

Fig. 2-1. High-pressure measurement system. (A) Component of high-pressure measurement system. A high-pressure cell is set in a fluorometer. High-pressure is generated with a hand pump. (B) A high-pressure cell. A sample is put in the quartz inner cell. High-pressure generated with the pump is conveyed by the water medium. The water medium pushes up the silicon gum and the sample in the inner cell is compressed. The whole cell is cooled with cold water.

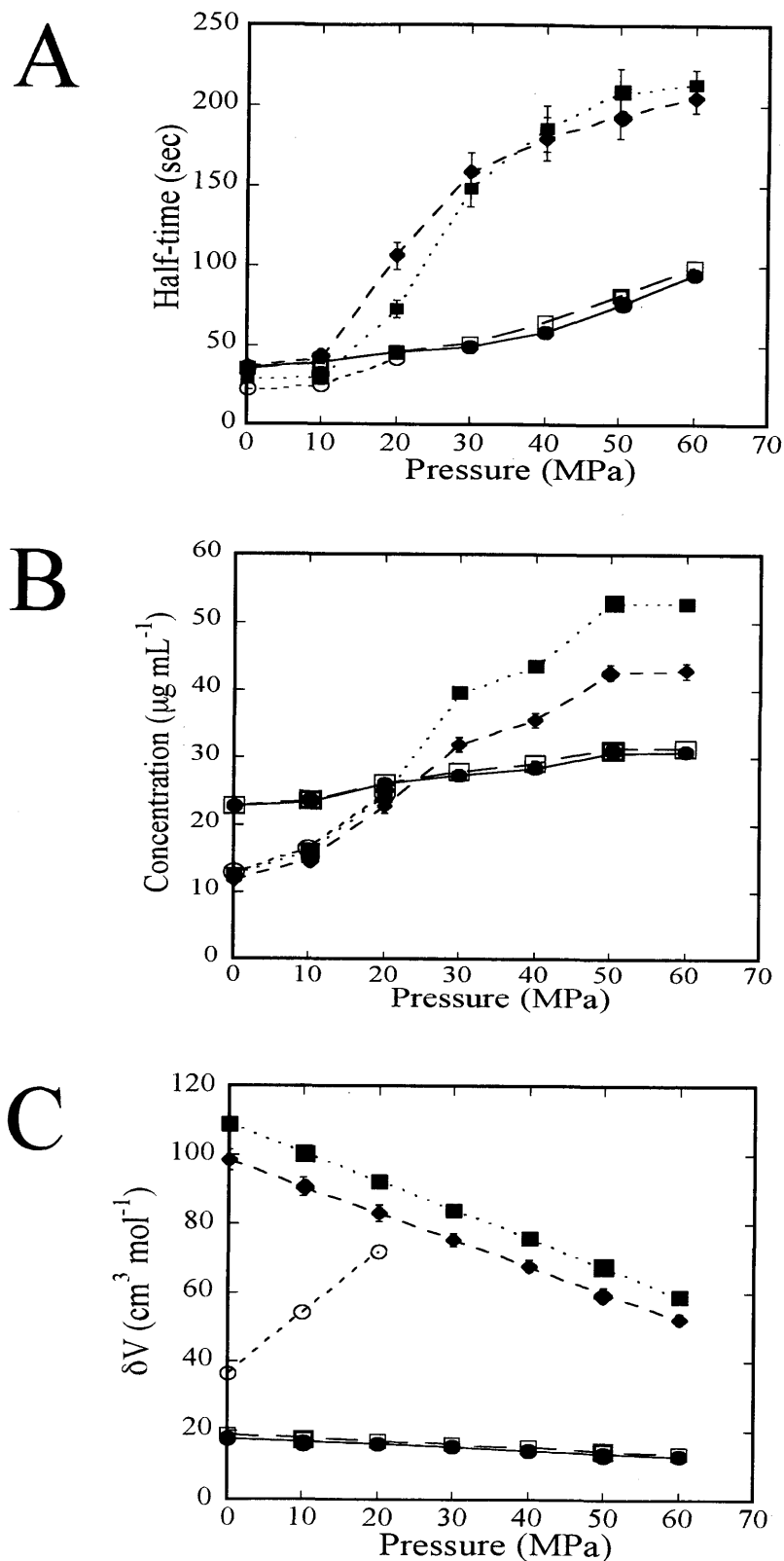


Fig. 2-2. Effects of pressure on actin polymerization. (A) Half-time to steady state in polymerization of actin. (B) Critical concentration of actin at various pressures. (C) Volume change ( $\delta V$ ) associated with polymerization of actin at various pressures. Symbols are as follows: *C. yaquinae* (●); *C. armatus* (□); Chicken (◆); carp (○); *C. acrolepis* (■). Values are means  $\pm$  S.D. of four independent experiments. Errors for several experiments are too small to show error bars.

	-49	
<i>C. acrolepis</i> -1	TTGGGTCTT TCTCTCTCCA AGCCGCAGAC ACTCACCTAA GAAAGCCATC	
<i>C. acrolepis</i> -2a	TTAC... GT.....GAT CC..CGGAG. TAC..A..G. AT...T..A.	
<i>C. cinereus</i> -1		G.....
<i>C. cinereus</i> -2a		..T..A.
<i>C. armatus</i> -2a	T CC..CGGAG. TAC..A..G. AT...T..A.	
<i>C. armatus</i> -2b	.....	.....G.....T..
<i>C. yaquinae</i> -2a	T CC..CGGAG. TAC..A..G. AT...T..A.	
<i>C. yaquinae</i> -2b	.....	.....G.....T..
	<b>START</b>	50
<i>C. acrolepis</i> -1	ATGTGTGACG ATGAGGAGAC TACCGCCCTT GTGTGCGACA ACGGCTCCGG	
<i>C. acrolepis</i> -2a	..... .C..A..... C.....C .....	
<i>C. cinereus</i> -1	.....	.....
<i>C. cinereus</i> -2a	..... .C..A..... C.....C .....	
<i>C. armatus</i> -2a	..... .C..A..... C.....C .....	
<i>C. armatus</i> -2b	.....	.....
<i>C. yaquinae</i> -2a	..... .C..A..... C.....C .....	
<i>C. yaquinae</i> -2b	.....	.....
		100
<i>C. acrolepis</i> -1	CCTGGTGAAG GCTGGGTTCG CCGGAGACGA TGCCCCCAGG GCTGTCTTCC	
<i>C. acrolepis</i> -2a	...C..... ..C.... .....	C..A..... ..C.....
<i>C. cinereus</i> -1	.....	.....
<i>C. cinereus</i> -2a	...C..... ..C.... .....	C..A..... ..C.....
<i>C. armatus</i> -2a	...C..... ..C.... .....	C..A..... ..C.....
<i>C. armatus</i> -2b	...C..... ..C.... .....	C..A..... ..C.....
<i>C. yaquinae</i> -2a	...C..... ..C.... .....	C..A..... ..C.....
<i>C. yaquinae</i> -2b	...C..... ..C.... .....	C..A..... ..C.....
		150
<i>C. acrolepis</i> -1	CCTCCATCGT CGGCCGCCCC CGTCACCAGG GTGTCATGGT CGGTATGGGT	
<i>C. acrolepis</i> -2a	.A..... ..T .....	.C..... G..C....G
<i>C. cinereus</i> -1	.....	.....
<i>C. cinereus</i> -2a	.A..... ..T .....	.C..... G..C....G
<i>C. armatus</i> -2a	.A..... ..T .....	.C..... G..C....G
<i>C. armatus</i> -2b	.A..... ..T .....	.C..... G..C....G
<i>C. yaquinae</i> -2a	.A..... ..T .....	.C..... G..C....G
<i>C. yaquinae</i> -2b	.A..... ..T .....	.C..... G..C....G
		200
<i>C. acrolepis</i> -1	CAGAAAGACT CCTACGTCGG CGACGAGGCC CAGAGCAAGA GAGGTATCTT	
<i>C. acrolepis</i> -2a	.....G.... ..G.. .....	.....C.
<i>C. cinereus</i> -1	.....G.... ..G.. .....	.....
<i>C. cinereus</i> -2a	.....G.... ..G.. .....	.....G.....C.
<i>C. armatus</i> -2a	.....G.... ..G.. .....	.....G..C...C.
<i>C. armatus</i> -2b	.....G.... ..CG.. .....	.....G.....C.
<i>C. yaquinae</i> -2a	.....G.... ..G.. .....	.....G..C...C.
<i>C. yaquinae</i> -2b	.....G.... ..G.. .....	.....A. .G.....C.
		250
<i>C. acrolepis</i> -1	GACTCTTAAG TACCCCATTTG AGCACGGCAT CATCACCAAC TGGGACGACA	
<i>C. acrolepis</i> -2a	...C..C... ..C. ....	.....
<i>C. cinereus</i> -1	.....	.....
<i>C. cinereus</i> -2a	...C..C... ..C. ....	.....
<i>C. armatus</i> -2a	...C..C... ..C. ....	.....
<i>C. armatus</i> -2b	...C..C... ..C. ....	.....T .....
<i>C. yaquinae</i> -2a	...C..C... ..C. ....	.....
<i>C. yaquinae</i> -2b	...C.CC... ..C. ....	.....T .....

