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Biochemical and Biophysical Research Communications 332 (2005) 149–156
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Two membrane juxtaposed signaling modules in ANF-RGC are interlocked

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Received 8 April 2005
Available online 28 April 2005

Abstract

Atrial natriuretic factor (ANF) receptor guanylate cyclase ANF-RGC is a single transmembrane spanning modular protein. Juxtaposed to each side of the transmembrane module is a Cys^{423}–Cys^{432} disulfide ANF signaling module motif and the ATP-regulated transduction module (ARM) motif. The signaling module motif is conserved in nearly all membrane guanylate cyclases and is believed to be critical in the signaling activities of all membrane guanylate cyclases. The present study with the model system of the olfactory membrane guanylate cyclase shows that this concept is not valid. Furthermore, the study shows that in ANF-GC the signaling motif works through the ARM domain. A new signaling model is proposed where in its natural state the disulfide structural motif represses the ARM domain activity, which, in turn, represses the catalytic module activity of ANF-RGC. ANF signaling relieves the disulfide structural motif restraint on the ARM inhibition and stimulates the catalytic module of the cyclase.

Keywords: ANF; ANF-RGC membrane guanylate cyclase; ATP-regulated module; ONE-GC membrane guanylate cyclase; ANF signaling

Atrial natriuretic factor (ANF) is one of the three members of the natriuretic peptide family. The other two are brain natriuretic peptide (BNP) and type C natriuretic peptide (CNP) (reviewed in: [1]). The residential sites of ANF and BNP are in the atrial and ventricular granules of the heart. With each atrial stretch, defined doses of these peptides are pulsated into the bloodstream and carried to their target tissues where they exhibit biological activities. This mode of action was first demonstrated for ANF in the heart, which was classified as an exocrine gland and, thereby, showed that it is not merely a blood pump [2]. To varying degrees, all three forms of natriuretic peptides exhibit vasodilatory activities and lower blood pressure by accelerating the renal sodium and water secretion [3–5]. Gene knockout studies link ANF and its receptor guanylate cyclase (ANF-RGC) with salt-sensitive and salt-insensitive hypertensions [6,7]. These studies show that ANF and ANF-RGC are closely linked with the renal and cardiovascular physiology.

ANP and BNP exhibit their biological activities through ANF-RGC [8–14]. Like other members of the membrane guanylate cyclase family, ANF-RGC is a single transmembrane-spanning protein, composed of modular blocks [15]. The transmembrane module separates the protein into two regions, extracellular and intracellular (Fig. 1). The extracellular region contains the ANF-binding domain [16]. Recent evidence indicates that the ANF-binding signal domain exists as a homodimer and is composed of a hinge region with a signature Cys^{423}–Cys^{432} disulfide structural motif [17–20]. Barring one, enterotoxin-receptor guanylate cyclase, this structural motif exists in all members of the membrane guanylate cyclase family. It has, therefore, been

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doi:10.1016/j.bbrc.2005.04.102

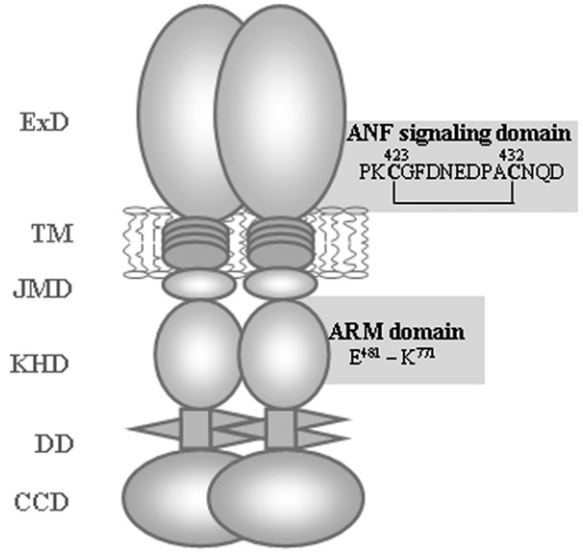

Fig. 1. Topographical model of receptor guanylate cyclase, ANF-RGC. ANF-RGC is a single transmembrane-spanning protein, composed of modular blocks: ExD, extracellular domain; TMD, transmembrane domain; JMD, juxtamembrane domain; KHD, kinase homology domain; DD, dimerization domain; and CCD, cyclase catalytic domain. The TM domain dividing the protein into extracellular and intracellular portions is flanked by two functional domains, ANF signaling domain at its N-terminus and ARM domain at its C-terminus. A signature Cys^{423}–Cys^{432} disulfide structural motif defines the ANF signaling domain. The ARM domain is comprised of the amino acid region Glu^{481} to Lys^{771}.

predicted that this motif participates in the transmembrane signaling activity of all members of the membrane guanylate cyclase family [17,18].

