J. Biochem. 120, 996-1001 (1996)

Importance of N-Terminal Regions of G Protein α Subunits for the Activation of Phospholipase C in *Xenopus* Oocytes[1]

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Received for publication, July 1, 1996

The α subunits of G_q family G proteins, $G_{L1}\alpha$ and $G_{L2}\alpha$, are bovine homologues of mouse $G_{14}\alpha$ and $G_{11}\alpha$, respectively, and are closely related to each other. When expressed in *Xenopus* oocytes together with metabotropic glutamate receptors, $G_{L2}\alpha$ activates endogenous phospholipase C (PLC*x*) in response to glutamate stimulation, whereas $G_{L1}\alpha$ inhibits the activation of PLC*x*. By examining the properties of 10 chimeras between $G_{L1}\alpha$ and $G_{L2}\alpha$ and their mutants, we tried to identify the regions on the $G\alpha$ proteins that are important for the activation of PLC*x*. The results indicated that a necessary (but not sufficient) condition for a chimeric $G\alpha$ protein to be able to clearly activate PLC*x* was that its N-terminal quarter portion should be derived from $G_{L2}\alpha$. No correlation was found between the origin ($G_{L1}\alpha$ or $G_{L2}\alpha$) of C-terminal regions of the chimeras and the ability of chimeras to activate PLC*x*. One of the chimeras is different from $G_{L2}\alpha$ at only four amino acid residues in the N-terminal region, and yet it could not activate PLC*x*. When one of the four residues, Ser-59, in the chimera was mutated back to Ala as in the original $G_{L2}\alpha$, the resulting mutant became capable of activating PLC*x*. This residue is localized in the midst of the N-terminal linker connecting the two major domains in the $G\alpha$ proteins. These results indicate that Ala-59 is critical for the activation of PLC*x*, and that the linker may play important roles in determining functions of $G\alpha$ proteins.

Key words: chimera, glutamate receptor, G protein, oocyte, phospholipase C.

Heterotrimeric guanine nucleotide-binding proteins (G proteins) are composed of α, β, and γ subunits. Various regulatory sites of the G proteins, such as the sites responsible for guanine nucleotide binding, GTPase activity, $\beta\gamma$ subunits binding, receptor recognition and effector interaction, are localized on the α subunit (*1*). G protein-effector interactions have been examined by constructing $G_i\alpha$-$G_s\alpha$ chimeras (*2-5*) and by using synthetic peptides of transducin (*6*) and $G_q\alpha$ (*7*). The results suggest that the C-terminal half of the α subunit is important for the effector activation. The N-terminal region is thought to be involved in the interaction with the $\beta\gamma$ subunit (*1*). However, its participation in the effector interaction has not been ruled out, because it shows analogies to the ras proteins ($p21^{ras}$) and bacterial elongation factor Tu (EF-Tu) (*8*); and its role in the activation of phospholipase C (PLC) remains to be studied.

When metabotropic glutamate receptor subtype 1 (mGluR1) is expressed in *Xenopus* oocytes, chloride current responses are produced by the application of L-glutamate (Glu) as a result of the activation of oocyte endogenous G proteins and PLC (termed here Gp and PLC*x*, respectively), the production of inositol 1,4,5-trisphosphate, the mobilization of intracellular calcium, and the opening of chloride channels (*9-11*). Although its composition is not fully known, oocyte PLC*x* comprises, among others, a PLCβ isoform homologous to mammalian PLCβ3 (*12*), which can be activated by $G_q\alpha$, $G_{11}\alpha$, and $G_{16}\alpha$ (*13*). We previously reported (*14*) that $G_{L2}\alpha$, a bovine version of $G_{11}\alpha$, potentiated the chloride current responses by interacting with mGluR1 and activating PLC*x*, whereas $G_{L1}\alpha$, a bovine version of $G_{14}\alpha$, inhibits the Glu-induced activation of PLC*x*. $G_{L1}\alpha$ (or $G_{14}\alpha$) has been shown to activate PLCβ1 or β4, but not β2 (*15, 16*), and its ability to activate PLC seems to depend on the types of the target enzyme. Therefore, according to the proposed mechanism of the inhibition by $G_{L1}\alpha$ (*14*), activated $G_{L1}\alpha$ interacts with PLC*x* but can not activate it, just as a pharmacological antagonist acts at a receptor site, thereby allowing the inhibition of its activation by endogenous G_p in the oocytes. Thus the two closely related G protein α subunits, $G_{L1}\alpha$ and $G_{L2}\alpha$, which show an 83% amino acid homology to each other (*17*), exhibit opposite physiological effects, one activating and the other blocking the activation of PLC*x*. They provide a useful model to examine possible regions of the G protein α subunit responsible for the activation of PLC*x*.

