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Relocation of Cys374 of Actin Induced by Labeling with Fluorescent Dyes

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Cys374 is an important landmark of actin, because its sulfhydryl group is reactive and can be labeled with various reagents. The atomic coordinates of actin Cys374 have been determined by X-ray crystallography of co-crystals of actin with either profilin or gelsolin. However, the positions of Cys374 in the crystals determined were not consistent with the data obtained through fluorescence resonance energy transfer. Here, we examined its position by means of probabilistic distance geometry using published fluorescence resonance transfer data and found that Cys374 of actin was relocated when Cys374 was labeled with fluorescent dyes that caused steric hindrance. The atomic coordinates of Cys374 after heavy atom labeling have been found to be different from those for the native crystal. This is consistent with our results. Therefore, the position of Cys374 is sensitive to chemical modification that introduces a bulky reagent. This also suggests that the conformation of the C-terminal region of actin could also be sensitive to the environment.

Key words: actin, Cys374, fluorescence resonance energy transfer.

Actin Cys374 is an important landmark of the actin structure, because Cys374 can be labeled by various chemical reagents, and the distances from Cys374 to other fluorescent dyes have been used to detect the conformational changes in actin filaments, thin filaments or myosin-S1–decorated actin filaments by means of a fluorescence resonance energy transfer (FRET) method. The position of actin Cys374 has been determined by FRET measurements (*1–7*), electron microscopy (*8*), and X-ray methods (*9–14*). However, the positions obtained were inconsistent: Moens *et al.* (*1*) reported that Cys374 was at the radius of 17 Å (S.D. 5 Å) from the axis of the actin helix, whereas it is located at the radius of 27 Å in an atomic model of F-actin (*12*). The location of Cys374 of actin from smooth muscle was found to be different from that in other crystal structures (*14*): This is puzzling because (i) the amino acid sequences of actin from various sources are highly conserved, and (ii) the main-chain structures of actin from rabbit skeletal muscle, bovine spleen and *Dictyostelium* were almost identical (*9–11*). Here, to elucidate the cause of such a difference, we re-examined the position of Cys374 by analyzing the distances between fluorophores obtained by FRET measurements with a probabilistic distance geometry method we developed (*15, 16*). It was found that actin Cys374 was relocated due to the introduction of bulky fluorescent dyes.

Through FRET measurements (*2–4*), the four distances from Cys374 to Lys61, Tyr69, C5 of an adenine nucleotide, and a divalent cation have been determined, as summarized in Table I. The probability distribution function of actin Cys374 was calculated by means of probabilistic distance geometry (*15, 16*). This method is an extension of a conventional triangulation method for determining the positions of fluorophores in three-dimensional space through combination of the distances between fluorophores with atomic coordinates of proteins and other structural information. The distribution functions can be calculated by assuming that (i) the distances between fluorophores determined with a FRET method can vary around the measured value as though the points are connected by springs, of which the spring constant is consistent with the experimental error of distances measured by means of FRET, and (ii) restriction of the distribution functions of fluorophores is due to mutual steric exclusion between fluorophores and the atoms of proteins. The atomic coordinates (pdb file 2btf) of residues 1–350 of actin bound to profilin (*11*) were used to construct an actin model: Residues 351–375 were not included to avoid bias in determining the position of Cys374. The three-dimensional space was divided into voxels of 2 Å × 2 Å × 2 Å. The fluorescent molecule attached to Cys374 of actin was assumed to be a small sphere with a diameter of 2 Å. According to the probabilistic distance geometry method (*15*), each of four points (Lys-61, Tyr-69, C5 of an adenine nucleotide, and a divalent cation), of which the positions are determined from the crystal structure, was assumed to be connected with the fluorophore of Cys374 by a spring with a spring constant of $k_BT/(5\ Å)^2$ to accommodate experimental errors. After 8 cycles of iterative calculations, the effective spring constants became $k_BT/(1.8\ Å)^2$. Four types of calculations on the basis of four sets of assumptions were performed: Four distances calculated from atomic coordinates (*11*) were used in cases I and II, whereas that calculated by FRET were used in cases III and IV, as shown in Table I and Fig. 1. It is reasonable to assume that fluorescent dyes should be outside of the actin structure: The mutual exclusion between the dye and actin

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Abbreviations: FRET, fluorescence resonance energy transfer; S1, subfragment 1.

