THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 2, Issue of January 9, pp. 1262–1268, 2004
Printed in U.S.A.

Mapping the Arrestin-Receptor Interface

STRUCTURAL ELEMENTS RESPONSIBLE FOR RECEPTOR SPECIFICITY OF ARRESTIN PROTEINS*

Received for publication, August 11, 2003, and in revised form, October 1, 2003
Published, JBC Papers in Press, October 6, 2003, DOI 10.1074/jbc.M308834200

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Arrestins selectively bind to phosphorylated activated forms of their cognate G protein-coupled receptors. Arrestin binding prevents further G protein activation and often redirects signaling to other pathways. The comparison of the high-resolution crystal structures of arrestin2, visual arrestin, and rhodopsin as well as earlier mutagenesis and peptide inhibition data collectively suggest that the elements on the concave sides of both arrestin domains most likely participate in receptor binding directly, thereby dictating its receptor preference. Using comparative binding of visual arrestin/arrestin2 chimeras to the preferred target of visual arrestin, light-activated phosphorylated rhodopsin (P-Rh*), and to the arrestin2 target, phosphorylated activated m2 muscarinic receptor (P-m2 mAChR*), we identified the elements that determine the receptor specificity of arrestins. We found that residues 49–90 (β-strands V and VI and adjacent loops in the N-domain) and 237–268 (β-strands XV and XVI in the C-domain) in visual arrestin and homologous regions in arrestin2 are largely responsible for their receptor preference. Only 35 amino acids (22 of which are nonconservative substitutions) in the two elements are different. Simultaneous exchange of both elements between visual arrestin and arrestin2 fully reverses their receptor specificity, demonstrating that these two elements in the two domains of arrestin are necessary and sufficient to determine their preferred receptor targets.

Preferential interaction of a heterotrimeric G protein with an activated receptor is a surprisingly uniform mechanism initiating the first round of signaling by a vast and diverse superfamily of G protein-coupled receptors (GPCRs).[1] The two-step mechanism that terminates signaling via G proteins and often redirects it to other pathways is also well conserved. First, an activated GPCR is phosphorylated by a cognate receptor kinase. Next, arrestin selectively binds to the active phosphorylated receptor covering its cytoplasmic tip and essentially crowding out G proteins (1, 2). Mammals have only four arrestin proteins, which apparently participate in the regulation of over 1000 different GPCRs (1). The expression of two of the four arrestins (visual arrestin, or arrestin1, and cone arrestin, or arrestin4)[2] is limited to rod and cone photoreceptors, where they quench the signaling of rhodopsin and cone opsins, respectively. Thus, only two remaining nonvisual arrestins (arrestin2 and arrestin3) regulate the vast majority of GPCRs. Obviously, a one-to-one receptor specificity of nonvisual arrestins is out of the question, although arrestins 2 and 3 do demonstrate a detectable preference for particular groups of receptors (3, 4). On the other hand, both visual arrestins are highly specialized; arrestin1 demonstrates clear preference for rhodopsin over any other receptor tested (5, 6), whereas arrestin4 prefers cone opsins (7). Therefore, to delineate the structural elements responsible for arrestin receptor specificity, we took advantage of a dramatic difference between arrestin1 and arrestin2 binding to rhodopsin (Rh) and the m2 muscarinic cholinergic receptor (m2 mAChR), which is easily measurable in a direct binding assay *in vitro* (6, 8). The comparison of the binding behavior of arrestin1/2 chimeras with that of parental arrestins allowed us to identify two elements in arrestin proteins largely responsible for their receptor preference. Interestingly, each arrestin domain has such an element, in agreement with earlier indications that both domains are involved in receptor binding (6, 8–13). These two elements along with several positively charged residues implicated in phosphate interaction (13–18) define the arrestin side of receptor-arrestin interface.

EXPERIMENTAL PROCEDURES

Materials

[γ-^{32}P]ATP, [^{14}C]leucine, and [^{3}H]leucine were purchased from R. K.PerkinElmer Life Sciences. All restriction enzymes were purchased from New England Biolabs. Sepharose 2B and all other chemicals were from sources described previously (19). Rabbit reticulocyte lysate and SP6 RNA polymerase were prepared as described previously (20). 11-*cis*-Retinal was generously supplied by Dr. R. K. Crouch (Medical University of South Carolina).

Construction of Arrestin1/2 Chimeras and Mutagenesis

Bovine visual arrestin cDNA (21) was a gift from Dr. T. Shinohara (Brigham and Women's Hospital, Boston, MA). The plasmids pARR-VSP and pBARRE were constructed and modified as described (6, 22). These pGEM2-based plasmids encode bovine wild type arrestins with an "idealized" 5′-untranslated region (20) under the control of a SP6 promoter. The following restriction sites (either existing or engineered by silent mutagenesis) were used for chimera construction (corresponding codons in the arrestin1 open reading frame followed by homologous codons in the arrestin2 open reading frame are shown in parentheses):

* This work was supported in part by National Institutes of Health Grants EY11500 and GM63097 (to V. V. G.) and GM44944 and GM47417 (to J. L. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: GPCR, G protein-coupled receptor; P-m2 mAChR*, phosphorylated carbachol-activated m2 muscarinic cholinergic receptor; P-Rh*, light-activated phosphorhodopsin; Rh*, light-activated unphosphorylated rhodopsin.

