Change of Reactivity of Lysine Residues upon Actin Polymerization†

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ABSTRACT: The reactivity of lysine residues of actin was measured by a surface labeling method—limited reductive methylation. After labeling, actin was subjected to CNBr and enzymatic cleavage, and all lysines were obtained either singly in a peptide or as a free residue. The specific activity of each lysine was taken as the measure of its reactivity. In actin denatured in 8 M urea, the reactivity of each lysine residue is approximately equal whereas those in G-actin fall into three categories: Lys-61 and Lys-113 are the most reactive ones; Lys-18, -213, -215, -314, and -358 are hardly reactive; the remainder, including Lys-50, -68, -84, -118, -191, -237, -283, -290, -325, -327, -335, and -372, are moderately reactive. The least reactive ones are probably buried in the native G-actin and all the others are most likely on the surface. Upon actin polymerization the reactivities of Lys-61, -68, -113, and -283 are significantly reduced while that of Lys-335 is strikingly enhanced. The decrease in reactivity could be readily explained if these residues were located in the monomer–monomer contact area although a polymerization-induced conformational change cannot be excluded. Such a conformational change may be invoked to explain the increase in the reactivity of Lys-335. Alternatively, the latter may be interacting with the bound ATP of G-actin, and the increased reactivity might be directly attributable to the loss of γ-P for ATP accompaning polymerization.

Actin is a globular protein with M_r 42 000, and it can exist as a monomer (G-actin) in the absence of salt or as a helical polymer (F-actin) at physiological concentration of salt. F-Actin together with tropomyosin and troponin constitutes the thin filament. It is now generally accepted that the force-generating event during muscle contraction involves an interaction between the cross bridges projecting from the myosin filaments and the thin filament and the hydrolysis of ATP. Thus, actin filaments serve as the site of cross bridge attachment and also bind the regulatory proteins tropomyosin and troponin.

Various observations have suggested that conformational changes accompany the G → F transition. Among the techniques used are ultraviolet spectrophotometry (Higashi & Oosawa, 1965), fluorescence (Weltman, 1972; Lehrer & Kerwar, 1972), electron paramagnetic resonance (Stone et al., 1970; Burley et al., 1971; Harwell et al., 1980), circular dichroism in the near-ultraviolet region (Murphy, 1971), chemical modification (Barany, 1956; Martonosi & Gouvea, 1961; Tonomura et al., 1962; Gerber & Ooi, 168; Muhlrad et al., 1968; Muhlrad, 1968), and proteolytic susceptibility (Rich & Ester, 1976). CD spectra in the far-ultraviolet region and infrared spectra, however, indicate that no gross changes in the secondary structure of actin take place as a result of the G → F transition (Hegyi & Venyaminov, 1980).

A number of attempts have been made to define regions of, or residues within, actin molecules that are in some way involved in the polymerization process. Sulfhydryl groups (Barany, 1956; Martonosi, 1968; Lusty & Fasold, 1969) histidine (Martonosi & Gouvea, 1961; Muhlrad et al., 1968; Hegyi et al., 1974), tyrosine (Martonosi & Gouvea, 1961; Muhlrad et al., 1968), and lysine residues (Tonomura et al., 1962; Muhlrad, 1968) have been suggested to play a role in the G → F transition; these results, however, are not conclusive. Since the primary structure of actin (Elzinga et al., 1973) is now known, it seemed possible to identify some residues of known location in the primary structure that undergo a change upon polymerization. Therefore, we decided to use an improved method of reductive methylation (Dottavio-Martin & Ravel, 1978; Jentoft & Dearborn, 1979) to study the reactivity of the 19 lysine residues of actin in the denatured, monomeric, and polymeric states. Our study indicates that all lysines in actin denatured in 8 M urea have approximately the same reactivity, whereas in G-actin they fall into three categories: Lys-61 and -113 are extremely reactive, Lys-18, -213, -215, -314, and -358 are slightly reactive, and the remainder, including Lys-50, -68, -84, -118, -191, -237, -283, -290, -325, -327, -335, and -372, are moderately reactive. Upon polymerization, the reactivities of Lys-61, -68, -113, and -283 are significantly reduced, while that of Lys-335 is strikingly enhanced.

Experimental Procedures

Protein Preparation. Actin was extracted from acetone powder according to the procedure of Spudich & Watt (1971).

