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A Novel Actin Label: A Fluorescent Probe at Glutamine-41 and Its Consequences†

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ABSTRACT: By peptide isolation and analysis, it has been shown that the dansyl fluorophore of dansylcadaverine [*N*-(5-aminopentyl)-5-(dimethylamino)naphthalene-1-sulfonamide] transfers to Gln-41 of actin from rabbit skeletal muscle when the reaction is catalyzed by guinea pig liver transglutaminase. As a function of time, the degree of labeling asymptotically approaches 1 mol of dansyl/1 mol of actin. About 80-85% of the attached dansyl fluorophore was found at Gln-41. Such labeled G-actin polymerizes to the same extent as control actin, but the polymerization rate is greater and the critical concentration is less than for control actin. Complete polymerization is accompanied by a 1.5-2.0-fold increase in the emission intensity of the attached fluorophore. Labeled F-actin thus obtained activates myosin subfragment 1 (S-1) Mg^{2+}-ATPase activity with the same K_{app}, and to the same V_{max}, as control actin; moreover, when such labeled F-actin is cross-linked to S-1 by 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, the resulting superactivation of Mg^{2+}-ATPase is the same as that attained with control actin. The attributes of this label thus make it an ideal reporter of events in the N-terminal 10-kilodalton region of actin, and a new topological point for proximity mapping.

Actin is one of the most important proteins of eukaryotic cells; it is essential in cytoskeletal phenomena and in muscle cells; it is, along with myosin, a partner in the contractile event. Domain structure has been ascribed to actin in two senses: Crystallographic studies (Kabsch et al., 1985) show that actin monomer consists of two somewhat unequal lobes; on the other hand, proteases of different specificity all cut initially between two quite unequal fragments of actin (Jacobson & Rosenbusch, 1976; Mornet & Ue, 1984; Konno, 1987). For example, Konno (1988) found chymotrypsin to cleave between Met-44 and Val-45, thus generating isolatable, N-terminal "10-kilo-

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dalton (kDa)"[1] and C-terminal "33-kDa" fragments. It seems likely that the 10-kDa segment resides in the smaller of the two crystallographically defined lobes. The 10-kDa segment contains important functionalities, e.g., the anionic residues participating in EDC-induced cross-linking with myosin subfragment 1 (S-1) (Mornet et al., 1981; Sutoh, 1982), at least a part of the Me^{2+} binding site (Mornet & Ue, 1984; Barden & dos Remedios, 1984), and a binding site for myosin subfragment 1 that has been suggested by both immunological (Mêjean et al., 1981) and ^1H NMR studies (Levine & Moir, 1985). Because of these functionalities, it is of interest to place a reporter group or label on the N-terminal 10-kDa region. Once placed, the label can also serve in "proximity mapping" of acto(S-1) (Botts et al., 1984). Ideally, a label is uniquely placed and reports on neighboring events without significantly influencing them. It has been reported (Barden et al., 1986) that for such labeling a fluorophore can be placed on Cys-10, but Konno and Morales (1985) had earlier found that Cys-257 in the 35-kDa segment is labeled along with Cys-10. In this paper, we show that Gln-41 of the N-terminal 10-kDa region can be almost uniquely labeled, without significant effect on the functional properties of actin. This labeling is achieved with the aid of guinea pig liver transglutaminase and fluorophoric derivatives of cadaverine. A preliminary note about this work has appeared (Takashi & Kasprzak, 1985), and new proximity data employing actin with a fluorophore at Gln-41 have been reported (Takashi & Kasprzak, 1987).

Materials and Methods

Protein Preparation. Actin was extracted and purified from acetone powder (Spudich & Watt, 1971), myosin subfragment 1 (S-1) was prepared according to published procedures (Weeds & Taylor, 1975), and the S-1 isozymes, S-1(LC_1) and S-1(LC_3), were fractionated on an SE-cellulose column (Trayer & Trayer, 1983). S-1(LC_3) was used in the experiments. Transglutaminase was prepared as in Connellan et al. (1971). The specific activities of transglutaminase were typically 20–25 units/mg at 37 °C.