Fig. 2-3. Comparison of cDNA nucleotide sequences encoding  $\alpha$ -actin 1,  $\alpha$ -actin 2a and  $\alpha$ -actin 2b. The dot indicates an identical nucleotide with that of *C. acrolepis*  $\alpha$ -actin 1. 5' primer-2, 3, 5' primer-4, 5 and polyadenylation signals are indicated by red, blue and boldfaced letters, respectively. These sequences are available from the DDBJ/EMBL/GenBank databases. The accession numbers are AB021649, AB021650, AB021651, AB021652, AB086240, AB086241, AB086242, and AB086242 from the top.

					300
<i>C. acrolepis</i> -1	TGGAGAAGAT	CTGGCACCAC	ACCTTCTACA	ATGAGCTGCG	CGTGGCCCCC
<i>C. acrolepis</i> -2a	.....	.....	.....	.C.....	T.....
<i>C. cinereus</i> -1	.....	.....	.....	.....	.....
<i>C. cinereus</i> -2a	.....	.....	.....	.C.....	T.....
<i>C. armatus</i> -2a	.....	.....	.....	.C.....	T.....
<i>C. armatus</i> -2b	.....	.....	.....	.C.....	.....
<i>C. yaquinae</i> -2a	.....	.....	.....	.C.....	T.....
<i>C. yaquinae</i> -2b	.....	.....	.....	.C.....	.....
					350
<i>C. acrolepis</i> -1	GAGGAGCACC	CCACCCTGCT	CACTGAGGCC	CCCCTGAACC	CCAAGGCTAA
<i>C. acrolepis</i> -2a	.....	.....	..C.....	....C....	.....C..
<i>C. cinereus</i> -1	.....	.....	.....	.....	.....
<i>C. cinereus</i> -2a	.....	.....	..C.....	....T....	.....C..
<i>C. armatus</i> -2a	.....	....T....	..C.....	....C....	.....C..
<i>C. armatus</i> -2b	.....	.....	.....	....C....	.....
<i>C. yaquinae</i> -2a	.....	.....	..C.....	....C....	.....C..
<i>C. yaquinae</i> -2b	.....	.....	.....	....C....	.....
					400
<i>C. acrolepis</i> -1	CCGTGAAAAG	ATGACCCAGA	TCATGTTTGA	GACCTTCAAC	GTCCCCGCCA
<i>C. acrolepis</i> -2a	.A.G..G..	.....	.....C..	.....	..G.....
<i>C. cinereus</i> -1	.....	.....	.....	.....	.....
<i>C. cinereus</i> -2a	.A.G..G..	.....	.....C..	.....	..G.....
<i>C. armatus</i> -2a	.A.G..G..	.....	.....C..	.....	..G.....
<i>C. armatus</i> -2b	.A....G..	....A....	.....	.....	..G.....
<i>C. yaquinae</i> -2a	.A.G..G..	.....	.....C..	.....	..G.....
<i>C. yaquinae</i> -2b	.A....G..	....A....	.....	.....	..G.....
					450
<i>C. acrolepis</i> -1	TGTATGTGGC	CATCCAGGCT	GTGCTGTCCC	TGTACGCTTC	CGGCCGTACC
<i>C. acrolepis</i> -2a	....C.....	.....C	.....	.....C..	.....
<i>C. cinereus</i> -1	.....	.....	.....	.....	.....
<i>C. cinereus</i> -2a	....C.....	.....C	.....	.....C..	.....
<i>C. armatus</i> -2a	....C.....	.....C	.....	.....C..	.....
<i>C. armatus</i> -2b	.....	....A....C	.....	.....	.....C..
<i>C. yaquinae</i> -2a	....C.....	.....C	.....	.....C..	.....
<i>C. yaquinae</i> -2b	.....C..	....A....C	.....	.....	....A..C..
					500
<i>C. acrolepis</i> -1	ACCGGTATTG	TGCTGGATGC	TGGTGATGGT	GTCACCCACA	ACGTCCCAGT
<i>C. acrolepis</i> -2a	..T....C.	.....CT.	C.....C...	..G.....	....G..G..
<i>C. cinereus</i> -1	.....	.....	.....	.....	.....
<i>C. cinereus</i> -2a	..T....C.	.....CT.	C.....C...	..G.....	....G..G..
<i>C. armatus</i> -2a	..T....C.	.....CT.	C.....C...	..G.....	....G..G..
<i>C. armatus</i> -2b	..T....C.	.....CT.	C..G..C...	..G....T.	....G..G..
<i>C. yaquinae</i> -2a	..T....C.	.....CT.	C.....C...	..G.....	....G..G..
<i>C. yaquinae</i> -2b	..A....C.	.....CT.	C..G..C...	..G....T.	.....C..
					550
<i>C. acrolepis</i> -1	GTATGAGGGT	TACGCCCTGC	CCCACGCCAT	CATGCGTCTT	GACCTGGCTG
<i>C. acrolepis</i> -2a	.....C	....G....	.....	.....C..G	.....C.
<i>C. cinereus</i> -1	.....	.....	.....	.....	.....
<i>C. cinereus</i> -2a	.....C	....G....	.....	.....C..G	.....C.
<i>C. armatus</i> -2a	...C....C	....G....	.....	.....C..G	.....C.
<i>C. armatus</i> -2b	...C....	....T....	.....	....A.G..G	..T....C.
<i>C. yaquinae</i> -2a	...C....C	....G....	.....	.....C..G	.....C.
<i>C. yaquinae</i> -2b	A..C....C	....T....	.....	....A.GT.G	..T....C.
					600
<i>C. acrolepis</i> -1	GTCGCGACCT	GACCGACTAC	CTGATGAAGA	TCCTGACCGA	GCGTGGCTAC
<i>C. acrolepis</i> -2a	.C.....	C.....	..C.....	....C.....	...C..A...
<i>C. cinereus</i> -1	.....	.....	.....	.....	.....
<i>C. cinereus</i> -2a	.C.....	C.....	..C.....	....C.....	...C..A...
<i>C. armatus</i> -2a	.C.....	C.....	..C.....	....C.....	...C..A...
<i>C. armatus</i> -2b	.....	C.....	..C.....	....C.....	..A.G..G...
<i>C. yaquinae</i> -2a	.C.....	C.....	..C.....	....C.....	...C..A...
<i>C. yaquinae</i> -2b	....G.....	C..A.....	..C.....	.....	..A.G.....

Fig. 2-3. Continued.



					650
<i>C.acrolepis</i> -1	TCTTTCGTTA	CCACCGCCGA	GCGTGAGATC	GTGCGCGACA	TCAAGGAGAA
<i>C.acrolepis</i> -2a	..C.....C.	.....	.....	.....	.....
<i>C.cinereus</i> -1	.....	.....	.....	.....	.....
<i>C.cinereus</i> -2a	..C.....C.	.....	.....	.....	.....A..
<i>C.armatus</i> -2a	..C.....C.	.....	...G.....	.....	.....
<i>C.armatus</i> -2b	.....C.	.....	.....	.....	.....
<i>C.yaquinae</i> -2a	..C.....C.	.....	...G.....	.....	.....
<i>C.yaquinae</i> -2b	AG.....C.	.....	.....	.....	.....
					700
<i>C.acrolepis</i> -1	GCTGTGCTAT	GTGGCTCTGG	ACTTCGAGAA	CGAGATGGCC	ACCGCCGCCT
<i>C.acrolepis</i> -2a	.....C	..C..C....	.....	.....A	.....
<i>C.cinereus</i> -1	.....	.....	.....	.....	.....
<i>C.cinereus</i> -2a	.....C	..C..C....	.....	.....A	.....
<i>C.armatus</i> -2a	.....C	..C..C....	.....	.....G	.....
<i>C.armatus</i> -2b	.....	.....G....	.....	.....G	.....G.
<i>C.yaquinae</i> -2a	.....C	..C..C....	.....	.....G	.....
<i>C.yaquinae</i> -2b	.....	.....G....	.....	.....G	.....T.
					750
<i>C.acrolepis</i> -1	CCTCCTCCTC	CCTGGAGAAG	AGCTACGAGC	TTCCCACGCG	TCAGGTCATC
<i>C.acrolepis</i> -2a	.....	.....	.....T....	.G.....	.....
<i>C.cinereus</i> -1	.....	.....A	.....	.....	.....
<i>C.cinereus</i> -2a	.....	.....	.....	.G.....	.....
<i>C.armatus</i> -2a	.....	.....	.....	.G.....	.....
<i>C.armatus</i> -2b	.....A..	T.....	.....	.G.....	.....
<i>C.yaquinae</i> -2a	.....	.....	.....	.G.....	.....
<i>C.yaquinae</i> -2b	.....A..	.....	..T.....	.G.....	.....
					800
<i>C.acrolepis</i> -1	ACCATAGGAA	ACGAGCGTTT	CCGTTGCCCT	GAGACCCCTCT	TCCAGCCCTC
<i>C.acrolepis</i> -2a	.....C..C.	.....G..	...C.....C	.....	.....C..
<i>C.cinereus</i> -1	.....C....	.....	.....	.....	.....
<i>C.cinereus</i> -2a	.....C..C.	.....C..	...C.....C	.....	.....C..
<i>C.armatus</i> -2a	.....C..C.	.....C..	...C.....C	.....	.....C..
<i>C.armatus</i> -2b	.....C..	.....C..	...C.....	.....	.....
<i>C.yaquinae</i> -2a	.....C..C.	...A..C..	...C.....C	.....	.....C..
<i>C.yaquinae</i> -2b	.....C..	.....C..	...C.....	.....	.....
					850
<i>C.acrolepis</i> -1	CTTCATCGGA	ATGGAGTCCG	CCGGTATCCA	TGAGACCGCC	TACAACAGCA
<i>C.acrolepis</i> -2a	.....C	.....T.	...C.....	C.....	.....
<i>C.cinereus</i> -1	.....	.....	.....	.....	.....
<i>C.cinereus</i> -2a	.....C	.....T.	...C.....	C.....	.....
<i>C.armatus</i> -2a	.....C	.....	...C.....	C.....	.....
<i>C.armatus</i> -2b	.....T	.....	.....	.....	.....
<i>C.yaquinae</i> -2a	.....C	.....	...C.....	C.....	.....
<i>C.yaquinae</i> -2b	.....T	.....G.	.....	.....	.....
					900
<i>C.acrolepis</i> -1	TCATGAAGTG	CGACATTGAC	ATCCGCAAGG	ACCTGTACGC	CAACAACGTG
<i>C.acrolepis</i> -2a	.....	.....C...	.....	.....	.....
<i>C.cinereus</i> -1	.....	.....	.....	.....	.....
<i>C.cinereus</i> -2a	.....	.....C...	.....	.....	.....
<i>C.armatus</i> -2a	.....	.....C...	.....	.....	.....
<i>C.armatus</i> -2b	.....	.....C...	.....	.....	.....T
<i>C.yaquinae</i> -2a	.....	.....C...	.....	.....	.....
<i>C.yaquinae</i> -2b	.....	.....C...	.....	.....	.....
					950
<i>C.acrolepis</i> -1	CTCTCCGGTG	GTACCACCAT	GTACCCTGGT	ATTGCTGACC	GTATGCAGAA
<i>C.acrolepis</i> -2a	..G.....C.	C.....	.....	.....	..C.....
<i>C.cinereus</i> -1	.....	.....	.....	.....	.....
<i>C.cinereus</i> -2a	..G.....C.	.C.....	.....	..C.....	.C.....
<i>C.armatus</i> -2a	..G.....C.	.C.....	.....C...	..C.....	.C.....
<i>C.armatus</i> -2b	..G.....	.....	.....	..C.....	.....
<i>C.yaquinae</i> -2a	..G.....C.	.C.....	.....C...	..C.....	.C.....
<i>C.yaquinae</i> -2b	..G.....	.....	.....G...	..C..C...	.....