The intracellular region of ANF-RGC is composed of the following sequential modular blocks: juxtaposed 40 amino acids to the transmembrane module are the ATP-regulated module (ARM), the kinase homology domain (KHD), the hypothetical dimerization domain, and the catalytic module (reviewed in: [12,15,21]) (Fig. 1). ARM is a critical transduction module, which is directly regulated by ATP and stringently controls the ANF-dependent activity of the catalytic module [22]. Its three-dimensional structure has been simulated and the critical residues involved in the formation of the folding pattern of the ATP-binding pocket have been predicted, and for some, it has been experimentally validated [23,24]. Point mutation studies show that one central architectural motif of the ARM module is $Gly^{505}Ser^{506}XXGly$ [23,24]. The Ser^{506} activity is Gly^{505}-dependent; upon ATP binding to ARM, Gly^{505} pivots Ser from the buried to the exposed state [23,24].

Before the independent identity of the ARM domain was recognized [22], ARM domain was considered a part of the KHD. An original study proposed an ANF signal transduction model, in which KHD, under natural conditions, is a negative regulator of the ANF-RGC's catalytic module [25]. ATP overcomes the negative constraint and, thereby, causes the ANF-dependent stimulation of the catalytic moiety [25]. This model has become a central theme for some laboratories to explain the role of the ANF signaling Cys^{423}–Cys^{433} disulfide structural motif in signaling of ANF-RGC [19]. In an alternative model, KHD is not a direct natural negative regulator of ANF-RGC's catalytic module [22–24]. ANF functions through ATP allosteric regulation of ARM, which, in turn, stimulates the catalytic module of ANF-RGC [22–24].

The present study was designed to address the following issues. (1) Is the disulfide structural motif necessary for the signal transduction activities of all membrane guanylate cyclase family members? As a test model, a Ca^{2+}-modulated membrane guanylate cyclase, ONE-GC [26] (alternatively named GC-D) [27], has been used in the present studies. (2) What are the detailed events involved in exhibiting the control of the disulfide structural motif in ANF signaling of ANF-RGC and what is the role of the ARM domain in transmission of this signal? (3) Which of the two proposed ATP-transduction models is valid: is KHD the natural negative regulator of the catalytic module of ANF-GC? Or, alternatively, does ATP via its ARM positively regulate ANF-RGC?

Materials and methods

Materials

ANF (rat, 8–33) was purchased from Penninsula Laboratories; nucleotides (ATP, AMP-PNP, and ATPγS) were from Boehringer–Mannheim. Oligonucleotides for mutagenesis were synthesized by Integrated DNA Technologies.

Mutants and expression in COS cells

Point mutations were introduced into ANF-RGC and ONE-GC cDNAs subcloned into pSVL or pcDNA3 expression vectors, respectively, using "Quick change" mutagenesis kit (Stratagene). ANF-RGCkin$^-$ mutant with the deleted fragment, aa 473–745, was constructed as described in [28]. A noteworthy feature of this mutant is that except for the 10 aa fragment 463–472, it lacks the entire ARM and the KHD domain starting from juxtamembrane domain to the beginning of the C-terminal dimerization domain. The mutated recombinants were sequenced to confirm their identities. They were then expressed in COS cells according to the previously published protocols [23,28]. At 60 h post-transfection, the cells were washed twice with 50 mM Tris–HCl (pH 7.5)/10 mM $MgCl_2$ buffer, scraped into 2 ml of the buffer, sonicated, centrifuged for 15 min at 5000*g*, and washed with the same buffer. The pellet represented the crude membranes.

Guanylate cyclase activity assay

ANF-RGC and its mutants. The crude membranes were pre-incubated with or without 10^{-7} M ANF and ATP (AMP-PNP or ATPγS) for 10 min on an ice-bath [23]. The reaction was initiated by the addition of the substrate solution containing 1 mM $MgCl_2$ and 4 mM GTP (final concentration). Incubation (37 °C, 10 min) was terminated by the addition of 50 mM sodium acetate buffer, pH 6.25, followed by

heating on a boiling water-bath for 4 min. The amount of cyclic GMP formed was measured by radioimmunoassay [29].

