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Partial Amino Acid Sequence of Brain Actin and Its Homology with Muscle Actin†

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ABSTRACT: Actin was purified from calf brains by chromatography on DEAE-Sephadex and hydroxylapatite. The protein was then subjected to amino acid sequence analysis by isolating and sequencing its cyanogen bromide peptides. CB-1, 3, 4, 5, 6, 9, 10, and 12 correspond to equivalent segments of rabbit skeletal muscle actin, while subsitutions involving methionines give rise to some new peptides. The region that corresponds to CB-13 in muscle actin becomes two peptides in the brain protein because of a Leu → Met replacement at position 16, while Met → Leu substitutions at positions 176 and 298 give rise to two larger peptides, CB-15+7 and CB-8+2, which correspond to muscle actin CB-15 fused with CB-7 and CB-8 fused with CB-2, respectively. The peptides that have been isolated from brain actin contain 267 of the 374 residues in actin, of which 157 have been unequivocally identified. When the data are compared with those for rabbit skeletal muscle actin, 11 replacements are seen; thus the two actins differ at about 7% of the positions examined.

The basic elements of the muscle contractile apparatus exist in many types of cells and seem to be responsible for the generation of force in a variety of loci; this fact justifies the use of data on the muscle system as a frame of reference for characterization of analogous proteins in cells from tissues other than muscles. During the past few years, the body of information about the basic structure and organization of the myofibrillar proteins has developed rapidly; in particular the amino acid sequences of all of the major proteins, except the myosin heavy chain, have been determined, and the functional roles of the various components have in general been identified. Thus, the contractile apparatus of a nonmuscle cell can be dissected, and rigorous comparisons of the components with corresponding proteins from muscle, carried out at several levels, including organizational, functional, structural, and genetic are instructive in identifying relationships between muscle and nonmuscle contraction.

At this time, the protein that provides the greatest degree of continuity among the various contractile systems is actin. Its role in the thin filament of muscle is well understood, and it is now clear that most eukaryotic cells, and possibly prokaryotic cells as well, contain actin. Actin probably functions in force generation only in combination with myosin, and actin–myosin interaction seems to be controlled by proteins that respond to variable levels of intracellular calcium; myosin and control proteins have been identified in some nonmyofibrillar contractile systems, and a reasonable assumption is that these proteins exist in some form wherever actin is found. Thus, when actin is identified in a given cell, it seems likely that a

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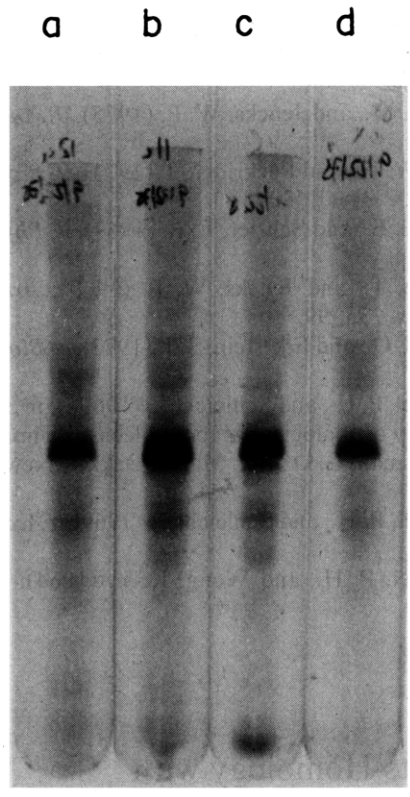

FIGURE 1: The 7.5% polyacrylamide gels that contain 0.1% $NaDodSO_4$–25 mM Tris–glycine, pH 8.3 (Lu and Elzinga, 1977). (a) Carboxymethylated bovine brain actin prepared by our method, 10 μg; (b) carboxymethylated bovine cardiac muscle actin, 20 μg; (c) a plus b; (d) a plus 5 μg of bovine brain actin prepared by polymerization method (Moring et al., 1975).

Ca^{2+}-regulated actomyosin system is present, which is responsible for the generation of force to carry out a biological function within that cell. Since many different types of processes are involved, it seems logical that the characteristics of different actomyosin systems should vary.

