Role of Disulfide Bonds in Acrp30/Adiponectin Structure and Signaling Specificity

**DIFFERENT OLIGOMERS ACTIVATE DIFFERENT SIGNAL TRANSDUCTION PATHWAYS**

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Acrp30/adiponectin is an adipocyte-derived serum protein with important roles in regulation of lipid and glucose metabolism, but which of its isoforms are biologically active remains controversial. We addressed this issue by first characterizing the structure of each individual Acrp30 oligomer and the determinants responsible for multimer formation. Freeze etch electron microscopy showed the trimer to exhibit a ball-and-stick-like structure containing a large globular sphere, an extended collagen stalk, and a smaller sphere on the opposite end of the stalk. The hexamer consists of two adjacent trimeric globular domains and a single stalk composed of collagen domains from two trimers. Although not necessary for trimer formation or stability, two of the three monomers in an Acrp30 trimer are covalently linked by a disulfide bond between cysteine residues at position 22. In contrast, assembly of hexameric and higher molecular weight (HMW) forms of Acrp30 depends upon formation of Cys22-mediated disulfide bonds because their reduction with dithiothreitol or substitution of Cys22 with alanine led exclusively to trimers. HMW and hexamer isoforms of Acrp30 activated NF-κB in C2C12 cells, but trimers, either natural, formed by reduction of Acrp30 hexamer, or formed by the C22A mutant, did not. In contrast, incubation of isolated rat extensor digitorum longus with naturally formed Acrp30 trimers or trimeric C22A Acrp30 led to increased phosphorylation of AMP-activated protein kinase-α at Thr172 and its activation. Hexameric and HMW Acrp30 could not activate AMP-activated protein kinase. Thus, trimeric and HMW/hexameric Acrp30 activate different signal transduction pathways, and Acrp30 represents a novel example of the control of ligand signaling via changes in its oligomerization state.

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Apical complement related protein of 30 kDa (Acrp30), or adiponectin, is an adipocyte-secreted hormone found abundantly in serum (1). Its expression and serum concentration are decreased in obese or diabetic humans and animals (2, 3). In Pima Indians, occurrence of diabetes later in life is accompanied by decreased Acrp30 levels before onset of diabetes (4). Acrp30 exerts multiple metabolic actions at multiple tissue sites. The isolated globular domain of Acrp30 (gAcrp30) simulates fatty acid oxidation in skeletal muscle, whereas full-length Acrp30 synergizes with insulin to inhibit hepatic glucose production (5–7). In mice, disruption of Acrp30 locus leading to its ablation resulted in impaired fatty acid clearance, increased tumor necrosis factor α levels, and aggravated insulin resistance in animals fed a high fat diet (8, 9).

How Acrp30 acts as a hormone to regulate these physiological processes remains unknown. We addressed this issue by analyzing the structure of Acrp30 secreted from cells and in serum. Acrp30 purified from transfected human embryonic kidney (HEK) 1293T cells or Escherichia coli exists as trimers and hexamers (10). Transfected HEK cells also secrete an even higher molecular weight (HMW) isoform of Acrp30 (10). All three isoforms of Acrp30 are present in mouse serum and the conditioned medium of differentiated 3T3-L1 adipocytes, albeit with different relative abundances (10). Purified isoforms are stable in PBS and do not interconvert (10). The HMW and hexameric Acrp30 can activate transcription factor NF-κB in undifferentiated or differentiated C2C12 cells, but trimeric Acrp30 or gAcrp30 cannot. Rather, gAcrp30, but not full-length Acrp30 hexamer, enhances muscle fatty acid oxidation by inactivating acetyl-CoA carboxylase following stimulation of AMP-activated protein kinase (AMPK) (11, 12).

