Deep-sea fishes occur at depths of several thousand meters, and at these abyssal depths encounter pressures that shallower living fishes cannot tolerate. Tolerance of abyssal pressures by deep-sea fish is likely to depend in part on adaptive modifications of proteins. However, the types of structural modifications to proteins that allow function at high pressure have not been discovered. To elucidate the mechanisms of protein adaptation to high pressure, we cloned the α-skeletal actin cDNAs from two abyssal Coryphaenoides species, C. armatus and C. yaquinae, and identified three amino acid substitutions, V54A or L67P, Q137K, and A155S, that distinguish these deep-sea actins from orthologs of α-actin from non-abyssal Coryphaenoides. These substitutions, Q137K and A155S, prevent the dissociation reactions of ATP and Ca²⁺ from being influenced by high pressure. In particular, the lysine residue at position 137 results in a much smaller apparent volume change in the Ca²⁺ dissociation reaction. The V54A or L67P substitution reduces the volume change associated with actin polymerization and has a role in maintaining the DNase I activity of actin at high pressure. Together, these results indicate that a few amino acid substitutions in key functional positions can adaptively alter the pressure sensitivity of a protein.

The deep-sea is typified by low temperature (1–4°C), extremely high hydrostatic pressure, a lack of sunlight and a relatively low influx of utilisable organic material derived from primary production in surface waters. Among such environmental factors, hydrostatic pressure is thought to have the most influence on the vertical distribution of organisms and speciation in deep-sea (1–3). Hydrostatic pressure increases by approximately 0.1 megapascal (MPa) with every 10 m of depth in the ocean (4) and influences organism functions, especially those involving the formation of protein complexes, e.g., enzyme-substrate or protein-protein interaction (5, 6). Many previous studies have identified proteins from deep-sea fish that function at high pressure (5–10), and hypothetical models for protein adaptation to deep-sea pressure have been proposed (5); however, the primary structures of these unique proteins have not yet been determined.

Marine fish belonging to the genus Coryphaenoides known as rattails or grenadiers have been studied extensively as an excellent model in which to elucidate adaptation to the deep sea (5–12), because of their widespread bathymetric distribution up to a depth of about 6000 m (13). An important parameter to study in the context of pressure is the change in volume that accompanies such events as protein-ligand interactions and protein-protein interactions, for the sign and magnitude of the reaction volume change determine the reaction’s sensitivity to pressure. Swezey and Somero (14) have investigated the volume change (δV) that is associated with the polymerization of G-actin to F-actin α-skeletal actin from C. armatus (abyssal species) and C. acrolepis (non-abyssal species). The δV of actin from C. armatus was much smaller, which is advantageous for a deep-sea habitat, than that from C. acrolepis (14).

Actin is the main component of the microfilament system in all eukaryotic cells and plays a central role in maintaining the cytoskeletal structure, cell motility, cell division, intracellular movements, and contractile processes (15, 16). It is one of the most conserved proteins in eukaryotic cells, for example, α-skeletal actin proteins in carp and rat share 99.4% homology at the amino acid sequence level (17, 18). It is therefore surprising that differences in the δV of this highly conserved protein have been found between two species of Coryphaenoides that inhabit different niches.

In this study, we have cloned and sequenced the α-skeletal actin cDNAs from two abyssal Coryphaenoides, C. armatus and C. yaquinae, of which C. yaquinae inhabits greater depths. These actins contain three unique amino acid substitutions compared with the previously sequenced α-skeletal actin from two non-abyssal Coryphaenoides, C. acrolepis and C. cinereus (19). Biochemical analyses of the α-actin molecules purified from the skeletal muscles of C. armatus, C. yaquinae, C. acrolepis, carp, and chicken show that these amino acid substitutions are responsible for the adaptation of α-actin to high pressures in abyssal species. Here we describe, for the first time, the mechanism of adaptation of deep-sea fishes to high pressures at the amino acid sequence level.

