestin1 is a dynamic interaction with several loops on arrestin moving when binding with enolase. Knowing how enolase
and arrestin interact with each other will provide us with the opportunity to further study the interaction by narrowing in on those residues to determine the direct link between the two proteins.

One complication of this assay is when there is a shift in fluorescence emission in the presence of enolase1 compared to the absence of enolase1, there is no way of telling if the enolase is shielding the bimane or if the arrestin loop is actually moving. The next step to be taken is to determine which loops in arrestin1 actually change their conformational structure when binding with enolase1 and which residues were being shielded by enolase1. This study is currently in progress and the results are promising.

These results along with other results from our lab show enolase1 and arrestin1 primarily interact through the final 100 residues on the N-terminus of arrestin1. Further studies are being done to determine the interacting domains of enolase1 when arrestin1 binds to it. With these two sets of data, the enolase-arrestin interaction can be decoded which will provide an avenue to then determine a way to decrease the interaction when enolase1 activity is most needed.

**Conclusions**

In conclusion, these, along with other studies done by our lab provide the first evidence for direct interaction between enolase1 and arrestin1. Showing that arrestin modulates the enzymatic activity of enolase proves to be pretty significant in the photoreceptors. Light driven translocation of arrestin may regulate the metabolic activity of the photoreceptor cells. This research also provides an insight to the structural understanding of this interaction by showing that multiple loops of arrestin have
significant conformational mobility which is induced by enolase binding. Further study of the link between enolase1 and arrestin1 in the photoreceptor cells of the mammalian retina may provide a mechanism to manipulate the metabolic rate of photoreceptors to extend to their lifetime in age-related retinal diseases.
References