TABLE I. **The location of actin Cys374 calculated with a probabilistic distance geometry method.**

	Distances calculated from 2btf (Å)	Distances measured by FRET (Å)	Expected distances calculated from the distances from 2btf (Å)		Expected distances calculated from the distances from FRET (Å)	
			No mutual exclusion (Case I)	Mutual exclusion (Case II)	No mutual exclusion (Case III)	Mutual exclusion (Case IV)
C374-K61(NZ)	37	46	37 (37)	38 (40)	44 (44)	43 (43)
C374-Y69	29	25	28 (28)	25 (25)	28 (28)	27 (27)
C374-NucC5	30	29	30 (31)	32 (32)	29 (29)	32 (31)
C374-Cation	18	23	18 (18)	27 (24)	23 (24)	24 (24)
Rms			0.1 (0.4)	4.7 (3.7)	1.8 (1.7)	2.5 (2.3)
C374 coordinates (Å)	(32, 75, 61)		(34, 73, 60) [(36, 74, 60)]	(52, 57, 60) [(52, 58, 60)]	(49, 54, 70) [(50, 52, 70)]	(52, 57, 64) [(52, 56, 66)]

The values in the upper four rows show the averaged distances between the probable position of Cys374 and the four known points, whereas the values in parentheses indicate the distances between the points with maximum probability and the known coordinates. The rms values show the root mean squares of the differences between the distance given for examination and that calculated by the examination. In the bottom row, the expected coordinates of Cys374 on our analysis are shown, whereas the values in square brackets indicate the coordinates with maximum probability. The fluorescent dyes attached to the Cys374 of actin for measurement of distances by FRET (*4*) were *N*-[4-[[4-(dimethylamino)phenyl]azo]phenyl]maleimide (DABMI), *N*-[4-(dimethylamino)-3,5-dinitrophenyl]maleimide (DDPM), and 5-{2-[(iodoacetyl)-amino]ethyl}amino naphthalene-1-sulfonic acid (1,5-IAEDANS). The size of the fluorescent dyes was ~10 Å.

due to steric hindrance was assumed in cases II and IV. As a control, it was not assumed in cases I and III.

Table I shows the computed coordinates and distances in four cases. In Fig. 1 the probability distribution functions of the fluorophore-labeled Cys374 are represented by surface rendering superimposed on a backbone model of actin. The position of Cys374 could be determined in the vicinity of one of the β-strands in subdomain 1 of actin (*10–12*) with an accuracy of 2 Å in case I (Fig. 1a): The fluorophore should be inside of the contour surface with 80% probability and the extent of surface along the long axis was ~2 Å. This shows that the position of Cys374 revealed by the algorithm could be consistent with that determined with a conventional triangulation method. The distance between a sulfur atom in the sulfhydryl group (Sγ) of Cys374 of actin and the nearest atom (C_δ of Y133) in a β-strand in subdomain 1 of actin was ~4 Å and the labeled Cys374 would bump to the β-strand, when it is labeled with a bulky fluorescent dye. When mutual exclusion was incorporated in the assumptions (case II, Fig. 1b), Cys374 could not be at an original position, but was found to be shifted by 24 Å towards the back of the outer domain of actin. This indicates that the location of a fluorescent dye labeling Cys374 cannot be near that in crystal structures without labeling (*9–12*).

In case III, where the distances determined through FRET were used, the probabilistic distribution function of Cys374 converged to a different position, which is about 28 Å from that in the crystal structure without labeling, even though mutual exclusion was not assumed. Moreover, the distribution function of actin Cys374 was confined to a more limited region (< 6 Å in diameter) in case IV (Fig. 1d) than in the other cases. Interestingly, the Cys374 location calculated from FRET data was in the neighborhood of that in case II, in which the distances from the crystal structure were used. These findings suggest that the bulky fluorescent dye labeling Cys374 could not be accommodated near the original position of Cys374 in the crystal structure and the Cys374 had to change its location in actin.