[2] In this paper we use systematic names of arrestin proteins, with the exception of arrestin1, which is historically called visual arrestin. The synonyms for arrestin2 are β-arrestin and β-arrestin1; arrestin3 is also called β-arrestin2.

EcoRI (upstream of start codon), AvrII (25–27; 21–23), BamHI (47–49; 43–45), PmlI (61–63; 57–59), AatII (73–74; 69–70), MluI (101–102; 98–99), SalI (145–146; 142–143), BsiWI (169–171; 163–165), BspEI (174–177; 168–170), ApaI (185–186; 179–180), XbaI (294–296; 288–290), XhoI (307–309; 301–303), BstXI (345–349; 340–344), and HindIII (downstream of stop codon) (Fig. 1). Certain other sites were eliminated by silent mutagenesis to make these sites unique. In some cases (inside regions 5 and 14) in which it was impossible to introduce convenient restriction sites, arrestin coding sequences were joined as follows. An oligonucleotide with the 5′ half matching the arrestin1 sequence and the 3′ half matching arrestin2 was used as a forward primer, and an arrestin2 oligonucleotide downstream from the far restriction site intended for use in subcloning as a reverse primer on the arrestin2 template. The resulting fragments, containing a short arrestin1 sequence at one end along with an arrestin1 primer upstream of the near restriction site, were then used as the reverse and forward primers, respectively. This second round of PCR on the arrestin1 template generated a fragment in which the sequence switched from arrestin1 to arrestin2 between restriction sites. Combination chimeras were constructed using the same sites. In this paper we named visual arrestin-based and arrestin2-based chimeras ChV and ChB, respectively, with a number following these letters to designate the region exchanged (*cf.* Fig. 1). The sequence of all constructs was confirmed by dideoxy sequencing.

In Vitro Transcription, Translation, and Evaluation of Mutant Stability

Plasmids were linearized using the HindIII site downstream of the coding sequence (Fig. 1) before *in vitro* transcription to produce mRNAs encoding full-length arrestin proteins. *In vitro* transcription and translation were performed as described previously (6, 19, 20). All arrestin proteins were labeled by incorporation of [^{3}H]leucine and [^{14}C]leucine with the specific activity of the mix 1.5–3 Ci/mmol, resulting in the specific activity of arrestin proteins within the range of 66 to 85 Ci/mmol (150–230 dpm/fmol). The translation of every mutant used in this study produced a single labeled protein band with the expected mobility on SDS-PAGE. Two parameters were used for the assessment of mutant relative stability as described (12); its yield was multiplied by the percentage of the protein remaining in the supernatant after incubation for 10 min at 37 °C followed by centrifugation. This integral parameter calculated for a mutant was expressed as a percent of that for wild type arrestin (22). The relative stability of all mutants used in this study exceeded 80%.

Receptor Preparations

Rhodopsin—Urea-treated rod outer segment membranes were prepared, phosphorylated with rhodopsin kinase, and regenerated with 11-*cis*-retinal as described (6). The stoichiometry of phosphorylation for the rhodopsin preparations used in these studies was 3.8 mol of phosphate/mol of rhodopsin (see Ref. 14 and references therein).

Muscarinic Receptor—The human m2 mAChR was expressed in Sf9 cells, purified by affinity chromatography, and reconstituted into chick heart phospholipids as described (6, 8). The reconstituted m2 mAChR was phosphorylated by purified GRK2 to a stoichiometry of 3.1–3.7 mol of phosphate/mol of m2 mAChR as described (6, 8).

Arrestin Binding to Receptors

Arrestin binding to receptors was performed as described (6, 8, 19).

Rhodopsin—Briefly, *in vitro* translated tritiated arrestins (50 fmol) were incubated in 50 mM Tris-HCl, pH 7.5, 0.5 mM $MgCl_2$, 1.5 mM dithiothreitol, and 50 mM potassium acetate, with 7.5 pmol (0.3 μg) of the various functional forms of rhodopsin in a final volume of 50 μl for 5 min at 37 °C either in the dark or in room light.

Muscarinic m2 Receptor—Tritiated arrestins (50 fmol) were incubated with 50 fmol of phosphorylated receptor in the presence of 100 μM carbachol for 35 min at 30 °C. After incubation with either receptor, the samples were immediately cooled on ice and loaded onto 2-ml Sepharose 2B columns equilibrated with 10 mM Tris-HCl, pH 7.5, 100 mM NaCl. Bound arrestin eluted with receptor-containing membranes in the void volume (between 0.5 and 1.1 ml). Nonspecific binding determined in the presence of 0.3 μg of liposomes (less than 10% of the total binding and less than 0.5% of the arrestin present in the assay) was subtracted.