Reductive Methylation. Since the aim was to determine the reactivities of individual lysine residues of G-actin and the effect of polymerization upon them, ^{14}C- and ^{3}H-containing reagents were used on G-actin and F-actin, respectively, in order to isolate the Lys-containing peptides from the two forms of actin simulataneously. Similarly, the dual-labeling technique was used when native G-actin and denatured actin were compared.

The procedure of Jentoft & Dearborn (1979) for reductive methylation was adopted. Native G-actin (4.5 mg/mL) in a solution containing 0.2 mM ATP, 0.2 mM $CaCl_2$, and 5 mM Hepes, pH 7.5, was treated with 1 mM [^{3}H]HCHO (New England Nuclear; 85.0 μCi/mmol) in the presence of 5 mM $NaCNBH_3$ (Aldrich; used without recrystallization) for 8 h at 0° C and then quenched with 5 mM glycine.

F-actin (4.5 mg/mL) in a solution containing 50 mM KCl, 25 mM Hepes, pH 7.5, 1 mM $MgCl_2$, 0.2 mM ATP, and 0.2 mM $CaCl_2$ was methylated with 1 mM [^{14}C]HCHO (10 μCi/mmol) and 5 mM $NaCNBH_3$ for 8 h. The reaction was quenched with 5 mM glycine, and labeled F-actin was sedimented by ultracentrifugation (120000*g* for 2 h) and resuspended in 50 mM KCl and 25 mM Hepes, pH 7.5.

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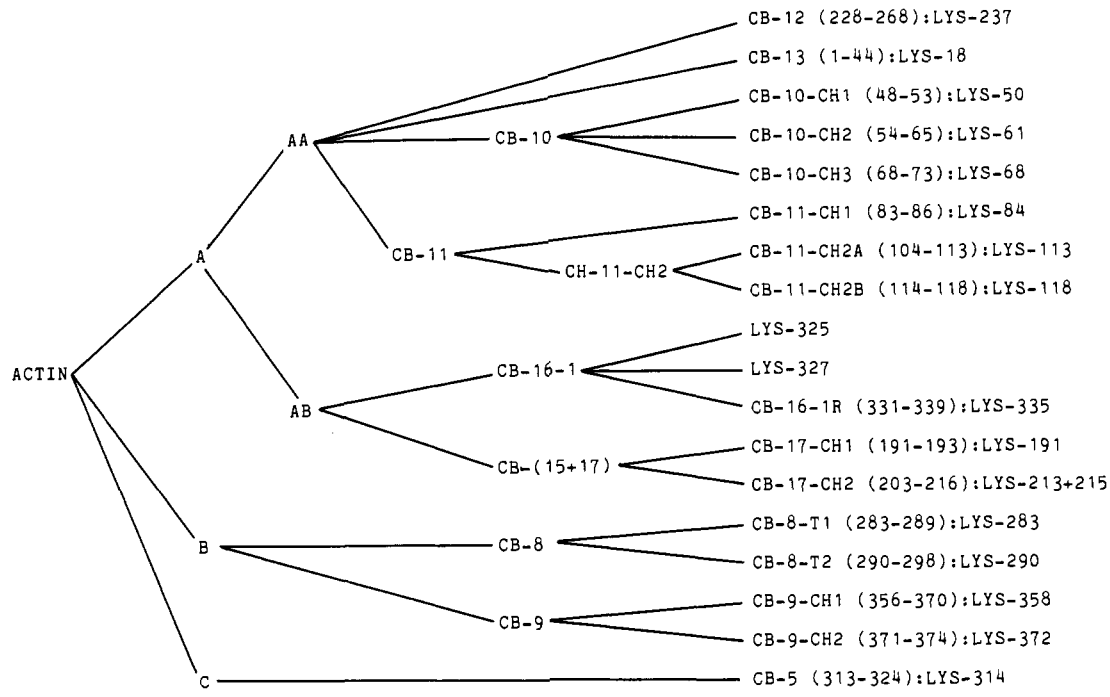

FIGURE 1: Outline of the procedure of the isolation of lysine-containing peptides.

Denatured actin (2.5 mg/mL) was first carboxamidomethylated to prevent disulfide formation (as described later) and then methylated in the presence of 8 M urea and 25 mM Hepes, pH 7.5, adding 1 mM [^{14}C]HCHO (New England Nuclear; 7.5 μCi/mmol) and 5 mM $NaCNBH_3$, for 4 h at 0 °C and then quenched with 5 mM glycine.