ONE-GC and its mutants. The crude membranes of COS cells expressing ONE-GC or its Cys-mutants were assayed for basal guanylate cyclase activity in the presence of 1 mM EGTA (without Ca^{2+}) or 100 μM Ca^{2+} (with Ca^{2+}). To determine the Ca^{2+}-dependent activity of ONE-GC and its Cys-mutants, membranes of appropriately transfected COS cells were incubated with increasing concentrations of neurocalcin-δ (0–8 μM) in the presence of 100 μM Ca^{2+}.

Western blotting

Western blotting was carried out according to the previously published protocols [30,31].

Results and discussion

The disulfide structural motif is not necessary for the signal transduction activity of ONE-GC, a member of the Ca^{2+}-modulated ROS-GC membrane guanylate cyclase subfamily

The membrane guanylate cyclase family is composed of two subfamilies (reviewed in. [12]). One subfamily is stimulated by the extracellularly generated peptide hormone signals and the other is stimulated by the intracellularly generated Ca^{2+} signals within the neurons. With the exception of the peptide hormone receptor subfamily member, enterotoxin receptor guanylate cyclase, all other family members contain a conserved hinge region juxtaposed to the N-terminal side of the transmembrane domain (Fig. 1). This region contains an intrachain disulfide structural motif [17]. This is a signature structural motif in all members of the membrane guanylate cyclases. And it is assumed that the motif also bears the intrachain structural feature in all membrane guanylate cyclases. The disulfide bridge bearing residues in ANF-RGC are positioned at 423 and 432, Cys^{423} and Cys^{432}. Studies with the recombinant (rANF-RGC) expressed in heterologous cell systems have shown that the Cys-structural motif is critical in ANF signaling of ANF-RGC [17–20]. Based on these studies and the conserved nature of this structural motif, it has been predicted that this motif is involved in the signaling of all members of the membrane guanylate cyclase family, including members of the ROS-GC subfamily [17,18]. In the present study, this hypothesis has been tested for ONE-GC, a member of the ROS-GC subfamily. The predicted intramolecular disulfide structural motif in ONE-GC is Cys^{462} and Cys^{470}.

The Cys-based three ANF-RGC and three identical ONE-GC mutants were constructed. They all involved point mutations, which changed the indicated cysteine residue/s to serine. The resulting ANF-RGC mutants were: $C^{423}S$; $C^{432}S$; and $C^{423}S$,$C^{432}S$, the last mutant named as double Cys-mutant. The corresponding ONE-GC mutants were: $C^{462}S$; $C^{470}S$; and $C^{462}S$,$C^{470}S$ (double Cys mutant). The mutants and their parent wild-type (wt) membrane guanylate cyclases were expressed in COS cells and it was ascertained that the protein levels in their membrane fractions were equal or nearly equal (Fig. 2A). The membrane fractions were assessed for their specific guanylate cyclase activities.

The results showed that compared to the wt-ANF-RGC, all of its mutants contained higher basal guanylate cyclase activity (Fig. 2A). Both single-Cys mutants contained more than 2-fold and the double mutant