The studies described in this paper were undertaken in order to compare in detail a nonmyofibrillar actin, isolated from calf brain, with skeletal muscle actin. Actin was originally isolated from brain by Berl and Puszkin (Berl and Puszkin, 1970; Puszkin and Berl, 1972; Berl et al., 1973), and has subsequently been studied by others (Fine and Bray, 1971; Bray, 1973; Moring et al., 1975; Gruenstein and Rich, 1975; Pardee and Bamburg, 1976). The overall question that we wish to address is whether there exist functionally important differences among actins. The method of comparison that we have used is amino acid sequence analysis, and this approach gives information that permits one to address several specific questions about actin. For example, the existence of chemically distinct actins in a given species was demonstrated by isolating and sequencing selected peptides from human muscle and platelet actins (Elzinga et al., 1976).

In conjunction with measurements of the intrinsic functional properties of the appropriate actins, sequence analyses should eventually permit the identification of substitutions that are responsible for variations in the properties of actins. Such analyses are not yet possible, and will presumably require both sequence information and extensive knowledge of the tertiary structure of the protein. This information may come from x-ray diffraction studies on crystals that have recently been prepared from spleen (Carlsson et al., 1976a) and muscle (Mannherz et al., 1977) actins. The sequence analyses also permit evaluations of the genetic relationships among actins from different cells, and, by comparing actins from different tissues and different species, one can assess the evolution of the actin molecule.

Materials and Methods

Preparation of Proteins and Peptides. Actin was purified as described previously (Lu and Elzinga, 1976, 1977). The method used is adapted from the procedure developed by Weisenberg et al. (1968) for the isolation of tubulin. The first step involves a batch adsorption of the supernatant of a brain homogenate to DEAE[1]-Sephadex-A-50 (Pharmacia); gel electrophoresis of the eluate indicates that it contains the subunits of tubulin as well as a polypeptide having a molecular weight similar to that of actin. The proteins in the eluate were alkylated with iodoacetic acid and chromatographed on a hydroxylapatite column; the protein that emerged at the breakthrough moved with muscle actin on $NaDodSO_4$ gel electrophoresis and had an amino acid composition similar to that of muscle actin, including one residue of N^{τ}-methylhistidine. This material was digested with cyanogen bromide (CNBr) and peptides were isolated by gel filtration on Sephadex G-50 (Elzinga, 1970) followed by chromatography on SP-Sephadex C-25 (Collins and Elzinga, 1975).

Sequence Determinations. Edman degradations were carried out using either a Beckman 890C sequencer operated with the 0.1 M Quadrol program, or a solid phase sequencer (Laursen, 1971) in which peptides were covalently attached to resins (Horn and Laursen, 1973). Anilinothiazolinones were converted to the corresponding phenylthiohydantoins (Pth) and identified by two independent methods: thin-layer chromatography (Laursen, 1971) and amino acid analysis after regeneration of free amino acids by hydrolysis in 56.6% hydriodic acid at 150 °C for 4 h (Smithies et al., 1971). Pth-carboxymethylcysteine was identified as a spot that moves with Pth-aspartic acid upon thin-layer chromatography and yields only alanine (50–70% yield) upon hydriodic acid hydrolysis and amino acid analyses. Histidine and arginine Pth's were identified by hydriodic acid hydrolysis of the aqueous phase followed by amino acid analysis.

Enzyme Digestions. Trypsin (TRTPCK), chymotrypsin (CDI), carboxypeptidase A (COADFP), and carboxypeptidase B (COBDFP) were purchased from Worthington, Freehold, N.J. Tryptic and chymotryptic digestions (at enzyme:substrate ratios of 1:50 and 1:100, respectively) were carried out in 0.2 M *N*-ethylmorpholine–acetic acid buffer, pH 8.0, for 16 h at 23–25 °C. Carboxypeptidase A and/or B digestions were performed in 0.2 M phosphate buffer, pH 7.5, at 23–25 °C (or for release of amino acids including dicarboxylic acid residues, pH 5.2, at 40 °C; Ambler, 1967). The enzyme:substrate ratios and times of incubation varied with the peptide being studied. Thermolysin (Calbiochem) digestions were carried out in 0.2 M *N*-ethylmorpholine buffer, pH 8.0, for 1 h and at 37 °C, at enzyme:substrate ratios between 1:50 and 1:20.