Labeling of Actin Gln-41. G-Actin at a concentration of 50 μM was incubated with a 5-fold molar excess of dansylcadaverine in a solution containing 5 mM Tris-HCl (pH 8.0), 1 mM $CaCl_2$, 1 mM DTT, 0.4 mM ATP, 1 mM NaN_3, and 1 μM transglutaminase at 4 °C. At arranged time intervals, an aliquot was withdrawn, and the addition of NaCl, $MgCl_2$, and EGTA was made to give final concentrations of 50 mM, 2 mM, and 1 mM, respectively. The actin solution was then allowed to polymerize for at least 2 h at 4 °C and centrifuged at 160000*g* for 60 min. The labeled F-actin pellet was homogenized in a glass homogenizer with a solution containing 0.1 M NaCl, 25 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 0.5 mM DTT, and 10% (v/v) dimethylformamide and was dialyzed against the aforementioned buffer with at least two changes over a 20-h period. Subsequently labeled F-actin was collected by centrifugation at 160000*g* for 60 min and was homogenized with appropriate volumes of depolymerizing buffer (buffer A: 2 mM Tris-HCl, pH 8.0, 0.2 mM ATP, 0.2 mM $CaCl_2$, and 0.25 mM DTT) in a glass homogenizer so as to obtain actin of ca. 2.0 mg/mL. The subsequent solution was dialyzed against buffer A at 4 °C for 2 days with four changes and centrifuged at 160000*g* for 60 min to obtain G-actin labeled with dansylcadaverine (DNC-G-actin). The purity of this prepared actin was routinely checked on $NaDodSO_4$–PAGE. No labeling occurs in the absence of transglutaminase.

Labeling conditions of F-actin were essentially the same as those of G-actin as described above. F-Actin used here was prepared as follows: G-actin in buffer A was polymerized by the addition of 1 mM $CaCl_2$ for 2 h at 25 °C. The subsequent solution was further allowed to stand overnight at 4 °C prior to its use.

Alkylation of Actin. Reaction of the actin with iodoacetamide was performed following the general procedure of Crestfield et al. (1963). In a typical experiment, the sulfhydryl groups of DNC-actin (50 mg) in 8 M urea and 0.1 M Tris-HCl (pH 8.0) were reduced with 20 mM β-mercaptoethanol under N_2 gas for at least 2 h, and iodoacetamide was added to 50 mM; the pH was maintained at 8.0 by addition of 1 M KOH. The reaction was allowed to proceed for 1 h at room temperature in the dark. A large excess of β-mercaptoethanol over iodoacetamide was added to quench any free iodoacetamide prior to exhaustive dialysis against 1% acetic acid. Finally, the protein was lyophilized.

Cyanogen Bromide Cleavage. Salt-free and lyophilized actin (50 mg) was dissolved in 2 mL of 70% formic acid, and an equal volume of freshly prepared 2 M cyanogen bromide in 70% formic acid was added. After 16–18 h at room temperature, the solution was diluted with 20 volumes of ice-cold water and lyophilized.

Peptide Isolation. Separation of peptides was achieved as shown in Figure 2 by gel filtration on Sephadex G-50 and Sephadex G-10 and ion-exchange chromatography on SP-Sephadex C-25 following cyanogen bromide cleavages on the basis of previous work (Elzinga et al., 1973; Lu & Szilagyi, 1981).

Analyzing the Peptides. The amino acid composition of the peptides was analyzed on a Durrum Model D-500 amino acid analyzer after hydrolysis in vacuo in 5.7 N HCl at 110 °C for 24 h. N-Terminal analysis was performed with dansyl chloride as described by Gray (1972).

Other Procedures. $NaDodSO_4$–PAGE was conducted in a 10–18% polyacrylamide gradient containing 0.1% $NaDodSO_4$ (Laemmli, 1970).

Actin was cross-linked to S-1 using EDC in a solution containing 5 μM S-1, 10 μM actin, 20 mM imidazole (pH 7.0), 2 mM $MgCl_2$, and 2.5 mM EDC (Mornet et al., 1981).