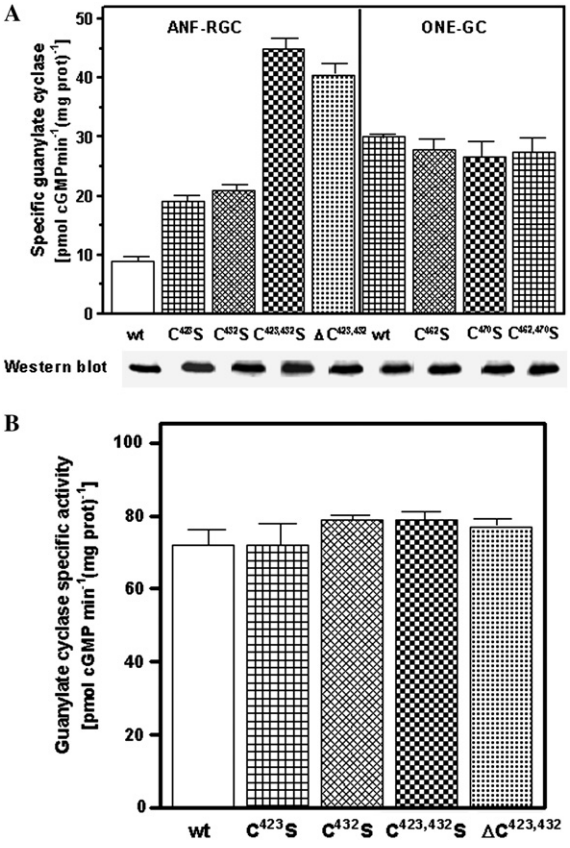

Fig. 2. Effect of Cys mutations on ANF-RGC and ONE-GC guanylate cyclase activity. (A) ANF-RGC mutants: $C^{423}S$, $C^{432}S$, $C^{423}S$,$C^{432}S$, and Δ(deleted)C^{423},C^{432}; and ONE-GC mutants: $C^{462}S$, $C^{470}S$, and $C^{462}S$,$C^{470}S$ were expressed in COS cells. Their membranes were assayed for guanylate cyclase activity as described in Materials and methods. The COS cells expressing wt-ANF-RGC and wt-ONE-GC were processed in parallel as control. The experiment was done in triplicate and repeated three times with different membrane preparations. The results presented are from one experiment. (Western blot) The solubilized membranes of COS cells expressing wt-ANF-RGC and its mutants, and ONE-GC and its mutants (equivalent of ~150 μg protein) were subjected to SDS–6% PAGE. After electrophoresis the proteins were transferred to Immobilon membranes and the blot was probed with anti-ANF-RGC or anti-ONE-GC antibody as described in Materials and methods. Immunoreactive bands were visualized by enhanced chemiluminescence. (B) Membranes of COS cells expressing wt-ANF-RGC or its Cys-mutants were assayed for guanylate cyclase activity in the presence of 100 pM ANF and 0.6 mM ATP. The experiment was done in triplicate and repeated three times with different membrane preparations. The results presented are from one experiment.

about 5-fold higher activity. In contrast, the wt-ONE-GC and all of its mutants contained almost the same [30 pmol cGMPmin^{-1} (mg prot)$^{-1}$] cyclase activity. The basal cyclase activity was unaffected with and without Ca^{2+}, a feature already established for ONE-GC [26]. And, the Cys-mutations had no effect on the Ca^{2+}-dependent modulation by neurocalcin-δ, the Ca^{2+}-sensor modulator of ONE-GC (data not shown). In accord with earlier conclusions [17–20], these data demonstrate that the intrachain disulfide bond motif consisting of Cys^{423} and Cys^{432} is critical for the signaling of ANF-RGC. It, however, is not essential for the signaling of ONE-GC. It is thus concluded that the hypothesis predicting the universal role of this structural motif in all membrane guanylate cyclases is not valid. The results suggest that it may be valid for transmembrane signaling of the peptide hormone receptor subfamily but not for the Ca^{2+}-modulated ROS-GC subfamily.

In its natural state, the disulfide structural motif represses the ANF-GC activity

There is now convincing evidence that the hinge region containing intrachain Cys^{423}–Cys^{432} bond is one of the critical ANF signaling structural motifs in ANF-RGC [17–20]. However, the precise details of the participation of these Cys residues in ANF-RGC signal transduction are lacking. Earlier point mutation studies involving $C^{423}S$ and $C^{423}S$,$C^{432}S$ concluded that these mutations cause constitutive activation of ANF-RGC [17,20]. However, biochemical explanations for the activation have been different [17,19,20]. The original group ascribed the activation to the interchain bonding of Cys^{423} and Cys^{432} [19]. The group concluded that this bonding caused dimerization of the extracellular domain of ANF-RGC and the dimerization event stimulated the ANF-RGC activity. This group conducted their studies with the model mutant $C^{423}S$ system and used ANF-RGC-transfected HEK cells. The total cyclic GMP formed in the intact cells was considered to represent the ANF-RGC activity. The other group conducted their studies with the double mutant model $C^{423}S$, $C^{432}S$ [17]. In accord with the conclusions of the previous group, this group concluded that the Cys residues are involved in the constitutive activation of ANF-RGC, and affirmed that these residues constitute the important ANF signaling motif [17]. However, the biochemical explanation for the constitutive activation by this group was different. These investigators concluded that the constitutive activation of ANF-RGC did not involve dimeric interchain bond formation between Cys^{423} and Cys^{432} [17]. Rather, it was the intrinsic property of the intrachain disulfide bridge structural motif [17]. Like the other group, this group also conducted their studies with the ANF-transfected intact cells and the cyclic GMP formed in the intact cells was considered to be reflective of the membrane guanylate cyclase activity [17]. Significantly, the investigators commented that ANF stimulates the membrane fraction of ANF-RGC only 2- to 3-fold [17]. This probably was the reason for both groups not to assess the direct membrane guanylate cyclase activity.