**EXPERIMENTAL PROCEDURES**

**Materials**—C. acrolepis, C. armatus, and C. yaquinae were collected in large live-traps made from netting material by the R/V Soyomaru of the National Research Institute of Fisheries Science. The sampling locations were 41–40.20′ N, 142–57.40′ E, 180 m for C. acrolepis (habitat depth, about 180–2000 m) and 44–00.70′ N, 145–22.20′ E, 3940 m for C. armatus (habitat depth, about 2700–5000 m), and 39–58.10′ N, 154–59.50′ E, 5600 m for C. yaquinae (habitat depth, about 4000–6400 m). Carp (Cyprinus carpio) and chicken samples were purchased from local stores. All samples were stored below −80°C until use.

**Actin Protein**—Actin was isolated from the skeletal muscle of each species according to Spudich and Watt (20) and purified by gel-filtration chromatography over Sephadex G-200 in G buffer (0.2 mM ATP, 0.2 mM CaCl₂, 0.5 mM β-mercaptoethanol, Tris-HCl, pH 7.8). Actins were converted into the Mg²⁺-G form as described previously (21, 22). Mg²⁺-G-actin was used immediately after its conversion from Ca²⁺-G-actin. Actins were stored in G buffer at 4°C after purification and used within 3 days. The concentration of G-actin was determined spectrophotometrically using an absorption coefficient of 0.63 ml/mg at 290 nm.

**Polimerization, Critical Concentration, and δV Assembly—Polymer-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AB086240, AB086241, AB086242, and AB086243.

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ization of Mg\(^{2+}\)-actin was initiated by adding KCl and MgCl\(_2\) to final concentrations of 50 and 2 mM, respectively. Polymerization was monitored by light scattering, with excitation and emission wavelengths both set at 400 nm at 4 °C in a high pressure cell with a high pressure pump (PCI-400 cell and TP-500 pump Teramecs Co. Ltd., Kyoto, Japan). The change in light scattering was recorded as a function of time. The critical concentration was determined as described previously (23, 24). The volume change (\(\delta V\)) in assembly of G-actin into F-actin was calculated by the method of Sweezy and Somero (14).

Isolation of \(\alpha\)-Skeletal Actin cDNAs from \(C.\) armatus and \(C.\) yaquinae—\(C.\) armatus and \(C.\) yaquinae muscle cDNA libraries were constructed, and \(\alpha\)-skeletal actin cDNAs from each library were cloned as described previously (19). Structures of actin were prepared using the program MOLSCRIPT (25) with data from the Protein Data Bank (accession number 1ATN).

Phylogenetic Analysis—A molecular phylogenetic tree was constructed from the sequences of the actin coding regions. The DNADIST program in the PHYLIP version 3.5 program package (26) were used for neighbor joining (27). Bootstrap analyses with 1000 replicates were performed to examine the confidence of nodes within the resultant topology. Molecular phylogenetic analysis showed that a-actin genes from the C. borealisoides species were categorized into three types, actin 1, actin 2a, and actin 2b (see “Results”). The GenBank\(^\text{TM}\) accession numbers of actin nucleotide sequences used in this study are given in parentheses: \(C.\) armatus 2a (AB086240), \(C.\) armatus 2b (AB086241), \(C.\) yaquinae 2a (AB086242), \(C.\) yaquinae 2b (AB086243), \(C.\) acrolepis (AB021649), \(C.\) acrolepis 2a (AB021650), \(C.\) cinereus 2a (AB021651), \(C.\) cinereus 2a (AB021652); carp, \(C.\) carpio (D50025); medaka, \(Oryzias\) latipes (DS7740); fugu 1, Fugu rubripes (U38580), fugu 2 (U38585); goldfish, \(Carassius\) auratus (D50029); tilapia, \(Oreochromis\) mossambicus (AB079866); zebrafish, \(Danio\) rerio (AF180987); channel catfish, \(Oncorhynchus\) keta (AF030446); Atlantic salmon, \(Salmo\) salar (AF030446); chicken, Gallus gallus (K02257); human, \(Homo\) sapiens (M20543); mouse, \(Mus\) musculus (M12234); rat, \(Rattus\) norvegicus (V01218); and bovine, \(Bos\) taurus (U02285). Medaka \(\beta\)-actin (D89627) was used as the outgroup gene.