Because different isoforms of Acrp30 have different activities, it is important to understand how they are formed. Acrp30 contains three easily recognizable domains: an N-terminal signal sequence, a collagen domain, and a C-terminal globular domain. Because the crystal structure of the Acrp30 globular domain shows a trimeric fold similar to tumor necrosis factor α, the collagen domain of Acrp30 may mediate multimerization into hexamer and HMW isoforms. Alternatively, disulfide bonds may hold Acrp30 oligomers together because they are required for multimerization of Acrp30 homologs C1q and emilin (13, 14). Acrp30 contains two cysteine residues that are conserved among all species with available sequence information. One is located in the globular domain at position 138 of...
Acrp30 Structure, Oligomerization Mechanism, and Signaling

To understand mechanisms used by Acrp30 to multimerize, we employed deep etch cryo-electron microscopy to determine the structures of each oligomer. We then tested whether disulfide bonds are required for Acrp30 oligomerization and which cysteine residues are involved in their formation. Acrp30 trimer is best described as a “ball on a stick” model, whereas the hexamer consists of two adjacent parallel trimers that resemble a “Y.” The HMW structure is currently not interpretable. Reduction of HMW or hexameric Acrp30 with dithiothreitol (DTT) collapsed them into trimers. In parallel, site-directed mutagenesis of each of the two cysteines in Acrp30 showed cysteine at position 22 to be crucial for formation of the HMW and hexamer species. In contrast, cysteine at position 138 in the globular domain had no role in directing the formation of Acrp30 oligomers. Consistent with our prior results that naturally produced Acrp30 trimers cannot activate NF-κB, we show that neither trimeric C22A Acrp30 nor trimers formed by the reduction of Acrp30 hexamers can activate NF-κB. Previously we showed that trimeric gAcrp30 could activate AMPK, and here we show that full-length C22A trimers as well as C138A trimers activated AMPK in rat extensor digitorum longus (EDL) muscle, as measured by phosphorylation at Thr172 of AMPKα. In contrast, hexameric and HMW C138A Acrp30 could not. Thus, trimeric Acrp30 activates AMPK and hexameric/HMW Acrp30 activates NF-κB, and we propose that the oligomerization state of Acrp30 determines the signaling pathway it can activate.

EXPERIMENTAL PROCEDURES

Cysteine Mutagenesis—Site-directed mutagenesis of Acrp30 in pcDNA3.1 vector was performed using a QuikChange Mutagenesis kit (Stratagene) according to the manufacturer’s instructions. Primers used to construct C22A mutant have the sequences 5′-CCC AAG GTA ACT GCT GCA GTG TGG ATG GC-3′ and its reverse complement. Primers used to construct C138A mutant are 5′-CAG GCC ACA TAC GGC AAG TTC and its reverse complement. The mutations were confirmed by DNA sequencing.

Production and Gel Filtration Analysis of Wild Type and Mutant Acrp30—As previously described, Acrp30 (residues 1–230, without the 17-residue signal sequence) was purified from E. coli strain BL21 (DE3) as a His6-tagged precursor that was subsequently treated with PreScission protease (Amersham Biosciences) to remove the tag (10). Wild type (GenBankTM accession number U37222), C22A, and C138A Acrp30 (nucleotides 46–789 of U37222) were purified from conditioned serum-free medium of transfected HEK 293T cells as described previously (5, 10). Purified protein samples were loaded into an Akta fast protein liquid chromatography system and fractionated either through a 16/60 or a 10/30 Superdex 200 column (Amersham Biosciences) and eluted with PBS.

Electron Microscopic Imaging of Acrp30 Oligomers—HEK 293T Acrp30 oligomers purified by gel filtration chromatography were suspended with finely ground mica flakes and quickly frozen using liquid helium in a cryopress as described previously (15, 16). The samples were then fractured using a cryomicrotome and partially freeze-dried with finely ground mica flakes, we only obtained what we believe to be the tail end views of the HMW isoform (Fig. 1C). More apparent in promoter (19) and β-galactosidase driven by the cytomegalovirus promoter using FuGene 6 (Roche Applied Science) according to the manufacturer’s instructions. Following overnight or 8 h of incubation with various forms of Acrp30 or lipopolysaccharide, the cells were washed, and the luciferase and β-galactosidase activities were tested using kits from Promega and Clontech.