Results

The protein prepared by the method described here was identified as actin by two criteria. It comigrates with rabbit muscle actin as well as with bovine brain actin prepared by the polymerization method of Moring et al. (Figure 1), and it yielded peptides that were similar to or identical with those

[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; DEAE, diethylaminoethyl; Pth, phenylthiohydantoins.

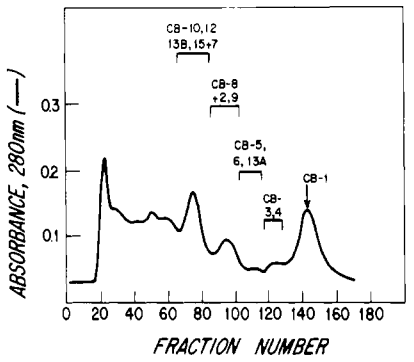

FIGURE 2: Gel filtration of a CNBr digest of brain actin on Sephadex G-50 fine. Column size was 400 × 1.9 cm, 23 °C, and the solvent was 25% (v/v) acetic acid. Fraction size was 4 mL, and a 200-mL forefraction was collected.

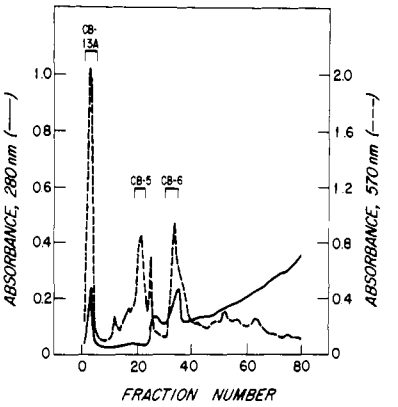

FIGURE 3: Chromatography of a Sephadex G-50 fraction on a 0.9 × 15 cm column of SP-Sephadex C-25. Fraction size was 5 mL. A gradient in pyridine concentration was established by using vessels containing 600 mL of 25% (v/v) acetic acid and 230 mL of 10:25:65 (v/v/v) of pyridine, acetic acid, and water. Absorbance at 570 nm resulted from reaction of an alkaline-hydrolyzed aliquot with ninhydrin (Hirs, 1967).

obtained from rabbit muscle actin (Elzinga, 1970; Adelstein and Kuehl, 1970).

Characterization of Cyanogen Bromide Peptides. Gel filtration of a cyanogen bromide digest of brain actin is illustrated in Figure 1. Pooling of the fractions was based partly upon the distribution of the peaks and partly upon a comparison of the pattern with that seen for rabbit skeletal muscle actin. The various fractions were rechromatographed and the peptides were pooled, analyzed, and sequenced, as follows.

CB-1: The peak marked CB-1 (Figure 2) contained some contaminating material having 280-nm absorption. This fraction was purified on a Bio-Gel P-2 column and amino acid analysis indicated that the amino acids present in significant quantities were valine, glycine, and homoserine. Solid phase sequence analysis indicated the sequence Val-Gly-Hse, identical with that seen for CB-1 in muscle actin.

CB-3 and CB-4: The peptides in this peak were chromatographed on SP-Sephadex, and two fractions were obtained. One had a composition and sequence (by solid phase) identical with that of CB-3 from rabbit muscle. The other was like CB-4, except for a Val → Thr substitution at residue 6; thus its sequence is Phe-Glu-Thr-Phe-Asn-Thr-Pro-Ala-Hse.