The distance between Cys10 (Val10 in 2btf) and Cys374 was ~31 Å on our analysis. This is consistent with FRET (*2–4*) or the atomic coordinates of actin with labeled Cys374 (*14*). However, the distance between Gln41 and Cys374 was 42 Å, *i.e.* different from that determined by means of FRET (30 Å). This may be because the DNase I binding loop, which contains Gln41, is flexible with high temperature factors in the crystal (*10–12*).

The Cys374 position estimated for one actin protomer only (2btf) in the F-actin configuration is 10 Å (S.D. 3 Å) from the axis of the actin helix. In contrast, when other protomers in the F-actin configuration (*12*) were used for calculations, the position was more restricted due to mutual exclusion by other actin protomers: The probable position shifted to 15 Å (S.D. 4 Å) from the axis of the actin helix. This is comparable with the radial position of fluorescently labeled Cys374 (17 Å, S.D. 5 Å), as reported by Moens *et al.* (*1, 5*). The position appeared to be close to that in the crystal structure of actin from smooth muscle, in which Cys374 was labeled (*14*). On the other hand, it is different from that in an F-actin model (27 Å), in which Cys374 is not labeled (*13*). Therefore, it is plausible that the position of Cys374 in the crystal of actin from smooth muscle differed from that in other crystal forms due to the labeling of Cys374 in the former but not due to species specificity. Indeed, in all crystal structures except for that of smooth muscle actin, Cys374 was either absent due to proteolysis or unlabeled.

On our calculation, the fluorescent dye-labeled Cys374 in F-actin was found to be positioned in the groove of the actin helix, where three actin protomers meet, as shown in Fig. 1 (e, f). The residues of the three protomers, (i) residues 72–77, 112, and 177–179 of the actin with the fluorophore, (ii) residues 193–206 and 237–249 of the barbed-end actin, and (iii) residues 276–290 and 316–327 of the pointed-end actin, were within 10 Å from the fluorophores. Phalloidin also binds to a similar region of actin filaments. This is consistent with cross-linking between phalloidin and Cys374, as reported by Faulstich *et al.* (*17*). The distance between Gly366 and the determined position of the fluorophore-labeled Cys374 was 30 Å. When the nine residues starting from Gly366, *i.e.* 366–374, are extended, they can be 27 Å in length. Thus we anticipate that the C-terminal region of actin would be in an extended form, when Cys374 is labeled with a bulky reagent. Also, the secondary structure of the sequence containing 12 residues of the C-terminal end was predicted to be either extended or looped. Furthermore, labeling of Cys374 and deletion of the last residue, Phe375, induced destabilization of F-actin as to shearing