RESULTS

In the basal (inactive) conformation arrestins are elongated molecules consisting of N- and C-domains (9, 10, 23, 24). Overall, the N-domain is considerably more flexible than the C-domain (9, 10, 13). Numerous studies suggest that receptor-binding elements are present in both domains (6, 11, 12, 25). We have recently identified two phosphate-binding elements in the N-domain comprising Lys-14, Lys-15, Arg-171, Arg-175, and Lys-176 that together constitute the phosphate sensor that ensures the destabilization of the basal arrestin conformation in response to receptor-attached phosphates (14, 15). Our previous studies also localized to the N-domain yet another arrestin sensor element responsible for the preferential binding to the active receptor (termed activation-recognition site), which is distinct from the phosphate-binding site (11). Based on enhanced specificity of the binding of phosphorylation-independent mutants to activated unphosphorylated receptors (7, 16, 17) we reasoned that the activation recognition site is most likely responsible for the ability of arrestin to discriminate between different receptors. Therefore we targeted the arrestin N-domain first. Using engineered restriction sites (Fig. 1) we constructed a series of visual arrestin-based chimeras containing nine different elements of the arrestin2 N-domain (Fig. 2). These proteins were expressed in cell-free translation, and their binding to phosphorylated light-activated rhodopsin (P-Rh*) and phosphorylated carbachol-activated m2 mAChR (P-m2 mAChR*) was compared with parental arrestins 1 and 2. We expected the introduction of any arrestin2 element playing a role in receptor specificity to decrease the binding of the chimera to P-Rh* and increase its binding to P-m2 mAChR* as compared with arrestin1. Indeed, the exchange of three consecutive elements spanning residues 45 through 98 reduced the binding of the chimeras to P-Rh* while enhancing their binding to P-m2 mAChR* at the same time (Fig. 2). We constructed and tested three additional chimeras with arrestin2-derived sequences in this region and found that the incorporation of arrestin2 element 45–86 in place of the homologous part of arrestin1 yields a chimera (ChV11) with arrestin2-like receptor preference, high binding to P-m2 mAChR* and low binding to P-Rh* (Fig. 2). According to the crystal structures of both

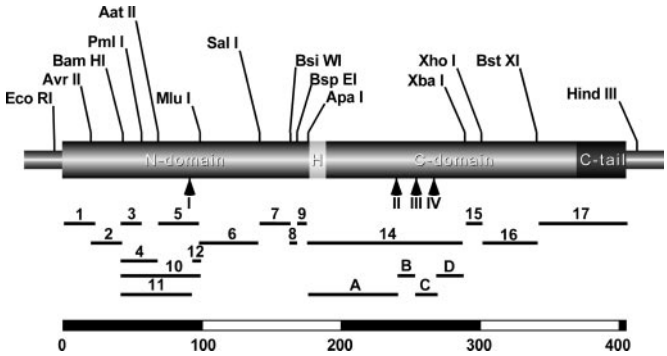

FIG. 1. **Construction of arrestin1/2 chimeras.** Coding and untranslated sequences are shown as *thick* and *thin rods*, respectively. The N-domain, inter-domain hinge (*H*), C-domain, and C-tail are shown according to the solved crystal structure (15, 16). Restriction sites used for chimera construction are shown *above* the rods, positions I–IV; where the sequences were joined by PCR without use of restriction sites are marked by *arrowheads below* the rods. The relative sizes of the exchanged arrestin elements are shown between the schematic and the scale in amino acid residues. The names of visual arrestin- and arrestin2-based chimeras begin with ChV and ChB, respectively. The designation above each fragment is used as the second part of the respective chimera name. Amino acid *position numbers* in the regions exchanged in chimeras (regions 1–17, with region 14 subdivided into parts *A*, *B*, *C*, and *D*) were as follows (visual arrestin residues are followed by corresponding arrestin2 residues in parentheses): 1, 1–25 (1–21); 2, 26–48 (22–44); 3, 49–62 (45–58); 4, 63–73 (59–68); 5, 74–101 (69–98); 6, 102–145 (99–142); 7, 146–170 (143–164); 8, 171–175 (165–169); 9, 176–185 (170–179); 10, 49–101 (45–98); 11, 49–90 (45–86); 12, 91–101 (87–98); 14, 186–295 (180–289); 14A, 186–236 (180–230); 14B, 237–250 (231–244); 14C, 251–268 (245–262); 14D, 269–295 (263–289); 15, 296–308 (290–302); 16, 309–346 (303–342); 17, 347–404 (343–418).