Fig. 2-3. *Continued.*

					1000
<i>C.acrolepis</i> -1	GGAGATCACC	GCCCTGGCCC	CATCCACCAT	GAAGATCAAG	ATCATTGCTC
<i>C.acrolepis</i> -2a	.....	.....G.	.T.....	.....	.....C..C.
<i>C.cinereus</i> -1	.....	.....	.T.....	.....	.....
<i>C.cinereus</i> -2a	.....	.....G.	.T.....	.....	.....C..C.
<i>C.armatus</i> -2a	.....	.....G.	.T.....	.....	.....C..C.
<i>C.armatus</i> -2b	.....	.....A.	.C.....	.....	.....
<i>C.yaquinae</i> -2a	.....	.....G.	.T.....	.....	.....C..C.
<i>C.yaquinae</i> -2b	.....A	.....A.	.G.....	.....	.....
					1050
<i>C.acrolepis</i> -1	CCCCGAGAG	GAAGTACTCC	GTCTGGATCG	GTGGCTCCAT	CCTGGCTTCC
<i>C.acrolepis</i> -2a	....A.....	.....T	.....	.A.....	.....C...
<i>C.cinereus</i> -1	.....	.....	.....	.....	.....
<i>C.cinereus</i> -2a	....A.....	.....T	.....	.A.....	.....C...
<i>C.armatus</i> -2a	....A.....	.....T	.....	.A.....	.....C...
<i>C.armatus</i> -2b	....A.....	.....	.....	.....	.....
<i>C.yaquinae</i> -2a	....A.....	.....T	.G.....	.A.....	.....C...
<i>C.yaquinae</i> -2b	....A.....	.....	.....	.....	.....
					1100
<i>C.acrolepis</i> -1	CTGTCCACCT	TCCAGCAGAT	GTGGATCTCC	AAGCAGGAGT	ACGACGAGGC
<i>C.acrolepis</i> -2a	.....	.....	.....AG.	.....	.....
<i>C.cinereus</i> -1	.....A.	.....	.....	.....	.....
<i>C.cinereus</i> -2a	.....	.....	.....AG.	.....	.....
<i>C.armatus</i> -2a	.....	.....	.....AG.	.....	.....
<i>C.armatus</i> -2b	.....	.....	.....	.....	.....
<i>C.yaquinae</i> -2a	.....	.....	.....AG.	.....	.....
<i>C.yaquinae</i> -2b	.....	.....	.....	.....	.....
				<b>STOP</b>	1150
<i>C.acrolepis</i> -1	AGGCCCCAGC	ATTGTCCACA	GGAAGTGCTT	CTAAATCCTC	AACATTTGTC
<i>C.acrolepis</i> -2a	C.....TC.	..C.....	.....	.....AC.	C..CCAGCGT
<i>C.cinereus</i> -1	.....	.....	.....	.....	.....
<i>C.cinereus</i> -2a	C.....TC.	..C.....	.....	.....AC.	C..CCAGCGT
<i>C.armatus</i> -2a	C.....TC.	..C.....	.....	.....AC.	C..CCAGCGT
<i>C.armatus</i> -2b	.....	.....	.....	.....AC.	CG.CCAGCGT
<i>C.yaquinae</i> -2a	C.....TC.	..C.....	.....	.....AC.	C..CCAGCAT
<i>C.yaquinae</i> -2b	.....	.....	.....	.....AC.	CG.CCAGCGT
					1200
<i>C.acrolepis</i> -1	TCCATCATTT	TCGCCATCAC	CATCATCACC	ACCTGTCACC	AGGAGATCGG
<i>C.acrolepis</i> -2a	CTGC..TCAG	GACA..A.CA	ACCGG.GG.A	CGAC.G.G..	.CCCAGGA.C
<i>C.cinereus</i> -1	.....	.....	...T.....	.....	.....
<i>C.cinereus</i> -2a	CTGC..TCAG	GACA..A.CA	ACCGG.GG.A	CGAC.G.G..	.CCCAGGA.C
<i>C.armatus</i> -2a	CTGC..TCAG	GACA..A.CA	ACCGG.GG.A	CGAC.G.G..	.CCCAGGA.C
<i>C.armatus</i> -2b	CTGC..TCAG	GACA..A.CA	ACCGG.GG.A	CGAC.G.G..	.CCCAGGG.C
<i>C.yaquinae</i> -2a	CTGC..TCAG	GACA..A.CA	ACCGG.GG.A	CGAC.G.G..	.CCCAGGA.C
<i>C.yaquinae</i> -2b	CTGC..TCAG	GACA..A.CA	ACCGG.GG.A	CGAC.G.G..	.CCCAGGA.C
					1250
<i>C.acrolepis</i> -1	GCGAGGAGGA	GGACCACCCC	ATGCGGCACA	GCGACACCCT	GCTGCTCATA
<i>C.acrolepis</i> -2a	CGCCTAGT.G	AC.TTTTGTT	G.TGTTGTTG	TT.TTGTGGA	TG..G.AG..
<i>C.cinereus</i> -1	.....	.....	.....	.....	.....
<i>C.cinereus</i> -2a	CGCCTAGT.G	AC.TTTTGTT	G.TGTTGTTG	TT.TGGATG.	.G.AG.AG.T
<i>C.armatus</i> -2a	CGCCTA.T.G	AC.TTTTGTT	G.TGTTGTTG	TT.TTGTGGA	TG..G.AG..
<i>C.armatus</i> -2b	CGCCTAGT.G	AC.TTTTGTT	G.TGTTGTTG	TT.TTGTGGA	TG..G.AG..
<i>C.yaquinae</i> -2a	CGCCTAGT.G	AC.TTTTGTT	G.TGTTGTTG	TT.TTGTGGA	TG..G.AG..
<i>C.yaquinae</i> -2b	CGCCTAGT.G	AC.TTTTGTT	G.TGTTGTTG	TT.TGGATG.	.G.AG.AG.T
					1300
<i>C.acrolepis</i> -1	TGCATTTTTT	TTTATGATTC	TTGAATCTGC	ATATCGTACT	GGCTGTCTGT
<i>C.acrolepis</i> -2a	GTTG..G..G	..GTG.TAGT	GGA.GAAGTG	T.TGGTGGTG	T.G.CAACCG
<i>C.cinereus</i> -1	.....	.....	..AG.....	.....TCT..	.....
<i>C.cinereus</i> -2a	GTTG..G..G	..GGTG.TGGA	AGAGG.G.TT	GGTGGTGTGG	TCACCGAA.C
<i>C.armatus</i> -2a	GTTG..G..G	..GTG.TACT	GGA.GAAGTG	T.TGGTGGTG	T.G.CA.C.A
<i>C.armatus</i> -2b	GTTG..G..G	..GGTG.TGGT	GA...GG..T	T.TGG.CTG.	..TTG...ACC
<i>C.yaquinae</i> -2a	GTTG..G..G	..GTG.TACT	GGA.GAAGTG	T.TGGTGGTG	T.G.CAACCG
<i>C.yaquinae</i> -2b	GTTG..G.GG	..GGTG.TGGA	GGAGG.G.TT	GGCGGTGTG.	TCACCGAA.C