[1] This research was supported in part by grants from the Ministry of Education, Science, Sports and Culture of Japan.

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Abbreviations: PLC, phospholipase C; PLC*x*, endogenous PLC in *Xenopus* oocytes; PTX, pertussis toxin; mGluR1, metabotropic glutamate receptor subtype 1.

In the present study, we constructed different $G_{L1}\alpha/G_{L2}\alpha$ chimeras and their mutants, and expressed them in the oocytes together with mGluR1 and, in some cases, exogenous G protein β_1 and γ_2 subunits. By examining their effects on the mGluR1-stimulated, PLC*x*-mediated current responses, we tried to identify the α subunit regions that are important for the activation of PLC*x*.

MATERIALS AND METHODS

Construction of $G_{L1}\alpha/G_{L2}\alpha$ Chimeras and Mutants—The $G_{L1}\alpha/G_{L2}\alpha$ chimeras were constructed between bovine $G_{L1}\alpha$ and $G_{L2}\alpha$ cDNAs (*17*). The recombinant pSPA2 plasmid (pSPGL1) (*14*) carrying the SP6 promoter and protein-coding sequence for $G_{L1}\alpha$ was cleaved with *Xho*I, treated with T4 DNA polymerase with the four dNTPs, and digested with *Nco*I. The 4.2 kilobase-pair (kb) *Xho*I-*Nco*I fragment was ligated with the 390 base-pair (bp) *Sma*I-*Nco*I fragment excised from pGL7 (*17*) to yield plasmid pSPB-2. The 880-bp *Nco*I-*Hin*dIII fragment from the recombinant pSPA2 plasmid (pSPGL2), a transcription vector for $G_{L2}\alpha$ (*14*), was ligated with the 3.9-kb *Nco*I-*Hin*dIII fragment from pSPGL1. The resulting plasmid was digested with *Hin*dIII, blunted with T4 DNA polymerase and circularized with T4 DNA ligase to yield plasmid pSPW-2. Plasmid pGL7 was digested with *Hin*cII, ligated with the *Xho*I linker, dCCTCGAGG, and cleaved with *Xho*I and *Sca*I. The 740-bp *Xho*I-*Sca*I fragment was ligated with the 4.0-kb *Xho*I-*Ssp*I fragment from pSPGL1 to yield plasmid pSPB-3. The 4.1-kb *Ssp*I-*Hin*dIII fragment from pSPGL1 was ligated with the 690-bp *Sca*I-*Hin*dIII fragment from pSPGL2. The resulting plasmid was digested with *Hin*dIII, blunted with T4 DNA polymerase and circularized with T4 DNA ligase to yield plasmid pSPW-3. The 890-bp *Sma*I-*Dra*I fragment from pGL1 (*17*) was inserted into the *Sma*I site of pUC119 to obtain plasmid pUCGL1. This plasmid was digested with *Kpn*I, blunted with T4 DNA polymerase, and cleaved with *Sma*I. Plasmid pSPGL2 was partially digested with *Bsp*EI and *Hin*dIII, and blunted with T4 DNA polymerase. The 3.7-kb *Bsp*EI-*Hin*dIII fragment from pSPGL2 was ligated with the 890-bp *Sma*I-*Kpn*I fragment from pUCGL1 to construct plasmid pSPW-4. The 4.3-kb *Bsp*EI-*Hin*dIII fragment from pSPGL2 was ligated with the 310-bp *Dra*I-*Dra*I fragment from pGL1 to yield plasmid pSPB-4. The 3.2-kb *Sac*I-*Sac*I fragment from pSPGL1 was ligated with the 1.4- or 1.3-kb *Sac*I-*Sac*I fragment from pSPGL2 or pSPB-2 to yield plasmid pSPW-1 or pSPWB-1, respectively. The 3.4-kb *Sac*I-*Sac*I fragment from pSPGL2 was ligated with the 1.3- or 1.5-kb *Sac*I-*Sac*I fragment from pSPGL1 or pSPW-2 to yield plasmid pSPB-1 or pSPBW-2, respectively.