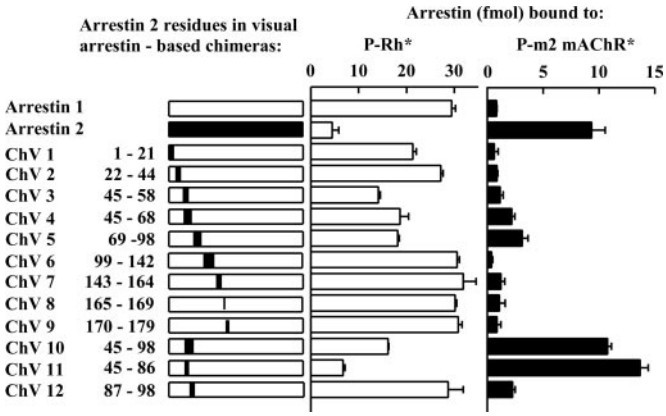

FIG. 2. **Binding characteristics of visual arrestin-based chimeras with arrestin2-derived N-domain elements.** Wild type arrestins 1 and 2 and chimeras are shown as *bars* with elements of arrestin2 origin in *black*. In a direct m2 mAChR binding assay, 100 fmol of m2 mAChR that was purified, reconstituted into liposomes, and phosphorylated by GRK2 was incubated in 50 μl with 50 fmol of the indicated tritiated arrestin (specific activities, 80–200 dpm/fmol) in the presence of 100 μM m2 agonist carbachol (P-m2 mAChR*) as described under "Experimental Procedures." In rhodopsin binding, 0.3 μg of rhodopsin kinase-phosphorylated rhodopsin, P-Rh*, was incubated with the same amounts of arrestins under room light. In both cases, membrane-bound arrestins were separated by gel filtration on Sepharose 2B and quantified in a liquid scintillation counter. Means ± S.D. from three experiments each performed in duplicate are shown.

proteins, this region encompasses β-strands V and VI and adjacent loops (9, 10, 23, 24).

To further test the role of the whole 55-amino acid element in the N-domain, we constructed arrestin2-based chimeras in which all (ChB10) or parts (ChB11, ChB12) of corresponding visual arrestin-derived elements were placed into the context of arrestin2 (Fig. 3). We reasoned that if this element were necessary and sufficient to switch receptor preference of arrestin proteins (as appears to be the case with ChV10 and ChV11), ChB10 and ChB11 would behave like visual arrestin. However, we found that although these substitutions decrease arrestin2 binding to P-m2 mAChR*, they fail to increase its binding to P-Rh*, suggesting that additional elements likely localized in the C-domain are also involved in arrestin receptor recognition.

Therefore we "scanned" the arrestin C-domain in the same fashion, constructing visual arrestin-based chimeras with arrestin2-derived parts (Fig. 4*A*). We found that the replacement of residues 186–295 with arrestin2 residues 180–289 (ChV14) dramatically changes arrestin specificity, whereas all three downstream replacements (in ChV15, 16, and 17) do not have this effect. In fact, ChV14 behaves almost like arrestin2, whereas symmetrical arrestin2-based ChB14 demonstrates binding behavior that is intermediate between that of visual arrestin and arrestin2 (Fig. 4*B*). Interestingly, either the N- or the C-domain element derived from arrestin2 in the context of visual arrestin (in ChV11 (Fig. 3) and ChV14 (Fig. 4)) effectively decreases its ability to bind P-Rh* and enhances its binding to P-m2 mAChR* to the level of arrestin2 or higher, whereas symmetrical substitutions in the context of arrestin2 (Figs. 3 and 4, ChB11 and ChB14, respectively) are a lot less effective in "converting" it into visual arrestin. Apparently, it is a lot easier to destroy the exact fit of visual arrestin to P-Rh* and create a chimera that can accommodate P-m2 mAChR* than it is to reconstruct highly specific visual arrestin on arrestin2 "backbone."

Because the element exchanged in ChV14 and ChB14 is relatively large, we divided it into four parts (termed A, B, C, and D) and constructed visual arrestin-based chimeras with these smaller arrestin2-derived elements (Fig. 5*A*). We found that none of these parts alone gives the full effect of the larger element on P-Rh* binding. The largest, part A (arrestin2 residues 180–230), appears to be the least potent, whereas two smaller parts, in B (residues 231–244) and D (residues 263–289), yield stronger effects (Fig. 5*A*), none of which is dramatic. Because the C-domain acts in concert with the N-domain in determining arrestin receptor preference, we constructed several visual arrestin-based chimeras with the identified N-domain element from arrestin2 (as in ChV11) combined with smaller C-domain elements (Fig. 5*B*), as well as arrestin2-based chimeras combining the identified N-domain element from visual arrestin (as in ChB11) with small C-domain elements and their combinations (Fig. 5*C*). The combination of the N-domain and large (A+B+C+D) C-domain elements yields a complete switch from visual arrestin-like to arrestin2-like receptor specificity. Although no single arrestin2-derived C-domain element in the context of ChV11 is as potent as the large element, the combination of elements B+C mimics the effect of the large element (Fig. 5*B*). Similarly, the combination of the visual arrestin-derived N-domain element with the large C-domain piece fully switches the specificity of arrestin2 to visual arrestin-like (Fig. 5*C*). The combinations of smaller C-domain elements that mimic it are the ones containing both B and C parts, whereas combinations of either B or C element with other elements appears less effective. Importantly, in these sets of 7 visual arrestin-based and 10 arrestin2-based chimeras (as well as in other chimeras described above) the increase in P-Rh* binding relative to parental protein is invariably accompanied by a decrease in P-m2 mAChR* binding, and vice versa, suggesting that the changes in binding reflect the shift in receptor preference of these chimeras rather than variations in their stability or folding problems. Collectively, these data suggest that parts B and C, *i.e.* visual arrestin residues 237–268 (and homologous residues 231–262 in arrestin2) in the C-domain are most important for receptor specificity. According to the crystal structures of both proteins, these residues encompass β-strands XV and XVI and the loop between them (9, 10).