Fig. 2-3. *Continued.*

1350

*C.acrolepis*-1 TGTCTGTGTT GTGCGGAGAA CAAGCTGTGA ACAAACAACA CTGAACCATG  
*C.acrolepis*-2a AAG.GCAAG. ...TA.C.C. A.CCGGAGCG .GC.G.GGGG GGCG.G..G.  
*C.cinereus*-1 .....G. ....G A.....  
*C.cinereus*-2a GCAAGTGTG. AGCGCA.ACC GG...GAGC. G.GGGGGG.G AGC.GGG.G.  
*C.armatus*-2a A.CGCAA..G TGTA.CGC.. .CG.ACGCA. CAGCGGGGGG .GAGCAAGGA  
*C.armatus*-2b GAAGC.CAAG CGTGTACCGC A.CCGGAGCG .AC.CGCGGG GG.GGAGCA.  
*C.yaquinae*-2a AAG.GCAAG. ...TA.C.C. A.CCGGA.CG CGGGGGGGGG GG.G.G..G.  
*C.yaquinae*-2b GCAAGCGTG. AGCGCA.CCG G.GCGA.CAG .AC.CGCGGG GC.G.G..GT

1400

*C.acrolepis*-1 CATCTAACCC CCAAAAAATT **AATAAAAAAA** TCAAAACAAA AAAAAAAAAA  
*C.acrolepis*-2a G.GG.C.G.A .GTG..CGCG TGA.G..CTG .AC.T.TG.T T.TTT.TTG.  
*C.cinereus*-1 ....C..... .....ATC...AC.. C.....  
*C.cinereus*-2a TCAGC.CGTG AACGCGTGAA G.ACTGT.C. .ATG.TT.TT T.TTG.TT.T  
*C.armatus*-2a AG..AGCA.G TG..CGCT.G ..AG...CTG .AC.T.ATG. .T.TTT.TTG  
*C.armatus*-2b TGAGGTCAG. A.GTG..GAA .CA.C..... .A....ACC. CC.....  
*C.yaquinae*-2a G.AG.C.G.A ..TG..CGCG TGA.G..CTG .AC.T.TG.. T.TTT.TTG.  
*C.yaquinae*-2b G.GG.C.G.A .GTG..G.AA C.AC.....T AA.....C.C C.....

1450

*C.acrolepis*-1 AAAAA  
*C.acrolepis*-2a TT.TGCAGGC CTACATGGAG GGATTGTTTT GTAAGGACAG GGGCCCGTGG  
*C.cinereus*-1 .....AAAAA A  
*C.cinereus*-2a GC.GGCCTAC ATGGAGGGAT TGTTTTGTA GACAGGGGC CCGTGGCCCC  
*C.armatus*-2a .TT.TGCAGG CCTACATGGA AGGATGTTTG TTAGAACGGG GGCCCCACGG  
*C.armatus*-2b .  
*C.yaquinae*-2a TT.TGCAGGC CTACATGGAG GGATTGTAG AAAAGGGGCC CACGGCCCCG  
*C.yaquinae*-2b .

1500

*C.acrolepis*-1  
*C.acrolepis*-2a CCCCCGAGGC GATGAGTTGG CTGTTGGCTT CGAGGTCGAG GAAAACCCCG  
*C.cinereus*-1  
*C.cinereus*-2a GCAGGCGATG AGTTGGCTGT TGGCTTCGAG GTCGAGGAAA ACCCCGAACA  
*C.armatus*-2a CCCCCGAGGA GACGAGTTGG CTGTTGGCTT CGAGGTCGAG GAAAACCCCG  
*C.armatus*-2b  
*C.yaquinae*-2a CAGGAGACGA GTTGGCTGTT GGCTTCGAGG TCGAGGAAAA CCCCGAACAA  
*C.yaquinae*-2b

1550

*C.acrolepis*-1  
*C.acrolepis*-2a AACAAAGAAA TGAAAAAGAC CAACAAAAAA AAAAGAACAA AGCA**AAATAAA**  
*C.cinereus*-1  
*C.cinereus*-2a AAGAAATGAA AAAGACCAAC AAAAAAAAAA AAGAACAAAG CA**AAATAAAAT**  
*C.armatus*-2a AACAAAGAAA TGAAAAAGAC CAACAAAAAA AAAAAGAAC AAAGC**AAATA**  
*C.armatus*-2b  
*C.yaquinae*-2a AGAAATGAAA AAGACCAACA AAAAAAAAAA AGAACAAAGC **AAATAAAATA**  
*C.yaquinae*-2b

1587

*C.acrolepis*-1  
*C.acrolepis*-2a ATAATTTATT TTCTAAAAAA AAAAAAAAAA AAAAAA  
*C.cinereus*-1  
*C.cinereus*-2a AATTTATTTT CAAAAAAAAA AAAAAAAAAA  
*C.armatus*-2a **AAATAATTTA** TTTTCAAAAA AAAAAAAAAA AAAAAA  
*C.armatus*-2b  
*C.yaquinae*-2a ATTTATTTTC AAAAAAAAAA AAAAAAAAAA AA  
*C.yaquinae*-2b

Fig. 2-3. *Continued.*

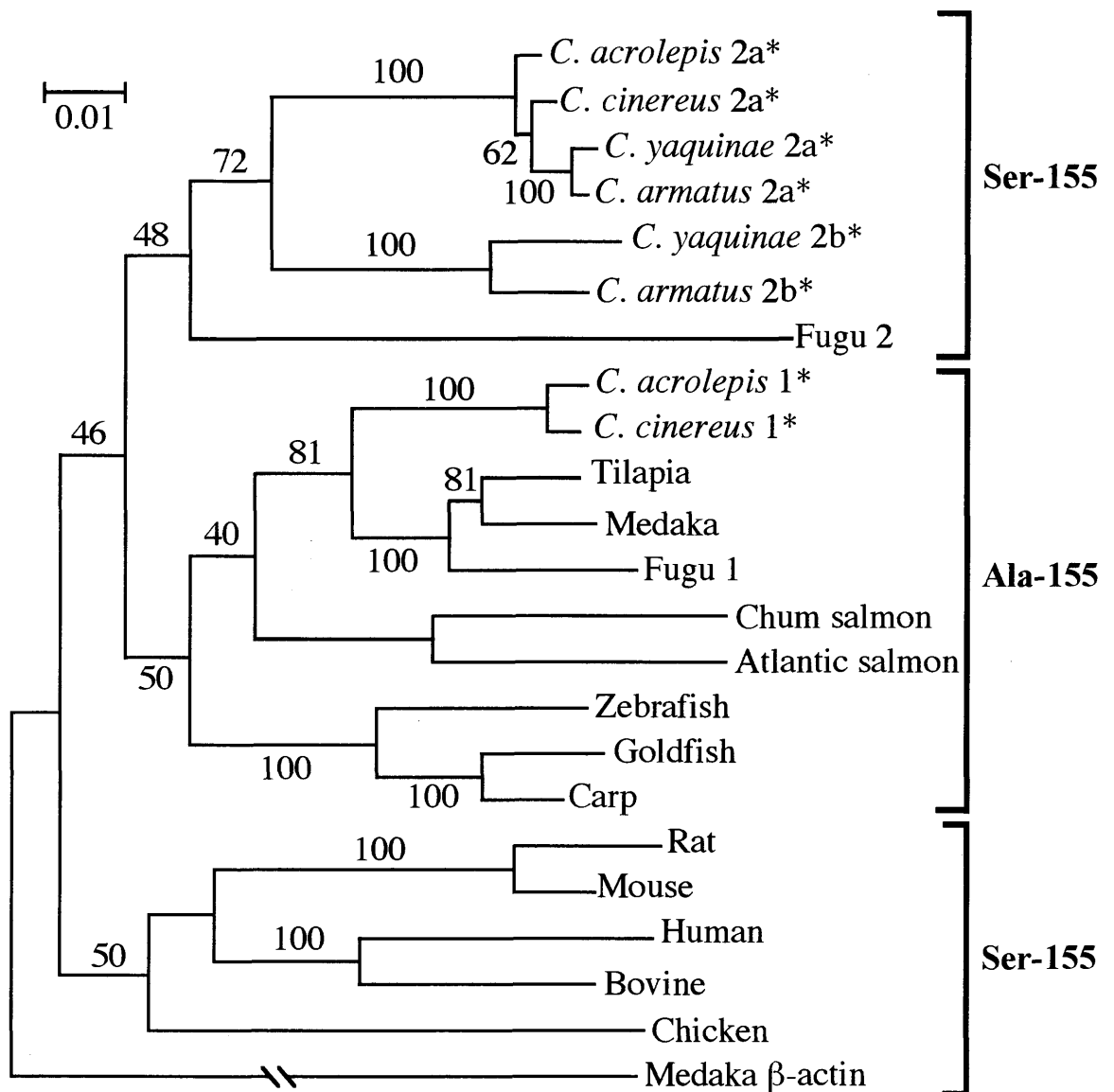


Fig. 2-4. Molecular phylogenetic tree constructed by neighbor-joining method based on nucleotide sequences in the coding regions of actin cDNA from various species. Numbers at internal branches denote the bootstrap percentages of 1000 replicates. The scale indicates the evolutionary distance of the base substitution per site, estimated by the Kimura two-parameter method. The Medaka β-actin gene was used as the outgroup gene. Asterisks indicate the cDNAs that were cloned in this study.