The Mg^{2+}-ATPase activity of S-1 cross-linked to actin was measured in a solution containing 0.6 M KCl, 2.5 mM $MgCl_2$, and 50 mM Tris-HCl (pH 8.0) at 25 °C. The actin-activated Mg^{2+}-ATPase of S-1 was measured in a medium containing 10 mM KCl, 10 mM Tris-HCl (pH 7.9), 5 mM $MgCl_2$, and 2 mM ATP at 25 °C. The P_i liberated was determined by the method of Fiske and Subbarow (1925).

The time course of polymerization was measured by either fluorometry or light scattering at 20 °C in a Perkin-Elmer MPF-4 spectrofluorometer. Light-scattering measurements were made at right angles to the incident beam using 450-nm light. For fluorescence measurements, the excitation and emission wavelengths were 350 and 510 nm, respectively. The critical concentrations of actin were measured essentially by the method of Brenner and Korn (1983) wherein actin is polymerized at high concentration ($\approx$20 μM) and then diluted in the same buffer to a series of lower actin concentrations.

[1] Abbreviations: $NaDodSO_4$–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; EDC, 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide; DNC, dansylcadaverine; DNC-actin, actin labeled with dansylcadaverine; S-1, myosin subfragment 1; S-1(LC_1) and S-1(LC_3), myosin subfragment 1 containing either light chain 1 or light chain 3; DTT, dithiothreitol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; kDa, kilodalton(s); Tris-HCl, tris-(hydroxymethyl)aminomethane hydrochloride; TLC, thin-layer chromatography.

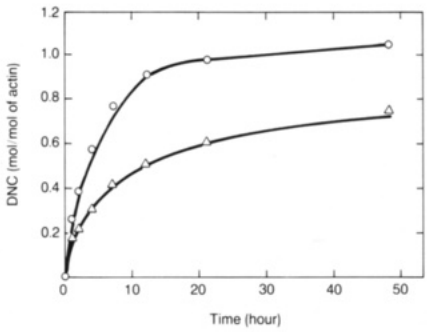

FIGURE 1: Kinetics of the transglutaminase-catalyzed labeling of actin. G-Actin (○) or F-actin (△) was incubated with dansylcadaverine and transglutaminase under the conditions described in the text.

After a 15–18-h incubation at 20 °C to reach a new steady state, both the steady-state fluorescence and light scattering of each sample were measured at 20 °C. Only the light-scattering method was used to determine the critical concentrations of unlabeled actin.

Protein Concentration. S-1 concentrations were estimated by using $A^{1\%}_{280nm} = 7.5$ (Wagner & Weeds, 1977) while G-actin concentrations were measured by the absorbance at 280 nm ($A^{1\%}_{280nm} = 11.1$) (Houk & Ue, 1974) or at 290 nm ($A^{1\%}_{290nm} = 6.30$) (Lehrer & Kerwar, 1972).

Concentrations of G-actin labeled with dansylcadaverine were measured either by the method of Lowry et al. (1951), with unlabeled G-actin as a standard, or by the absorbance at 280 and 350 nm. In the latter method, the dansyl probe itself had an absorbance at 280 nm; therefore, it was necessary to subtract its contribution from the measured absorbance. This contribution was estimated to be $A_{280nm} = 0.528A_{350nm}$.

Molecular weights of S-1, G-actin, and transglutaminase were assumed to be 1.10×10^5 (Margossian et al., 1981), 4.2×10^4 (Elzinga et al., 1973), and 8.5×10^4 (Connellan et al., 1971), respectively.

The amounts of dansylcadaverine bound to actin were estimated from its absorbance at 326 nm in buffer A, using $\epsilon_{326nm} = 4.64 \times 10^3$ (Lorand et al., 1968), or by its fluorescence at 500 nm when excited at 350 nm in 6 M guanidine hydrochloride, using the concentration of dansylcadaverine as a standard.

Chemicals. ATP and dansylcadaverine were purchased from Sigma Chemical Co. $NaDodSO_4$ and acrylamide (Bio-Rad Laboratories) were of electrophoretic grade. Sephadex G-50 (fine), Sephadex G-10 (fine), and SP-Sephadex C-25 were from Pharmacia Chemical Co. Ultrapure guanidine hydrochloride was from Schwarz/Mann. Polyamide TLC plates, constant-boiling HCl, dansyl chloride, and dansyl amino acid standards were purchased from Pierce Chemical Co. EDC was from Calbiochem. All other chemicals were of reagent grade.