Western Blot Analysis—Twenty-five μg of crude muscle homogenate were fractionated by SDS-PAGE and transferred to polyvinylidene difluoride membrane (Bio-Rad). Following transfer, the membranes were blocked with 5% bovine serum albumin in 25 mM Tris, pH 7.5, 135 mM NaCl, 2.5 mM KCl, and 0.05% Tween 20 for 1 h at room temperature. The membranes were incubated with Phospho-AMPKα antibodies from Cell Signaling Technology (Beverly, MA) and then with secondary antibody conjugated to horseradish peroxidase from Amersham Biosciences. The bands were visualized by enhanced chemiluminescence and quantified by laser scanning densitometry. The immunoblots were performed under conditions in which autorigraph detection was in the linear response range.

RESULTS

We performed freeze etch electron microscopy to visualize the architecture of HEK 293T cell-produced Acrp30 trimer, hexamer, and HMW isoforms following their separation by gel filtration chromatography (Fig. 1). The trimer exhibits a ball-and-stick structure with the globular domain forming the ball and the collagen triple helix forming the rigid stick (Fig. 1A). In the majority of the pictures a ball-like structure smaller than the globular head domain was also observed. This probably represents the N-terminal region of Acrp30 upstream of the collagen domain. The length of the collagen domain is ~15 nm. Because the collagen triple helix has 10 Gly-X-Y repeats per turn and a pitch of 86 Å, the expected length of the Acrp30 collagen domain with 21 repeats is ~18 nm, similar to what was observed. The architecture of hexameric Acrp30 shows two trimers lying adjacent to each other in parallel head-to-head fashion and is reminiscent of the letter Y (Fig. 1B). Possibly because of strong interactions between the globular domain with the mica flakes, we only obtained what we believe to be the tail end views of the HMW isoform (Fig. 1C). More apparent in...
the stereo views in supplemental Fig. S1, these HMW molecules resemble cones standing upside down, with the globular heads adhering to the mica and the tails sticking up into the air. However, we cannot infer the precise structure of HMW Acrp30 from these images.

To investigate the possibility that disulfide bonds are responsible for formation or maintenance of hexamer or HMW Acrp30, we analyzed elution profiles of isolated hexamers and trimers purified from *E. coli* in gel filtration columns following incubation with 100 mM DTT. In the absence of DTT, the apparent molecular masses of *E. coli*-produced Acrp30 hexamer (Fig. 2A) and trimer (Fig. 2B) are 450 and 218 kDa, respectively. Similarly, the molecular masses of hexamer (Fig. 2C) and HMW (Fig. 2D) isoforms secreted from transfected 293T cells are 775 and 418 kDa. The trimer shown in Fig. 2B was referred to as trimer A in our previous publication (10). Because trimer B consists of two full-length Acrp30 polypeptides and one polypeptide missing the collagen domain and is not found in serum (10), it is an artifact of the bacterial production process and was not studied further. In the present study we refer to the trimer A species simply as trimer. As shown in Fig. 2, 100 mM DTT treatment reduced the apparent molecular masses of *E. coli*-derived hexamer (Fig. 2A) and HEK cell-derived hexamer (Fig. 2C) and HMW Acrp30 (Fig. 2D) to that of a trimer (Fig. 2B). Reduction of *E. coli* hexamer into trimer could also be achieved with 10 mM of DTT or 1 mM βME (data not shown).

As shown in Fig. 3, Acrp30 contains two conserved cysteines, one in the conserved region between the signal sequence and the collagen domain (Cys138) and the other in the globular domain (Cys22). To investigate whether one or both of these cysteines are responsible for oligomerization of Acrp30, each was substituted with alanine. Size exclusion chromatography of the C138A Acrp30 secreted from transfected HEK cells revealed a migration profile similar
to that of wild type protein (Fig. 4A). Three peaks correspond-
ing to HMW, hexamer, and trimer in proportions analogous to
those of wild type Acrp30 were observed (Fig. 4A). Gel filtration
analysis of purified C22A Acrp30 demonstrates that this mu-
tant migrates only as a single, apparently trimeric species (Fig.
4B). Equilibrium sedimentation in the analytical ultracentri-
fuge confirmed this species to be a trimer (Fig. 5A); the molec-
ular mass determined by this analysis is 78 kDa, identical to
that of the wild type Acrp30 trimer A (10). C22A Acrp30 was
also visualized by freeze etch electron microscopy (Fig. 5B).
Similar to wild type Acrp30 trimer (Fig. 1A), a large sphere
consisting the globular domain, a collagen stalk, and a smaller
sphere most likely representing the N-terminal region could be
seen in images of C22A Acrp30. These results indicate that the
C138A mutation has no observable effect on oligomer formation
and distribution, whereas the presence of Cys22 is critical for
formation of hexamer and HMW complexes.