Carboxamidomethylation. ^{3}H-Labeled G-actin (10 mg) and ^{14}C-labeled F-actin (10 mg) were mixed with 100 mg of unlabeled actin and subjected to carboxamidomethylation (Crestfield et al., 1963). Actin in 8 M urea and 100 mM Tris, pH 8.0, was reduced with 25 mM dithiothreitol (Sigma Chemical Co.) under N_2 for at least 2 h, and then iodoacetamide (Sigma Chemical Co.) was added to 50 mM; the pH was kept at 8.0 by addition of 5 M KOH. The mixture was protected from light by aluminum foil. After 30 min β-mercaptoethanol (10 mM) was added, and the solution was exhaustively dialyzed against distilled water (at least 60 h; five to seven changes of 200 volumes of water) and finally lyophilized. In one experiment actin was carboxamidomethylated first and then methylated with [^{14}C]HCHO (see above). ^{14}C-Labeled denatured actin and ^{3}H-labeled native G-actin were mixed with unlabeled actin and were subjected to carboxamidomethylation.

Cyanogen Bromide Treatment. Lyophilized protein was dissolved in 5 mL of 70% formic acid, and cyanogen bromide (Pierce) was added in 5-fold excess (w/w). After 16 h at room temperature, formic acid and excess reagent were removed by rotary evaporation.

Enzymatic Digestion. Peptides were dissolved in 2 mL of 0.1 M *N*-methylmorpholine–acetic acid buffer, pH 8.0, and 100–300 μg of chymotrypsin (CDI; Worthington) was added. The digestion was performed at room temperature for 16 h and terminated by acidifying the solution. In the case of tryptic digestion, 100 μg of trypsin (Worthington; TRTPCK) was used, and the digestion was allowed to proceed for 4 h at room temperature.

Peptide Isolation. Separation of peptides was achieved by gel filtration on Sephadex G-50 and Sephadex G-10 and ion-exchange chromatography on SP-Sephadex C-25 (Pharmacia) following cyanogen bromide treatment and enzymatic digestion as outlined in Figure 1.

Analyzing the Peptides. The amino acid composition of the peptides was analyzed on a Beckman 119 CL analyzer after hydrolysis in vacuo in 6 N HCl at 100 °C for 20–24 h. In some cases, peptides were subjected to Edman degradation on a Beckman 890C sequenator by using program no. 030176. Phenylthiohydantoin of amino acids was identified by 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).

Determination of Incorporation of Methyl Groups. Effluent of columns was routinely monitored by counting aliquots in 5 mL of a scintillation solution containing 25% (v/v) Triton X-114, 75% (v/v) xylene, 0.3% PPO (w/v; New England Nuclear), and 0.02% POPOP (w/v; New England Nuclear) as dscribed by Anderson & McClure (1973). Appropriate amounts of water were added so the total volume of the aqueous phase was 0.5 mL. When the purified peptide contained only a single lysine, the quantity of lysine was determined on an amino acid analyzer, and aliquots of the hydrolysate were counted in the same solution on a Beckman LS-230. In dual-isotope experiments the ^{3}H counts were corrected for counts originating in ^{14}C; 29% of the counts in the ^{14}C channel were subtracted from the counts in the ^{3}H channel based on the results of a standard curve. The counting efficiency has been determined as 63% for the ^{14}C label and 18.6% for the ^{3}H label. cpm is converted to methyl group based on the specific activity and counting efficiency of each isotope.

Results

Effect on Polymerizability. For examination of the effect of methylation on the polymerizability, G-actin solutions were treated with different concentrations of [^{14}C]formaldehyde and sodium cyanoborohydride. After the reaction was quenched with glycine, actin was polymerized and F-actin was sedimented. Control experiments showed that no detectable amount of protein or radioactivity can be sedimented before actin polymerization. As the concentration of reagents in-

Table I: Polymerizability of Methylated Actin[a]

[HCHO] (mM)	A_s	polymerizability	Δ polymerizability
0	0.129	84	
0.5	0.140	82	2
1.0	0.144	81	2
2.0	0.195	75	8
3.0	0.223	72	12
5.0	0.287	64	20