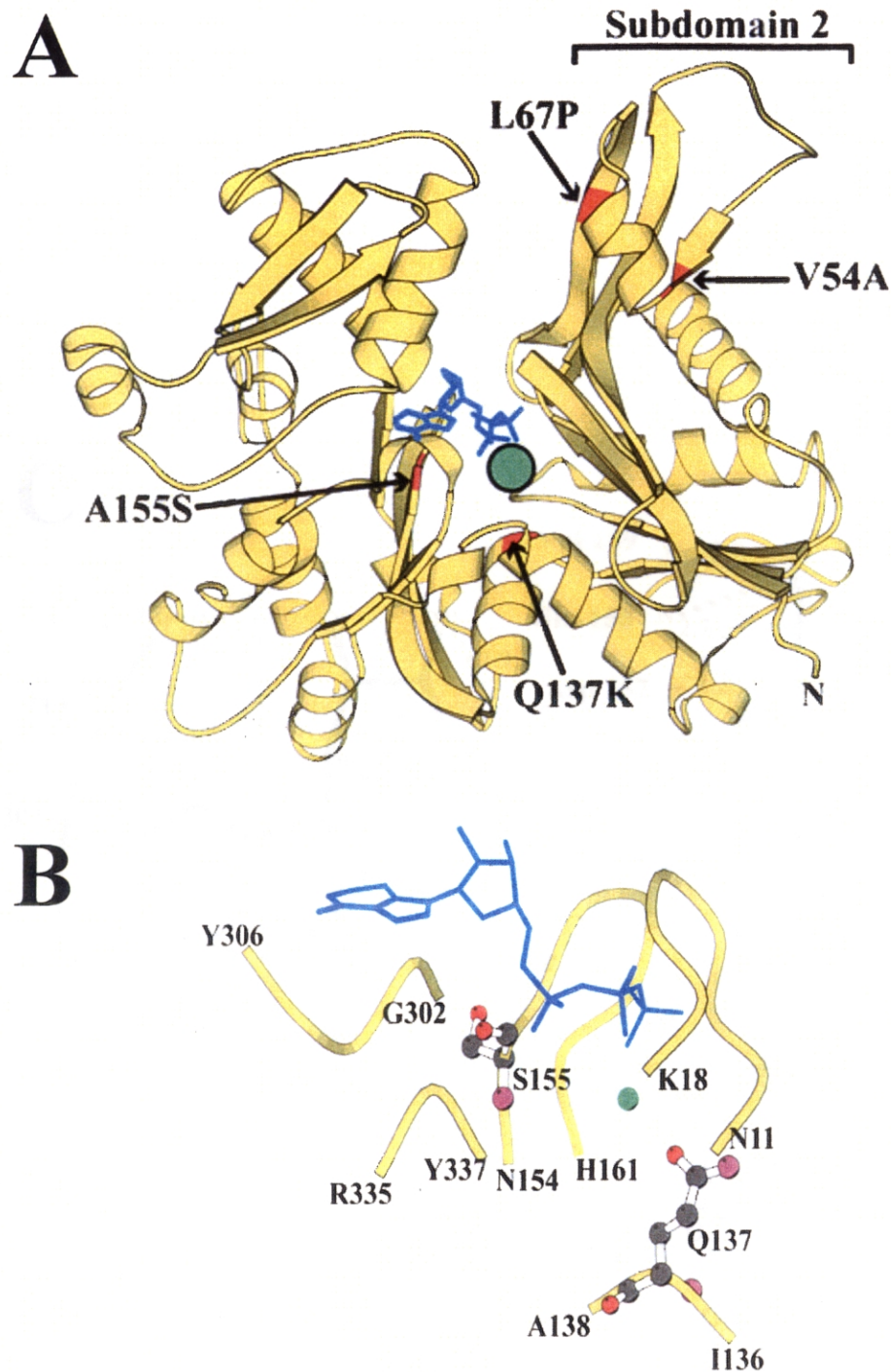
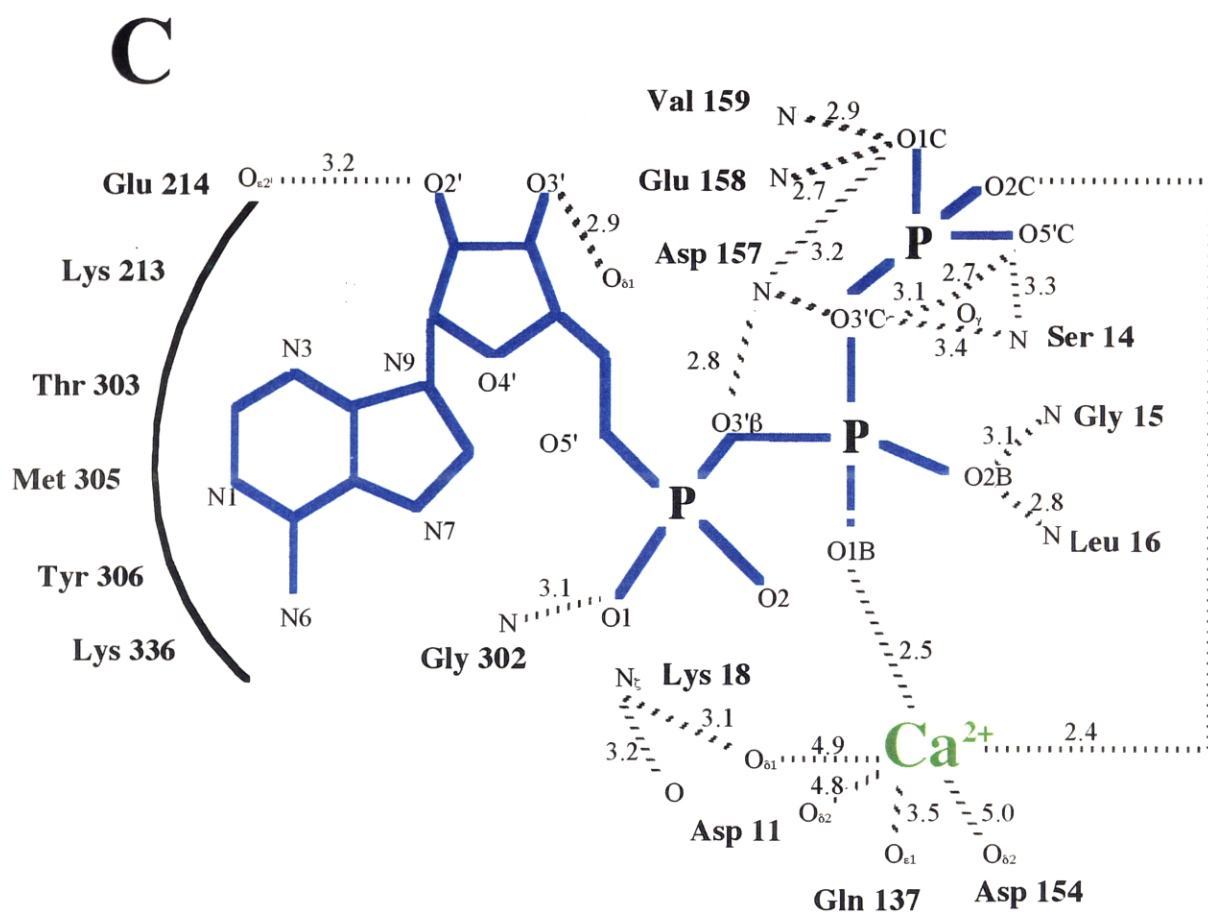


Fig. 2-5. The structure of the actin bound to ATP and  $\text{Ca}^{2+}$ . Blue sticks and a greenball represent ATP and  $\text{Ca}^{2+}$ , respectively. (A) Ribbon drawing of actin (Kabsch et al., 1990). The substitutions found in this study are colored red. (B) Environment of ATP and  $\text{Ca}^{2+}$  bound to the actin. Gln-137 and Ser-155 are represented by ball and stick. Atoms C, O and N are colored grey, red and purple, respectively. Images A and B are created using MOLSCRIPT (Kraulis, 1991). (C) Schematic drawing for interactions of ATP with actin and  $\text{Ca}^{2+}$  (Kabsch et al., 1990). Distances between atoms are given in Å. Amino acids of actin are specified near the circular segment in the left part of the drawing from a pocket covering the adenine.