The genetic mutation of the $G_{L1}\alpha/G_{L2}\alpha$ chimera BW2 was performed by the polymerase chain reaction (PCR) using the sense primer M1 (TGAGAATCATCCACGGGGCTGGGTACAGCGAGGAAGAC) or M2 (TGAGAATCATCCACGGGTCTGGGTACAGCGACGAAGACAAAAGGGGGTTC), and the antisense primer M3 [ATCTTCAG(GC)GT(GT)TCCATGGC(GT)C]. The primers M1, M2, and M3 correspond to amino acid residues *55* to *67*, *55* to *71*, and *88* to *95* of $G_{L1}\alpha$, respectively (in the text and the figures, all numbers of amino acid residues are based on the positions in $G_{L2}\alpha$, except that numbers in italics represent the positions in $G_{L1}\alpha$). The primer M1 contains point mutations (G) replacing the first codon TCT coding for Ser-*61* and the third codon GAC coding for Asp-*65* of $G_{L1}\alpha$ by GCT and GAG coding for Ala and Glu; the primer M2 contains point mutations (A and G) replacing the second codon AGA and AAG for Arg-*68* and Lys-*69* of $G_{L1}\alpha$ by AAA and AGG coding for Lys and Arg (these 4 amino acid positions *61*, *65*, *68*, and *69* in G_{L1} correspond to positions 59, 63, 66, and 67 in G_{L2} or BW2). Plasmid pSPBW-2 was used as a template. PCRs were carried out for 1 min at 94°C, 2 min at 50°C, and 2.5 min at 72°C for 30 cycles. The 120-bp products amplified by PCR using a set of primers of M1/M3 or M2/M3 were digested with *Tfi*I and *Nco*I, and purified. Plasmid pSPBW-2 was partially digested with *Sac*I, blunted with T4 DNA polymerase and circularized to delete a *Sac*I site on the vector. The resulting plasmid was cleaved with *Sac*I and *Nco*I, and the 4.7-kb *Sac*I-*Nco*I fragment was then ligated with the 60-bp *Sac*I-*Tfi*I fragment from pGL1 and the purified 100-bp *Tfi*I-*Nco*I fragment from PCR product amplified by using primers M1/M3 or M2/M3 to obtain plasmid pSPBW2AE or pSPBW2KR, respectively. This 4.7-kb *Sac*I-*Nco*I fragment was also ligated with the 80-bp *Sac*I-*Rsa*I fragment from pSPBW-2AE and the 80-bp *Rsa*I-*Nco*I fragment from pGL1 to yield plasmid pSPBW2A.

Subcloning and mutagenesis procedures were verified by restriction enzyme analysis and DNA sequencing. cRNAs specific for $G_{L1}\alpha/G_{L2}\alpha$ chimeras and mutants were synthesized *in vitro* using plasmids pSPB-1 to pSPB-4, pSPW-1 to pSPW-4, pSPWB-1, pSPBW-2, pSPBW2AE, pSPBW-2KR, and pSPBW2A as described previously (*14*).

Functional Analyses—All experimental procedures for the functional analyses of chimeras and mutants using *Xenopus* oocytes were as described previously (*14*). The amounts of specific mRNAs injected were 50 pg (mGluR1α), 5 ng (Gα proteins, including chimeras and mutants), 5 ng (Gβ1), or 1 ng (Gγ2) per oocyte. The current responses of the oocytes elicited by 100 μM Glu were measured under the voltage-clamp conditions at −60 mV. The maximum amplitudes of the responses were used as estimates of the reactivity (*14*).

In some experiments, oocytes were treated with pertussis toxin (Seikagaku Kogyo, Tokyo), 2 μg/ml, at 20°C for 24 h preceding electrophysiological measurements.

RESULTS

$G_{L1}\alpha$ and $G_{L2}\alpha$ are G protein α subunits of G_q class and show 83% identity in amino acid sequences with each other (*17*). When $G_{L2}\alpha$ was expressed together with mGluR1 in *Xenopus* oocytes, mGluR1-mediated Cl^- current responses were potentiated (*14*). In contrast, when $G_{L1}\alpha$ was expressed together with mGluR1, Glu responses were greatly suppressed (*14*). We concluded that this inhibition is due to the antagonistic effects of $G_{L1}\alpha$ on the regulatory sites of PLC*x* (*14*). In other words, activated $G_{L1}\alpha$ may associate with and occupy the regulatory sites of PLC*x*, but fails to activate it, thereby allowing the blockade of its activation by endogenous G_p.

The opposite effects of $G_{L1}\alpha$ and $G_{L2}\alpha$ on mGluR1-mediated PLC*x* reactions in the oocytes probably derive from structural differences between $G_{L1}\alpha$ and $G_{L2}\alpha$ which cause these G proteins to interact differently with PLC*x*. To

investigate specific regions responsible for such differences, we constructed ten different $G_{L1}\alpha/G_{L2}\alpha$ chimeras by recombining $G_{L1}\alpha$ and $G_{L2}\alpha$ cDNAs at four corresponding restriction enzyme sites (referred to as the sites a, b, c, and d; Fig. 1). The effects of these chimeras on mGluR1-mediated Cl^- current responses were examined by injecting *in vitro* transcribed mRNAs of these chimeras together with mGluR1-specific mRNA into the oocytes.