To ascertain that exchange of the 41-amino acid-long N-domain element together with this 32-amino acid-long C-domain element fully switches the receptor specificity of both arrestins, we constructed two corresponding visual arrestin- and arrestin2-based chimeras (ChVNBC and ChBNBC, respectively) and compared their binding to P-Rh* and P-m2 mAChR* with that of parental proteins. We found that simultaneous exchange of these two elements completely reverses the receptor preferences of both arrestins (Fig. 6). Thus, these two elements together are necessary and sufficient to determine receptor specificity of arrestin proteins.

DISCUSSION

Four arrestin proteins expressed in mammals clearly fall into two distinct categories; specialized rod and cone arrestins demonstrate high specificity for their cognate receptors and remarkable species specificity, whereas both nonvisual arrestins are relatively promiscuous, readily interacting with various receptor types of different origin (1, 3, 6–8). The structural basis of receptor preference displayed by specialized arrestins or of the ability of other arrestin proteins to accommodate a wide variety of receptors is not known. The model of sequential multisite binding, proposed in 1993 (11), which is consistent with crystal structures solved later on (9, 10, 23, 24), logically explains arrestin selectivity for the phosphorylated active form of the receptor. The model posits that arrestin functions as a molecular coincidence detector. It has two sensor sites; one of these detects receptor phosphorylation, whereas the other one detects the receptor activation state. Simultaneous engagement of both sensors, which can occur only when arrestin encounters the active phosphorylated form of the

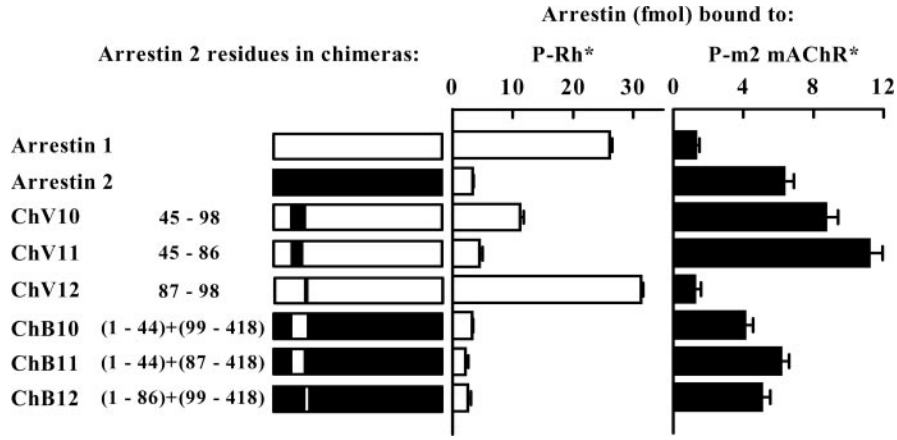

FIG. 3. **Short arrestin2-derived element enables high visual arrestin binding to P-m2 mAChR* but fails to enhance the binding to P-Rh* of a symmetrical arrestin2-based chimera.** The schematic representation of proteins and binding assays is as described in the legend to Fig. 2. Means ± S.D. from three experiments each performed in duplicate are shown.