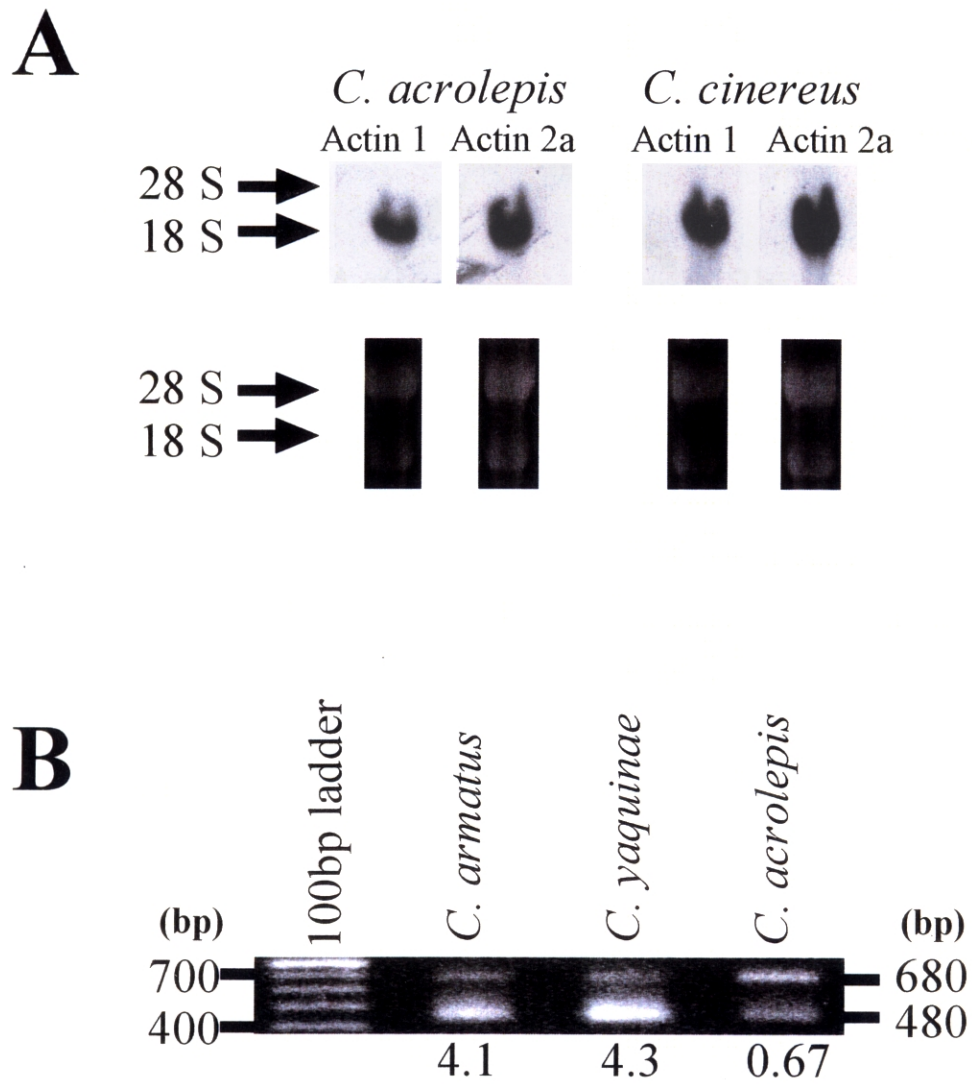


Fig. 2-6. Northern blot analysis and quantification of actin isoform mRNAs. (A) Northern blot analysis of *C. acrolepis* and *C. cinereus*  $\alpha$ -skeletal actin isoform mRNA. The ethidium bromide-stained gels show 28S and 18S rRNA (lower panel), and the separated RNA bands were blotted onto nylon membranes and hybridized with the specific probe for  $\alpha$ -skeletal actin 1 and  $\alpha$ -skeletal actin 2a (upper panel). (B) RT-PCR analysis for the actin genes from *C. armatus*, *C. yaquinae* and *C. acrolepis*. Numbers indicate the ratios of actin 2b to actin 2a for *C. armatus* and *C. yaquinae*, and actin 1 to actin 2a for *C. acrolepis*.

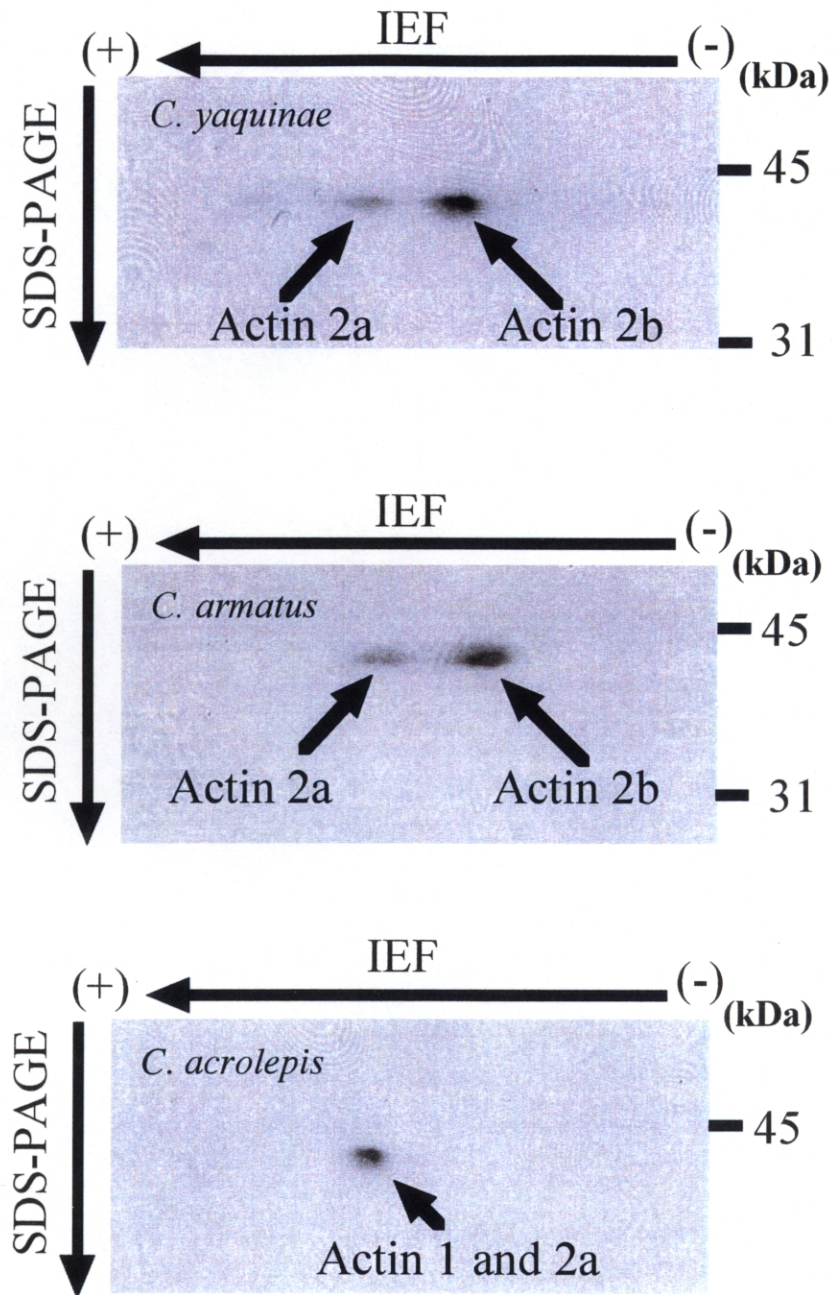


Fig. 2-7. Quantification of actin isoforms. Two-dimensional electrophoretic patterns of actin from *C. yaquinae*, *C. armatus* and *C. acrolepis*.

```

-2++++          ++  +          # #####
MCDDEETALVC DNGSGLVKAG FAGDDAPRAV FPSIVGRPRH QGVMVGMGQK 50

      54      ##### 67          + +
DSYVGDEAQS KRGILTLYKP IEHGIITNWD DMEKIWHHTF YNELRVAPEE 100

          # ##
      137
HPTLLTEAPL NPKANREKMT QIMFETFNVP AMYVAIQAVL SLYASGRTTG 150

      155      ##### # #          ##
IVLDSGDGVT HNPVYEGYA LPHAIMRLDL AGRDLTDYLM KILTERGYSF 200

#####          ###
VTTAEREIVR DIKEKLCYVA LDFENEMATA ASSSLEKSY ELPDGQVITI 250

          ##### ###          #####
GNERFRCPET LFQPSFIGME SAGIHETAYN SIMKCDIDIR KDLYANNVLS 300

          #####          +
GGTTPYPGIA DRMQKEITAL APSTMKIKII APPERKYSVW IGGSILASLS 350

          #
TFQQMWISKQ EYDEAGPSIV HRKCF 375

```

Fig. 2-8. Amino acid sequences of *Coryphaenoides* actin 1. Symbols # and + represent the binding sites to other actin monomers and myosin, respectively. Amino acid residues constructing subdomains 1, 2, 3 and 4 are indicated by red, blue, green and orange characters, respectively.