The drawbacks with the whole cell studies are that they are not the direct measurement of the membrane guanylate cyclase activity and that other multiple indirect factors, including the soluble guanylate cyclase/s and their modulators, can seriously affect the results. Most significantly, the ANF signaling of ANF-RGC is a multi-event phenomenon that involves the multi-module domains of ANF-RGC. Thus, it is not possible to dissect the individual events involved in the phenomenon. For instance, ARM is a critical transduction domain [23,24] and the individual events modulated by the ARM domain cannot be dissected out from the ANF signaling events mediated by the disulfide-structural motif of ANF-RGC in intact cell studies. For these reasons the present study was conducted with the membrane fractions of the ANF-RGC-transfected cells. These membranes were remarkably sensitive, responding 8-fold over the basal value to the saturating (100 pM) level of ANF (Fig. 2B). To fully evaluate the role of the intact intrachain disulfide Cys^{423}–Cys^{432} structural motif and of the individualized Cys^{423} and Cys^{432} residues, four mutants, two single, one double, and one double deletion were used. They were: (1) $C^{423}S$; (2) $C^{432}S$; (3) $C^{423}S$,$C^{432}S$ (double); and (4) double deleted (DD) Cys^{423},Cys^{432}, this mutant lacked both Cys residues and thus there were two less residues in the mutant. This mutant was created to assess the direct role of the disulfide-dependent conformational change on the ANF signaling module. The membranes of the wt-ANF-RGC constituted the control.

Earlier studies have established that ANF signaling of ANF-RGC is dependent on the transduction event/s mediated by ATP [32,33]. Neither ANF nor ATP alone signaled ANF-RGC. The two together are required to elicit the signaling process. To determine the role of the ANF signaling disulfide structural motif module on ANF-RGC activity, the wt- and the Cys-mutants were incubated with the saturating concentrations of ANF (100 pM) and ATP (0.6 mM). The results showed that under these conditions the wt-ANF-RGC was stimulated 8-fold over the basal value (compare wt basal activity in Fig. 2A with the stimulated activity in Fig. 2B), indicating that the stimulation range of ANF-RGC from basal to the saturation level was 8-fold. Comparison of these results with the mutants showed that all of them also achieved saturation, the saturation level being equal to that of the wt-ANF-RGC. However, the range of their saturations from their basal levels to the saturation levels was different from the

wt-ANF-RGC (Fig. 2B). It was about 4-fold for the $C^{423}S$ and for the $C^{432}S$ mutants, and about 2-fold for the double Cys- and DD Cys-mutants (compare basal activity of the mutants in Fig. 2A with their stimulated activity in Fig. 2B). These results show that the individualized Cys^{423} and Cys^{432} residues cause ~25% of the constitutive saturation of ANF-RGC; and ANF/ATP signaling fully saturates ANF-RGC with its over all 4-fold stimulation from its basal activity. The double Cys- and DD Cys-mutants, however, are half-saturated. They, therefore, are only 2-fold stimulated by the ANF/ATP signaling. The following interpretations are drawn from these results. (1) Both Cys^{423} and Cys^{432} residues independently and equally control the catalytic activity of ANF-RGC. (2) The residues together forming the intrachain disulfide structural motif stringently control the basal activity of ANF-RGC. (3) The constitutive activation event of ANF-RGC does not involve Cys-related interchain-disulfide bond formation. It is, therefore, concluded that in its natural state the disulfide structural motif module keeps the ANF-RGC in its repressed catalytic state and ANF/ATP signaling brings it to the fully active state.