[a] The reaction mixture contained 1.2 mg of actin/mL in 25 mM Hepes, pH 7.5, and various amounts of HCHO as indicated in each case. The concentration of $NaCNBH_3$ was kept as twice that of HCHO. After 2 h at 0 °C the reaction was stopped by the addition of glycine in a 5:1 ratio to HCHO. Actin was polymerized by adding KCl to 100 mM. Polymerizability is expressed as $(0.792 - A_s)/0.792$; 0.792 is the absorbance at 290 nm of the reaction mixture before polymerization and A_s represents the absorbance of the supernatant. Δ polymerizability is the difference in polymerizability between a sample and the control (no reagents were added).

creased, more molecules became nonpolymerizable as indicated by the increase of absorbance at 290 nm of the supernatants (Table I). Nevertheless, at low concentrations of the reagent (≤2 mM), the decrease in polymerizability appears insignificant, suggesting that the most reactive lysines are not essential for polymerization.

Isolation of Lysine Peptides. On the basis of previous work (Elzinga et al., 1973; Collins & Elzinga, 1975; Lu & Elzinga, 1977), a scheme was developed to isolate the lysine-containing peptides so that each lysine would be located in a different peptide and thus identified unambiguously (Figure 1). Following cyanogen bromide cleavage of actin, the mixture of peptides was gel filtrated on a Sephadex G-50 column (Figures 1 and 2) and pools A–C were made. CB-5, CB-8, and CB-9 were obtained in pure form after pool B and C were further chromatographed on SP-Sephadex C-25 columns (Figures 1 and 2). Pool A was resolved into two fractions, AA and AB, based on its solubility of the peptides in 0.01 M pyridine–acetate buffer at pH 6.0 [Figure 2 and Collins & Elzinga (1975)]. Fraction AA was chromatographed on SP-Sephadex C-25 and four peptides, CB-10, CB-11, CB-12, and CB-13, were isolated (Figure 2). Fraction AB containing CB-15, CB-16, and CB-17, which were eluted together on a Sephadex G-50 column, was again treated with cyanogen bromide to cleave at tryptophan (Ozols & Gerard, 1977). This resulted in the formation of two smaller peptides, CB-16-1 and CB-16-2, which were separated from CB-15 and CB-17 by gel filtration (Figure 1).

Among seventeen cyanogen bromide peptides, three contain single lysines, viz., Lys-18, Lys-237, and Lys-314 in peptides CB-13, CB-12, and CB-5, respectively (Figure 1). For those peptides that contained two or three lysines, additional cleavages were required. CB-8 was subjected to tryptic digestion, and peptides containing Lys-283 and Lys-290 were obtained. CB-9 was further cleaved with chymotrypsin, and two peptides containing Lys-358 and Lys-372, respectively, were obtained. Lys-50, -61, and -68 were separated in the three chymotryptic fragments of CB-10. Two lysine-containing peptides were obtained from the chymotryptic digests of CB-11; CH_1 contains Lys-84 and CH_2 contains two lysines. Therefore, the latter was further degraded with trypsin to separate Lys-113 and Lys-118. Since CB-17 does not contain any lysine residues, the mixture of CB-15 and CB-17 was subjected to chymotryptic digestion, and two peptides, one containing Lys-191 and the other containing Lys-213 and -215, were obtained.

