Regulation of 5′-AMP-activated Protein Kinase Activity by the Noncatalytic β and γ Subunits*

(Received for publication, March 26, 1996, and in revised form, May 7, 1996)

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The mammalian 5′-AMP-activated protein kinase (AMPK) is a heterotrimer consisting of an α catalytic subunit and β and γ noncatalytic subunits, each of which is represented in a larger isoprotein family, related to the SNF1 kinase and its interacting proteins in yeast. In this study, we have used mammalian cell transfection to compare the activities of the two α subunit isoforms, α-1 and α-2, and to study the influence of the noncatalytic subunits on enzyme subunit association and activity. Expression of epitope-tagged protein subunits in COS7 cells indicates detectable but low level kinase activity for each of the two catalytic α subunits. Co-expression of α subunits with the β or γ subunits modestly increases kinase activity accompanied by the formation of αβ or αγ heterodimers. Co-expression of all three subunits, however, is accompanied by a 50–110-fold increase in kinase activity with the formation of a heterotrimeric complex. In addition to binding of each noncatalytic subunit to the α subunit, the β and γ subunits bind to each other, likely resulting in a more stable heterotrimeric complex. The increase in kinase activity associated with expression of this heterotrimer is due both to an increase in enzyme-specific activity (units/enzyme mass) and to an apparent enhanced α subunit expression. Co-expression of a catalytically defective α subunit or the βγ-binding COOH-terminal domain of the α subunit results in reduced heterotrimeric kinase activity. The synergistic positive regulatory roles for both the noncatalytic β and γ subunits of 5′-AMP-activated protein kinase contrasts with the Snf1p kinase, where only heterodimers of Snf1p and Snf4p seem to be required for maximum kinase activity.

The 5′-AMP-activated protein kinase (AMPK) is a heterotrimeric kinase involved in modulating several metabolic pathways during cellular stress (1–9). Initially identified as a kinase that regulates hydroxymethylglutaryl-CoA reductase, AMPK has also been shown to phosphorylate adipose hormone-sensitive lipase and hepatic acetyl-CoA carboxylase (1–9). Regulation of AMPK activity, through alterations in cellular 5′-AMP levels under conditions of varying nutrient availability, provides an adaptive mechanism to control cellular metabolism of glucose and fatty acid and to limit ATP utilization (4–8). The mechanism of 5′-AMP activation is complex, involving allosteric regulation of AMPK subunits and modulation of AMPK phosphorylation by AMPK kinase(s) and phosphatase(s) (10, 11).

Isolation of AMPK to homogeneity revealed that the catalytic subunit (α) co-purifies with two other noncatalytic subunits (β and γ) (12, 13). Recent cloning data indicate that for each of these subunits, there exists at least one other mammalian protein isoform (14–19). For example, we have recently reported the identification of two different catalytic subunits, α-1 and α-2, that are the products of unique genes with wide mammalian tissue expression (16). The α subunit of the mammalian AMPK is related to the yeast SNF12 protein kinase family (14–16, 18, 19). The Snf1p protein kinase is responsible for expression of the glucose-repressed genes during glucose starvation (20–24). The noncatalytic β and γ subunits of AMPK are related to proteins that interact with Snf1p. The AMPK-γ subunits (γ1, γ2, and γ3) are homologous to the yeast protein, Snf4p (CAT3), and the AMPK-β subunit is related to the yeast Sip1p/Sip2p/Gal83p family of proteins (14, 17). All of these yeast proteins have been shown to associate with Snf1p, as judged by two-hybrid analysis, immunoprecipitation, and enzyme isolation (12, 23, 24). Genetic evidence, derived from yeast mutants, suggests that both Snf4p and the Sip1p/Sip2p/Gal83p family of proteins positively regulate Snf1p kinase activity (20, 21, 23). However, direct demonstration by subunit recombination of these regulatory roles has not been reported.

Unsuccessful attempts to express an active α-2 isoform of the AMPK in bacteria, mammalian cells, and yeast have been reported (18, 19), but these experiments did not take into account α subunit heterogeneity and/or the possible necessary co-expression of the β and γ subunits. In the present report, we have investigated the expression of AMPK subunits in COS7 cells and find that co-expression of the noncatalytic β and γ subunits is required for optimal activity of the α catalytic subunits.