To assess the degree to which Acrp30 monomers are disul-
fide-linked in each of the three isoforms, we analyzed the pro-
portion of Acrp30 disulfide-linked dimers and monomers in
nonreducing SDS-PAGE (Fig. 5C). As control, after reduction all
of the Acrp30 species migrate as monomers on SDS-PAGE
(data not shown). Trimers from both wild type and C138A
Acrp30 contain dimers and monomers in an approximately 2:1
proportion, indicating one Cys22–Cys22 disulfide bond between
two monomers and a free Cys22 sulfhydryl group in the third
monomer. Both wild type and C138A HMW and hexamer iso-
forms contain virtually no monomers, indicating that all
Acrp30 polypeptides are linked together by disulfide bonds.
Because C138A HMW and hexamer have the same dimer to
monomer ratios as the corresponding wild type Acrp30 species,
the disulfide bonds linking monomers together must be formed
by Cys22. Indeed, the C22A mutant trimer contains no dimers
in nonreducing SDS-PAGE and consists only of monomers that
are not disulfide linked.

Previously we showed that HMW and hexameric Acrp30
activated NF-κB, but trimer could not (10). To confirm the
causal relationship between the oligomerization state and
NF-κB activation, we examined the ability of βME-reduced
E. coli-produced hexamer as well as C22A and C138A oli-
gomers produced in transfected mammalian cells to activate
NF-κB, as measured by an E-selectin promoter reporter gene
assay. Following treatment with 0.5 mM βME at 2 h at 37 °C
and centrifugation under vacuum for 15 min to reduce the
concentration of βME, E. coli-derived Acrp30 hexamer was
collapsed to trimer (data not shown) and lost its ability to
activate NF-κB (Fig. 6A). Reduction of βME concentration by
centrifugation under vacuum did not reoxidize Acrp30 (data
not shown). Consistent with results obtained using wild type
Acrp30, the C138A HMW and hexamer species potently acti-
vated NF-κB (Fig. 6B). Neither the C138A trimer nor the
mutant C22A trimer activated NF-κB (Fig. 6B), corroborating
the observation that only hexamer and higher order oligomers
of Acrp30 can activate NF-κB.

We previously reported that gAcrp30 activated AMPK in
isolated rat skeletal muscle but that full-length Acrp30 hex-
amer produced in E. coli did not (11). We thus tested whether
activation of AMPK also depends on the oligomerization state
of Acrp30. Incubation of isolated rat EDL muscles with trimeric
C22A Acrp30 or with purified C138A trimer led to 2.5- and
1.5-fold increases, respectively, in the proportion of AMPKα
phosphorylated at Thr172 (Fig. 7); phosphorylation at Thr172 is
a metric of AMPK activation. These changes in phosphoryla-
tion of AMPKα were not due to changes in AMPKα abundance
(Fig. 7). Neither HMW (Fig. 7) nor hexamer (data not shown)
isoform of C138A Acrp30 was able to increase AMPKα phos-
phorylation in isolated rat EDL muscles.

Thus, gAcrp30 and trimeric Acrp30, but not hexameric or
HMW Acrp30 isoforms, activate AMPK in skeletal muscle,
whereas hexameric or HMW Acrp30 isoforms, but not gAcrp30
or trimeric Acrp30, activate NF-κB (Table I). Clearly the state
of oligomerization of Acrp30 determines the signal transduc-
tion pathway it activates.