CB-5, CB-6, and CB-13A: This fraction yielded three peaks when chromatographed on SP Sephadex (Figure 3). The first appeared at the column volume and was ninhydrin negative. It was designated CB-13A because its amino acid composition (Table I) and subsequent sequence analysis indicated that it corresponded to the NH_2-terminal segment of muscle actin CB-13. It failed to adsorb to the SP-Sephadex because it contains no basic amino acid residues, and the NH_2 terminus was apparently blocked, presumably by acetylation as in muscle actin (Gaetjens and Barany, 1966; Alving and Laki, 1966). CB-13A was then digested with thermolysin, and SP-Sephadex chromatography of the digest yielded two fractions. Fraction I (containing Th-1) passed directly through the SP-Sephadex, and thus was identified as the blocked NH_2-terminal thermolytic peptide. Although it contains only 5 residues, unequivocal determination of its sequence was difficult because (1) the amino terminus was blocked and thus the Edman degradation could not be used and (2) the presence of 4 dicarboxylic acid residues made it partially refractory to the action of carboxypeptidase A. When Th-1 was digested with carboxypeptidase A at pH 7.5, only 1 mol of Ala was released, and when the digestion was carried out at pH 5.2, 1 mol of Ala and 1 mol of Glu were released; this indicated a partial sequence Ac-(Asp, Glu, Asp)-Glu-Ala. It also suggested that Asp was at position 3 since if Glu were there it should have been released by carboxypeptidase A at pH 5.2. The acetylated tripeptide (Ac(Asp, Glu, Asp)) was then isolated by releasing free Glu and Ala by carboxypeptidase A treatment at pH 5.2, and passing the digest over Dowex 50-X2. The tripeptide was recovered and then subjected to partial acid hydrolysis (0.03 N HCl, 110 °C, 4 h); amino acid analysis of the hydrolysate indicated that about 1 mol of Asp and negligible amounts of Glu were released. The most probable sequence of the pentapeptide that will accommodate these results is Ac-Asp-Glu-Asp-Glu-Ala.

TABLE I: Amino Acid Compositions of Peptides from Brain Actin.[a]

	CB-9	CB-13A	CB-13A Th-1	CB-13A TH-2+Th-3
Lys	2.0 (2)			
His	0.7 (1)			
Arg	1.3 (1)			
CMC	0.7 (1)	<0.1 (1)		
Asp	1.3 (1)	3.8 (4)	1.8 (2)	2.0 (2)
Thr	0.2 (1)	0.3 (2)	<0.1 (1)	0.3 (1)
Ser	2.5 (1)	1.1 (1)		1.1 (1)
Glu	3.3 (3)	1.9 (2)	2.1 (2)	
Pro	1.2 (1)			
Gly	1.5 (1)	2.0 (2)		2.0 (2)
Ala	0.5 (1)	2.3 (1)	1.1 (0)	1.7 (1)
Val	0.7 (1)	1.3 (1)		1.1 (1)
Ile	2.1 (2)	1.4 (0)		1.0 (0)
Leu		1.2 (2)		1.1 (2)
Tyr	1.0 (1)			
Phe	0.9 (1)			
Hse		1.0 (0)		

[a] Samples were hydrolyzed for 20-22 h in 6 N HCl at 110 °C. Numbers in parentheses are compositions of corresponding segments from rabbit muscle actin.

The presence of homoserine in fraction II (Th-3) indicated that it contained the COOH-terminal peptide of CB-13A. Fraction II (which contained 2 peptides, Th-2 and Th-3) was subjected to homoserine lactone coupling and sequenced on the solid phase sequencer. The sequence showed that the peptide that attached to the resin (Th-3) corresponded to residues 8-18 in rabbit skeletal muscle actin. At step 3 (residue 10 in actin), instead of Cys, approximately equal amounts of Val and Ile were seen (Table II). This presumably reflects microheterogeneity and means that our brain actin contains (at least) two species in about equal concentration. At step 5

TABLE II: Automated Sequence Analysis.[a]

Step	CB-6 Amino acid	CB-6 Yield (nmol)	CB-13A-Th-3 Amino acid	CB-13A-Th-3 Yield (nmol)	CB-13B Amino acid	CB-13B Yield (nmol)
1	Glu	242.0	Leu	234.0	Cys[c]	205.3
2	Ser[b]	86.6	Val	133.2	Lys	123.2
3	Cys[c]	158.7	Val/Ile[d]	66.7/49.5	Ala	163.3
4	Gly	142.3	Asp	99.6	Gly	120.6
5	Ile[d]	93.1	Asn[f]	93.5	Phe	86.1
6	His	69.5	Gly	66.5	Ala	150.3
7	Glu	104.4	Ser[b]	17.2	Gly	89.2
8	Thr[e]	27.9	Gly	36.3	Asp	56.5
9	Thr[e]	18.8	Hse	ND[g]	Asp	61.2
10	Phe	30.3			Ala	95.9
11	Asn[f]	24.9			Pro	28.1
12	Ser[b]	21.8			Arg	30.9
13	Ile[d]	18.1			Ala	39.5
14	Hse	ND[g]			Val	42.8
15					Phe	17.4