EXPERIMENTAL PROCEDURES

Plasmid Construction—For mammalian cell expression, AMPK subunit cDNAs were cloned into either pEBG vector (α-1 and α-2 subunits) or pMT2 vector (β and γ-1 subunits) (25). In the former vector, the cloned insert is preceded by a glutathione S-transferase (GST) se-
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...sequence, which enables isolation of expressed protein on glutathione-agarose (25). The pMT2 vectors employed also had 5′-end epitope tags (peptide sequences derived from either hemagglutinin (HA) or c-myc protein), which enabled detection of expressed proteins by immunologic techniques employing epitope-specific antibodies (25). In the current studies, rat DNAs for the α-1, α-2, and β subunits were employed; the γ subunit insert was sequenced from a human cDNA library, as previously reported (15–17). The latter represents the γ 1-subunit of the γ isoform family (17); it is referred to throughout this report simply as γ.

In addition to these native subunit sequences, two other pEBG expression plasmids were employed in efforts to generate a kinase inhibitor. The first was constructed after site-specific mutagenesis of the α-1 subunit whose catalytic Lys45, as below, was replaced by Arg to yield the following sequence, which enables isolation of expressed protein on glutathione-agarose and kinase assay, as described under “Experimental Procedures.” Kinase activity is expressed as pmol 32P transferred to the SAMS peptide/min/ml of lysate adsorbed and is represented as the mean (± standard deviation) of four independent transfections of each. Phosphotransferase activity has been corrected by subtraction of apparent kinase activity in adsorbates from mock transfected (pEBG only) cells; this value averaged less than 1% of 32P incorporation in the experimental cell samples.

with the appropriate secondary antibody conjugated with horseradish peroxidase and with enhanced chemiluminescence (Amersham Corp.). Exposed films (Kodak XAR5) were analyzed on a Molecular Dynamics Scanning Densitometer and quantitated using IPLab Gel software.

Materials—Cost7 cells were obtained from American Type Culture Collection. Media, fetal bovine serum, and lipofectamine were purchased from Life Technologies, Inc. (GAPDH was from Clontech).

RESULTS

Expression of the α-1 and α-2 Catalytic Subunits—The 5′-AMP-activated protein kinase consists of one catalytic subunit and two noncatalytic subunits (12). Two isoforms of the catalytic subunit of AMPK have recently been identified and have been designated α-1 and α-2 (15, 16). Previous attempts by others to express an active α-2 isoform in mammalian cells, bacteria, and yeast were unsuccessful (18, 19). In our initial experiments, we therefore examined the expression of these two isoforms as GST fusion proteins by transient transfection of Cost7 cells and assessed kinase activity after the adsorption of expressed protein to glutathione-agarose. As shown in Fig. 1A, a 90-kDa fusion protein for each isofrom is readily detected in the glutathione-agarose adsorbates using an anti-GST antibody. This molecular mass is that predicted from α sequence (63 kDa) and the GST fusion sequence (27.7 kDa). SAMS peptide phosphotransferase activity of each of the expressed catalytic subunits is readily detectable in the glutathione-agarose adsorbates (Fig. 1B). Although the α-2 expressed activity appears to be lower in these representative experiments, correction for the mass of the expressed subunit indicates nearly equal specific activities (units kinase activity corrected for fusion protein expression) for each under these assay conditions (data not shown). These data indicate that the free catalytic subunits of AMPK possess phosphotransferase activity that can be expressed in mammalian cells.

Activation of the α Subunit by β and γ Binding—As isolated from rat liver by substrate affinity chromatography and immu-
Fig. 2. Co-expression of AMPK-α subunits with AMPK-β or AMPK-γ subunits. A, COS7 cells transfected with either pEBG-α-1 (α1, left-hand panels) or pEBG-α-2 (α2, right-hand panels) were simultaneously co-transfected with either pMT2-HA-β (β), pMT2-HA-γ (γ), or the pMT-HA vector (control). Cell lysates (upper panels) or glutathione-agarose adsorbates (lower panels) were analyzed following SDS-polyacrylamide gel electrophoresis and immunoblot analysis using anti-HA antibodies. The position of the ovalbumin (43 kDa) standard is indicated. B, the phosphotransferase activity of α-1 in glutathione-agarose adsorbates was determined after co-transfection of pEBG-α-1 with pMT2-HA vector (α1), pMT2-HA-β (α1 β), or pMT2-HA-γ (α1 γ). Kinase activity (means ± S.D. of three separate transfections) is expressed as indicated in the legend to Fig. 1. A representative immunoblot probed with an anti-GST antibody demonstrates the relative levels of α-1 present in the glutathione adsorbate under each condition of transfection (upper panel).