RESULTS

Incorporation of Dansylcadaverine into Actin. Labeling of G-actin with dansylcadaverine was performed as described under Materials and Methods. The amount of label incorporated into actin increases asymptotically with time, approaching ca. 0.98 mol of label per actin monomer in 20 h; thereafter, it levels off at 1.0–1.05 mol of label per actin (Figure 1), indicating that labeling occurs in a highly specific manner.

On the other hand, labeling F-actin is considerably slower. The rate ($t_{1/2} = \sim 12$ h; $t_{1/2}$ is the time required for 1 mol of actin to incorporate 0.5 mol of label) and the extent (0.6–0.7 mol of label per actin monomer) are about 3.6-fold slower and

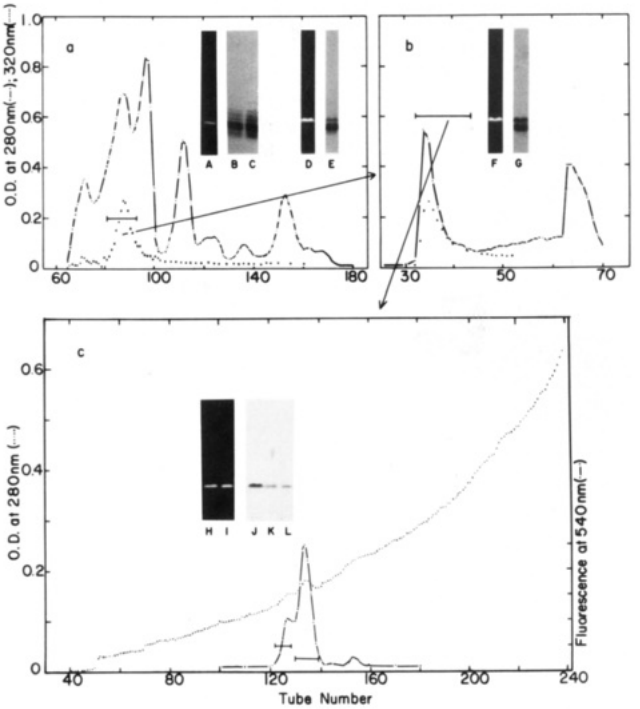

FIGURE 2: Chromatography of the cyanogen bromide peptides of DNC-actin. (a) Gel filtration of the entire digest on a Sephadex G-50 column (1.8 × 200 cm) equilibrated with 25% acetic acid. Fractions that contained DNC fluorescence as indicated by the bar were pooled. (b) Separation of peptides on a Sephadex G-10 column (1.8 × 100 cm) equilibrated with 0.01 M pyridine–acetic acid, pH 6.0. Peptides obtained in (a) were dissolved in 2.0 mL of 70% formic acid and applied to a Sephadex G-10 column. The region enclosed by the bar was pooled. (c) Chromatography of the fractions in (b) on a SP-Sephadex C-25 column (0.9 × 14 cm). The gradient was made of 650 mL of 25% acetic acid and 250 mL of 10:25:65 (v/v/v) pyridine–acetic acid–water. The eluate was routinely monitored by the absorbance at 280 and 320 nm and/or by the fluorescence at 540 nm. At each step, fluorescent polypeptides were subjected to $NaDodSO_4$–PAGE and detected by illuminating gels with a UV lamp (lanes A, D, F, H, and I) or by staining them with Coomassie Brilliant Blue R-250 (lanes B, C, E, G, J, K, and L). Lanes A and C, the entire digest of DNC-actin; lane B, the entire digest of control actin; lanes D and E, the major fluorescent fractions (82–92); lanes F and G, the fluorescent fractions; lanes H and K, fractions 122–129; lanes I and L, fractions 130–139; lane J, isolated CB-13 from control actin.

40–50% less than those of G-actin ($t_{1/2} = \sim 3.3$ h, 1.0 mol of label per actin). The difference implies that G-actin has a more favorable conformation for labeling and that its labeling sites are more exposed to solvent than that of F-actin.