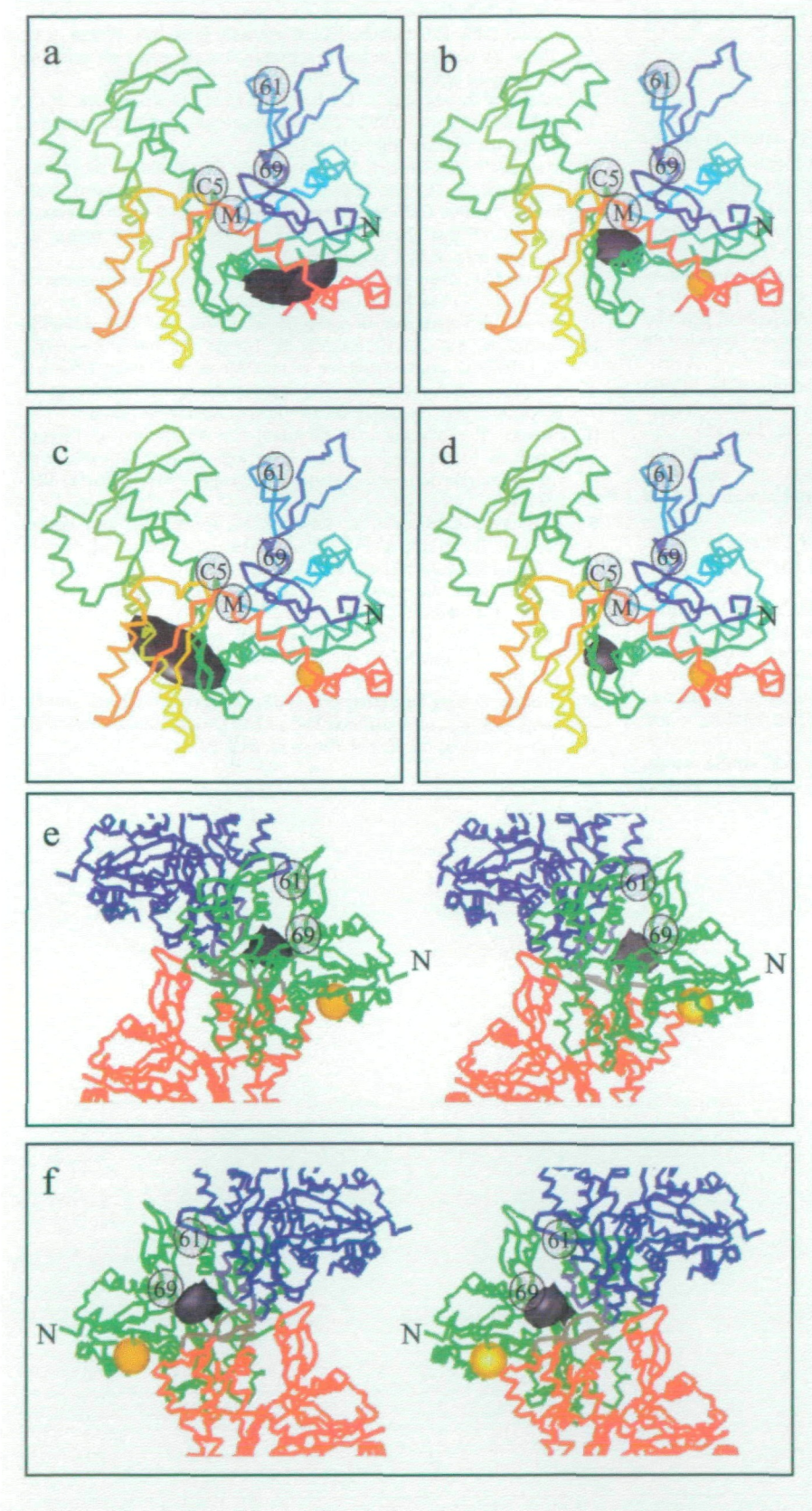

Fig. 1. **The probable location of Cys374 of actin.** In (a–d), the distribution functions of the fluorophore-labeled Cys374 of actin in the four cases, cases I–IV in Table I, are shown as solid contour surfaces. The four distances calculated from atomic coordinates by X-ray crystallography were used in case I (a) and case II (b), whereas those calculated by FRET were used in case III (c) and case IV (d). Mutual exclusion was assumed in case II (b) and case IV (d), whereas it was not assumed in case I (a) and case III (c). The backbone structure of actin (*11*) is shown in colour (N-terminus in blue, and C-terminus in red). The orange spheres indicate the position of Cys374 of actin in the crystal (*11*). Stereo pairs in (e) and (f) show the probable location of the fluorophore-labeled Cys374 in F-actin. The solid contour surfaces (in dark blue) indicate the location of the fluorophore-labeled Cys374 of the green actin protomer, with the yellow sphere indicating the position of Cys374 in F-actin determined in X-ray studies (*13*). The backbone models in red, green, and blue represent three actin protomers. The parts of backbone models in light gray represent amino residues (72–77, 112, and 177–179 in green actin; 193–206 and 237–249 in red actin; 276–290 and 316–327 in blue actin), which were within 10 Å from the determined location of Cys374. The contour level of solid surfaces (dark blue) indicates 80% confidence as to the location of actin Cys374.

stress and an increase in the critical concentration, as reported by Drewes and Faulstich (*18*). In the crystal (*12*), the carboxyl group at the C-terminus forms hydrogen bonds with waters and the other residues to stabilize the C-terminal region. Therefore, the introduction of a bulky reagent to Cys374 could disrupt such hydrogen bonds at the

C-terminus of actin and induce conformational changes in the C-terminal region of actin.