ARM domain mediates the ANF signaling disulfide structural motif activity

Previous studies have shown that the ATP-dependent transduction activity of the ANF signaling occurs through ARM [22,23]. A three-dimensional ARM model has been simulated and the key residues involved in the folding pattern of the ATP-binding pocket have been depicted (Fig. 3 and [23,24]). Through point mutation studies validity of the folding pattern of some of the

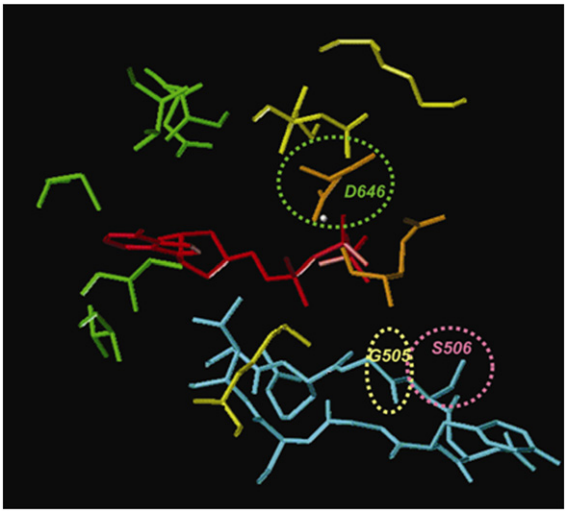

Fig. 3. The ATP-binding pocket in the ARM domain. Amino acid residues that participate in binding of the nucleotide (AMP-PNP) are color-coded based on the component of AMP-PNP with which they interact: β1 and β2 strands, which make the floor of the ATP-binding pocket are shown in cyan; amino acid residues surrounding the ribose ring are in green; residues surrounding the triphosphate moiety are in yellow; residue coordinating Mg^{2+} is in gold; the residue that stabilizes the pocket is in magenta; AMP-PNP is in red; and Mg^{2+} is a silver sphere. The three amino acid residues analyzed are indicated. The identity of the other residues involved in the ATP-binding pocket is described in [25,26]; the ARM structural model has been deposited (PDB ID 1T53).

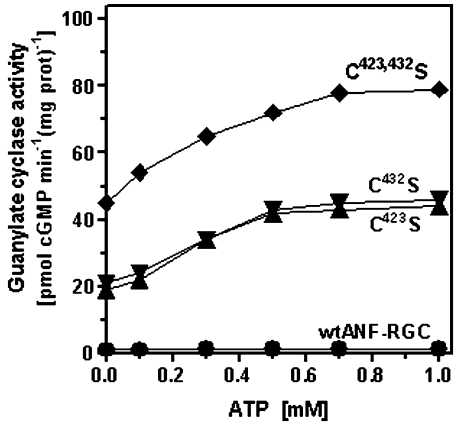

Fig. 4. ATP effect on guanylate cyclase activity of ANF-RGC and its Cys-mutants. COS cells were transfected with appropriate expression constructs and their membranes were prepared as described in Materials and methods. These were assayed for guanylate cyclase activity in the presence of indicated concentrations of ATP. The guanylate cyclase activity was calculated as the cyclase specific activity. Each experiment was done in triplicate and repeated at least two times with separate membrane preparations. The results presented are from one typical experiment. Error bars are within the size of the symbols.