The expression of these chimeric α subunits as well as $G_{L1}\alpha$ and $G_{L2}\alpha$ subunits were confirmed by Western blot analyses of the oocyte membranes using specific antibodies raised against the C-terminal pentadecapeptide of $G_{L2}\alpha$ proteins, which recognize both $G_{L1}\alpha$ and $G_{L2}\alpha$ (*17*) (data not shown). Typical examples of current responses of the oocytes expressing some of these chimeras are shown in Fig. 2.

Figure 3 shows the effects of four chimeras, W-1, B-1, W-3, and B-3, in which $G_{L1}\alpha$ and $G_{L2}\alpha$ were recombined at the sites a or c (Fig. 1). All four chimeras suppressed mGluR1-mediated Cl^- current responses, as did $G_{L1}\alpha$, when expressed in the oocytes together with mGluR1 (Figs. 2A and 3A). It is possible that the suppression by these four chimeras is caused by an indirect mechanism in which the chimeric α subunits compete with the endogenous α subunits for the endogenous $\beta\gamma$ subunits to form functional heterotrimers, as exemplified by $G_s\alpha$ (*14*). To examine this possibility, we expressed exogenous β_1 and γ_2 subunits together with each of the four chimeric α subunits. As shown in Fig. 3B, the suppressions by these four chimeras were not reversed by the co-expression of exogenous $\beta_1\gamma_2$ subunits, which stands in contrast to the case of $G_s\alpha$ (*14*). These results suggest that the inhibitory mechanisms of these four chimeras may be more direct ones, as in the case of $G_{L1}\alpha$ (*14*): these four chimeras may be activated by mGluR1 and interact with, but fail to activate, PLC*x* in the oocytes.

Previous results indicated that G protein-effector interaction sites reside in the C-terminal half of the α subunits (*2, 4, 5, 7*). However, chimera W-3, in which two C-terminal blocks (IV and V) of $G_{L1}\alpha$ were substituted by the corresponding blocks of $G_{L2}\alpha$, suppressed the responses. Furthermore, chimera W-1, in which four C-terminal blocks (II-V) of $G_{L1}\alpha$ are substituted by the corresponding blocks of $G_{L2}\alpha$, suppressed the responses. Therefore, activation or inhibition of mGluR1-mediated PLC*x* reactions cannot be explained by differences in the C-terminal half of their sequences. All four chimeras, W-1, B-1, W-3, and B-3, suppressed the responses in the presence or absence of exogenous $\beta_1\gamma_2$ subunits. It is possible that the construction of chimeric cDNAs at site a or site c affected

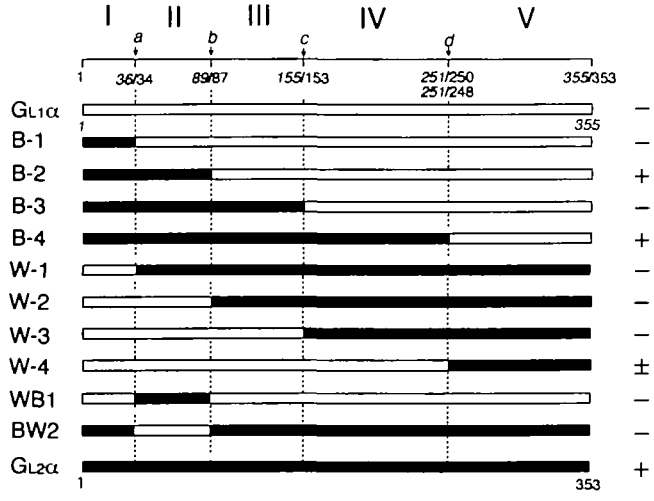

Fig. 1. **The names and compositions of the chimeras examined.** The open and closed boxes indicate the sequences derived from $G_{L1}\alpha$ and $G_{L2}\alpha$, respectively. The numbers (other than 1) represent the numbers of amino acid residues at the carboxyl ends of the blocks I to V in the original $G_{L2}\alpha$. The italic numbers represent the numbers of amino acid residues at the corresponding position in $G_{L1}\alpha$. At junction d, *251*/250 is for B-4 and *251*/248 is for W-4. The compositions of the individual chimeric Gαs are as follows [the numbers in brackets indicate amino acid numbers (*17*)]: B-1, $G_{L2}\alpha$ [1-34] and $G_{L1}\alpha$ [*37-355*]; B-2, $G_{L2}\alpha$ [1-87] and $G_{L1}\alpha$ [*90-355*]; B-3, $G_{L2}\alpha$ [1-153] and $G_{L1}\alpha$ [*156-355*]; B-4, $G_{L2}\alpha$ [1-250] and $G_{L1}\alpha$ [*252-355*]; W-1, $G_{L1}\alpha$ [*1-36*] and $G_{L2}\alpha$ [35-353]; W-2, $G_{L1}\alpha$ [*1-89*] and $G_{L2}\alpha$ [88-353]; W-3, $G_{L1}\alpha$ [*1-155*] and $G_{L2}\alpha$ [154-353]; W-4, $G_{L1}\alpha$ [*1-251*] and $G_{L2}\alpha$ [249-353, Phe-249 (TTC) was replaced with Gly (GGC)]; WB1, $G_{L1}\alpha$ [*1-36*], $G_{L2}\alpha$ [35-87] and $G_{L1}\alpha$ [*90-355*]; BW2, $G_{L2}\alpha$ [1-34], $G_{L1}\alpha$ [*37-89*] and $G_{L2}\alpha$ [88-353]. The effects of these α subunits on the mGluR1-evoked current responses are summarized on the right by + (potentiation) or − (inhibition). ± means weak potentiation observed only after pertussis toxin treatment.