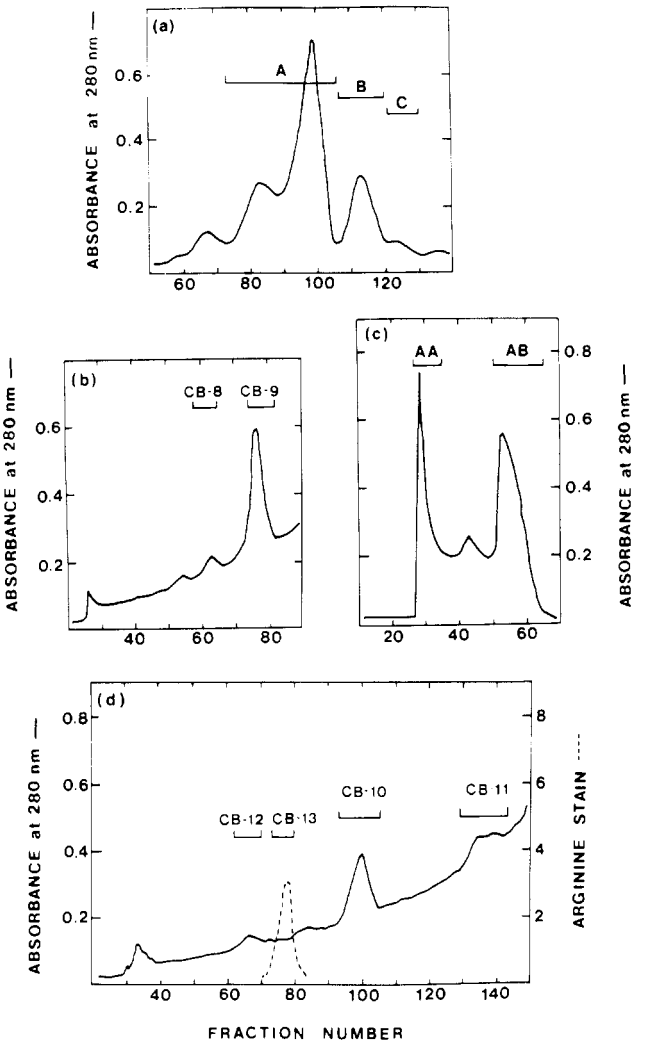

FIGURE 2: Chromatography of the cyanogen bromide peptides of actin. (a) Gel filtration of the entire mixture of cyanogen bromide peptides on a Sephadex G-50 column (1.9 × 200 cm) equilibrated with 25% acetic acid, and fractions were pooled as indicated. (b) Chromatography of fraction B on a column of Sp-Sephadex C-25 (0.9 × 10 cm). The peptides wre eluted with a gradient made of 400 mL of 25% (v/v) acetic acid and 200 mL of 10:25:65 (v/v/v) pyridine–acetic acid–water. (c) Separation of peptides in fraction A based on their solubility (Collins & Elzinga, 1975). Fraction A was dissolved in 5 mL of 70% formic acid and then passed through a Sephadex G-10 column (2.5 × 100 cm) which was equilibrated with 0.01 M pyridine–acetic acid, pH 6.0. (d) Chromatography of fraction AA on a column of Sp-Sephadex C-25 (0.9 × 15 cm). The gradient was made of 650 mL of 25% (v/v) acetic acid and 225 mL of 10:25:65 (v/v/v) pyridine–acetic acid–water. The eluate of each column were routinely monitored by absorbance at 280 nm, radioactivity, ninhydrin, and/or arginine staining (Yamada & Itano, 1966).

CB-16-1 can be resolved from CB-16-2 on a Sp-sephadex column, and Lys-325, -327 and -335 were obtained together in CB-16-1. On the basis of its amino acid composition, the sequence of CB-16-1 should be Lys-Ile-Lys-Ile-Ile-Ala-Pro-Pro-Glu-Arg-Lys-Tyr-Ser-Val-Trp (Elzinga et al. 1973). The best way to measure the reactivity of them individually was to subject this peptide (CB-16-1 in Figure 1) to six cycles of Edman degradation. As expected, Lys-325 was recovered from the first cycle and Lys-327 from the third cycle; two additional cycles were carried out to ensure that no residual Lys-325 or Lys-327 but only Lys-335 remained in the parent peptide (Table II). The reliability of this method was checked by a second method. When CB-16-1 was subjected to chymotryptic digestion, two peptides were produced, one containing Lys-325 and Lys-327 and the other representing residues 329–