REFERENCES

1. Moens, P.D., Yee, D.J., and dos Remedios, C.G. (1994) Determination of the radial coordinate of Cys374 in F-actin using fluorescence resonance energy transfer spectroscopy: effect of phalloidin on polymer assembly. *Biochemistry* **33**, 13102–13108
2. dos Remedios, C.G. and Moens, P.D. (1995) Fluorescence resonance energy transfer spectroscopy is a reliable "ruler" for measuring structural changes in proteins. Dispelling the problem of the unknown orientation factor. *J. Struct. Biol.* **115**, 175–185
3. Miki, M. (1991) Detection of conformational changes in actin by fluorescence resonance energy transfer between tyrosine-69 and cysteine-374. *Biochemistry* **30**, 10878–10884
4. Miki, M., O'Donoghue, S.I., and dos Remedios, C.G. (1992) Structure of actin observed by fluorescence resonance energy transfer spectroscopy. *J. Mus. Res. Cell Motil.* **13**, 132–145
5. Moens, P.D. and dos Remedios, C.G. (1997) A conformational change in F-actin when myosin binds: fluorescence resonance energy transfer detects an increase in the radial coordinate of Cys-374. *Biochemistry* **36**, 7353–7360
6. Taylor, D.L., Reidler, J., Spudich, J.A., and Stryer, L. (1981) Detection of actin assembly by fluorescence energy transfer. *J. Cell Biol.* **89**, 362–367
7. Kasprzak, A.A., Takashi, R., and Morales, M.F. (1988) Orientation of actin monomer in the F-actin filament: radial coordinate of glutamine-41 and effect of myosin subfragment 1 binding on the monomer orientation. *Biochemistry* **27**, 4512–4522
8. Milligan, R.A., Whittaker, M., and Safer, D. (1990) Molecular structure of F-actin and location of surface binding sites. *Nature* **348**, 217–221
9. Kabsch, W., Mannherz, H.G., Suck, D., Pai, E.F., and Holmes, K.C. (1990) Atomic structure of the actin: DNase I complex. *Nature* **347**, 37–44
10. McLaughlin, P.J., Gooch, J.T., Mannherz, H.G., and Weeds, A.G. (1993) Structure of gelsolin segment 1-actin complex and the mechanism of filament severing. *Nature* **364**, 685–692
11. Schutt, C.E., Myslik, J.C., Rozycki, M.D., Goonesekere, N.C., and Lindberg, U. (1993) The structure of crystalline profilin-beta-actin. *Nature* **365**, 810–816
12. Matsuura, Y., Stewart, M., Kawamoto, M., Kamiya, N., Saeki, K., Yasunaga, T., and Wakabayashi, T. (2000) Structural basis for the higher Ca^{2+}-activation of the regulated actin-activated myosin ATPase observed with Dictyostelium/Tetrahymena actin chimeras. *J. Mol. Biol.* **296**, 579–595
13. Lorenz, M., Popp, D., and Holmes, K.C. (1993) Refinement of the F-actin model against X-ray fiber diffraction data by the use of a directed mutation algorithm. *J. Mol. Biol.* **234**, 826–836
14. Sasaki, K., Sakabe, K., Sakabe, N., Kondo, H., and Shimomura, M. (1992) Crystal structure of smooth muscle actin DNase I complex at 2 Å resolution. *The 4th International Conference on Biophysics and Synchrotron Radiation,* Abstracts F1-03
15. Suzuki, Y., Yasunaga, T., Ohkura, R., Wakabayashi, T., and Sutoh, K. (1998) Swing of the lever arm of a myosin motor at the isomerization and phosphate-release steps. *Nature* **396**, 380–383
16. Yasunaga, T., Suzuki, Y., Ohkura, R., Sutoh, K., and Wakabayashi, T. (2000) ATP-induced transconformation of myosin revealed by determining three-dimensional positions of fluorophores from fluorescence energy transfer measurements. *J. Struct. Biol.* **132**, 6–18
17. Faulstich, H., Zobeley, S., Heintz, D., and Drewes, G. (1993) Probing the phalloidin binding site of actin. *FEBS Lett.* **318**, 218–222
18. Drewes, G. and Faulstich, H. (1993) Cooperative effects on filament stability in actin modified at the C-terminus by substitution or truncation. *Eur. J. Biochem.* **212**, 247–253