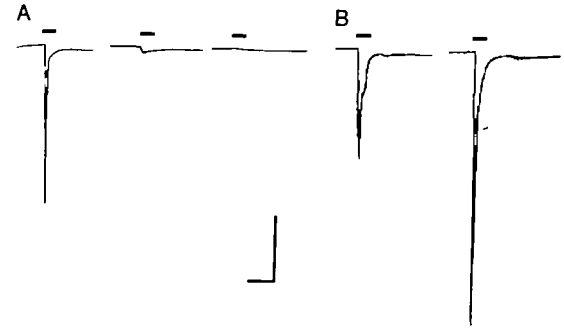

Fig. 2. **Typical examples of the mGluR1-evoked current responses of the oocytes injected with mRNAs specific for mGluR1 with or without mRNAs for $G_{L1}\alpha/G_{L2}\alpha$ chimeras and G protein $\beta_1\gamma_2$ subunits.** (A) Left, control; center, chimera B-3; right, B-3 and $\beta_1\gamma_2$. (B) Left, control; right, chimera B-2. In this and all the other figures, "control" oocytes mean the oocytes in which only mGluR1 mRNA was injected. Thick bars represent Glu (100 μM) application. Calibrations: 500 nA and 1 min (for A and B).

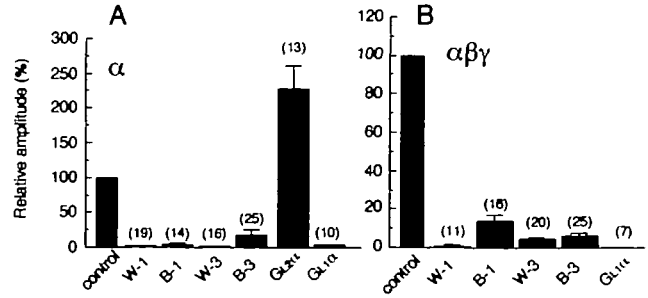

Fig. 3. **The effects of chimeric Gα proteins on the mGluR1-evoked current responses of the oocytes.** The maximum amplitudes of responses were measured and expressed relative to the control, as means±SEM (number of oocytes examined). A: The responses of the oocytes injected with the indicated Gα mRNAs together with mGluR1. B: The responses of the oocytes injected with the indicated Gα mRNAs together with G protein $\beta_1\gamma_2$ and mGluR1.

the structures of the proteins, and resulted in the failure to acquire the ability to activate PLC*x*.

Figure 4 shows the results for four other chimeras, W-2, B-2, W-4, and B-4, in which the cDNAs were recombined at sites b or d (Fig. 1). When chimera B-4, in which the N-terminal four blocks (I-IV) of $G_{L2}\alpha$ are connected at site d to the fifth block of $G_{L1}\alpha$, was expressed in the oocytes together with mGluR1, Glu responses were potentiated by approximately twofold compared to the control. In the oocytes treated with pertussis toxin (PTX), which inactivated endogenous G proteins coupled to PLC*x*, the extent of potentiation by chimera B-4 was much more prominent (Fig. 5). These potentiations were comparable to that of $G_{L2}\alpha$. Furthermore, chimera B-2, in which the N-terminal two blocks (I and II) of $G_{L2}\alpha$ were connected at the site b to the C-terminal three blocks of $G_{L1}\alpha$, also potentiated Glu responses by approximately twofold compared to the control (Figs. 2B and 4). Glu responses were also potentiated greatly by chimera B-2 in PTX-treated oocytes to a comparable extent to those by chimera B-4 and $G_{L2}\alpha$ (Fig. 5). These results suggest that both chimera B-4 and chimera B-2 can associate with the endogenous $\beta\gamma$ subunits of oocytes to form a functional heterotrimer, couple with mGluR1 and activate PLC*x* in the same manner as in $G_{L2}\alpha$ (*14*). In contrast, chimera W-2, the reversed construction of chimera B-2, suppressed Glu responses and the suppression was enhanced by the expression of exogenous $\beta_1\gamma_2$ subunits (Fig. 4), similarly to the case of $G_{L1}\alpha$ (Fig. 3).