Quantification of Actin Isoforms—To identify the ratio of the a-actin isoform, quantitative reverse transcriptase polymerase chain reaction (RT-PCR\(^1\)) and two-dimensional electrophoresis were performed. These analyses were repeatedly done four times using total RNAs, and actin proteins were isolated from four individuals in each species. The conditions for RT-PCR were as described previously (19), except for the cycle number. The cycle number within a linear range of PCR amplification was determined to be 25 on the basis of the signal intensity of PCR products with sequential cycles. The primer 5'-ATGGTGAAGCAGGATGCGAGAAA-3' and NOT-1 d(T)\(_{18}\) primer amplified \(-480\) bp and 680 bp, for actin 1 and actin 2b, respectively. RT-PCR products were subjected to 1.5% agarose gel electrophoresis. Two-dimensional electrophoresis was performed by Multiphor II electrophoresis system with a pH range of 4.0–7.0 or 5.0–6.0 gel (24 cm) and 12.5% SDS-PAGE gel (Amersham Biosciences). The ratios of actin 2b to actin 2a, or actin 1 to actin 2a, were quantified using a computerized image analysis scanner STORM 880 (Amersham Bioscience).

Quin 2 Assay—The dissociation rate constant of Ca\(^{2+}\) from actin was determined by assaying the fluorescence intensity increase of Quin 2 (8-amino-2-[2-amino-5-methylphenoxo]methyl]-6-methoxyquinoline-N,N,N',N'-tetraacetic acid) (28). Ca\(^{2+}\)-actin (5 \(\mu\)M) prepared by dialysis against G-buffer (free CaCl\(_2\)) was added to 100 \(\mu\)M Quin 2 and 0.2 mM MgCl\(_2\), and the fluorescence was measured at respective excitation and emission wavelengths of 340 and 410 nm at 4 °C in a high pressure cell. The critical concentrations of actin also increased with high pressure, and at atmospheric pressure the polymerization half-time was shortest for carp. The half-times for chicken and non-abyssal actin species increased markedly above 20 MPa and, at 60 MPa were, respectively, about 5.6- and 7.3-fold higher than at atmospheric pressure. By contrast, the half-time of actin polymerization for the two abyssal actin species had increased only about 2.7-fold at 60 MPa (Fig. 1A).

The critical concentrations of actin also increased with high pressure for each species (Fig. 1B). The critical concentrations of the two abyssal actin species were higher than those of other species at 20 MPa and lower pressures, and increased slightly from 0.1 to 60 MPa.

The volume change (\(\delta V\)) associated with polymerization at each pressure was determined from the respective critical concentrations (Fig. 1C). The two abyssal actin species had a much smaller \(\delta V\) at each pressure than did the other species, in agreement with previous reports (14). The \(\delta V\) of actins from chicken and non-abyssal species decreased at high pressure, whereas those from the abyssal species showed little variation.

These observations indicated that, for chicken and non-abyssal species, the coefficient of compressibility for F-actin was larger than that for G-actin and that the space produced by actin-actin interactions was reduced by high pressure. Clearly, there was no such reduction in space in actins from the abyssal species. Unexpectedly, the \(\delta V\) of carp actin, unlike that of other species, increased with high pressure, which indicated that for carp the coefficient of compressibility for G-actin was larger than that for F-actin; in other words, carp G-actin is softer than the G-actin of the other species. Thus, this would explain why carp actin was able to polymerize at pressures of only 20 MPa or less.