To assess possible mechanisms underlying this marked increase in the expressed kinase activity of both α-1 and α-2, as determined in glutathione-agarose adsorbates, after co-transfection of COS7 cells with pMT2-HA vector (α1 and α2) or with both pMT2-HA-β and pMT2-HA-γ (α1 β γ and α2 β γ). Activities expressed as in Fig. 1 represent the means (± S.D.) of three separate transfection experiments. In the right-hand panel is shown a representative immunoblot of glutathione-agarose adsorbates from each of these transfection conditions, as probed with an anti-HA antibody. The top and middle immunostained bands are the expressed HA-β protein, which migrates at two distinct molecular sizes (discussed in Ref. 17); the bottom band is the expressed HA-γ protein. The migration of the ovalbumin standard (43 kilodaltons (K)) is indicated.

Shown in the upper panel of Fig. 2B, co-expression of either β or γ did not significantly alter the expression of the α-1 polypeptide. These data therefore demonstrate that the β or γ individually have a modest stimulatory effect on activity upon binding to the α catalytic unit of AMPK.

Triple transient transfection was employed to assess the effects of β and γ in combination on α subunit activity. In all instances, 2 μg of total DNA was transfected per well, and the amount of each plasmid type was kept constant by the addition, where appropriate, of plasmid lacking the cDNA insert. When co-transfected with either α-1 or α-2, both HA-β and HA-γ bind to the glutathione-adsorbed GST α fusion protein (Fig. 3, right-hand panel). The HA-β subunit is expressed as two closely migrating bands at 43 and 46 kDa, whereas HA-γ is expressed as a single band at ~41 kDa as previously reported (17). The total binding of both noncatalytic subunits to α-1 or α-2 under these conditions is roughly equivalent.

The impact of co-expression of both β and γ on the catalytic activity of the α subunit is large in contrast to the relatively small effects of individual noncatalytic β or γ subunits (Fig. 3, left-hand panel). α-1 kinase activity in these experiments is stimulated about 110-fold by co-expression of both β and γ, whereas α-2 activity is stimulated about 50-fold under the same conditions of transfection. This striking increase in kinase activity on triple transfection ranged from 50- to ~500-fold in other experiments (see, for example, Fig. 6). Thus, β and γ have a pronounced synergistic effect on the expressed activity of both isoforms of the α subunit.

To assess possible mechanisms underlying this marked increase in SAMS peptide phosphotransferase activity, the glutathione-agarose adsorbates from these experiments were analyzed for GST-α content by immunoblotting. As shown in Fig. 4A, as analyzed with an anti-GST antibody, co-expression of β and γ result in increased recovery (9-9.6-fold, as determined by quantitative densitometry) of α-1 or α-2 GST fusion protein in the adsorbate. This same fold increase in α-1 and α-2 expression was also observed on immunoblot analysis of the cell lysates prior to glutathione-agarose adsorption, indicating that co-expression of βγ subunits increases the overall level of cellular α subunit expression under these conditions (data not shown). However, increased α subunit expression does not entirely account for the increased in expressed kinase activity. When SAMS peptide kinase activity was corrected for expressed α protein in the same glutathione-agarose adsorbate (determined by immunoblotting and scanning densitometry), 123- (α-1) and 5-fold (α-2) increases in kinase activity due to

A

| α | Lysates | Adsorbates |
|---|---------|------------|
| α1 | α1 | α1 |
| α2 | α2 | α2 |

B

| α | α1-GST |
|---|--------|
| α1 | α1 |
| α1/β | α1/β |
| α1/γ | α1/γ |

Fig. 3. Co-expression of AMPK-α, AMPK-β, and AMPK-γ. In the left-hand panel is shown the kinase activity of both α-1 and α-2, as determined in glutathione-agarose adsorbates, after co-transfection of COS7 cells with pMT2-HA vector (α1 and α2) or with both pMT2-HA-β and pMT2-HA-γ (α1 β γ and α2 β γ). Activities expressed as in Fig. 1 represent the means (± S.D.) of three separate transfection experiments. In the right-hand panel is shown a representative immunoblot of glutathione-agarose adsorbates from each of these transfection conditions, as probed with an anti-HA antibody. The top and middle immunostained bands are the expressed HA-β protein, which migrates at two distinct molecular sizes (discussed in Ref. 17); the bottom band is the expressed HA-γ protein. The migration of the ovalbumin standard (43 kilodaltons (K)) is indicated.