Table II: Amino Acid Compositions of the Lysine-Containing Peptides

lysine position:	18	50	61	68	84	113	118	191	213 + 215	237	283	290	314	335	358	372
peptide position:	1–44	48–53	54–65	68–75	83–86	106–113	114–118	191–193	203–216	228–268	283–289	290–298	313–324	330–339	356–370	371–374
Asx	5.7 (6)[d]	1.1 (1)	1.2 (1)	1.8 (1)		1.2 (1)	1.1 (1)		1.3 (1)	2.4 (2)	1.8 (2)	3.0 (3)			1.1 (1)	
Thr	1.9 (2)			1.0 (1)		0.9 (1)			1.8 (2)	2.8 (3)			2.1 (2)		0.9 (1)	
Ser	1.9 (2)	0.9 (1)	1.0 (1)							5.6 (6)			1.0 (1)	0.9 (1)	0.9 (1)	
Glx	3.4 (3)	1.3 (1)	2.1 (2)	1.3 (1)	1.3 (1)	1.1 (1)	1.0 (1)		3.4 (3)	6.3 (6)			2.3 (2)	1.2 (1)	3.1 (3)	
Pro	2.6 (3)			0.6 (1)		1.9 (2)				2.5 (3)			1.0 (1)	1.8 (2)	0.8 (1)	
Gly	5.6 (6)	1.0 (1)	2.0 (2)	1.1 (1)						2.8 (3)					0.9 (1)	
Ala	5.3 (5)		0.9 (1)			1.1 (1)	1.0 (1)		1.1 (1)	3.2 (3)	0.8 (1)	1.1 (1)	2.0 (2)	1.0 (1)	1.0 (1)	
Cys[a]	0.8 (1)									0.9 (1)						1.1 (1)
Val	5.3 (5)		0.9 (1)						1.7 (2)	0.8 (1)		1.1 (1)		1.2 (1)	0.7 (1)	
Met[b]	0.8 (1)									0.8 (1)		0.7 (1)	0.9 (1)			
Ile	0.9 (1)		1.0 (1)	1.8 (3)	0.8 (1)	0.9 (1)		1.0 (1)	1.7 (2)	2.6 (3)	1.9 (2)		0.9 (1)	1.0 (1)	2.1 (2)	
Leu	2.1 (2)		0.9 (1)					1.2 (1)	1.0 (1)	3.0 (3)		0.9 (1)	0.8 (1)			
Tyr		0.8 (1)		1.3 (1)						0.9 (1)		1.0 (1)		0.9 (1)	1.4 (1)	
Phe	2.1 (2)									3.0 (3)						1.1 (1)
Trp					ND[e] (1)									ND (1)		ND (1)
His[c]	1.1 (1)			1.0 (1)											1.0 (1)	
Lys	1.1 (1)	0.9 (1)	1.1 (1)	1.0 (1)	0.8 (1)	0.8 (1)	0.8 (1)	0.8 (1)	1.8 (2)	1.1 (1)	0.9 (1)	1.2 (1)	0.9 (1)	1.0 (1)	0.9 (1)	0.9 (1)
Arg	3.4 (3)		0.9 (1)				1.0 (1)		2.0 (2)	2.1 (2)	1.0 (1)			1.0 (1)		0.9 (1)

[a] Measured as (carboxymethyl)cysteine. [b] Measured as homoserine plus homoserine lactone. [c] Includes N^{τ}-methylhistidine. [d] Values in parentheses are based on the amino sequence (Elzinga et al., 1973). [e] ND, not determined.

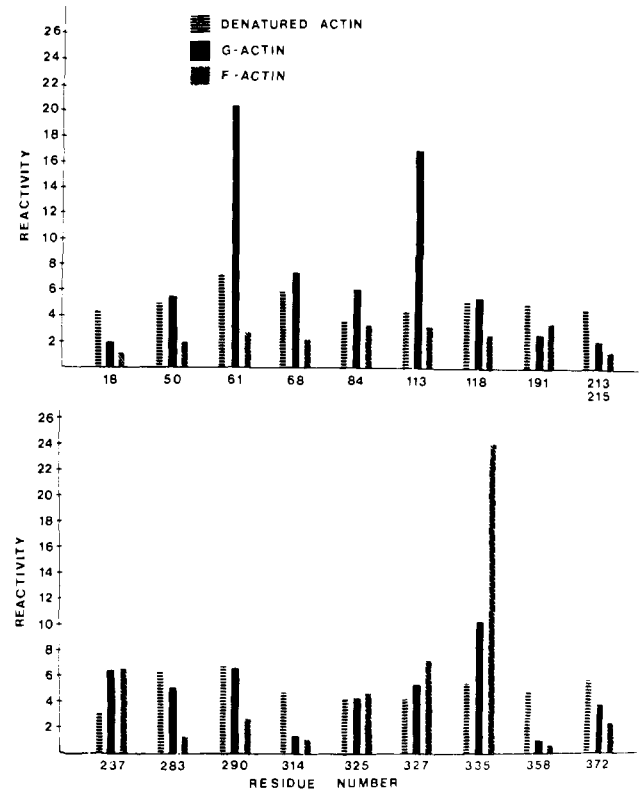

FIGURE 3: Reactivity of individual lysine residues in G-actin, F-actin, and denatured actin. Reactivity is expressed as moles of methyl groups incorporated × 10^2 per mole of lysine. Lys-213 and Lys-215 were measured as an average. Reactivity of lysine residues in G-actin and F-actin were measured simultaneously, whereas those in denatured actin were obtained separately. (See Experimental Procedure for details.) The reactivity of lysine residues of G-actin obtained from the denatured actin/G-actin experiment were very similar to those obtained from the F-actin/G-actin experiment.