\(^1\)The abbreviations used are: RT, reverse transcription; \(\delta V\), volume change; Quin 2, 8-amino-2-[2-amino-5-methylphenoxo]methyl]-6-methoxyquinoline-N,N,N',N'-tetraacetic acid; \(V^\alpha\), activation volume; e-ATP, 1,5'-etheno-ATP.
Consequently, the non-abyssal actin 2 was re-designated actin 2a. The amino acid sequences of actin 1 and 2a differ by one amino acid residue at position 155, which is Ala-155 in actin 1 and Ser-155 in actin 2a (19). The sequence of actin 2b differs from that of actin 2a by two amino acids (either V54A or L67P and Q137K) (Table I). The x-ray crystallography structure of rabbit skeletal muscle actin shows that residues 54 and 67 are located in a β-sheet of subdomain 2 (residues 33–69), whereas residues 137 and 155 are located in the Ca\(^{2+}\)- and ATP-binding sites (Fig. 3, A and B) (33).

### Quantification of Actin Isoforms—To identify the expression ratio of the α-actin isoforms, the expression of isoform mRNA was investigated using quantitative RT-PCR. Quantitative mRNA was investigated using quantitative RT-PCR. Quantitative RT-PCR was carried out with one primer set, which amplified different length products from the two isoforms, about 480 bp for actin 1 and actin 2b, and about 680 bp for actin 2a, owing to the different lengths of their 3’ non-coding regions (Fig. 2B). Direct sequencing confirmed that the RT-PCR products were the expected actin isoforms. The results showed that there was...
A dot indicates that the amino acid residue is identical to that in actin-1. Numbers above the sequence indicate the residue position. *yaq* actin-2b and *aer* actin 2b are actin 2b cloned from *C. yaquinae* and *C. armatus*, respectively.

| Actin type    | Position |
|---------------|----------|
|               | 2       | 3       | 54      | 67      | 137     | 155     | 165     | 278     | 299     | 358     |
| Actin 1       | D       | E       | V       | L       | Q       | A       | V       | A       | L       | S       |
| *yaq* actin 2b| -       | -       | -       | -       | -       | S       | -       | -       | -       | -       |
| *aer* actin 2b| -       | -       | A       | -       | K       | S       | -       | -       | -       | -       |
| Carp          | -       | D       | -       | -       | -       | -       | -       | -       | -       | -       |
| Chicken       | E       | D       | -       | -       | -       | S       | I       | T       | M       | T       |

Fig. 3. *Structure of actin with bound ATP and Ca\(^{2+}\)*. Blue sticks and a green ball represent ATP and Ca\(^{2+}\), respectively. A, ribbon drawing of actin (33). The positions of substitutions found in this study are colored red. B, environment of ATP and Ca\(^{2+}\) in actin. Gln-137 and Ser-155 are indicated by ball-and-stick representation. The atoms carbon, oxygen, and nitrogen are colored gray, red, and purple, respectively. These images are created using MOLSCRIPT (25).

differential expression of the two isoforms in each species. The expression ratio of actin 2b to actin 2a, or actin 1 to actin 2a, was 4.3 ± 0.18 for *C. yaquinae*, 4.1 ± 0.083 for *C. armatus*, and 0.67 ± 0.034 for *C. acrolepis*, respectively. We examined further the ratio of protein isoforms using two-dimensional electrophoresis. The abundance ratio of actin 2b to actin 2a was 4.8 ± 0.087 for *C. yaquinae* (Fig. 2C) and 4.5 ± 0.18 for *C. armatus* (data not shown), respectively. The protein isoforms from *C. acrolepis*, which have the same isoelectric point estimated, could not be separated by this electrophoresis method (data not shown). These results of the electrophoresis were not affected by dephosphorylation using *Escherichia coli* alkaline phosphatase (data not shown). These ratios reflect higher expression of the isoform that is more essential for the species habitat, as described below.

