

ESPS Peer-review Report

Name of Journal: World Journal of Biological Chemistry

ESPS Manuscript NO: 7552

Title: Comparative Study on the Binding of Rhodopsin, and Rhodopsin Analogues Containing the 9-cis and 13-cis Isomers of Retinal, to Transducin, Rhodopsin kinase and Arrestin-1

Reviewer code: 00069496

Science editor: Qi, Yuan

Date sent for review: 2013-11-24 14:02

Date reviewed: 2013-11-29 02:57

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input checked="" type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)		<input type="checkbox"/> No records	<input type="checkbox"/> Major revision

COMMENTS TO AUTHORS

General Comments: This manuscript by Araujo et al is aimed to study if reconstituted rhodopsin, 9-cis-retinal-rhodopsin and 13-cis-retinal-rhodopsin interact with transducin, rhodopsin kinase and arrestin-1. The authors isolated rod outer segments (ROS) from bovine retinas, generated rhodopsin and rhodopsin analogues with the different retinal isomers, purified transducin and arrestin-1 to homogeneity, and obtained an enriched-fraction of rhodopsin kinase by extracting freshly prepared ROS. The authors characterized the reconstituted rhodopsin and rhodopsin analogues through three sets of experiments: activation of transducin, ability to serve as substrates for rhodopsin kinase, and binding to arrestin-1. Different approaches including column chromatography, guanine nucleotide binding assay, in vitro phosphorylation, etc. were used. They found that rhodopsin analogue harboring the 13-cis isomer of retinal is capable of activating transducin in a light-independent way. They concluded that the rhodopsin analogue containing the 13-cis isomer of retinal seems to fold in a pseudo-active conformation that mimics the active photointermediate of rhodopsin. The manuscript is well written.

Specific Comments: Major: 1. Page 15. "Basal amounts of arrestin-1 interacted with rhodopsin, isorhodopsin and the 13-cis-retinal-rhodopsin complex, both in the dark and under illumination (data not shown)". The data should be shown and quantified to compare the effect of phosphorylation on binding to arrestin-1 in Fig. 6. 2. Discussion. A section should be added to discuss the effect of phosphorylation on binding to arrestin-1 and the potential molecular mechanism. Minor: 3. Fig. 3, A, B & C were not labeled 4. Fig. 4, Black and white bar graphs are not labeled. 5. Fig. 5A, Dark and Light labeling is apparently not labeled appropriately since the 13-Cis-Rho lane under Dark is missing, but is duplicated under Light. 6. Fig. 5C, Black



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and white bar graphs are not labeled.

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Title: Comparative Study on the Binding of Rhodopsin, and Rhodopsin Analogues Containing the 9-cis and 13-cis Isomers of Retinal, to Transducin, Rhodopsin kinase and Arrestin-1

Reviewer code: 02618391

Science editor: Qi, Yuan

Date sent for review: 2013-11-24 14:02

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input checked="" type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Minor revision
<input checked="" type="checkbox"/> Grade E (Poor)		<input type="checkbox"/> No records	<input type="checkbox"/> Major revision

COMMENTS TO AUTHORS

In current manuscript titled "Comparative Study on the Binding of Rhodopsin, and Rhodopsin Analogues Containing the 9-cis and 13-cis Isomers of Retinal, to Transducin, Rhodopsin kinase and Arrestin-1", Araujo and co-authors examine the interaction of reconstituted rhodopsin, 9-cis-retinal-rhodopsin and 13-cis-retinal-rhodopsin with transducin, rhodopsin kinase and arrestin-1. In particular, investigators found that the 13-cis isomer of retinal appears to fold in a pseudo-active conformation that mimics the active photointermediate of rhodopsin in dark condition. Major concerns: 1.The authors failed to suggest the physiological importance of such study. As the authors said, "the rod visual pigment rhodopsin uses the 11-cis form of retinal exclusively as the chromophore", then what's the potential importance to study the 9-cis and 13-cis Isomers? It is difficult to appeal the audience without clearly emphasis the scientific significance of the study. 2.The purified Components, such as transducin, rhodopsin and arrestin-1, as well as the regenerated rhodopsin (analogues) should be examined and the results should be provided. Otherwise, it is hard to judge. 3.The discussion section, instead of being simply used as a review of the literature in the field, should be used to discuss the presented results, their meaning, importance and suggestion. 4.Figure legends should provide sufficient explanation including experiment conditions that can let readers understand the figure independent of the text. 5.Each experiment should be repeated independently and the number of repeat should be indicated in figure legends. Minor concerns: 1.It will be more convincing if the investigators can show the co-IP results of transducing, opsin and rhodopsin. 2.In Figure 5 and 6, these experiments should be repeated multiple times and then error bars and statistical significance should be indicated. An internal control should be shown and in



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panel C, the ratio of interest protein against internal control should be used instead of “arbitrary units”.