**DISCUSSION**

Here we defined the oligomeric structures of two Acrp30
isoforms and demonstrated the crucial role of Cys22 disulfide
bonds in formation of the hexamer and HMW species. Impor-
tantly, we showed that only hexameric and HMW Acrp30, but
not trimeric Acrp30 species, can activate NF-κB, and that only
gAcrp30 and trimeric Acrp30, but not hexameric or HMW
Acrp30 isoforms, activate AMPK in skeletal muscle. Our re-
results with the C22A mutant are broadly consistent with the
recent findings of Pajvani et al. (21). Although Pajvani et al.
(21) reported that substitution of Cys22 with serine resulted in
N-terminal truncation of Acrp30, we did not observe any pro-
teolysis in the C22A mutant. Together, these studies indicate
that individual Acrp30 oligomers have different biological ac-
tivities, because Pajvani et al. also showed that trimeric Acrp30
was the most potent isoform in suppressing hepatocyte glucose
production (21).

**Multiple Oligomeric Forms of Acrp30**—Table I summarizes
the activities of different Acrp30 oligomers purified from dif-
ferent sources. Because trimeric full-length Acrp30 and
gAcrp30 activate AMPK but not NF-κB and because hexa-
meric/HMW Acrp30 have the opposite actions in that they acti-
vate NF-κB but not AMPK, the signaling specificity of Acrp30
depends critically upon its oligomerization state. Acrp30 thus
represents a novel example where the signaling specificity of a
hormone is regulated by its oligomerization state.
All three oligomeric isoforms of Acrp30 are present in serum (10), but at least in mice the relative proportion of the different oligomers depends on gender; serum from females contains a higher proportion of HMW isoforms than that from males (21). Injection of mice with glucose or insulin causes a transient decrease of the HMW isoform (21). Little else is known to affect the distribution of Acrp30 oligomers. Given our observation that different oligomers activate different signaling pathways, current clinical data correlating the levels of Acrp30 with the degree of insulin resistance and body mass index (2, 3) should be reconsidered. In particular, we do not know whether the current enzymes used to measure total Acrp30 levels (22, 23) exhibit any bias toward one or more isoforms. Our data suggest that only some of these oligomers affect insulin sensitivity, presumably via activation of AMPK. A comparison of oligomer distribution between normal and insulin resistant individuals might elucidate some of these oligomers affect insulin sensitivity, presumably via activation of AMPK. A comparison of oligomer distribution between normal and insulin resistant individuals might elucidate different oligomers activate different signaling pathways, currently not known whether these differences are significant.

**Disulfide Bonds and the Structure of Acrp30 Oligomers**—In deep etched electron micrographs the collagen stalks of Acrp30 trimers and hexamers appeared to be extended and straight deep etched electron micrographs the collagen stalks of Acrp30 trimers and hexamers appeared to be extended and straight. The crystal structure of the trimeric Acrp30 globular domain (24) shows a remarkable degree of amino acid sequence conservation of the primary sequences of Acrp30 orthologs (Fig. 3), but at least in mice the relative proportion of the different oligomers depends on gender; serum from females contains a higher proportion of HMW isoforms than that from males (21). Injection of mice with glucose or insulin causes a transient decrease of the HMW isoform (21). Little else is known to affect the distribution of Acrp30 oligomers. Given our observation that different oligomers activate different signaling pathways, current clinical data correlating the levels of Acrp30 with the degree of insulin resistance and body mass index (2, 3) should be reconsidered. In particular, we do not know whether the current enzymes used to measure total Acrp30 levels (22, 23) exhibit any bias toward one or more isoforms. Our data suggest that only some of these oligomers affect insulin sensitivity, presumably via activation of AMPK. A comparison of oligomer distribution between normal and insulin resistant individuals might elucidate different oligomers activate different signaling pathways, currently not known whether these differences are significant.