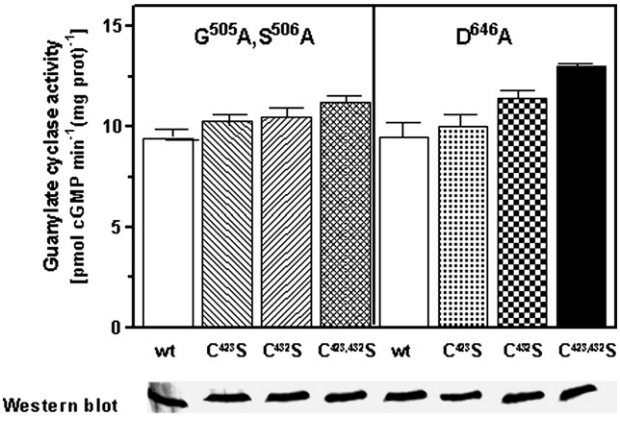

Fig. 5. Effect of $G^{505}A,S^{506}A$ and $D^{646}A$ mutations within the ARM domain on the basal guanylate cyclase activity of ANF-RGC and its Cys-mutants. $G^{505}A,S^{506}A$ or $D^{646}A$ mutations were introduced into each of the ANF-RGC Cys-mutants. The constructs were transfected into COS cells. Particulate fractions of transfected cells were individually assayed for basal guanylate cyclase activity (no ANF and/or ATP was added to the assay mixture) as described in Materials and methods. The experiment was done in triplicate and repeated two times with different membrane preparations. The results presented are from one experiment. (Western blot) Solubilized membranes of COS cells (~150 μg protein) expressing the above mutants were subjected to SDS–6% PAGE. After electrophoresis, the proteins were transferred to Immobilon membranes and the blot was probed with anti-ANF-RGC antibody as described in Materials and methods. Immunoreactive bands were visualized by enhanced chemiluminescence.

residues involved in the ATP-binding pocket has been validated [23,24]. To determine the mediatory role of the ARM in transmission of the ANF signal initiated by the disulfide structural motif, two strategies were used. First, the ATP effect by itself was assessed on the three Cys mutants: $C^{423}S$; $C^{432}S$; and $C^{423}S,C^{432}S$ (double). The prediction was that if the interpretations in the previous section were correct, then the range of stimulation observed by ATP alone would reflect the extent of the disulfide-dependent conformational change in ANF-RGC. In the second strategy, the ARM folding pattern in the Cys mutants was disrupted by mutation of the $G^{505}A,S^{506}A$ and of the $D^{646}A$ (Fig. 3). The G^{505} linked S^{506} motif controls the ATP-dependent rotation of the ATP-binding pocket and D^{646} residue through Mg^{2+} coordinates the linkage with ATP [23,24]. If the interpretation in the first strategy were correct, then the total constitutive activation of the mutants should be lost and the loss should directly reflect the mediatory role of the ATP-dependent ARM module in the transmission of the ANF-dependent disulfide structural motif signaling of ANF-RGC. The mutants' basal values should then equal the basal value of the wt-ANF-RGC.

To evaluate the first strategy, the effect of ATP alone on the activity of the mutants was assessed. The wt-ANF-RGC constituted the control. As anticipated, the wt-ANF-RGC did not respond to ATP (Fig. 4). However, in contrast all mutants responded to the ATP effect. The EC_{50} values for ATP for all mutants were the same, about 0.3 mM. The V_{max} achieved by the single $C^{423}S$ and $Cys^{432}S$ mutants was half to that of the double Cys-mutant (Fig. 4). These results indicate that signaling initiated by the disulfide structural motif is mediated by the ATP-dependent ARM domain. This conclusion was supported by the second strategy experimentation. The mutations in the residues involved in the folding pattern of ARM brought the basal value to that of the wt-ANF-RGC (Fig. 5). It is, therefore, concluded that the ATP-dependent ARM domain mediates the disulfide structural motif-dependent configurational changes in the catalytic module.

To determine the detailed effects of the ANF/ATP signaling the ARM domain of the wt-ANF-RGC and of the Cys-mutants, dose-dependent ATP effects were assessed. In the presence of 100 pM ANF, ATP stimulated the wt-ANF-RGC activity in a dose-dependent