*Quin 2 and Nucleotide Exchange Assay*—Sequence analysis showed that, although actin 2b from abyssal species has a lysine at position 137, the other isoform and all actins from the other species have a glutamine at this position, and all actin isoforms from abyssal species have a serine at residue 155. Actin binds ATP by sandwiching the ATP β- and γ-phosphates between two structurally equivalent β-hairpins (residues 11–18 and 154–161), which belong to homologous subdomains 1 and 3 (Fig. 3, A and B) (33, 34). Actin also contains a tightly bound divalent cation (Ca\(^{2+}\) or Mg\(^{2+}\)) in a deep hydrophilic pocket formed by the β- and γ-phosphates of the bound ATP and actin residues Asp-11, Gln-137, and Asp-154 (Fig. 3, A and B) (33, 35). Although actin has a higher affinity for Ca\(^{2+}\) than for Mg\(^{2+}\), the much higher cellular concentration of Mg\(^{2+}\) means that it will be the main occupant of the divalent cation site in vivo. We also investigated the effect of high pressures on the divalent cation and nucleotide binding of actin protein at various pressures (Fig. 4, A and B). Surprisingly, both dissociation rate constants of actin from abyssal species were much less affected by high pressures than those of the other actins, which increased rapidly at pressures greater than 20 MPa. The dissociation rate constant of Ca\(^{2+}\) in abyssal actins did not differ greatly from that of the other actins at atmospheric pressure, which means that the actins bind to Ca\(^{2+}\) with almost the same strength, whereas the differences in the dissociation rate constant of ATP suggest that Ser-155 actin binds ATP more tightly than Ala-155 actin, as pointed out previously (19). The apparent δV\(^\circ\) value in the Ca\(^{2+}\) dissociation reaction at pressures greater than 20 MPa was estimated as −4.27 ± 1.86 cm\(^3\) mol\(^{-1}\) for abyssal species and −154.3 ± 0.538 cm\(^3\) mol\(^{-1}\) for the other species. The apparent δV\(^\circ\) in the ATP dissociation reaction at more than 20 MPa was also determined to be −43.4 ± 0.47 cm\(^3\) mol\(^{-1}\) or more for abyssal actin, and as −80.4 ± 1.44, −90.0 ± 1.59, and −84.2 ± 0.995 cm\(^3\) mol\(^{-1}\) for chicken, carp, and non-abyssal actin, respectively. These results indicated that the smaller effect in both dissociation rate constants of pressure in abyssal actin results from Q137K and the differences in both constants among carp, chicken, and non-abyssal species from A155S.

*Effects of Pressure on Intrinsic Tryptophan Fluorescence Spectrum*—Actin contains four tryptophan residues (at positions 74, 86, 340, and 356), which are all located in subdomain 1. The emission maximum of fluorescing tryptophan residues is 350 nm in a neutral water solution but shifts to shorter wavelengths in a hydrophobic environment, such as the interior of a folded protein (36). To investigate effect of pressures on the actin structure, we measured the intrinsic tryptophan fluorescence spectrum of Ca\(^{2+}\)-G-actin at various pressures. Although
the emission maximum did not shift, the fluorescence intensity of actins from chicken, carp, and non-abyssal species began to decrease at only 10 MPa (data not shown), and in particular the decrease for carp actin was larger than that for chicken and non-abyssal actin; by contrast, the fluorescence of the abyssal actins did not change even at 60 MPa (Fig. 5, A and B). Actin affinity for Ca\(^{2+}\) is greater than for Mg\(^{2+}\), but there were no differences in fluorescence intensity between the Ca\(^{2+}\)- and Mg\(^{2+}\)-G-actin forms for all actin species (data not shown). These results indicate that high pressure changes the environment of the tryptophan residues in actin; in other words, the structure of actin subdomain 1, which includes one of the \(\beta\)-hairpins that sandwiches the ATP \(\beta\)- and \(\gamma\)-phosphates and the Ca\(^{2+}\)-binding sites.