[a] Amino acid recovered after hydriodic acid hydrolysis of Pth-amino acids obtained from sequencer degradation of three different peptides. [b,c] Recovered as alanine. [d] Recovered as isoleucine and alloisoleucine. [e] Recovered as α-aminobutyric acid. [f] Recovered as aspartic acid. [g] ND, not determined.

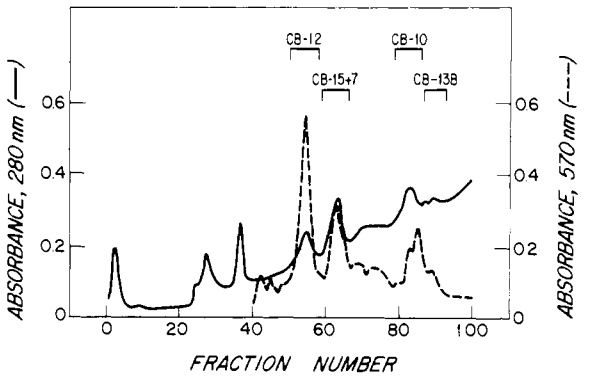

FIGURE 4: Chromatography of a Sephadex G-50 fraction on SP-Sephadex C-25. Conditions were identical with those described in legend to Figure 3.

(residue 12 in actin) Asn was found, whereas Asp had been reported in rabbit muscle actin. It is possible that rabbit muscle actin also has Asn at this position and that deamidation occurred during work-up of the peptide that was sequenced during that study. This residue will be reexamined in rabbit muscle actin. Since only the peptide with homoserine could be coupled to the resin, Th-2 would be left in the washing during coupling. Thus, one step of manual Edman degradation was carried out on 10% of the washing and 7.8 nmol of free Ile was found. The dipeptide Ala-Ile was placed between Th-1 and Th-3.

The sequence of residues 1–7, if compared with the rabbit muscle sequence, differs at 4 positions. Because of difficulties in determining this sequence (caused mainly by the presence of the blocked NH_2 terminus), we consider this sequence to be tentative, and have placed it in parentheses in Figure 5. The amino acid compositions of the corresponding peptides indicate that there must be at least two differences between them, but before accepting all four as substitutions we would like to reexamine the rabbit muscle actin sequence.

The next peptide to emerge from the SP-Sephadex column had an amino acid composition similar to that of muscle CB-5. It was thus assumed to have an NH_2-terminal glutamine which cyclized to pyrrolidonecarboxylic acid. It was digested with trypsin, the digest was passed over a column of Sephadex G-25, and two fractions, one having the composition Glx, Lys and the other the remaining amino acids, were obtained. Based upon the specificity of trypsin, the amino terminus was assumed to be Gln-Lys, and the larger fraction was subjected to solid phase sequence analysis. The sequence of brain CB-5 was identical with that of muscle actin CB-5. CB-6 was sequenced by solid phase methods, and the results are given in Table II. It contains two substitutions relative to muscle actin, Cys rather than Ala at residue 271, and Phe instead of Tyr at position 278.

CB-8+2 and CB-9: This fraction was chromatographed on SP-Sephadex and two peptides emerged. The first was obtained in low yield, and it was identified by amino acid composition and partial sequence analysis of its chymotryptic fragments to be CB-8 plus CB-2, with the fusion resulting from a Met → Leu substitution at position 298. There may be additional substitutions in the region that corresponds to CB-8 in muscle actin, but lack of sufficient material precluded a complete sequence determination. The amino acid composition of CB-9 was similar to that of muscle CB-9 (Table I) except for the presence of three (rather than one) Ser, and no Thr or Ala. Partial sequence analysis on the tryptic fragments indicated that a Thr → Ser occurs at position 3 in the peptide (residue 357). An Ala → Ser substitution probably occurs at position 10 in CB-9 (residue 364 in actin) but this was not proven directly because of lack of material. The sequence of the COOH-terminal region of CB-9 was identified by digestion with carboxypeptidase A plus B.