It should be noted that under the G-actin labeling conditions it was not possible to keep all the actin in the unpolymerized form for longer than 4–5 h, since the labeling medium contains 1.0 mM Ca^{2+}.

Identification of the Labeling Site. It is established that the Ca^{2+}-dependent transglutaminase from guinea pig liver catalyzes the insertion of aliphatic primary amines only into glutamine residues of peptides and proteins (Clarke et al., 1959; Folk, 1980). Therefore, we sought to find out which of the glutamine residues of actin had been labeled. This was performed by gel filtration and ion-exchange chromatography following cyanogen bromide fragmentation of dansylcadaverine-labeled actin prepared under the G-actin labeling conditions. The eluting positions of the labeled peptides on chromatography were followed, either by measuring the absorbance at 280 nm/360 nm or by monitoring the fluorescence at 540 nm (Figure 2). Elution profiles (Figure 2a) show that the second peak (indicated by the bar) contains the fluorescent peptides that are 80–85% of the total fluorescent peptides.

Table I: Amino Acid Composition (Residues per Mole) of DNC-Labeled Peptide of Actin

amino acid	DNC-peptide	N-terminal CNBr peptide[a]
Lys	0.98	1.04 (1)[b]
His	0.93	0.96 (1)
Arg	3.39	3.50 (3)
Asp	6.85	6.91 (6)
Thr	2.25	2.18 (2)
Ser	2.37	2.23 (2)
Glu	3.79	3.62 (3)
Pro	3.27	3.29 (3)
Gly	7.15	6.87 (6)
Ala	5.87	5.83 (5)
Val	5.40	5.34 (5)
Ile	1.08	0.99 (1)
Leu	2.25	2.26 (2)
Tyr		(0)
Phe	2.03	2.18 (2)
Cys	ND[c]	ND (1)
Hse	ND	ND (1)

[a] N-Terminal cyanogen bromide peptide (CB-13) from control actin that was isolated by the method as described in the text. [b] The values in parentheses are those based on the amino acid sequence determined by Elzinga et al. (1973). Data are normalized by setting the average of values of Lys, His, and Ile, present in equimolar amounts, equal to 1. The contents of Cys and Hse were not determined. Amino acid residues <0.1 are not listed in this table. [c] Not determined.

Tubes 82–92 containing the major fluorescent peak were combined and subjected to gel filtration on a Sephadex G-10 column. As shown in Figure 2b, the first peak (tubes 32–40) contains almost all the dansyl fluorescent peptides. These tube contents were pooled and further purified on a SP-Sephadex C-25 column. Only one major fluorescent peak, with a shoulder, was obtained (Figure 2c). In this final step, both fractions 122–129 and 130–139 pooled showed a single band showing the same mobility in $NaDodSO_4$–PAGE and virtually the same amino acid composition. Their partial separation might be attributed to minor differences in charge, possibly to the presence of both homoserine and homoserine lactone at the C-terminal position of the peptides [even under acidic conditions, these are known to be in equilibrium (Gross, 1967)].

Table I gives the amino acid composition of the isolated fluorescent peptide and of CB-13 peptide isolated from control actin treated in the same manner. The amino acid composition of the former is nearly identical with that of CB-13. The foregoing results strongly indicate that the cyanogen bromide peptide predominantly labeled with dansylcadaverine is CB-13, the N-terminal cyanogen bromide peptide in the actin sequence (Elzinga et al., 1973). CB-13 contains only one glutamine residue, at position 41. Therefore, we conclude that Gln-41 is the most probable candidate for the reaction in the presence of dansylcadaverine and transglutaminase.

Characterization of Labeled Actin. First we ask how the labeling at Gln-41 affects polymerization of actin. In all the experiments, polymerization was induced by the addition of 1 mM $MgCl_2$ at 20 °C.