No adsorption, both α-1 and α-2 catalytic subunits co-purify with β and γ noncatalytic subunits (12, 16). To document the binding characteristics of the three AMPK subunits, a series of transfection experiments were performed using both the α-1 and α-2 GST fusion expression constructs and expression plasmids encoding the β and γ subunits. These latter plasmids expressed the β and the γ subunits tagged on the 5'-end with a HA decapeptide. A series of co-transfection experiments in COS7 cells were performed with these plasmids to determine whether the individual α subunit proteins could both bind β and γ and to determine if this binding had an effect on kinase activity. As shown in Fig. 2A (upper panel), both HA-β and HA-γ fusion proteins can be expressed in COS7 cell lysates when co-expressed with either α-1 or α-2 GST fusion proteins. Analysis of glutathione-agarose adsorbates following the double transfection with αβ or αγ reveals that both HA-tagged noncatalytic subunit proteins can bind to the GST-α subunit (Fig. 2A, lower panel). The extent of binding of HA-β and HA-γ to α-1 or α-2 appears to be approximately equivalent under these conditions. No immunoblottable HA-β or HA-γ protein was adsorbed to glutathione-agarose in the absence of expressed α subunit (not shown).

As assayed in the glutathione-agarose adsorbed state, co-expression of the β subunit with α-1 causes a modest 1.5-fold increase in the SAMS peptide phosphotransferase activity (Fig. 2B). When γ is co-expressed with α-1, a 2.5-fold increase in the activity of α-1 was seen (Fig. 2B). Similar quantitative effects of co-expressed β or γ were also seen with α-2 (not shown).
these experiments, the HA epitope tag of the catalytic subunit was precipitated by incubation of lysate with the appropriate antibody for 3 h at 4°C with adsorption of immune complexes to protein G–agarose beads followed by SDS-polyacrylamide gel electrophoresis sample buffer. The migration of the molecular mass standard ovalbumin (43 kilodaltons (K)) is shown for each panel.

with β and γ into COS7 cells with or without the wild type form of α-1. When the kinase activities of these adsorbates were examined, no kinase activity of the Lys→Arg mutant could be detected even in the presence of βγ (Fig. 6A). As shown in Fig. 6B, the binding of βγ to the glutathione-adsorbed α-1 subunit is similar in the wild type and in the Lys→Arg mutant. Thus, an active kinase is not necessary for βγ binding. Because the Lys→Arg α-1 is still capable of binding β and γ and may also contain the activating phosphorylation site for the AMPK kinase (Thr172) (16), it is conceivable that the mutant could act to inhibit the expressed activity of wild type α-1 even in the absence of the presence of βγ. Indeed, when co-transfected with β, γ, and wild type α-1, the Lys→Arg mutant markedly diminishes (by 90%) expressed α-1 activity (as compared with its activity when co-transfected with equivalent amounts of pEBG vector) (Fig. 6A). Co-expression of wild type and Lys→Arg mutant did not alter the expression of total α subunit protein, as determined by immunoblotting either with anti-GST or anti-α-1 antibodies (not shown). Because these immunologic reagents cannot distinguish the two fusion protein products, we cannot assess the individual expression of wild type and Lys→Arg mutant by this analysis. In other experiments (not shown), the α-1 Lys→Arg mutant also inhibits the activity of the co-expressed α-2 catalytic subunit.