**DNase I Inhibition Assay**—Actin 2b of the two abyssal species contains either a V54A substitution or an L67P substitution (Table I). The x-ray crystallography structure shows that these residues are located in subdomain 2 (residues 33–69) (Fig. 3A). In actin-DNase I interactions, DNase I primarily contacts the DNase I-binding loop (residues 40–48) in subdomain 2 and interacts slightly with Thr-203 and Glu-207 in subdomain 4 (residues 181–269) (33, 37). To investigate whether these substitutions, V54A or L67P, affect the actin-DNase I interaction, we performed a DNase I Inhibition assay at various pressures (Fig. 6). Notably, we found that the abyssal actin inhibited DNase I even at 60 MPa, whereas the other actin species showed little activity at 60 MPa. In addition, carp actin was almost inactive at 30 MPa. The decrease in DNase I inhibition activity of actin proteins from 0.1 to 60 MPa was greater in *C. armatus* actin (23.9%) than in *C. yaquinae* actin (17.2%), in agreement with the fact that *C. yaquinae* lives at greater depths (38). These decreases should depend on the denatured state of actin 2a by high pressures, which exist
slightly in muscles of both *C. yaquinae* and *C. armatus* as shown by two-dimensional electrophoresis (Fig. 2C). Our finding clearly indicates that the actins from abyssal species were bound to DNase I even at 60 MPa, which suggests that the substitutions in subdomain 2 of actins from abyssal species are most likely to reduce the increased volumes that occur with the interaction of actin with DNase I. However, the mechanism underlying the reduction in volume remains unclear.

**DISCUSSION**

One of the most surprising characteristics of actin from deep-sea fishes, which enables them to inhabit deep-sea environments, was the extremely small ΔV associated with actin polymerization, which results in a lower critical concentration of actin at high pressure. Because the small ΔV was observed even at atmospheric pressure, it is due to actin-actin interactions and not to the relative compressibilities of G- and F-actin. On the basis of the x-ray crystallography structure of actin, however, there are no differences in the amino acids at actin-actin contact points between abyssal actin and other actin species (Table I and Fig. 3A).

Subdomain 2 (residues 33–69) is probably the most flexible of the actin subdomains (39), and its conformational change after ATP hydrolysis and P\(_\text{i}\) release from ADP-P\(_\text{i}\), during actin polymerization plays a critical role in filament dynamics (40, 41). Residues 41–45 in subdomain 2 of one actin molecule interact with two other actin molecules, one at residues 166–169 and the other around residue 375 (42). Our results show that the DNase I-binding loop (residues 40–48) in subdomain 2 of the abyssal actin can interact with DNase I at high pressure levels; therefore, we propose that abyssal actin also undergoes actin-actin interactions at high pressure without a large increase in volume. Clearly, such a possibility is provided by the V54A or L67P substitutions found in the actin-actin interactions at high pressure without a large increase in volume. Clearly, such a possibility is provided by the V54A or L67P substitutions found in the subdomain 2 of actin from the abyssal species. We speculate that subdomain 2 of abyssal actin is more compact and has reduced space in the β-sheet, because these substituted residues do not normally form β-sheets. However, the exact mechanistic details remain to be determined.

Our findings indicate that the dissociation of ATP and Ca\(^{2+}\) in particular in actins from abyssal species is less affected by pressure than in actins from other species. This tolerance to high pressure is due especially to the A155S and Q137K substitutions in actins of abyssal species. These substituted amino acids, which increase the negative value of the ΔV\(_\text{o}\) at the Ca\(^{2+}\) and the ATP dissociation reactions, prevent these reactions from being strongly accelerated by high pressures. The Ca\(^{2+}\) lies below the bound ATP on the pseudo-2-fold axis and is coordinated by four water molecules, which are held in place through interactions with the side chains of Asp-11, Gln-137, and Asp-154, and two O atoms from the β- and γ-phosphates of the bound ATP (33, 34). Actin, Hsp70 molecular chaperones, hexokinase, and sugar kinases share structural homology and can, therefore, be considered members of a superfamily (43, 44). In this superfamily, the amino acid in the equivalent position to Gln-137 in most actin is usually Asp or Glu, i.e. a negatively charged residue. Surprisingly, actins from abyssal species have the positively charged residue Lys at position 137. The Q137K substitution thus changes the coordination of Ca\(^{2+}\) with the protein. The repulsion between Ca\(^{2+}\) and Lys-137 would prevent the Ca\(^{2+}\) from being pushed into the bottom of the interdomain cleft by high pressures. On the other hand, another study showed that the rate of ligand entry from the solvent to the protein interior is increased if the space available to the ligand in the protein pocket is increased by substituting an amino acid residue with a smaller side chain (45). The substitution of amino acids with larger side chains (46), Q137K and A155S, might prevent both the Ca\(^{2+}\) and the ATP dissociation reactions from being accelerated by high pressures. Previous studies have reported that high pressure can slightly affect the apparent \(K_m\) of substrate values of some dehydrogenases from deep-sea fishes (5–9). Our results suggest a mechanism for stabilizing enzyme-substrate interactions under elevated pressure.