Polymerization of DNC-G-actin was accompanied by a ca. 2-fold increase in fluorescence intensity and a shift in peak emission from 520 to 512 nm, indicating that the fluorescent probe at Gln-41 is highly sensitive to the polymerization process. We also found that the rate of polymerization of DNC-G-actin is considerably faster than that of control actin; polymerization of DNC-actin is complete in less than 10 min with $t_{1/2} = \sim 2.8$ min, while polymerization of control actin takes somewhat over 40 min with $t_{1/2} = \sim 18.7$ min under the same conditions, i.e., 12 μM actin, 1 mM $MgCl_2$, 2 mM Tris-HCl (pH 8.0), 0.2 mM $CaCl_2$, 0.2 mM ATP, and 0.25 mM DTT at 20 °C. It should be noted that polymerization of DNC-actin apparently proceeded without the initial lag phase observed with control actin under our conditions (Figure 3). This suggests that nuclei are present at the initiation of the experiments.

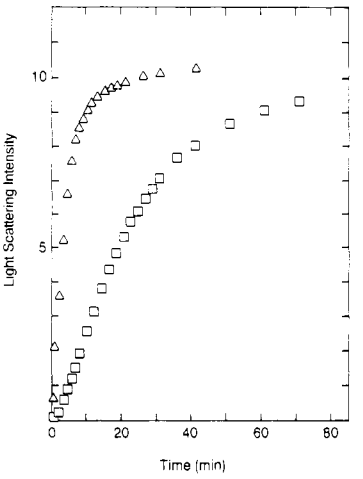

FIGURE 3: Polymerization of actin. G-Actin (12 μM) was polymerized by addition of 1 mM $MgCl_2$ in a solution containing 0.2 mM ATP, 0.2 mM $CaCl_2$, 2 mM Tris, pH 8.0, and 0.25 mM DTT, 20 °C. DNC-actin (△); unlabeled actin (□). Light scattering was observed at right angles to the incident beam, using 450-nm light.

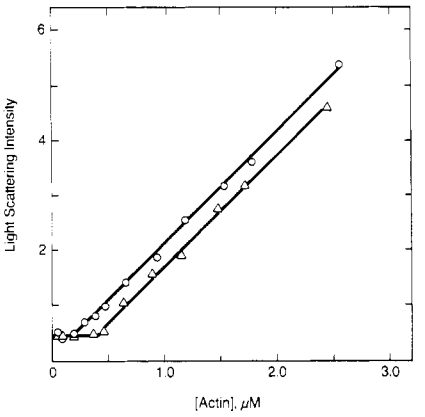

FIGURE 4: Critical concentrations for DNC-actin and unlabeled actin. Conditions: 50 mM NaCl, 1 mM $MgCl_2$, 2 mM Tris, pH 8.0, 0.2 mM $CaCl_2$, 0.2 mM ATP, and 0.25 mM DTT, 20 °C. DNC-actin (○); unlabeled actin (△). The critical concentration of DNC-actin obtained by fluorescence measurements, approximately 0.18 μM, agrees with those determined by the light-scattering method.

Although it is hardly noticeable in $NaDodSO_4$–PAGE, careful inspection of a labeled G-actin gel revealed the traces of actin oligomer, presumably products cross-linked by the enzyme. It is conceivable that these minute amounts of actin oligomer act as "seeds" to allow actin to polymerize faster and this effect could be at least in part responsible for accelerating the rate of polymerization of the labeled actin. We also measured the critical concentrations of DNC-actin and of unlabeled actin. In other experiments, we verified that the critical concentrations of DNC-actin determined by fluorescence were indistinguishable from those obtained by light scattering. Figure 4 shows that the critical concentrations for the labeled actin (0.18–0.2 μM) are ca. 2-fold less than those for unlabeled actin (0.38 μM). The latter is in good agreement with literature values (Gordon et al., 1977; Brenner & Korn, 1983).