Experiments above have shown that an active α-1 subunit is not necessary for the binding to the noncatalytic subunits, β and γ. We hypothesized that the noncatalytic region of the α-1 protein was responsible for the binding to β and γ and thus might serve as an inhibitor of kinase activity by competition for available βγ subunits. Therefore, the catalytic domain of the α-1 subunit was removed, and the carboxyl terminus (α-1-C; amino acid residues 262–548) was expressed as a GST-fusion protein in the presence of wild type α-1/βγ. Co-expression of α-1-C with wild type α-1 leads to a 50% decrease in kinase activity (Fig. 7A) and similar diminution in the expressed level of the wild type protein (about 50% as determined by scanning densitometry) (Fig. 7B, upper panel). It should be noted in this panel that an α-1-specific antibody raised against an α-1 peptide (amino acids 339–358) was used for analysis, because the anti-GST antibody recognized a nonspecific protein that migrated to the same location as the truncated α-1 subunit. As determined by anti-HA immunoblotting, the total amount of β plus γ subunits bound to glutathione-adsorbed proteins (wild type α-1, α-1-C, or both) remains constant, as expected (Fig. 7B,
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Fig. 6. Inhibition of wild type α-1 subunit expression by α-1 Lys → Arg. A, shown is kinase activity expressed as in Fig. 1 (means ± S.D. of three separate transfections) measured in glutathione-agarose adsorbates after cell transfection with various combinations of pEBG-wild type α-1 (α1WT), pMT2-HA-β and pMT2-HA-γ (βγ), pEBG-α-1 Lys → Arg (α1K→R), and pEBG vector (pEBG). The α1Lys → Arg mutant plasmid was present in 2-fold excess over wild type in all instances, but total pEBG vector DNA was kept constant. B, shown is a representative anti-HA blot of glutathione-agarose adsorbates from cells transfected with pEBG-wild type α-1 (α1WT)pMT2-HA-βpMT2-HA-γ (lane 1), pEBG-wild type α-1 (lane 2), pEBG-α-1 Lys → Arg (α1K→R)pMT2-HA-βpMT2-HA-γ (lane 3), and pEBG-α-1 Lys → Arg (lane 4).

lower panel). Thus, α-1-C can bind both the β and γ subunits and can also serve as an inhibitor of kinase activity under these co-transfection conditions, although it appears that its major effect is to diminish expression of the wild type α-1 subunit.

DISCUSSION

The AMPK, as purified from pig or rat liver, is a heterotrimeric enzyme consisting of an α, β, and γ subunits (12). Two isoforms of the α subunit, α-1 and α-2, have recently been identified, although the former appears to account for about 90% of expressed SAM5 peptide kinase activity in rat liver (16). Sequence analysis of these α subunits has revealed that each contains the conserved domain present in all serine/threonine-directed protein kinases, indicating that α is the catalytic subunit (12, 15, 16, 18, 26). However, efforts by others to express an active α subunit (α-2) have been unsuccessful in bacteria, yeast, and mammalian systems (18, 19). This present report establishes that both the α-1 and α-2 subunits indeed contain phosphotransferase activity against the SAM5 peptide, which represents one of the sites of phosphorylation of acetyl-CoA carboxylase by AMPK (9). The present study did not reveal any catalytic differences between these two α subunit isoforms.

However, we have previously shown that the α-2 isoform present in rat liver does not bind to an α-1 peptide substrate affinity column, possibly because the α-2-containing AMPK enzyme isolated from rat liver is not in its active phosphorylated form (16).

One major finding of the present investigation is that the β and γ subunits synergistically increase the expression of AMPK activity for both α subunit isoforms in transfected mammalian cells. β or γ alone are very weak activators of kinase activity (1.5–2.5-fold), but co-expression of β and γ with α leads to a minimum of 50–110-fold increases in expressed kinase activity. The positive regulatory effects of β and γ on AMPK activity have previously been predicted, based on their homologies to Snf1p protein kinase interacting proteins in yeast (14, 17, 23). These proteins may serve as adaptors that promote the activity of Snf1p toward specific targets (23). As analyzed in yeast mutants, some data suggest that these proteins may facilitate interaction of Snf1p with unique and different targets. Mutations of Gal83p can abolish most of the Snf1p kinase activity detectable in immune complexes precipitated with anti-Snf1p antibody (23). A Sip2Δ gal83Δ mutant shows reduced Snf1 protein kinase activity that is restored following expression of either Sip2p or Gal83p LexA fusion proteins in the yeast mutant strain (23). Analysis of SNF4 mutants in yeast suggests that Snf4p also positively regulates the activity of its associated catalytic subunit, Snf1p (20, 21). However, although both the Snf4p and Sip1p/Sip2p/Gal83p proteins have been shown to interact with the Snf1p kinase polypeptide in the yeast two-hybrid system and by immunoprecipitation (20–24), these studies of yeast mutants (and their complementation) do not establish the direct positive regulation of kinase activity of the catalytic unit by either of these Snf1p-interacting proteins. In the present study, we have successfully reassembled the AMPK heterotrimeric complex and isolated it by glutathione-agarose adsorption. These adsorbates demonstrate increased kinase activity, even when corrected for the amount of adsorbed catalytic subunit, demonstrating directly the positive regulation of the α subunit by the β and γ subunits. A parallel activating interaction for Snf1p and its β/γ homolog proteins in yeast remains to be demonstrated. It should be noted, however, that on isolation of SNF1 kinase from yeast by nickel affinity chromatography, only Snf4p is co-purified with the catalytic Snf1p polypeptide, suggesting perhaps that only a heterodimer is required for optimal activity of this kinase (12).