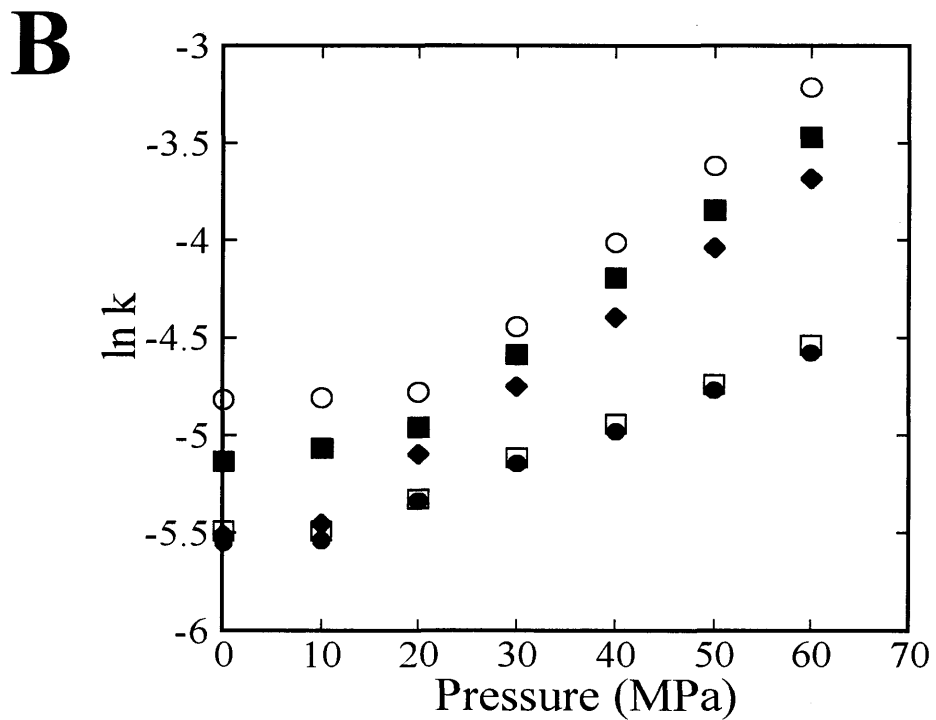
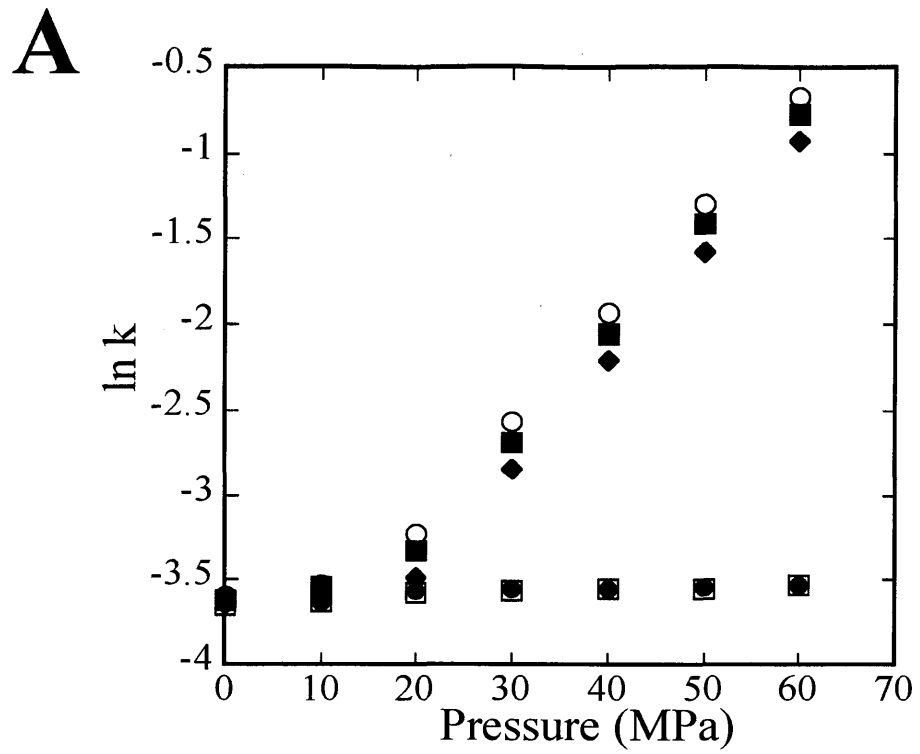


Fig. 3-1. Logarithmic plots of the dissociation rate constant of  $\text{Ca}^{2+}$  (A) and ATP (B) in actin at various pressures. Symbols are as follows: abyssal *C. yaquinae* (●); abyssal *C. armatus* (□); chicken (◆); carp (○); non-abyssal *C. acrolepis* (■). Values are means  $\pm$  S.D. of four independent experiments. Errors for all experiments are too small to show error bars.



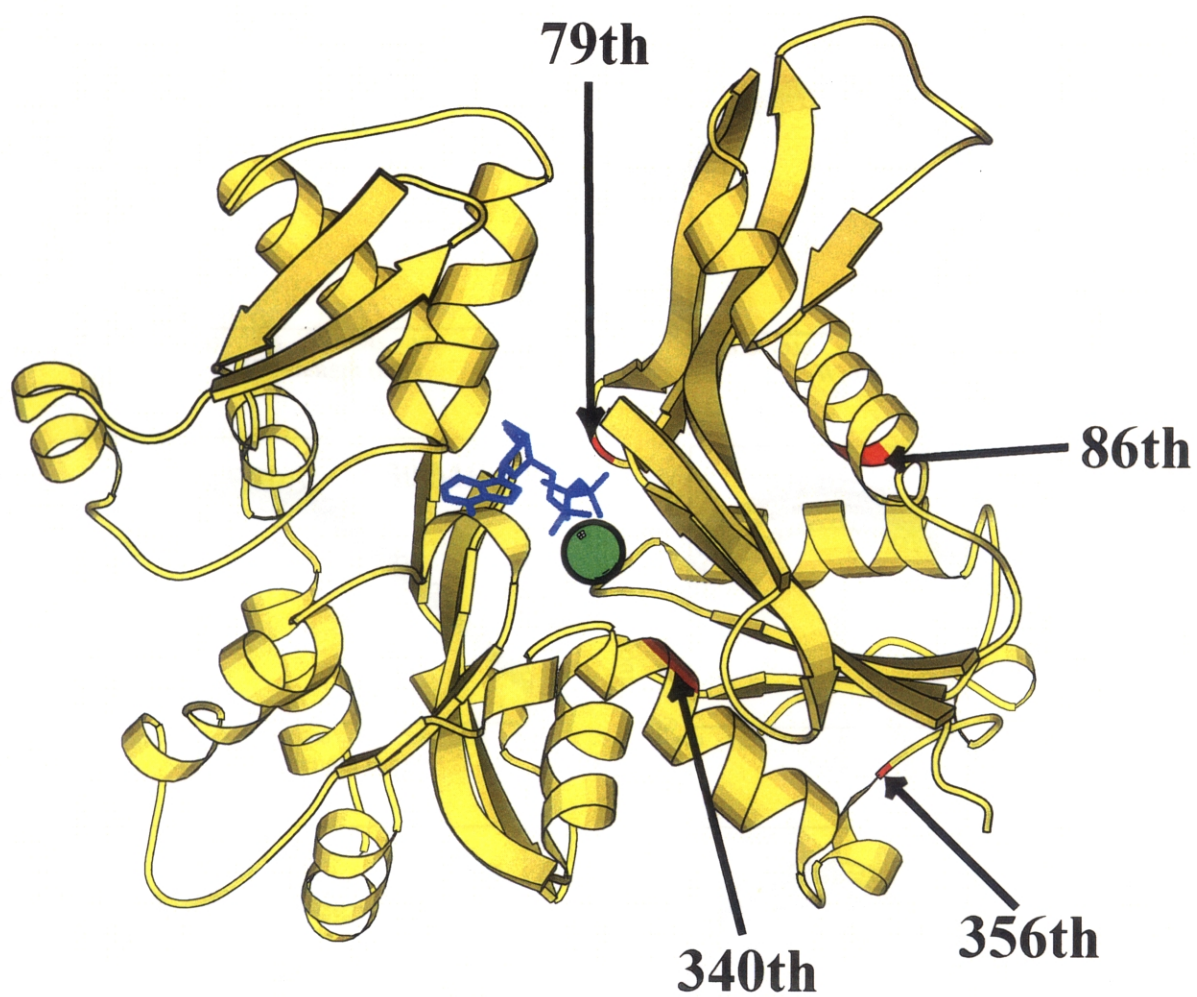


Fig. 3-2. The positions of tryptophan residues in G-actin. Tryptophan residues at 79, 86, 340, and 356 are colored by red. Blue bars indicate ATP, and a green ball does Ca.