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**Activation of the AMPK and NF-kB Pathways by Acrp30**—Either reduction of high order Acrp30 isoforms with βME or substitution of Cys⁵² with alanine abolished the ability of Acrp30 to activate NF-κB. This confirms our previous observation that only hexameric and HMW Acrp30 isoforms can effectively activate NF-κB. Perhaps one Acrp30 monomer binds one cell surface receptor, and receptor clustering is required for activation of NF-κB by Acrp30. The notion that a hexameric but not trimeric complex is required for receptor activation has recently been extended to Fas ligand, a member of the tumor necrosis factor α-C1q superfamily. Whereas trimeric soluble Fas ligand is unable to induce cell death, a soluble hexameric Fas ligand generated by fusion with the Acrp30 collagen domain could signal apoptosis (25).

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Figure 5. Oligomerization and disulfide states of cysteine-replaced Acrp30 species. A, sedimentation equilibrium trace of C22A Acrp30. Initial protein concentration was 3 μM in 5.7 mM phosphate (pH 7.5), 137 mM NaCl, 2.7 mM KCl. Experimental conditions and data fitting procedures are described under “Experimental Procedures.” The random residuals (top panel) indicated a good fit to a single ideal species model. B, freeze-etched rotary replicas of purified HEK 293T C22A Acrp30 visualized by electron microscopy. Procedures for sample preparation and electron microscopy are described briefly under “Experimental Procedures” and detailed in Refs. 15–17. Magnification is 70,000×. C, nonreducing SDS-PAGE analysis of C22A Acrp30 and the three different C138A Acrp30 oligomers purified from transfected HEK cells.
myotubes (26, 27). Interleukin-6 is produced at high levels by increased interleukin-6 gene expression and release from C2C12/H9251 state independent anti-AMPK gAcrp30, trimeric Acrp30 is not effective in NF-κB activation results in increased fatty acid oxidation but does not activate NF-κB (10, 11). Recently Pajvani et al. (21) reported that the trimer form of (full-length) Acrp30 does not activate NF-κB (10) instead it signals through AMPK (11, 12). Whether full-length Acrp30 can also stimulate AMPK is controversial. We found that Acrp30 hexamer could not activate AMPK (11), whereas Yamauchi et al. (12) reported that full-length Acrp30 could. One possible explanation for the difference is that “full-length Acrp30” used by Yamauchi et al. contained mixtures of different Acrp30 oligomers. In the present study we showed that trimeric full-length Acrp30, but not the higher molecular weight isoforms, could activate AMPK. The presence of some trimer in the preparation used by Yamauchi et al. could account for the apparent differences between these studies (11, 12). A recent report indicates that two heptahelical membrane-spanning proteins act as receptors, albeit with different binding affinities, for gAcrp30 and full-length Acrp30 to mediate activation of AMPK in muscle cell lines (29). It is unclear which full-length Acrp30 isoforms, trimer, hexamer, or HMW species, bind to each of these receptors.

The Role of the Collagen Domain in Acrp30—The use of disulfide bonds to stabilize oligomeric structures appears to be a common theme in the family of proteins bearing the C1q-like globular domains. Disulfide bonds near the N terminus help the six heterotrimers of C1q to assemble properly (14, 30). Similarly, emilin and multimerin both have a cysteine-rich segment at the N terminus that participates in multimerization of these molecules (13). Because neither emilin nor multimerin contains collagen repeats, it brings into question whether the collagen domains are needed for multimerization of this family of molecules. We demonstrated the necessity of disulfide bonds formed by Cys22 in the assembly of hexameric and HMW Acrp30 but have not shown that these disulfide bonds are sufficient as well. If disulfide bonds alone are sufficient, one might expect the trimer subunits of the hexamer to be more loosely associated rather than tightly linked in the

![Image](59x402)

**FIG. 6.** Differential activation of NF-κB by wild type and cysteine-mutated Acrp30 isoforms in C2C12 cells. A, loss of NF-κB activation by Acrp30 hexamer following reduction with βME. Luciferase activity was measured by E-selectin promoter-luciferase reporter gene in transfected C2C12 cells following overnight incubation with 4 μg/ml Acrp30 hexamer purified from E. coli, before or after a 2-h incubation at 37°C with 0.5 mM βME. As controls, lipopolysaccharide (LPS, 200 ng/ml, E. coli serotype 055:B5, Sigma) and PBS were similarly treated with βME. The samples containing βME were centrifuged in a Speed-vac for 15 min to reduce the concentration of βME before addition to the cells. B, activation of E-selectin promoter-luciferase in undifferentiated C2C12 cells following an 8-h incubation with 2 μg/ml of C22A Acrp30 or HMW, hexamer, and trimer isoforms of C138A Acrp30.