CB-10, CB-12, CB-13B, and CB-15+7: These peptides were obtained from an SP-Sephadex column (Figure 4). CB-10 was subjected to 26 steps of Edman degradation in the Beckman sequencer and no differences from muscle actin were observed. Step 26 was identified by hydriodic acid hydrolysis of the aqueous phase; the overall yield at this step was about 10%, and only N^{τ}-methylhistidine was observed, suggesting that in brain, as in muscle actin, this residue is fully methylated.

CB-12 was sequenced through 23 steps (Beckman 890C) and the only difference from the published rabbit muscle actin sequence is an additional Ser between Ser-234 and Leu-235. We have reviewed the original data on the rabbit muscle peptide, and it seems likely to us that this residue was overlooked (Elzinga and Collins, 1975). In order not to modify at this time the overall numbering of the rest of the sequence, we

TABLE III: Positions at Which Sequence Variations Have Been Observed in Actins.[a]

	10	16	17	129	176	271	278	298	357
Muscle									
Rabbit skeletal	Cys	Leu	Val	Val	Met	Ala	Tyr	Met	Thr
Bovine skeletal	—	—	—	—	—	—	—	—	Thr
Bovine heart	—	—	—	Val	—	—	—	—	Ser
Human heart	—	Leu	—	Val	—	Ala	Tyr	—	Ser
Cytoplasmic									
Human blood platelet	—	Met	—	Thr	Leu	Cys	Phe	Leu	Ser
Bovine brain	Val/Ile	Met	Cys	Thr	Leu	Cys	Phe	Leu	Ser

[a] The numbers above the columns identify positions at which variations in sequence occur in one or more of the actins that are listed. The amino acid abbreviations identify residues that were found at these positions. A dash indicates that the position has not yet been identified.

will designate this residue Ser-234a.

CB-13B was run for 15 steps in the Beckman 890C (0.1 M Quadrol program) and the only substitution observed was Cys → Val at position 1 (residue 16 in actin) (Table II).

The identity of the fraction marked CB-15+7 was inferred from amino acid analysis of this fraction. The fraction was digested with trypsin, and partial resolution of the digest yielded two peptides. A tetrapeptide having equimolar amounts of Ala, Ile, Leu, and Arg was sequenced by two steps of Edman degradation on the Beckman sequencer and by carboxypeptidase A+B digestion, and shown to be Ala-Ile-Leu-Arg. This peptide represents residues 174–177, and the presence of Leu at residue 176 (a substitution for Met in rabbit muscle actin) results in the fusion of CB-15 and CB-7. Another peptide obtained from the fraction CB-15+7 contained homoserine, and solid phase sequence analysis of this fragment gave a sequence identical with that of residues 184–190 in rabbit muscle actin.

FIGURE 5: Comparison of bovine brain and rabbit muscle actins. Dots (• • •) designate segments of sequence contained in peptides that were not isolated or studied in brain actin. Where two residues are shown, the upper one is from rabbit muscle actin, and the lower one from bovine brain actin. Where only one is indicated, it is the same in the two actins. Parentheses enclose sequences that are considered tentative. The asterisk (*) designates a residue which will be reexamined in rabbit muscle actin (see text).

Discussion

The actin used in this study was identified as such not by its biological properties, but because the denatured polypeptide chain behaved chemically and physically very much like muscle actin. One question that we wish to address is the extent of heterogeneity in actin from a given tissue and, in order to be definitive, it is important that the protein being studied be representative of the total population of actin molecules in the tissue. Since the method used depended basically upon binding of the actin to DEAE-Sephadex at pH 6.8 at low salt concentration, and releasing it at high salt, it seems likely that we would not distinguish between different forms of actin if they indeed exist in brain. If there is a brain actin that is tightly bound to membranes it could have remained in the precipitate when the brain homogenate was centrifuged. This point could perhaps be addressed by gel electrophoretic studies on the precipitated tissue.