Chimera W-4, the reversed construction of chimera B-4, neither potentiated nor inhibited the responses significantly in the presence or absence of exogenous $\beta_1\gamma_2$ subunits (Fig. 4). In PTX-treated oocytes, control responses were greatly suppressed (Fig. 6B) so that the potentiating effects of exogenous $G\alpha$ could be seen much more clearly. When examined under such conditions, chimera W-4 potentiated the responses by approximately 2.8-fold compared to the control. However, the stimulatory effects were much

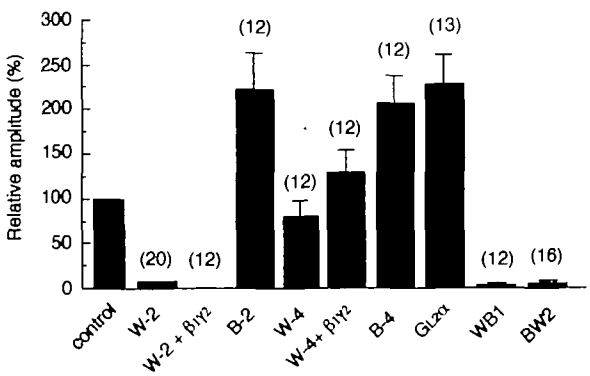

Fig. 4. **The effects of chimeric Gα proteins on the mGluR1-evoked current responses.** The maximum amplitudes of responses were measured and expressed relative to the control, as means ± SEM (number of oocytes examined). The G protein subunits injected are indicated under each column.

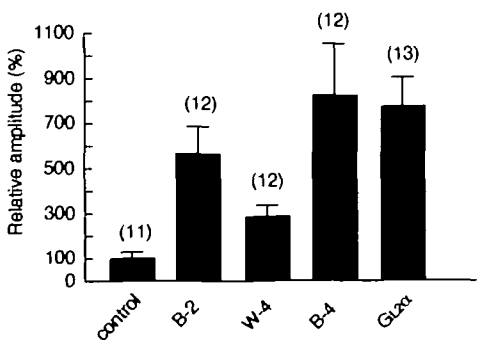

Fig. 5. **The effects of chimeric Gα proteins on the mGluR1-evoked current responses in the oocytes treated with pertussis toxin.** The maximum amplitudes of responses were measured and expressed relative to the control, as means ± SEM (number of oocytes examined). The G protein subunits injected are indicated under each column.