![Image](59x326)

**TABLE I**

| Acrp30 isoform          | NF-κB activation (ME) | AMPK activation |
|-------------------------|-----------------------|-----------------|
| E. coli-derived hexamer | +a                    | _b              |
| E. coli-derived trimer  | _a                    | +c              |
| E. coli-derived gAcrp30 | _a                    | +d              |
| Mammalian cell derived HMW (C138A) | _a      | _a              |
| Mammalian cell derived hexamer (C138A) | +a     | +e              |
| Mammalian cell derived trimer (C138A) | _a     | _a              |
| Mammalian cell derived trimer (C22A) | _a     | _a              |

a From Ref. 10.
b From Ref. 11.
c Tomas and Ruderman, unpublished observation.
d From Ref. 12.
e Present study.

skeletal muscle during exercise (28) and is thought to trigger increased fatty acid and glucose production from adipose tissue and liver, respectively, thus providing an increase in circulating fuel for use by muscles (28). Perhaps hexameric and HMW Acrp30 play a role in the release of IL-6 from skeletal muscle through NF-κB activation and thereby indirectly stimulate lipolysis from fat tissue and glucose production from liver.

As described above, unlike the hexamer and HMW isoforms, gAcrp30 does not activate NF-κB (10). Instead it signals through AMPK (11, 12). Whether full-length Acrp30 can also stimulate AMPK is controversial. We found that Acrp30 hexamer could not activate AMPK (11), whereas Yamauchi et al. (12) reported that full-length Acrp30 could. One possible explanation for the difference is that “full-length Acrp30” used by Yamauchi et al. contained mixtures of different Acrp30 oligomers. In the present study we showed that trimeric full-length Acrp30, but not the higher molecular weight isoforms, could activate AMPK. The presence of some trimer in the preparation used by Yamauchi et al. could account for the apparent differences between these studies (11, 12). A recent report indicates that two heptahelical membrane-spanning proteins act as receptors, albeit with different binding affinities, for gAcrp30 and full-length Acrp30 to mediate activation of AMPK in muscle cell lines (29). It is unclear which full-length Acrp30 isoforms, trimer, hexamer, or HMW species, bind to each of these receptors.
observed parallel, head-to-head, formation. This suggests that interactions other than disulfide bonds contribute to the high order structures of Acrp30.

Collagens form long fibrils that lend structural integrity to the extracellular matrix. Self-assembly of the fibrils is driven by hydration forces as well as entropy associated with hydrophobic forces (31–33). Because Acrp30 hexamer is composed of only two relatively short collagen triple helices, these forces may not be strong enough by themselves to hold the hexamer together. Nevertheless, these weak interactions may help to stabilize the tightness of the hexameric structure. These molecular forces that allow fibril formation may contribute significantly to the maintenance of the HMW form of Acrp30.

In conclusion, interchain disulfide bonds formed by residue Cys22 are necessary for the oligomerization of Acrp30 beyond significantly to the maintenance of the HMW form of Acrp30. These molecular forces other than the disulfide bonds may not be strong enough by themselves to hold the hexamer together. Nevertheless, these weak interactions may help to stabilize the tightness of the hexameric structure. These molecular forces that allow fibril formation may contribute significantly to the maintenance of the HMW form of Acrp30.

Acrp30 oligomers activate different signal transduction pathways in living animals must be determined. In particular, the physiological relevance of the different oligomerization states in living animals must be determined. In particular, measurements of the total level of Acrp30 in human serum are used in studies correlating Acrp30 levels with diabetes and obesity (2, 3), whereas the level of only one or more of these isoforms might actually be relevant.

Acknowledgments—We thank Drs. J. Bogan, L. J. S. Huang, and L. Rezende for valuable discussions throughout this study.

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