336, which contains Lys-335. The reactivity of Lys-335 measured by this method was comparable to that measured by the sequencing method.

The amino acid compositions of the lysine-containing peptides are in good agreement with the sequence data (Table II). Lys-213 and Lys-215 are only one residue apart and exhibit very low reactivity to reductive methylation. Therefore, they were not resolved and their reactivity was calculated as an average.

Reactivity of Lysine Residues. The reactivities of the lysine residues in G-actin fall into three categories: Lys-61 and -113 are the most reactive ones, each accounting for about 15% of the total incorporation; Lys-18, -191, -213, -215, -314, and -358 are unreactive, each accounting for about 1% of the labeling; Lys-50, -68, -84, -118, -237, -290, -325, -327, -335, and -372 are moderately reactive (Figure 2; Table III). Upon polymerization, the reactivity of most of the lysines decreases (Figure 3; Table III), and the distribution of radioactivity among the lysine residues changes: Lys-61 and Lys-113 only account for 4–5% of the methyl groups incorporated, but Lys-335 contains about 34% of the total incorporated reagent (Table III). As shown by the ratio of methyl groups incorporated into the G- and F-actin, the reactivity of most lysine residues decreases about 40–50% upon polymerization and that of Lys-61, -68, -113, and -283 decreased by 70–90%. Interestingly, the reactivity of Lys-191 increased about 30%, while that of Lys-335 increased about 130% (Table III).

Little variation was seen in the reactivity of the lysine residues in denatured actin (Figure 3; Table III), which indicates that the reactivity of the lysine residues is not influenced

Table III: Reactivity of Lysine Residue of G-Actin, F-Actin, and Denatured Actin[a]

residue no.	G-actin reactivity	G-actin I %	F-actin reactivity	F-actin I %	denatured actin reactivity	denatured actin I %	r
18	1.9	1.5	1.0	1.4	4.3	4.7	0.52
50	5.5	4.5	1.9	2.6	4.9	5.3	0.35
61	20.2	16.4	2.7	3.7	7.1	7.7	0.12
68	7.3	5.9	2.1	2.9	5.9	6.3	0.29
84	6.0	4.9	3.3	4.6	3.8	4.1	0.55
113	17.0	13.8	3.2	4.4	4.5	4.9	0.19
118	5.5	4.5	2.6	3.6	5.4	5.8	0.47
191	2.6	2.1	3.5	4.8	5.2	5.7	1.35
213[b]	2.1	1.7	1.2	1.7	4.7	5.1	1.06
237	6.6	5.4	6.6	9.1	3.2	3.6	1.00
283	5.2	4.2	1.3	1.8	6.5	7.0	0.25
290	6.8	5.5	2.7	3.7	6.9	7.5	0.40
314	1.3	1.0	1.1	1.5	4.8	5.3	1.18
325	8.8	7.1	4.6	5.7	4.3	4.7	0.52
327	10.8	8.8	7.2	9.9	4.3	4.7	0.66
335	10.4	9.2	24.2	33.5	5.6	6.1	2.32
358	1.1	1.0	0.6	0.8	4.9	5.3	0.55
372	4.0	3.5	2.4	3.3	5.9	6.1	0.60

[a] Reactivity is expressed as moles of methyl group incorporated $\times 10^2$ per mole of lysine; *I* % represents methyl groups incorporated into each residue normalized with the total incorporated methyl group, and *r* represents the ratio of the reactivity of a lysine residue in F-actin to that of the same residue in G-actin. [b] The value for Lys-213 is actually the average value of Lys-213 and Lys-215.

by the neighboring residues in the primary structure. Thus, it seems reasonable to assume that the reactivity of lysines in the native molecule is related to their accessibility and the nature of neighboring residues in the tertiary structure.

Discussion

The replacement of sodium borohydride with sodium cyanoborohydride (Jentoft & Dearborn, 1979) made the method of reductive methylation (Means & Feeney, 1968) a much more attractive technique to study the reactivity of lysine side chains of proteins. The facts that the modification is carried out under mild conditions (pH 7.5) and that the methyl group being introduced into the protein is relatively small and only changes slightly the p*K* of the lysine side chains will minimize the possibility of changing conformation by the labeling. Since on gel filtration or ion-exchange columns, modified peptides and unmodified peptides were eluted at nearly the same rate, it appeared more advantageous not to do a complete methylation after trace labeling to avoid side reactions which might be introduced by excess reagents. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and peptide analysis have demonstrated that neither inter- nor intramolecular weight cross-linkng has occurred in G-actin, F-actin, or denatured actin under our reductive methylation conditions. Since G-actin and F-actin were modified under conditions that the polymerizability of the molecules are not impaired, the reactivity of each lysine residue would reflect its local environment in the native structure.