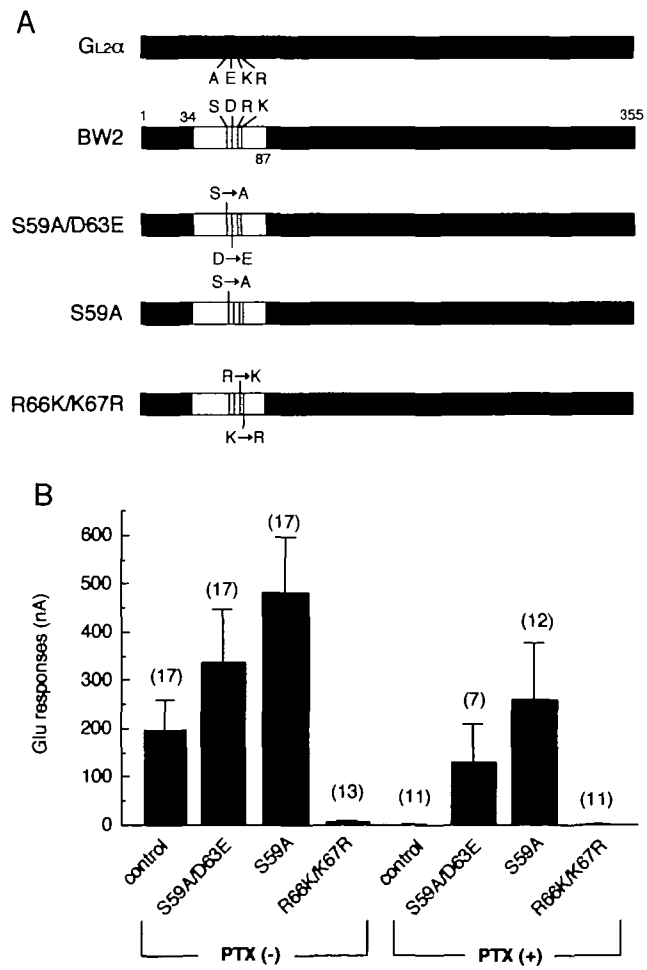

Fig. 6. **A: The names and structures of the mutants derived from the chimera BW2.** Ser-59 (TCT), Asp-63 (GAC), Arg-66 (AGA), or Lys-67 (AAG) in the $G_{L1}\alpha$ block (II) of chimera BW2 was mutated to Ala (GCT), Glu (GAG), Lys (AAA), or Arg (AGG), respectively. In the figure, the amino acid residues are shown by the vertical lines and single letter codes, and the mutations are indicated by the arrows. These four amino acids are coded by GCG (Ala-59), GAG (Glu-63), AAG (Lys-66), or CGG (Arg-67) in the original $G_{L2}\alpha$ protein. **B: The effects of mutant Gα proteins on the mGluR1-evoked current responses.** The maximum amplitudes of responses (nA) are shown as means ± SEM (number of oocytes examined). The G protein subunits injected are indicated under each column. The oocytes were analyzed without [PTX(−)] or after [PTX(+)] treatment with pertussis toxin.

weaker than those of chimeras B-2, B-4, and $G_{L2}\alpha$ (Fig. 5).

The results for chimeras WB1 and BW2, in which the cDNAs were recombined at both sites a and b (Fig. 1), are shown in Fig. 4. These two chimeras suppressed the responses, suggesting that the abilities of the $G\alpha$ proteins to activate PLCx depend on the two successive N-terminal blocks (I and II) of $G_{L2}\alpha$ but not on the C-terminal region.

$G_{L1}\alpha$ and $G_{L2}\alpha$ themselves are highly homologous to each other. Accordingly, the amino acid sequence of chimera BW2 is 99% identical with that of $G_{L2}\alpha$. Only four amino acid residues are different between these two α subunits (Fig. 6A). In BW2, the four residues, Ala-59, Glu-63, Lys-66, and Arg-67, of $G_{L2}\alpha$ were substituted by Ser, Asp, Arg, and Lys, respectively. Nevertheless, chimera BW2 suppressed Glu responses, whereas $G_{L2}\alpha$ potentiated them. Therefore, these four residues in the N-terminal region (block II) may be important for activation of PLCx in the oocytes. Thus, we induced point mutations to these four amino acid residues of chimera BW2, Ser-59, Asp-63, Arg-66, and Lys-67, to change them back to the original amino acids in $G_{L2}\alpha$, using PCR methods, and examined which residues were critical for PLCx activation.

When mutant R66K/K67R, in which Arg-66 and Lys-67 of chimera BW2 were changed back to Lys and Arg, respectively, as in $G_{L2}\alpha$, was expressed in the oocytes together with mGluR1, Glu responses were suppressed to a similar extent to the case of chimera BW2 and $G_{L1}\alpha$ (Fig. 6B). In contrast, when mutant S59A/D63E, in which Ser-59 and Asp-63 of chimera BW2 were changed back to Ala and Glu, respectively, was expressed together with mGluR1, Glu responses were potentiated by approx. 1.7-fold compared to the control (Fig. 6B). Furthermore, mutant S59A, in which Ser-59 of chimera BW2 was changed back to Ala, potentiated Glu responses by 2.4-fold compared to the control (Fig. 6B). PTX-treatment showed the potentiation by these mutants more clearly (Fig. 6B). These results indicated that the mutants S59A/D63E and S59A could couple with mGluR1 and activate PLCx as efficiently as $G_{L2}\alpha$.

DISCUSSION

When exogenous $G\alpha$ proteins are expressed in oocytes, the basal activity as well as the receptor-stimulated activation of PLCx may be affected. However, our electrophysiological methods under the voltage-clamp conditions do not detect a steady basal activity. Therefore, we analyzed only the mGluR-stimulated transient PLC responses. In the present study, all of the G_{L1}/G_{L2} chimeras or mutants examined showed either a potentiation or an inhibition of mGluR-mediated PLC responses. When the exogenous $G\alpha$ subunits showed inhibitory responses, the inhibition was not reversed by further supplying exogenous $\beta\gamma$ subunits. Therefore, the inhibition is not due to the arrest of $\beta\gamma$ subunits observed in the case of $G_s\alpha$ (*14*), but may be due to a competitive occupancy of PLCx sites without activation, as in the case of $G_{L1}\alpha$ (*14*). In that even synthetic peptides can interact with PLC (*6, 7*), it is highly likely that these chimeras and mutants can interact with PLCx, whether they can activate it (like pharmacological agonists) or not (like antagonists). Thus their properties are classified into either that of G_{L2} (activation) or G_{L1} (inhibition). The classification is summarized in Fig. 1.

