The guanylate cyclase signaling system in zebrafish photoreceptors

Karl-Wilhelm Koch*

Biochemistry Group, Faculty VI, Carl von Ossietzky University Oldenburg, D-26111 Oldenburg, Germany
Research Center Neurosensory Science, Carl von Ossietzky University Oldenburg, D-26111 Oldenburg, Germany
Center of Interface Science, Carl von Ossietzky University Oldenburg, D-26111 Oldenburg, Germany

1. Introduction

1.1. General

Excitation and adaptation of vertebrate photoreceptor cells is mediated by two second messengers, guanosine-3′,5′-cyclic monophosphate (cGMP) and Ca²⁺ [1–4]. Light absorption of rhodopsin triggers the hydrolysis of cGMP by activation of a well-known G-protein coupled pathway leading also with a short delay to a fall in cytoplasmic Ca²⁺-concentration ([Ca²⁺]). The mutual dependence of cytoplasmic concentrations in cGMP and Ca²⁺ critically controls the responsiveness of the photoreceptor cell under different illumination conditions and a change in cytoplasmic [Ca²⁺] is considered to be a critical step mediating light adaptation [1–4].

Key proteins involved in the interplay of cGMP and Ca²⁺ are membrane-bound guanylate cyclases (GCs) [4,5] and their Ca²⁺-sensitive regulators, named guanylate cyclase-activating proteins (GCAP) [6–8]. A combination of biochemical, genetic and physiological studies has established that photoreceptor GCs have a low basal cyclase activity at high intracellular [Ca²⁺] corresponding to the dark state of the cell and increase their activities, when the cytoplasmic [Ca²⁺] decreases after illumination. Although this general concept is well accepted and confirmed [1–8], several open questions remain. For example, what are the molecular mechanisms of protein–protein interaction during GC activation by GCAPs? Further, to what extents contribute other Ca²⁺-dependent or Ca²⁺-independent mechanisms to the regulation of phototransduction and the control of light adaptation [9]? Finally, zebrafish has attained increasing interest in vision research due to its fast development, amenability to genetic manipulation and to the fact that it is equipped with a set of cone photoreceptor cells, which are sensitive to the whole visible spectrum and to UV-light. The zebrafish retina expresses three isoforms of sensory GCs and six forms of GCAPs raising the question, whether this apparently redundant expression is of physiological meaning [10,11].

The present mini-review will discuss this topic, in particular by reflecting on the recently suggested Ca²⁺-relay model that accounts for differential properties of GCAP forms [4,12,13].

1.2. Sensory GCs in teleost fishes

Teleost DNA data bases harbour sequence information of several putative membrane bound GCs. These include four different sensory GCs denoted as OiGC3, OiGC4, OiGC5 and OiGC-R2 in medaka fish (Oryzias latipes) and four orthologous forms in pufferfish (Fugu rubripes) and three in carp (Cyprinus carpio) [10,14–19]. Transcripts of the three sensory GCs in the zebrafish (Danio rerio)
were detected in the photoreceptor layer of larval and adult fish denoted zGC1, zGC2, zGC3 [11,20].

Amino acid sequence alignments and a subsequent dot blot matrix analysis [21] between medaka and zebrafish GCs revealed high sequence homology in the cytoplasmic part and significant regions of conserved amino acids in the extracellular part confirming the conclusion that these GCs belong to the group of sensory GCs [22]. Sensory GCs operating in rod and cone cells of the vertebrate retina have been investigated in the past by numerous studies, which show collectively that these membrane bound GCs exist as dimers in photoreceptor membranes, form complexes with GCAPs and are regulated by GCAPs in a Ca\(^{2+}\)-dependent manner [for review see [3,4]]. So far, knowledge about the biochemical properties of zGCs is very limited and the cDNAs have not been heterologously expressed for functional studies. In situ hybridization experiments, however, showed that zGC1 and zGC2 were detected in rods and cones and no signals were seen in rods with zGC3 RNA probes. Instead, cones were labeled by zGC3 probes [11].

Screening of randomly mutagenized zebrasishes resulted in several behavioral mutants with mutations in photoreceptor specific genes including one mutant named zatoichi, which has a defect in the gene coding for zGC2 [23]. The zatoichi mutant larva shows no optokinetic response (OKR) and no optomotor response (OMR) measured at 6 days post fertilization (dpf), which points to the crucial role of GC expression for larval visual function. Further, in another study the gene gucy2f coding for the GC zGC1 (s. above) was overexpressed in zebrafish larvae in a gain-of-function approach yielding larvae with multiple defects including a loss of forebrain neurons [24]. Knocking down the expression of gucy2f by the morpholino antisense-oligomoleucleotide approach led to impaired visual function and shortening of photoreceptor cells in 6 day old larvae [25]. While these studies highlight the importance of genes coding for membrane bound GCs for normal visual performance they leave open questions that are related to regulatory features.