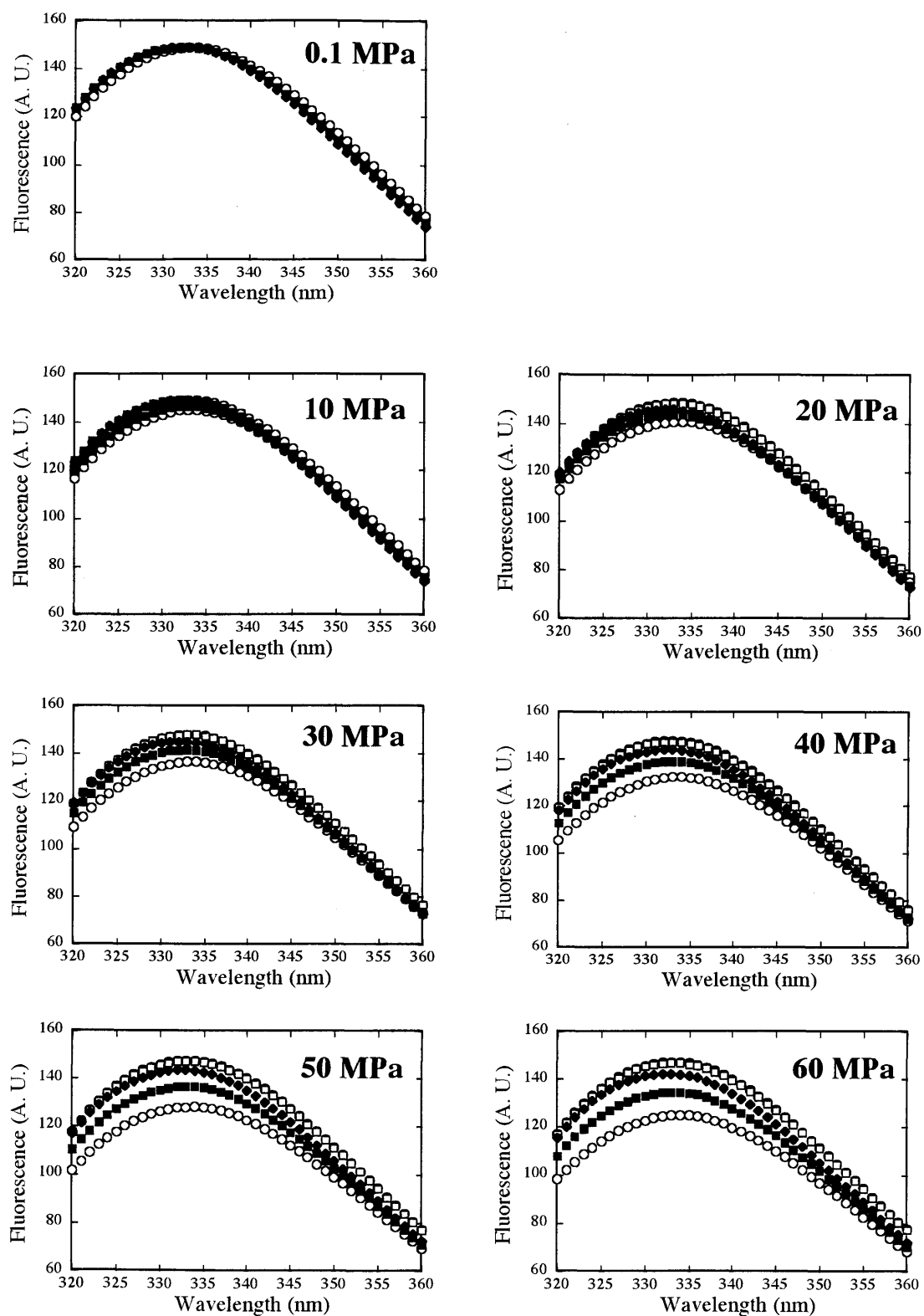


Fig. 3-3. Effects of pressures from 0.1 MPa to 60 MPa on the intrinsic tryptophan fluorescence spectrum of  $\text{Ca}^{2+}$ -G-actins. Symbols are as follows: abyssal *C. yaquinae* (●); abyssal *C. armatus* (□); chicken (◆); carp (○); non-abyssal *C. acrolepis* (■).



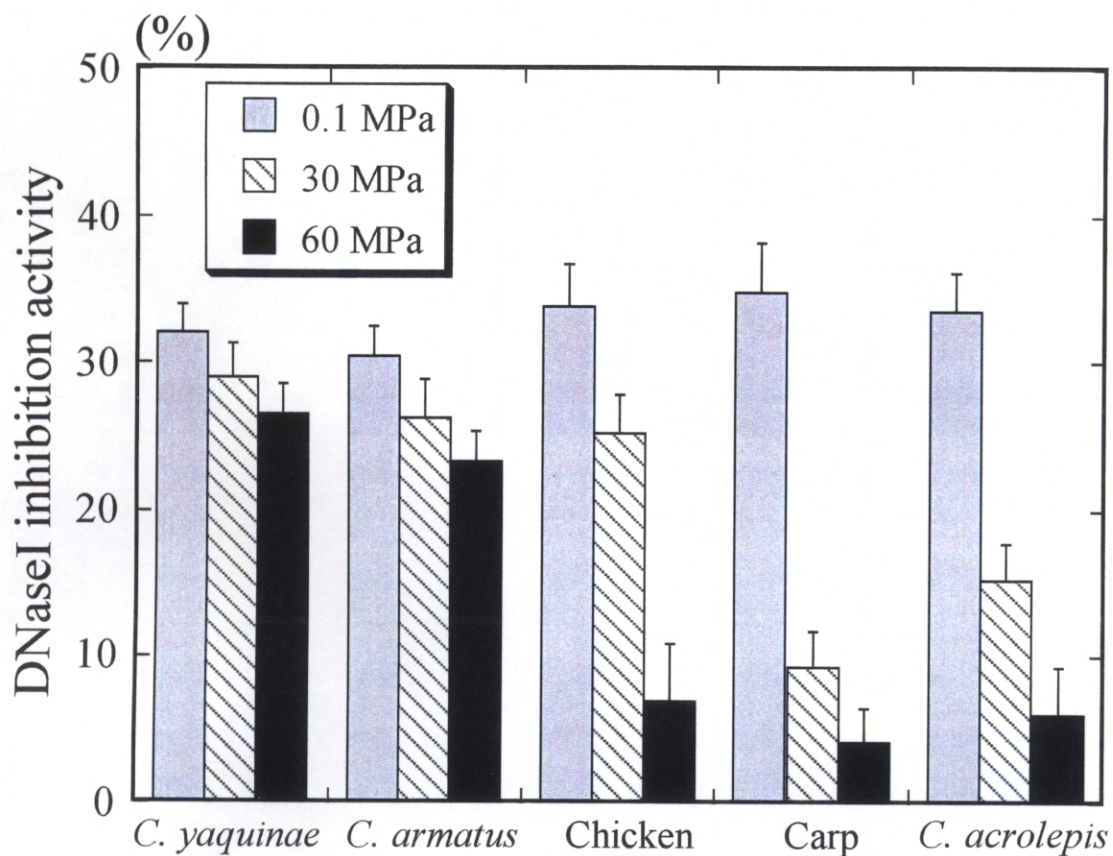


Fig. 3-4. Effects of pressures on the DNaseI inhibition activity of G-actin. The experiments for each actin were performed at the same ratios of actin/DNaseI at every pressure. Values are means  $\pm$  S.D. of four independent experiments.

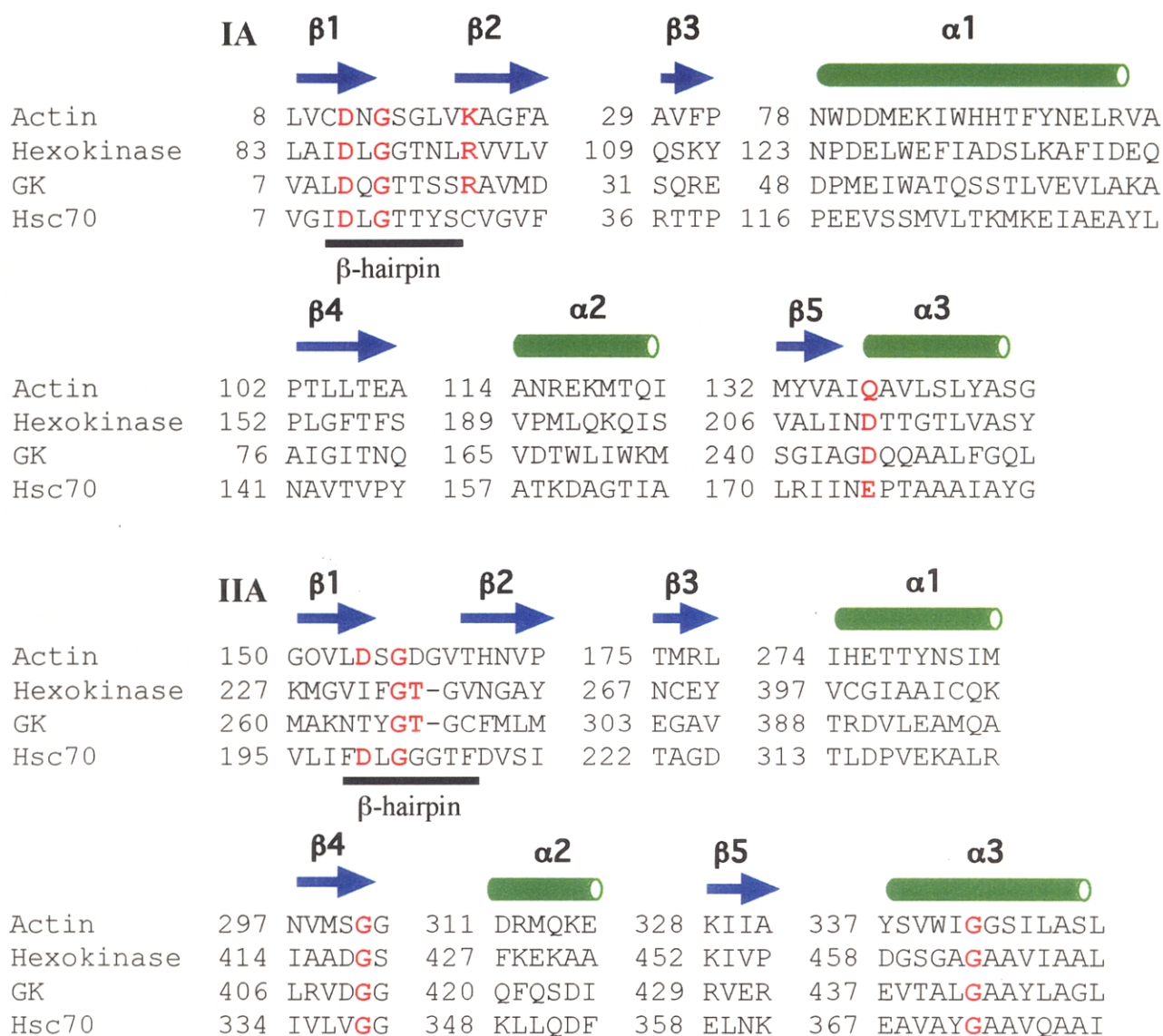


Fig. 3-5. Sequences of structurally conserved regions of actin related superfamily members (Hurley, 1996). The hexokinase sequence is numbered according to the translated gene sequence of yeast hexokinase B. The alignment is based solely on three-dimensional structural equivalence. Only regions that are equivalent structurally in all four proteins are shown. Red residues are either identical in all sequences or have structurally equivalent roles in the nucleotide binding site.