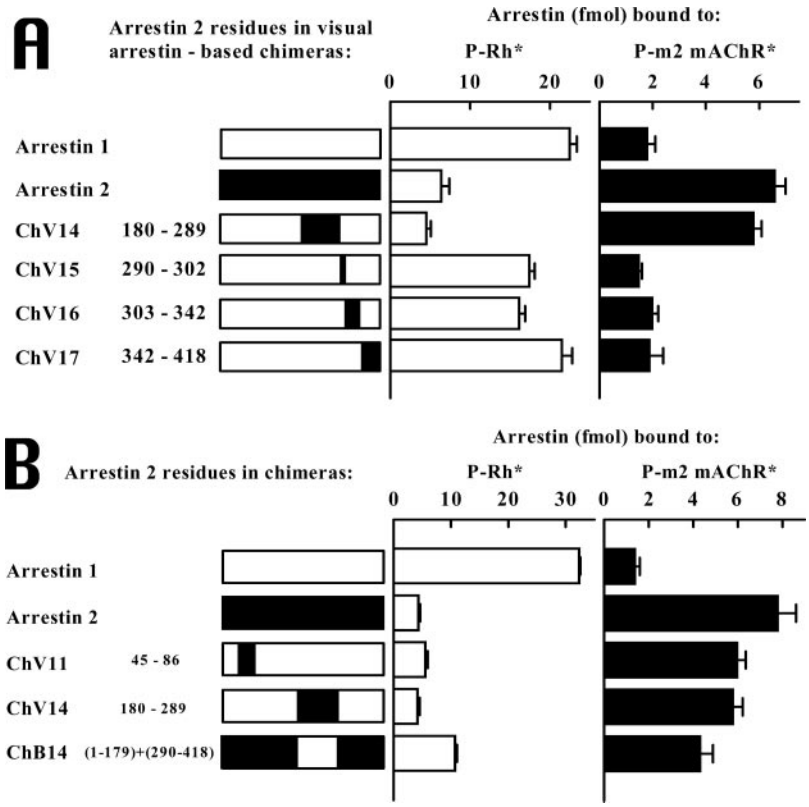

FIG. 4. **The large C-domain element enables high visual arrestin binding to P-m2 mAChR* (*A*) and enhances arrestin2 binding to P-Rh* (*B*).** The schematic representation of proteins and binding assays is as described in the legend to Fig. 2. Means ± S.D. from two experiments each performed in duplicate are shown.

receptor, releases molecular constrains that keep arrestin in its basal conformation. This allows its transition into the high affinity receptor-binding state, bringing into action additional binding sites. In the context of this model, receptor preference (be it species specificity or receptor subtype specificity) is determined by the activation sensor and/or additional sites mobilized by arrestin activation, not by phosphate-binding elements. The fact that arrestin mutants in which phosphate sensors are turned on constitutively demonstrate phosphorylation-independent binding only to their respective preferred receptors (7, 16, 17) supports this conclusion. Moreover, both the structure of phosphate-binding elements and the mechanism of phosphate sensor function are highly conserved in the arrestin family (7, 9, 10, 14–18, 22). Not surprisingly, swapping elements directly or indirectly involved in phosphorylation recognition (β-strands I, III, and X, α-helix I, lariat loop, C-tail; *cf.* Refs. 9 and 10) has no effect on receptor specificity (Figs. 2 and 4), even though a crucial role of β-strands I–III and X in arrestin binding to the receptor has been demonstrated by different laboratories using a variety of methods (12, 14–18, 25).

The elements important for receptor specificity identified here (Fig. 6) most likely interact with the cytoplasmic loop(s) and parts of the receptor C-tail that are not phosphorylated (4, 26–36). These regions are the most diverse (in terms of length and sequence) in the GPCR superfamily and most likely change conformation upon receptor activation (Refs. 37–48; recently reviewed in Refs. 49 and 50). Importantly, each domain carries a determinant of specificity (Figs. 6 and 7), supporting the idea that both arrestin domains are involved in receptor binding (6, 8–14, 25) and are brought into contact with the cytoplasmic tip of the receptor by a global conformational change in the arrestin molecule (9–11, 13, 18, 51, 52). The data are clearly inconsistent with speculative attempts to dock the arrestin N-domain alone to the receptor (23, 53). Interestingly, both elements are localized on the concave sides of their respective domains (Fig. 7), *i.e.* on the same arrestin surface as identified phosphate-binding residues (14, 15, 18), supporting the hypothesis that concave sides of the two domains face the receptor in the complex (9, 10, 13, 14, 18).