1.3. Expression of GCAPs and functional role in signaling

GCAPs belong to the family of neuronal calcium sensor (NCS) proteins that are mainly expressed in neurons, where they mediate as Ca\(^{2+}\)-sensors a wide range of physiological responses [26]. GCAPs are specifically expressed in photoreceptor cells and have been identified in different vertebrates like human, bovine, monkey, mice, chicken, fish and amphibians [6–8,10–12,27–29]. They are small compact proteins of approximately 200 amino acid length containing four EF-hand Ca\(^{2+}\)-binding motifs, of which the first EF-hand is non-functional and probably involved in protein–protein interaction [30,31]. GCAPs can bind Mg\(^{2+}\), when Ca\(^{2+}\) dissociates from its binding sites during light-induced changes of the cytoplasmic Ca\(^{2+}\)-concentration, which transforms GCAPs into their cyclase-activating conformation [32]. Mammalian rod and cone cells express two or three GCAP isoforms, but eight isoforms are known for functional studies. In situ hybridization of zGCs is very limited and the cDNAs have not been heterologously expressed for functional studies. In situ hybridization experiments, however, showed that zGC1 and zGC2 were detected in rods and cones and no signals were seen in rods with zGC3 RNA probes. Instead, cones were labeled by zGC3 probes [11].

2. GCAP regulatory modes in zebrasihish rods and cones

2.1. Ca\(^{2+}\)-binding and Ca\(^{2+}\)-induced conformational changes

All zGCAPs have successfully been purified as recombinant proteins from Escherichia coli allowing detailed investigations of their biochemical properties [38–40]. A non-radioactive chelator assay developed by Linse and co-workers [41–43] was used to investigate Ca\(^{2+}\)-binding affinities. All zGCAPs had at least three binding sites for Ca\(^{2+}\) with apparent affinity constants of high, medium and low affinity. However, these values differed among all investigated zGCAPs and are unique for every isoform [38].

Binding of Ca\(^{2+}\) triggers conformational changes in GCAPs and had been investigated thoroughly for the mammalian GCAP1 and GCAP2. Common spectroscopic methods include in particular intrinsic Tryptophan (Trp) fluorescence and circular dichroism (CD), which had been applied in studying the Ca\(^{2+}\)-induced conformational changes of GCAPs and another retina specific NCS protein, recoverin [31,41–47]. Mammalian GCAP1 typically shows a biphasic pattern in Trp emission during a Ca\(^{2+}\)-titration with decreasing emission from 0 to 1 μM Ca\(^{2+}\) and increasing emission above 1 μM Ca\(^{2+}\). The Trp emission studies therefore demonstrated that GCAPs undergo Ca\(^{2+}\)-induced conformational changes in the physiological range of cytoplasmic Ca\(^{2+}\). Interestingly, although all zGCAPs change their Trp emission on Ca\(^{2+}\)-binding or Ca\(^{2+}\)-dissociation, no zGCAP isoform followed the same pattern as GCAP1, which demonstrates significant differences in Ca\(^{2+}\)-sensing [38].

2.2. Activation profiles of GC signaling

One remarkable feature of zGCAPs is that each form has a distinct activation profile. Reconstitution of purified zGCAPs with membrane bound GCs using preparations of bovine rod outer segment (ROS) membranes as assay system showed that all zGCAPs could activate GCs with apparent affinities (EC\(_{50}\)-values) in the submicromolar or lower micromolar range [38], which is very similar to mammalian GCAPs that also have EC\(_{50}\) values around 1 μM. Most significant differences are however observed, when maximal GC activities are compared and calculated as x-fold activation from the definition: GC\(_{\text{max}}\) = GC\(_{\text{min}}\) GC\(_{\text{min}}\) (GC\(_{\text{max}}\): GC activity at maximum; GC\(_{\text{min}}\): GC activity at minimum). Maximal activities of zGCAP1, 5 and 7 are rather low (x-fold activation 1.5–3-fold), but zGCAP2, 3 and 4 are strong activators having an x-fold activation between 6 and 13 [38]. When these features are compared with the Ca\(^{2+}\)-sensitive regulatory properties it becomes apparent that zGCAPs can be classified into two groups having IC\(_{50}\) values either around 30 nM or around 400 nM [38–40]. The IC\(_{50}\) value is equivalent to the Ca\(^{2+}\)-concentration at which GC activation is half-maximal. Each of these groups contains further a combination of strong and weak GC activators (Fig. 1). If we look at the distribution of
zGCAPs in different photoreceptor cells, each cell type harbors strong and weak GC activators and all cone types express also a combination of zGCAPs having different IC₅₀ values (Fig. 2). Double cones and long single cones have two strong activators that both are also expressed at high rates (Fig. 2). Interestingly, short single cones have a high expression rate only for the zGCAP group with low IC₅₀ values. The only zGCAP with a high IC₅₀ value is zGCAP5, but this Ca²⁺-sensor shows the lowest degree of x-fold activation among all tested GCAPs [38] and probably does not contribute much to the overall regulation and activation of GCs. It is unclear at the moment, whether this special case of UV-cones reflects a different Ca²⁺-homeostasis. Direct measurements of cytoplasmic Ca²⁺-concentration in the dark have been performed so far only for UV-cones and yielded a value of 400 nM, which is suggested to decrease after illumination [48]. It will be a major challenge to further define exactly the changing pattern of cytoplasmic Ca²⁺ in zebrafish rod and cone cells. In any case, zGCAPs are operating as activating GC regulators in a specific time frame depending on the illumination state of the cell. Thus, zGCAPs exhibiting a combination of zGCAPs having different IC₅₀ values, zGCAP3 and 4 were purified as myristoylated and non-myristoylated forms; (b) and if so, whether these forms differ in their key properties. Heterologous expression and purification from bacteria revealed that zGCAP3 and zGCAP4 could be myristoylated by a co-expressed N-myristoyl-transferase from Escherichia coli and that zGCAP4, 5 and 7 would first be turned into an activating status followed by zGCAP 1, 2 or 3 that will become active at Ca²⁺-concentrations that are approx. one order of magnitude lower. This gradual step-by-step mode of action represents a Ca²⁺-relay mechanism of GCAP action.