There are at least 13 lysines, including the very reactive and moderately reactive ones, acessible to the reagent, if one regards lysine residues with reactivity at the level of those in denatured actin as accessible. The differences observed among the accessible ones are due to shielding effects and/or interaction with other residues in the vicinity. Amino groups in a hydrophobic environment or those near negatively charged groups would have lower pK_a s and thus higher reactivity. Conversely, those near positively charged residues or those being partially blocked would exhibit lower reactivity. The six lysines with very low reactivity are probably buried in native actin and thus not readily available for modification, although the possibility that some of them may be on the surface but having an unusually high pK_a can not be ruled out. The reactivity of lysine in urea-denatured actin could be regarded as corresponding to that of an unperturbed lysine on the surface of native actin. When G-actin and F-actin are compared, the reactivities of Lys-61, -68, -113, and -283 are significantly reduced upon actin polymerization, whereas those of Lys-191 and -335 are increased. The decrease of reactivity upon actin polymerization could be readily explained if these residues were located in the monomer–monomer contact area and made inaccessible to the reagent, although a polymerization-induced conformational change cannot be ruled out. The change in the reactivity of Lys-191, which appears to be buried in G-actin, could be attributed to its becoming exposed upon polymerization; such a conformational change may be invoked to explain the increase in reactivity of Lys-335 too. Alternatively, Lys-335 may be interacting with the bound ATP of G-actin, and the increase in reactivity might be directly attributable to the loss of γ-P of ATP that accompanies polymerization. Finally, Lys-335 may be located near the monomer–monomer interacting area, the hydrophobicity introduced by the neighboring molecule may create a favorable environment for reductive methylation.

The very high reactivity of Lys-335 in F-actin is intriguing: it accounts for 34% of the total incorporation into F-actin and it is twice as reactive as in the monomer form. Moreover, the reactivity of this residue is decreased by 50% when actin is complexed with myosin S-1 or tropomyosin which will be decribed in detail elsewhere (L. Sziligyi and R. C. Lu, unpublished experiments). The implication from the changes of reactivity of Lys-335 in these studies would be that (1) the microenvironment of Lys-335 changes either internally or because of the presence of neighboring molecules upon polymerization or (2) Lys-335 is located near the binding sites of myosin S-1 and/or tropomyosin; alternatively, the environment where Lys-335 is located changes due to the binding of myosin or/and tropomyosin.

Various pieces of evidence suggest that N^{τ}-methylhistidine at position 73, His-40, Tyr-53, Tyr-69, Tryp-74, and Lys-113 are located near or at the surface of G-actin (Elzinga & Collins, 1972; Hegyi et al., 1974; Bender et al., 1976; Elzinga, 1971; Lehrer & Elzinga, 1972; Collins et al., 1971). This is consistent with our finding that Lys-61, -68, and -113 are highly reactive in G-actin. The report that Cys-217 is buried in native actin (Lusty & Fasold, 1969; Elzinga & Collins, 1975) is also in agreement with our finding that Lys-213 and Lys-215 are hardly reactive in G- or F-actin. Lusty & Fasold (1969) found that in F-actin two sulfhydryl groups (Cys-284 and Cys-256) became inaccessible to the azo dye. This plus our finding that the reactivity of Lys-283 decreases significantly in F-actin suggests that this region is probably involved in polymerization. Tao and Cho have reported the fluorescence label *N*-(iodoacetyl)-*N'*(5-sulfonaphthyl)ethylenediamine at Lys-373 of G-actin became less accessible upon actin polymerization (Tao & Cho, 1979), which is consistent with our finding that the reactivity of Lys-372 decreased about 40% upon actin polymerization.

In conclusion, the study on the reactivity of lysine residues of actins by limited reductive methylation has shown that most of the lysine residues of G-actin are on the surface of the molecule and the immediate environment of regions containing

lysine-61, -68, -113, -191, -283, and -335 is changed upon actin polymerization.

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