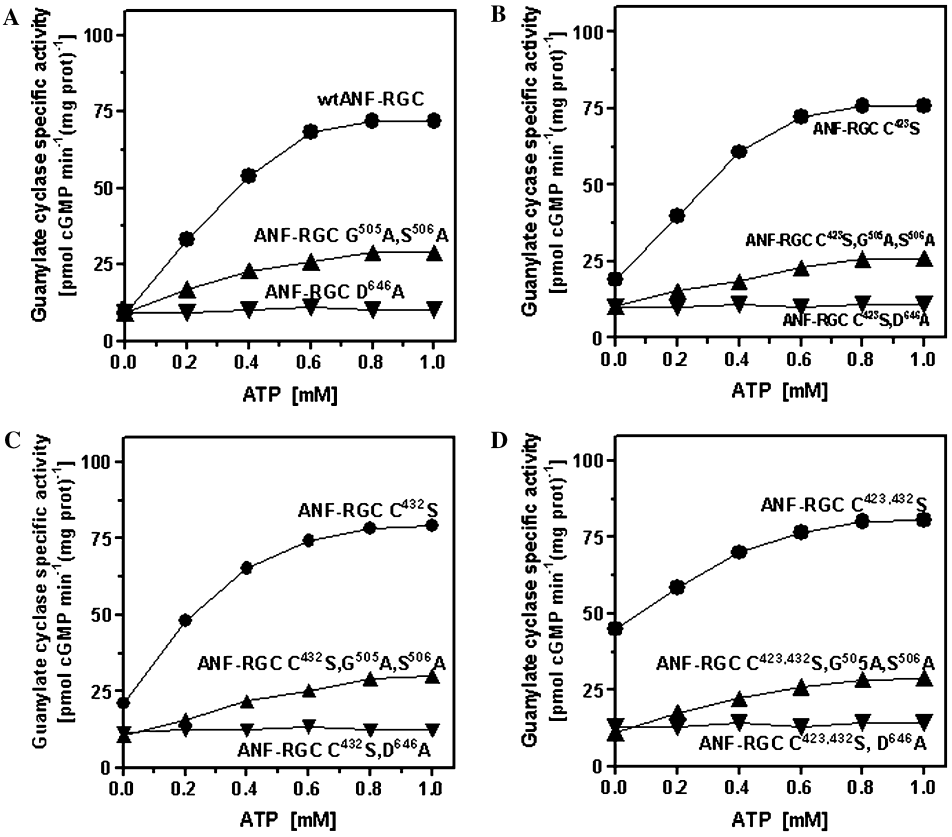

Fig. 6. Effect of $G^{505}A,S^{506}A$ and $D^{646}A$ mutations within the ARM domain on ANF/ATP-dependent activity of wt-ANF-RGC and its Cys-mutants. COS cells were transfected with appropriate expression constructs and the membranes were prepared as described in Materials and methods. These were assayed for guanylate cyclase activity in the presence of 10^{-7} M ANF and indicated concentrations of ATP. The guanylate cyclase activity is presented as the specific activity. Each experiment was done in triplicate and repeated at least two times with separate membrane preparations. The results presented are from one typical experiment. Error bars are within the size of the symbols.

fashion, but caused no or only minimal changes in the ARM-disrupted domain (Fig. 6A). Furthermore, the disruption of ARM lowered the activities of all Cys-mutants to the values of the parent ARM-disrupted mutants (Figs. 6B–D). These results establish the mediatory role of ARM in transmission of the ANF-dependent signaling of the Cys-disulfide structural motif in ANF-RGC.

ATP-regulated ARM domain model of ANF-RGC

To determine which of the two proposed ATP-transduction models is valid: KHD is the natural negative regulator of the catalytic module of ANF-GC [25] or, alternatively, ATP via its ARM positively regulates ANF-RGC [22], the expression studies with the ANF-RGC construct lacking the entire KHD domain were conducted. The wt-ANF-RGC served as the control. The KHD deletion has only minimal (~30%) stimulatory effect on the basal activity of ANF-RGC [12.7 ± 0.8 and 9.1 ± 1.3 pmol $cGMP min^{-1}$ (mg prot)$^{-1}$ for the KHD-deletion mutant and wt-ANF-RGC, respectively]. This is in contrast to the 800% stimulatory effect of the ANF signaling in ANF-RGC (Figs. 2 and 6A: wt-ANF RGC panels). These results are in agreement with the previous detailed study that has also shown that the KHD domain by itself has no role in repression of the ANF-RGC's catalytic activity. These findings together settle the issue that ARM domain by itself is not the repressor of the catalytic activity of ANF-RGC; and the findings support the model that ATP via ARM positively regulates ANF-RGC. In the current model, the repressor of the catalytic module of ANF-RGC is the disulfide structural motif. The mechanism of its repression is that the disulfide structural motif represses the ARM domain, which, in turn, inhibits the catalytic module of ANF-RGC. ANF signaling overcomes the disulfide structural motif-dependent inhibition of ARM, resulting in activation of the catalytic domain of ANF-RGC. In this context, it is noteworthy that the previous explanation linking the role of the Cys^{423} and Cys^{432} residues-motif with the "KHD-mediated guanylyl cyclase repression" [19] also needs revision.

Acknowledgments

These studies were supported by the NIH awards HL 070015 (T.D.) and DC 005349 (R.K.S.). We thank our laboratory colleagues, Dr. Ewa Fik-Rymarkiewicz and Ms. Beata Burczynska, for some help in the Western experiments, Dr. Venkateswar Venkataraman for help in artwork, and Mrs. Joan Sharma for the editorial assistance.

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