2.3. Comparison of zGCAP3 and zGCAP4

Two cone-specific Ca²⁺-sensors, zGCAP3 and 4, have been investigated in more detail [39,40]. Each of them represents a strong activator of GCs with high expression rate in cone cells, but with different operation profiles and Ca²⁺-sensitivities (Figs. 1 and 2). Most NCS proteins contain a consensus sequence for the attachment of a myristoyl group at the amino-terminus [26] and in particular recoverin and GCAPs from mammalian (ROS) exhibit differences in their functional properties, whether they are myristoylated or not. For example, recoverin undergoes a classical Ca²⁺-myristoyl switch, it binds to membranes, when it is saturated with Ca²⁺ and the myristoyl group is exposed. In its Ca²⁺-free form the myristoyl group is buried in a hydrophobic protein pocket making the protein less hydrophobic [49], which in turn leads to the release of recoverin into the cytoplasm. In contrast to recoverin, GCAP1 and 2 do not undergo a classical Ca²⁺-myristoyl switch [50–53], but presence of the myristoyl group in GCAP1 is essential to shift the Ca²⁺-sensitivity into the physiological range. In addition, the affinity of the GCAP1–ROS–GC1 interaction depends on the myristoyl modification [54–56]. In contrast, bovine GCAP2 shows an almost identical activation pattern in its myristoylated or non-myristoylated form, however, the myristoyl group in GCAP2 seems to be more flexible and is involved in stabilizing the Ca²⁺-bound conformation [57,58]. These findings raised the following questions, (a) whether zGCAPs are expressed in myristoylated or non-myristoylated forms; (b) and if so, whether these forms differ in their key properties. Heterologous expression and purification from bacteria revealed that zGCAP3 and zGCAP4 could be myristoylated by a co-expressed N-myristoyl-transferase from yeast, although a point mutant of zGCAP4 was necessary [39,40]. Probing larval and adult stages of zebrafish showed also that zGCAP3 is myristoylated in native retinae [40], but a similar study has not been conducted for zGCAP4 so far. In order to identify a possible influence of the myristoyl group on the functional properties of zGCAPs, zGCAP3 and 4 were purified as myristoylated and non-myristoylated recombinant proteins and investigated according to several criteria yielding the following results: they do not undergo a classical Ca²⁺-myristoyl-switch, although membrane binding of zGCAP3 is slightly improved by the myristoyl group [39,40]. Activation profiles of zGCAPs are nearly identical with similar IC₅₀ and EC₅₀ values for GC activation indicating no significant influence on Ca²⁺-sensor function. However, in zGCAP3 the myristoyl group has several other effects. It enhances the Ca²⁺-sensitivity of conformational changes, stabilizes the protein conformation in general and has a modulating effect on kinetic parameters of the GC target [40]. Interestingly, zebrafish larvae first express non-myristoylated zGCAP3 at 3.25 dpf, but myristoylated zGCAP3 was not detected before 7 dpf [40]. Apparently, the impact of the myristoyl group on zGCAP3 functions seems less important in the larval than in the adult stage.

In summary, GCAPs are NCS proteins that operate like their mammalian counterparts in a relay mode allowing rod and cone cells to detect incremental changes in cytoplasmic Ca²⁺. The biochemical complexity of zGCAPs reflects a fine-tuned system of
Ca²⁺-sensing and Ca²⁺-mediating proteins playing key roles in photoreceptor excitation and adaptation. However, many unresolved questions remain as for example to examine, whether the action mode of each zGCAP changes under varying illumination conditions. Furthermore, which other Ca²⁺-dependent control mechanisms targeting different enzymes or proteins are involved in adjusting the photoreceptor’s light adapting properties and forming the molecular basis for the dynamic range extension in cone adaptation of the fish retina.

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References