Our approach, based on the construction of visual-arrestin2

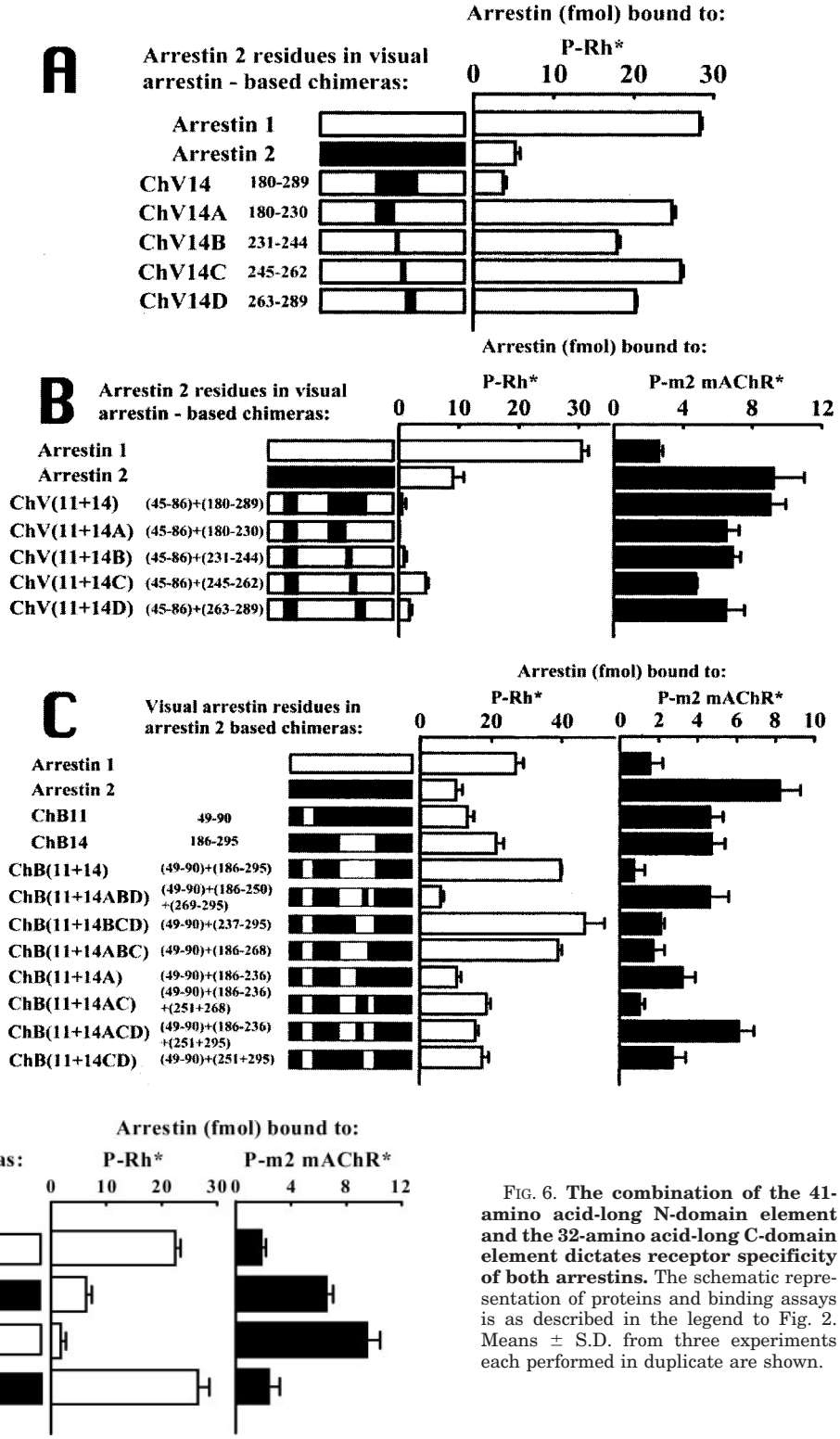

FIG. 5. **C-domain elements important for receptor preference of arrestin proteins.** *A*, none of the four small individual parts yields the full effect of the whole arrestin2 C domain element. *B*, combinations of the N-domain element and parts of the C-domain element more effectively change visual arrestin specificity toward that of arrestin2 than C-domain parts by themselves. *C*, combinations of N- and C-domain visual arrestin elements reverse receptor preference of arrestin2. The schematic representation of proteins and binding assays is as described in the legend to Fig. 2. Means ± S.D. from 2–3 experiments each performed in duplicate are shown.

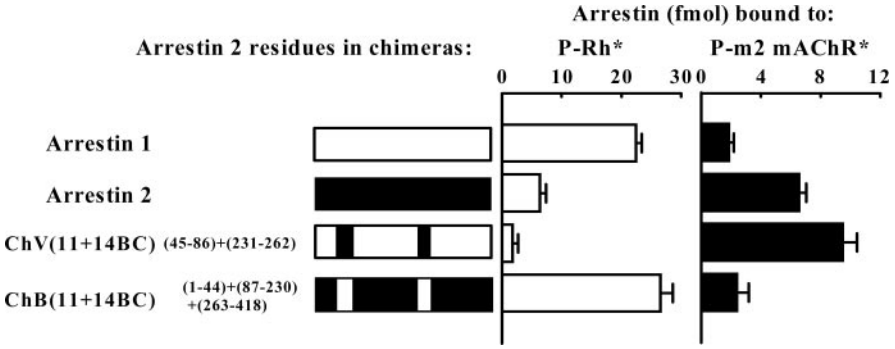

FIG. 6. **The combination of the 41-amino acid-long N-domain element and the 32-amino acid-long C-domain element dictates receptor specificity of both arrestins.** The schematic representation of proteins and binding assays is as described in the legend to Fig. 2. Means ± S.D. from three experiments each performed in duplicate are shown.

chimeras, was specifically designed to identify the elements that dictate receptor specificity by virtue of being different in the two members of the arrestin family and not to identify all arrestin elements involved in receptor binding. In particular, common, highly conserved receptor-binding elements, such as phosphate-binding residues, were clearly missed by this analysis, exactly as intended. So it is of particular interest to compare the elements we have identified with parts of visual arrestin identified by alternative methods designed to detect all arrestin elements involved in rhodopsin interaction. Two approaches proved most productive in this regard: differential chemical modification and hydrogen/deuterium exchange (25) and the use of synthetic arrestin peptides to inhibit arrestin and transducin binding to rhodopsin (12). Using differential modification, Palczewski and colleagues (25) found that lysines in β-strands I, X, XII, XV, and XVI and in the lariat loop (for consistency, here and below we use nomenclature from Ref. 15) are protected by P-Rh*. Hofmann and colleagues (12) found that peptides corresponding to β-strands I–III, V + adjacent loops, and XV+XVI inhibit arrestin and transducin binding.