In comparing the overall sequences of calf brain and rabbit muscle actins, it is apparent that they are very similar (Figure 5); 157 of the 374 residues in the protein have been unequivocally identified, and 11 replacements are seen; thus the two actins differ at about 7% of the positions examined. The substitutions are probably all tissue, and not species, differences, since a comparison of bovine and rabbit muscle actins at 55 positions revealed no differences between them (Elzinga and Lu, 1976). None of the replacements involves the type of substitution that one might expect to give a major change in biological activity.

The properties of brain actin have not been studied as rigorously as have those of *Acanthamoeba* (Gorden et al., 1976) and *Dictostelium discoideum* (Spudich and Cooke, 1976) actin, and these two have been shown to be virtually indistinguishable from muscle actin in polymerization and myosin activation. It has been reported that much of the actin in fibroblast cytoplasm is unpolymerized (Bray and Thomas, 1975, 1976) (or present as short oligomers), but it is not known whether this is due to intrinsic differences in polymerization properties or perhaps because of the presence of a low molecular weight protein (Carlsson et al., 1976a,b) that somehow modifies polymerization. Of the 11 substitutions seen, two are in the NH_2-terminal peptide and the substitutions are at this point not established. Five of the substitutions require a single base change, while four require two base changes (Lehninger, 1970); these four include the three which involve Cys, and a Val → Thr. All are chemically conservative, and their functional significance, while not yet clear, may be apparent when the location of these residues in the three-dimensional structure of actin can be seen.

The pattern (Table III) that emerges from comparisons of actin sequences is that in vertebrates there exists a type of actin which occurs in the thin filaments of striated muscle, and another which can be isolated from cells that are not muscles. In the muscle type there are at least two variants, one found in skeletal muscle and the other in heart, which are very similar, but differ in at least one position having either Thr or Ser at residue 357. There must be more than one gene for the cytoplasmic type as well, since in the work reported here there is microheterogeneity in one position. If cytoplasmic actins from different tissues are products of different genes, we would

expect to find tissue-specific differences in the sequences; yet preliminary results on platelet actin indicate that at seven positions where brain and muscle actins differ, platelet is always the same as brain. The nonmyofibrillar actins may thus be identical, and it seems likely to us that the spleen actin, a cytoplasmic actin which has yielded crystals that are being studied by x-ray diffraction (Carlsson et al., 1976a), is similar to brain and platelet actin.

Several investigators have reported that actin can be separated by isoelectric focusing into bands called α, β, and γ actins (Whalen et al., 1976; Garrels and Gibson, 1976; Rubenstein and Spudich, 1977). The α form is associated with myofibrillar actin, and the β and γ with cytoplasmic actin. It may be that the heterogeneity seen at residue 10 reflects the presence of β and γ forms in our brain actin. This point could presumably be tested by preparing pure β or γ actin and determining if it has only Val or Ile at residue 10. Since both cardiac and skeletal muscle actins from beef, which are slightly different in sequence (Lu and Elzinga, 1976), move as α-actins, it is not clear what the relationship is between electrophoretic bands and gene products. In a preliminary report, Pardee and Bamburg (1977) have suggested that brain actin consists of up to five (or possibly 8) electrophoretically distinguishable components, but the nature of the differences among them is not known.

Anderson (1976) has attempted to quantify cytoplasmic actin in fibroblasts by labeling the cysteine residues with a radioactive alkylating agent, digesting the protein with CNBr and trypsin, and isolating a peptide that would be equivalent to the NH_2-terminal 18-residue peptide of muscle actin. Fibroblast actin probably has the same substitutions as brain actin at residue 10 (Cys → Val/Ile) and 17 (Leu → Met); thus such an experiment would not yield the desired peptide, and the low estimates of actin content that were obtained are probably not valid.

The results reported here permit a comparison of much of the sequence of brain actin with that of muscle actin, but it remains essential to complete the sequences of the cytoplasmic actins. While it is clear that striated muscle actins and cytoplasmic actins represent different forms of the protein, further studies may provide a rigorous evaluation of the differences between the proposed β and γ actins. The results to date suggest that brain cells have one type of actin, perhaps containing two very closely related species, that can participate in several processes, rather than several cytoplasmic actins, each specific for a given function.

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