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Title: Comparative Study on the Binding of Rhodopsin, and Rhodopsin Analogues Containing the 9-cis and 13-cis Isomers of Retinal, to Transducin, Rhodopsin kinase and Arrestin-1

Reviewer code: 02619490

Science editor: Qi, Yuan

Date sent for review: 2013-11-24 14:02

Date reviewed: 2013-12-10 15:29

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input checked="" type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input checked="" type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

In this manuscript, the authors present that reconstituted rhodopsin, 9-cis-retinal-rhodopsin and 13-cis-retinal-rhodopsin interact with transducin, rhodopsin kinase and arrestin-1. Especially, investigator assert that the rhodopsin analogue containing the 13-cis isomer of retinal appears to fold in a pseudo-active conformation that mimics the active photointermediate of rhodopsin even though it is in the dark condition. This is an interesting topic and could represent a potentially important mechanism of rhodopsin using 13-cis isomer of retinal rhodopsin. However, this study was not properly performed because many positive and negative controls were missing. For these reasons, this manuscript it is not warranted for publication in World Journal of Biological Chemistry in its present form. Specific concern: 1. The purified proteins including transducin, rhodopsin kinase and arrestin-1 were used as substrates for in vitro assay. However, authors did not verify the purified proteins using immunoblot. 2. The figure legend was not properly described. It should be included more information, such as experimental conditions, labelling, and p-values in bar graphs. 3. Positive and negative controls should be included in all figures.

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Title: Comparative Study on the Binding of Rhodopsin, and Rhodopsin Analogues Containing the 9-cis and 13-cis Isomers of Retinal, to Transducin, Rhodopsin kinase and Arrestin-1

Reviewer code: 02770448

Science editor: Qi, Yuan

Date sent for review: 2013-11-24 14:02

Date reviewed: 2013-12-18 21:27

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input checked="" type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input checked="" type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input checked="" type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)		<input type="checkbox"/> No records	<input type="checkbox"/> Major revision

COMMENTS TO AUTHORS

In the present paper, Araujo and colleagues used biochemical approaches to compare the interaction between rhodopsin, 9-cis-retinal-rhodopsin and 13-cis-retinal-rhodopsin with signal transducers transducin, rhodopsin kinase and arrestin-1. As expected, reconstituted rhodopsin and 9-cis-retinal-rhodopsin behave similarly as they activate transducing, are substrate of the kinase and efficiently bind to arrestin-1 in a light dependent manner. The 13-cis-retinal-rhodopsin is also link to the downstream signaling partners however these interactions are mostly independent of the light. The reproducibility of the experiments should be validate by expressing the quantitative data as mean (+/- sd or sem) of several experiments. For instance no error bars in fig 5C and 6C and error bar in fig 4C are not defined. 1- Fig4 and Fig6C: The legend does not explain the nature of the white and dark bar of the histogram. 2- Fig 5 : Are the data from the same SDS-PAGE? Why the different lanes were separated?

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Title: Comparative Study on the Binding of Rhodopsin, and Rhodopsin Analogues Containing the 9-cis and 13-cis Isomers of Retinal, to Transducin, Rhodopsin kinase and Arrestin-1

Reviewer code: 02799802

Science editor: Qi, Yuan

Date sent for review: 2013-11-24 14:02

Date reviewed: 2013-12-21 21:35

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	<input checked="" type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	
		<input type="checkbox"/> No records	<input type="checkbox"/> Major revision

COMMENTS TO AUTHORS

The manuscript describes binding of reconstituted rhodopsin and rhodopsin analogues with transducin, rhodopsin kinase and arrestin1. The authors have shown that 13-cis isomer of retinal is capable of activating transducin independently of light and is highly phosphorylated by rhodopsin kinase in the dark. In addition, arrestin-1 was recognized by phosphorylated 13-cis-retinal-rhodopsin in the dark. Thus, 13-cis-retinal-rhodopsin folds in a pseudo-active conformation that mimics the active photointermediate of rhodopsin. However, couple of issues needs further address to improve the manuscript and make it fit for publication. 1. Interactions of rhodopsin and its analogs, especially 13-cis-retinal isomer with the transducin, rhodopsin kinase and arrestin1 should be further studied by other assays like binding anisotropy and binding constants and binding kinetics may be analyzed, since this is the key message of the manuscript. 2. Is 13-cis-retinal isomer the main or one of the natural rhodopsin photointermediates in the body? Why it behaves like an active rhodopsin? 3. It is mentioned in the discussion part that opsin can activate transducin 10 fold higher in the presence of retinal that resembles 13-cis isomeric form. It may be important to show that the same occurs with the reconstituted 13-cis retinal. 4. Absorption spectra of meta-I may be included in figure 3 as control.