The Q137K and A155S substitutions indicate that other actin species than abyssal actin have larger cavities in the protein pocket, owing to the presence of amino acid residues with smaller side chains. The results from the Quin 2 and nucleotide exchange assays show, however, that these cavities remain intact at high pressures. On the other hand, our observations here (Fig. 4, A and B) and previous studies show that abyssal actins form tighter associations with ATP and Ca\(^{2+}\) than do other actin species (19, 47). The structure of actin is maintained not only by its internal weak bonds but mainly by bound ATP and Ca\(^{2+}\) (28). Thus, the tightly bound ATP and Ca\(^{2+}\) in abyssal actins presumably maintain the actin structure from inside, rather like a pillar. In support of this, carp G-actin is much softer (Fig. 5, A and B), and the ΔV of carp actin increased with high pressure (Fig. 1C), which indicated that for carp the coefficient of compressibility for G-actin was larger than that for F-actin. Thus, Ala-155 actin would explain why carp actin was able to polymerize at pressures of only 20 MPa or less.

The Ala-155 actin variant is not disadvantageous for freshwater fishes such as carp, because they do not experience high pressures; however, it would be disadvantageous for many marine fishes. The molecular phylogenetic tree for actin genes clearly shows that actin 2a diverged from actin 1 (Fig. 2A). Because all α-actins cloned from the freshwater fishes so far are the Ala-155 variant, this suggests that Ala-155 actin is necessary for living in freshwater conditions and Ala-155 actin found in marine fishes may be a remnant from an ancestral fish. Deep-sea fishes, which live in an environment where actin 1 does not function, probably have the gene for actin 1 but do not express it. In the superfamily, including actin, Hsp70 molecular chaperones, hexokinase, and sugar kinases, the amino acid in the equivalent position 155 in actin is usually an uncharged polar or nonpolar amino acid (43, 44). Therefore, the Ala-155 actin found in many fishes would be accepted without the problem in the polarity. We do not, however, understand the full significance of the alanine residue at position 155 in actin in freshwater fishes.

A large portion of our data in this study was collected from a mixture of actin isoforms isolated from muscle, which we were unable to separate by biochemical techniques. Although the expression of recombinant proteins in microorganisms is a powerful tool, the system for actin, and in particular α-skeletal actin, has been not perfected as yet because of various problems, such as the formation of inclusion bodies and the low yield of expressed protein (48). We are therefore optimizing this system for future studies.

There are a multitude of actin-binding proteins (49), and, as a result, actin is one of the most conserved proteins with only limited variable positions. Our results have shown that a novel function that enables species to adapt to a new environment can evolve in a protein by a very few amino acid substitutions in key functional positions. Moreover, the molecular phylogenetic tree based on the α-actin coding region shows that actin 2a diverged from actin 1 and that actin 2b diverged from actin 2a, again by a very few amino acid substitutions (Table I). The previous study indicated that freshwater fishes had only Ala-155 actin, i.e. actin 1 (19). Thus, when the teleosts advanced from freshwater to sea and continuously from surface to
abyssal zone, they should duplicate the α-actin gene each time. These observations are consistent with Perutz’s theory of protein speciation (50), the theory of gene duplication (51), and the prediction from the molecular phylogenetic tree based on mitochondrial DNA analysis (3).

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Structure-based Analysis of High Pressure Adaptation of α-Actin
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