Our next concern was whether or not polymerized DNC-actin is able to bind to myosin heads. To examine this issue, we used two methods: The first method was to measure the

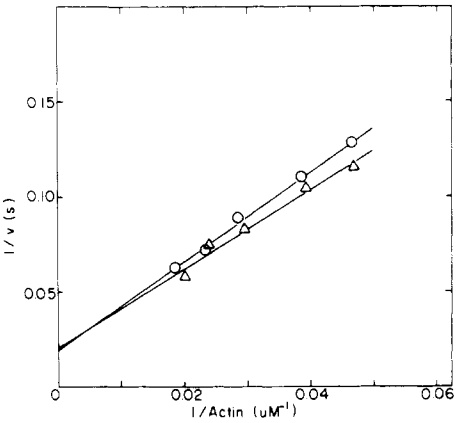

FIGURE 5: Double-reciprocal plot of the actin-activated Mg^{2+}-ATPase of S-1 as a function of actin concentration. Assays were performed as described under Materials and Methods; v is the observed rate of ATP hydrolysis by S-1 in the presence of actin minus that of S-1 alone. (Δ) DNC-actin (v_{max} = 47 s^{-1}, K_{app} = 105 μM); (O) control actin (v_{max} = 50 s^{-1}, K_{app} = 125 μM).

actin-activated Mg^{2+}-ATPase activity of S-1 as a function of [actin] and then to evaluate V_{max} and K_{app} from such data. Figure 5 shows double-reciprocal plots of the actin-activated Mg^{2+}-ATPase activity of S-1 with either control actin or DNC-actin. It is evident, from the values of V_{max} and K_{app} for actin (50 s^{-1} and 125 μM, respectively) and for DNC-actin (47 s^{-1} and 105 μM, respectively), that the labeling has virtually no effect on the binding of S-1 to actin in the presence of MgATP.

The second method was to cross-link actin to S-1 by EDC under rigor conditions (Mornet et al., 1981) and to ask how well the actin–S-1 contact sites are preserved. This was done by measuring the "superactivated" Mg^{2+}-ATPase activity of the cross-linked complex and by observing the formation of the polypeptide doublet with apparent molecular weights of 175K and 185K on $NaDodSO_4$–PAGE. For both control actin and DNC-actin, the Mg^{2+}-ATPase activity is greatly and similarly enhanced as a function of cross-linking time (Figure 6). In 30 min, the Mg^{2+}-ATPase activity reaches as high as 13 s^{-1}. Moreover, inspection of the cross-linked products on $NaDodSO_4$–PAGE clearly revealed a doublet being formed with DNC-actin and S-1, having apparent molecular weights of 175K and 185K (data not shown). These results indicate that upon labeling at Gln-41 the contact and potential cross-linking sites of actin to S-1 remain unaffected.

Discussion

The purpose of the present study was to develop the capability of introducing a variety of fluorophoric probes into a specific location in the 10-kDa region of actin. It was hoped that such probes might report on significant processes in which actin participates and also serve to identify a topological point in our proximity mapping of actomyosin. It was also necessary to show that such a probe once placed did not significantly interfere with the processes on which it was intended to report.

It has been shown here that of the 11 glutamine residues in actin (Elzinga et al., 1973) only a very small number can be selectively labeled with dansylcadaverine and guinea pig liver transglutaminase and that when ca. 1.0 mol of label per actin monomer has been incorporated, 80–85% of the label resides at Gln-41. This indicates a high, but by no means absolute, specificity. Minor amounts of label were also found in peptide CB-10 (data not shown) which contains two glutamine residues at positions 49 and 59 (Elzinga et al., 1973; Vandekerchove & Weber, 1978). At present, however, no sequence locations in this fragment have been made. In

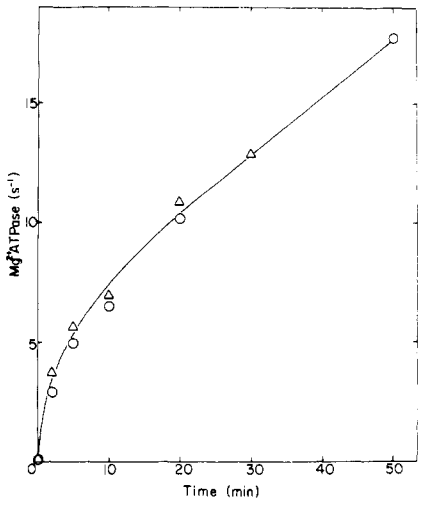

FIGURE 6: Mg^{2+}-ATPase activity of S-1 cross-linked to actin by EDC as a function of cross-linking time. S-1 was cross-linked to either control actin (O) or DNC-actin (Δ) under the conditions described in the text. At given times, aliquots were withdrawn from the reaction vessel and immediately diluted 100-fold with ATPase assay medium, ensuring a simultaneous quenching of the EDC reaction. The Mg^{2+}-ATPase assays were performed as described under Materials and Methods.