Of ten chimeras examined, B-2 and B-4 showed a potentiation comparable to that by the wild-type $G_{L2}\alpha$. A common feature of these chimeras as well as $G_{L2}\alpha$ is that their N-terminal regions are of $G_{L2}\alpha$-origin. Chimeras with C-terminal regions derived from $G_{L2}\alpha$ inhibited the responses (W-1, W-2, W-3, and BW2), or showed a weak potentiation observed only after the PTX-pretreatment (chimera W-4). These results strongly suggest that, for a chimeric $G\alpha$ to activate PLCx, the properties of the N-terminal region of the chimera are more important than those of the C-terminal. Of the five constituent blocks (I, II, III, IV, and V) of $G\alpha$ proteins shown in Fig. 1, it is important that at least the first two N-terminal blocks (I and II) should both be derived from G_{L2}. When only either the first (B-1 or BW2) or the second (W-1 or WB1) block is derived from $G_{L2}\alpha$, the chimeras cannot activate PLCx. However, the fact that B-3, in which the first three blocks (I–III) are from G_{L2}, is inhibitory means that this is not a sufficient condition. An additional condition may concern the third and fourth blocks. When they are derived from the same origin, either G_{L1} (B-2) or G_{L2} (B-4), the resulting chimeras can activate PLCx.

These results of the chimeras may thus be summarized as follows. When G_{L1} and G_{L2} cDNAs are recombined at sites a or c (Fig. 1), the resulting chimeras (B-1, B-3, W-1, W-3, WB1, and BW2) would be unable to activate PLCx. In other cases (B-2, B-4, W-2, and W-4), the activating ability is strong only when the first two N-terminal blocks (I and II) are derived from G_{L2}. Thus, the N-terminal two blocks are important for the determination of receptor-$G\alpha$-PLCx matching and the activation of PLCx.

$G_{L1}\alpha$ and $G_{L2}\alpha$ themselves are highly homologous to each other. The amino acid sequence of chimera BW2, in which only the second block (II) is from $G_{L1}\alpha$, is 99% identical with that of $G_{L2}\alpha$, differing in only four amino acid residues (Fig. 6A), and yet BW2 is unable to activate PLCx. To further clarify the critical amino acid residue(s), these four residues were modified. The results indicated that Ser-59 in BW2 was critical: when this residue was replaced by Ala, the original amino acid residue in G_{L2}, the resulting mutant (S59A) gained the ability to activate PLCx.

It has been reported that the G protein-effector interaction site is located in the C-terminal region in the case of $G_q\alpha$ (*7*), as well as $G_s\alpha$ (*2–5*) and transducin (*6*). Our results indicated that many chimeras in which these effector interaction sites are intact were still unable to activate PLCx. This was irrespective of whether this interaction site was derived from G_{L1} or G_{L2}. We found no direct relation between the origin (G_{L1} or G_{L2}) of the C-terminal region and the ability to activate PLCx. The activation of PLC in the oocytes was more closely correlated with the origin of the N-terminal region rather than the origin of C-terminal region. Together with the fact that all chimeras could suppress or potentiate the responses as wild-type $G_{L1}\alpha$ or $G_{L2}\alpha$, these results suggest that at least two regions in a $G\alpha$ protein perform critical functions for the activation of PLCx: The C-terminal region of $G\alpha$ interacts with PLCx, which is common to all $G\alpha$s, and then the N-terminal region of $G\alpha$ may perform other functions important to determine whether it activates PLCx or not. Although such functions are to be characterized further, it is possible that proper conformational changes of $G\alpha$ proteins may be required in order for the C-terminal

binding region to interact properly with PLC*x*, and for such functions, the N-terminal region may be critically important.

Previous crystallographic studies have shown that the α subunit proteins of heterotrimeric G proteins are composed of two major domains, the helical domain and the GTPase domain (*18-20*). These two domains are relatively rigid and are connected by two flexible linkers (*19, 20*). These two linkers are located in blocks II and IV shown in Fig. 1. Our results showed that when cDNAs are recombined at site a or site c, the resulting chimeras could not activate PLC*x*. Sites a and c each immediately precede one of the two linker regions. One can thus speculate that if mismatched chimeric recombinations occur close to the linkers, the structural environments surrounding the linkers may be spoiled, resulting in malfunction of linkers and inability of effector activation. In this context, it is intriguing that the first two N-terminal blocks (I and II) stretch over the GTPase domain and the helical domain of Gα proteins, and that the critical amino acid residue Ser-59 is located in the middle of linker 1. This strongly suggests the importance of the linkers for the effector-activating ability of Gα proteins, and indicates possible roles played by the N-terminal regions.

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