In addition to kinase activation, co-expression of β and γ leads to an increase in the cellular expression of the α subunit. Although several mechanisms might account for this, it seems most likely that the stabilization of the heterotrimeric complex by co-expression of the noncatalytic units might diminish protein turnover, allowing for higher levels of expression. This mechanism has previously been demonstrated in other multimeric protein complexes, including other protein kinases (27, 28).

Another major finding revealed by the present studies is the ability of the β and γ subunits to bind not only to the α subunit but also to each other. This was not anticipated based on analogy to the yeast system, because no interactions between Snf4p and Sip1p/Sip2p/Gal83p proteins have been reported. The α subunit, which is not involved in the independent binding of each to the α subunit may contribute to increased stabilization of the heterotrimer. This β/γ interaction could also contribute directly to the marked enhancement of kinase specific activity by altering the interactions of each with the α subunit (resulting in a different conformation for the catalytic unit) or by increasing the interactions of β and/or γ with kinase protein substrates. The demonstration of β/γ binding in the absence of α also raises the question as to whether this heterodimer might have some cellular function independent of its ability to stimulate kinase activity. Although our results indicate that heterodimers of αβ and αγ can be formed, thus far, we have not observed these heterodimers in liver extracts; only the heterotrimeric complex has been detected.

The β and γ-1 subunits used herein appear to have nearly
equal affinity for either the α-1 or α-2 subunit and to have similar effects on kinase activity catalyzed by each. We have previously identified a γ isomeric family consisting of at least three distinct γ proteins, and it seems likely that a β isomeric family exists as well (17). This raises the possibility that α-1 and α-2 could each associate preferentially with unique β/γ isomers in vivo. The β and γ subunits employed in the present study have been shown by direct peptide sequence analysis to be the noncatalytic subunits that associate with α-1 (14, 17). Whether the same or other isomers associate with α-2 remains to be determined. If unique noncatalytic subunits associate with each α isomeric, this could increase the diversity of functional interactions between the protein target and enzyme subunits.

Based on mutagenesis of the α-1 subunit, we have been able to generate two inhibitors of expressed enzyme activity. Mutation of Lys45 to arginine, as expected, abolishes α-1 protein kinase activity; however, the ability to bind β/γ in α-1 Lys→Arg mutant is fully preserved and thus does not require an active catalytic subunit. Co-expression of this mutant with wild type α-1 (and α-2) led to a marked decrease in expressed AMPK activity. This could result from competition for binding of β/γ between the wild type α subunit and the Lys→Arg catalytically incompetent mutant or competition of mutant with the wild type α-1 for AMPK kinase binding required for activation of α-1 subunit via phosphorylation. It is also possible that because β/γ binding appears to increase the expression of the α subunit, perhaps through alterations in protein turnover, competition for β/γ binding by the α-1 Lys→Arg mutant might increase the turnover of the free wild type α subunit. Less likely as an explanation for the inhibitory properties of α-1 Lys→Arg mutant are direct dominant negative effects of the α-1 Lys→Arg mutant on the wild type α by the formation of α heterodimers, because α subunit dimerization has not been demonstrable to date.

The second inhibitor of expressed kinase activity is the carboxy-terminal half of the α-1 subunit, which lacks the kinase catalytic subdomain (16). This mutant (α-1C) is capable of binding β/γ to approximately the same extent as the wild type α-1 polypeptide. It also inhibits co-expressed α-1 (in the presence of β/γ) by about 50%, an extent nearly equal to an observed diminution in the expression of wild type α-1 polypeptide. Although other mechanisms might contribute to the inhibitory properties of α-1C, it seems likely that its competition for β/γ binding destabilizes the wild type αββ heterotrimer, leading to increased wild type α-1 turnover.

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