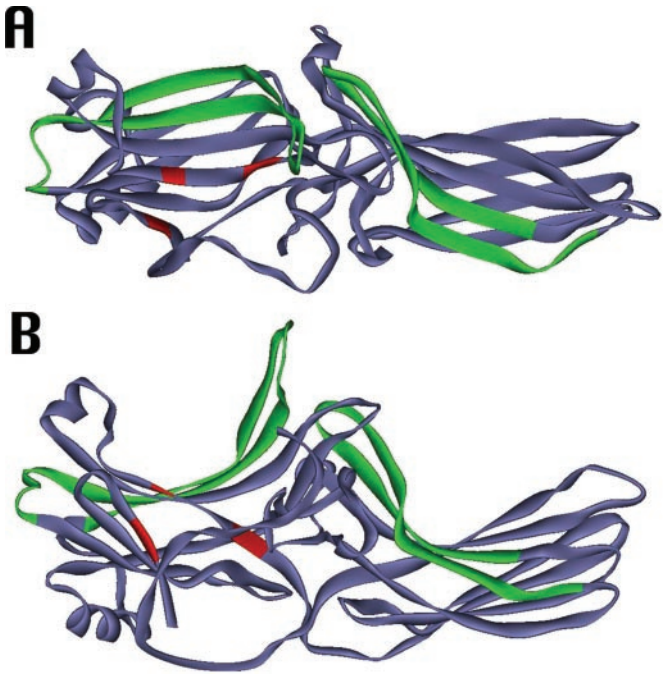

FIG. 7. **All arrestin elements implicated in receptor binding are localized on the concave sides of the two domains.** *A*, top view, down the cavities of both domains (from the "receptor viewpoint"). *B*, side view. In both panels the two receptor-binding elements identified in this study are shown in *green*, and phosphate-binding residues (13, 21, 25) are shown in *red*. Both views were generated with ViewerLite 5.0 (Accelrys, Inc., www.accelrys.com) based on the 2.8-Å crystal structure of visual arrestin (15).

Notably, the peptide encompassing β-strands I–III inhibited arrestin binding to P-Rh* much more effectively than transducin binding to Rh* (12). In addition, a peptide corresponding to β-strand X was found to bind P-Rh* (54). An antibody to this peptide was shown to interact with free arrestin, preventing its binding to P-Rh*, and it did not interact with rhodopsin-bound arrestin (54). The results of epitope insertion mutagenesis in visual arrestin suggest that the loop between β-strands V and VI and the one preceding β-strand X are involved in rhodopsin binding (55). It is noteworthy that all of these approaches point to essentially the same set of "suspects," which fall into two categories: elements that participate in phosphate interaction (β-strands I—III and X and lariat loop (9, 10, 13–18)) and those that likely bind other parts of the receptor. Apparently, only β-strands V+VI (with adjacent loops) and XV+XVI fall into this latter category. Notably, three absolutely different approaches (Refs. 12 and 25, and this study) are in remarkable agreement in identifying these two elements. In the context of our approach, this suggests that parts of arrestin interacting with any receptor elements other than phosphorylated sites are important for receptor specificity.

Obviously, receptor specificity must be determined by the differences in the sequence of these two elements. The overall identity and similarity scores between visual arrestin and arrestin2 are 59 and 75%, respectively (5). Interestingly, in the N-domain element, both identity (26 of 41 residues; 63%) and similarity (83%) are even higher than average, suggesting that the difference in as few as 15 residues (only seven of which are nonconservative substitutions) brings about a dramatic shift in receptor preference (compare visual arrestin and ChV11 on Fig. 3). The C-domain element is more divergent, with considerably lower identity (12 of 32 residues; 38%) and similarity (53%) scores. Still, our data indicate that just 20 different residues (15 nonconservative substitutions) of 170 in the C-domain dictate specificity. Thus, the difference in less then 10% of the residues (35 of 404 or 418), which includes nonconservative substitutions of only 5% (22 residues), is all it takes to completely reverse receptor preference of either of the two arrestins (Fig. 6). These findings set the stage for the identification of individual residues involved in receptor recognition and elucidation of the structural basis of high specificity of visual arrestins, as well as the ability of their nonvisual "cousins" to accommodate an amazing variety of GPCRs. We believe that ultimately this information will allow us to construct arrestins custom-designed to be specific for particular GPCR subfamilies or even individual receptors, which are likely to be useful in research and gene therapy of various congenital disorders associated with the malfunction of a particular mutant receptor.

Acknowledgments—We are grateful to Dr. R. K. Crouch for 11-*cis*-retinal, to Dr. T. Shinohara for bovine visual arrestin cDNA, to Judith Ptasienski for purified m2 mAChR, to students Jana E. Harris, Rebecca Liles, Lisa Olander, and Choya Yoon for the construction of several chimeras, and to Susan M. Hanson for critical reading of the manuscript.

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Mapping the Arrestin-Receptor Interface: STRUCTURAL ELEMENTS RESPONSIBLE FOR RECEPTOR SPECIFICITY OF ARRESTIN PROTEINS

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J. Biol. Chem. 2004, 279:1262-1268.
doi: 10.1074/jbc.M308834200 originally published online October 6, 2003