conjunction with glutamine donor sites in actin, it is of interest to note that actin from human blood platelets or rabbit skeletal muscle can also serve as substrate for factor XIIIa (Cohen et al., 1980), which is analogous to guinea pig liver transglutaminase but has differences in both structure and reactivity toward glutamine-containing peptide substrates (Gorman & Folk, 1984).

Ignoring the rather small nonspecific labeling, the following conclusions may be drawn. First, the ability of the labeled actin to undergo the polymerization/depolymerization process suggests that Gln-41 is not directly involved in this function, even though polymerization is affected to a certain extent in that the labeled actin polymerized more rapidly than the unlabeled actin and that the critical concentration of the labeled actin was also slightly less. In this regard, it has been recently demonstrated in detail by Tait and Frieden (1982) that the chemical modification of the C-terminal end of actin (Cys-374) promotes nucleation and that the promotion is dependent upon the derivatives employed. It should be mentioned that at present we are not certain if the high degree of nucleation observed in our experiments is due directly to the label at Gln-41 or rather to generation of trace amounts of cross-linked actin oligomer since no further attempts were made to purify the labeled actin by procedures intended to remove oligomers [e.g., see Rees and Young (1967) and Maclean-Fletcher and Pollard (1980)]. Second, the results that cyclic interactions of S-1 with actin and Mg^{2+}-ATP are virtually unimpaired by the labeling, and that the shape of the fluorescence emission curve of dansylcadaverine attached to actin exhibits no appreciable perturbation when S-1 binds, may imply that Gln-41 is not at the immediate S-1 binding sites. This would be consistent with recent studies that N-terminal residues 18–28 of actin are at least in part likely to participate in binding the myosin head (Mējean et al., 1986).

The present work also provides some additional information concerning the structure or environment around Gln-41. Since the labeling can readily be carried out in G-actin, the side chain of Gln-41 is likely to be exposed to the solvent. Solvent exposure would then make it simultaneously accessible to dansylcadaverine substrates and to the active sites of transglut-

aminase in a highly prescribed manner. Full exposure to solvent makes it likely that the side chain of the glutamine is located on the surface of G-actin. It is notable that, although the extent of labeling was much less, the same glutamine residue was available to dansylcadaverine and the enzyme even when actin was in polymerized form. This suggests that the side chain of Gln-41 is unlikely to be fully buried in the interior of actin. The accessibility of the residues to solvent and the proteolytic susceptibility of the region in the vicinity of Gln-41 have been noted by others. Konno (1987) has recently found that chymotrypsin cleaves preferentially between Met-44 and Val-45 of G-actin, leaving polymerizability unaffected, but it fails to do so when actin is in polymerized form. Earlier studies showed that carbethoxylation of G-actin at His-40 impaired polymerization (Hegyi et al., 1973). These studies suggest that the residues His-40 and Met-44/Val-45 are also likely to lie at or near the surface of G-actin and may be located rather closely to the actin–actin binding sites. The foregoing results seem consistent with our finding in this paper.

In conclusion, we have shown how to place a probe at Gln-41 in the N-terminal 10-kDa region of actin. This placement is specific and is accomplished harmlessly with an enzyme (guinea pig liver transglutaminase) and a fluorophoric derivative of cadaverine. By use of different derivatives, different fluorophores can be placed. The function of actin such as polymerization or myosin ATPase activation is virtually unimpaired. The DNC version of cadaverine reports the polymerization process very well (increasing emission intensity). The probe at Gln-41 functions well as a landmark in proximity mapping (Takashi & Kasprzak, 1985, 1987), and the possibility of placing various fluorophores at this point enables the measurement of its radial coordinate (Taylor et al., 1983; A. A. Kasprzak and R. Takashi, unpublished results); this would prove helpful for the complete determination of the orientation of actin monomer in the filament.

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