FEBS Letters 383 (1996) 105–108

# Conformational changes in subdomain I of actin induced by proteolytic cleavage within the DNase I-binding loop: energy transfer from tryptophan to AEDANS

Irina Kuznetsova*, Olga Antropova, Konstantin Turoverov, Sofia Khaitlina

*Institute of Cytology, Russian Academy of Sciences, Tikhoretsky av. 4, 194064 St. Petersburg, Russia*

Received 15 January 1996; revised version received 20 February 1996

**Abstract Alteration of the actin polypeptide chain within the DNase I-binding loop by cleavage with *E. coli* A2 protease or subtilisin was shown to increase the efficiency of energy transfer from tryptophan residues to AEDANS attached to Cys-374. Analysis of structural and fluorescence data suggested that only two of four actin tryptophan residues, namely, Trp-340 and/or Trp-356, can be energy transfer donors. It was also found that labelling with AEDANS induces perturbations in the environment of the tryptophan residues, these perturbations being smaller in the cleaved actin. These changes are consistent with a shift of the C-terminal segment of actin monomer upon cleavage and confirm the existence of high conformational coupling between subdomains 1 and 2 of actin monomer. We also suggest that tryptophan residues 340 and/or 356 are located in the focus of this coupling.**

*Key words:* Actin; Actin proteolysis; Tryptophan fluorescence; Energy transfer

## 1. Introduction

Cleavage of G-actin between residues 42 and 43 within the DNase I-binding loop in subdomain 2 with the *E. coli* A2 protease (ECP 32) [1] has been shown to induce changes in the structure of F-actin which involve the C-terminal part of the monomer. The excitation spectrum of the cleaved pyrenyl-F-actin is devoid of the peak at 365 nm arising in intact actin from interaction of the pyrenyl label attached to the penultimate Cys-374 with side chains of an adjacent polymer subunit [2]. Treatment of the cleaved F-actin with the bifunctional sulfhydryl reagent *N,N'*-1,2-phenylenebismaleimide does not produce dimers which appear in intact F-actin as a result of cross-linking of Cys-374 with Lys-191 of the adjacent subunit [2]. The three-dimensional reconstruction of actin filaments demonstrates a bridge of density formed in the cleaved but not in intact actin between the C-terminus of one monomer and subdomain 4 of another [3]. The aim of this work was to establish whether these changes appear only upon polymerization or take place in G-actin. For this purpose, we have used nonradiative resonance energy transfer from intrinsic tryptophan residues of actin to AEDANS attached to Cys-374. This allowed us to compare the position of the C-terminus in intact and in proteolytically modified actins.

*Corresponding author. Fax: (7) (812) 247-0341.

*Abbreviations:* IAEDANS, *N*-iodoacetyl-*N*-(5-sulfo-1-naphthyl)ethylenediamine; PMSF, phenylmethylsulfonylfluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

## 2. Materials and methods

### 2.1. Protein preparations

Actin was prepared from rabbit skeletal muscle as described in [1]. Labelling of Cys-374 with 1,5-IAEDANS was performed according to [4]. The concentration of G-actin was determined spectrophotometrically using an extinction coefficient of 0.63 mg $ml^{-1}$ $cm^{-1}$ at 290 nm [5]. The concentration of 1,5-AEDANS-labelled actin was calculated after correction for the absorbance of the label at 290 nm by subtracting $0.21 \times D_{337}$ [4]. The concentration of 1,5-AEDANS attached to actin was determined spectrophotometrically using an extinction coefficient of $6.0 \times 10^3$ $M^{-1}$ $cm^{-1}$ at 337 nm [6].

### 2.2. Proteolytic digestion

Actin was cleaved by *E. coli* A2 protease ECP 32 [1] at an enzyme/protein mass ratio of 1 : 100. The digestion was carried out for 14 h at 4°C. Cleavage of actin with subtilisin was performed at an enzyme/protein mass ratio of 1 : 1000 for 7 min at room temperature. The reaction was stopped by the addition of 1 mM PMSF. The cleavage products were analysed by SDS-PAGE [7]. Integrity of the cleaved molecules with which the C-terminal core and N-terminal 8 kDa fragments remain associated as long as the cleaved actin contains a tightly bound cation [2,8] was proved by the presence of both fragments in the samples obtained after a cycle of polymerization-depolymerization (Fig. 1). The native-like structure of ECP and subtilisin-cleaved actins was routinely controlled by intrinsic fluorescence using the parameter $A = I_{320}/I_{365}$ as a measure of actin nativity [1,9]. Upon cleavage, the parameter $A$ remained practically unchanged (2.56, 2.52 and 2.54 for intact, subtilisin-and ECP-cleaved actins, respectively).

### 2.3. Fluorescence measurements

Fluorescence measurements were carried out on the spectrofluorimeter described earlier [9]. Transfer efficiency ($E$) was determined from measurements of the relative decrease in donor fluorescence (Eq. 1) and of the acceptor sensitized fluorescence (Eq. 2):

$$E = \frac{1}{\alpha}\left(1 - \frac{I_D}{I_{D,0}} \cdot \frac{D_{D,0}}{D_D}\right) \quad (1)$$

$$E = \frac{1}{\alpha}\left(\frac{I_A(\lambda_2)}{I_A(\lambda_1)} \cdot \frac{D_A(\lambda_1)}{D_D(\lambda_2)} - \frac{D_A(\lambda_2)}{D_D(\lambda_2)}\right). \quad (2)$$

In both equations $\alpha$ is the labeling coefficient. In Eq. 1, $I_{D,0}$ and $I_D$ denote the fluorescence intensity of donor in the absence and presence of acceptor, respectively. $D_{D,0}$ and $D_D$ are the corresponding values of the optical density. In Eq. 2, $I_A(\lambda_i)$ and $D_A(\lambda_i)$ are the fluorescence intensity and optical density of acceptor at the wavelength where only acceptor absorbs ($\lambda_1$) and at the wavelength where both donor and acceptor absorb ($\lambda_2$), respectively, and $D_D(\lambda_2)$ is the donor absorption at the wavelength $\lambda_2$. These equations take into consideration two complicating factors: incomplete labelling of actin molecules by AEDANS and the impossibility of a separate donor excitation because of the overlap of the AEDANS absorption spectrum with that of tryptophan (Fig. 2, curves 1,2).

## 3. Results

To determine structural changes at the C-terminus of G-


*SSDI* S0014-5793(96)00238-4

actin caused by proteolytic modification within the DNase I-binding loop, nonradiative energy transfer from tryptophan residues of actin to AEDANS attached to Cys-374 was used. As seen in Fig. 2, the absorption spectrum of AEDANS (curve 2) overlaps with the fluorescence spectrum of intrinsic tryptophan residues of actin (curve 3), making nonradiative resonance energy transfer from tryptophan residues to protein-conjugated AEDANS possible. This process causes a decrease in tryptophan fluorescence intensity (curves 3,4) and leads to the appearance of sensitized fluorescence of AEDANS (curve 5). As structural modification is always possible on protein labelling, it is essential that in this work at least the donor is an intrinsic amino acid of the protein. At the same time, modification of the protein structure caused by the conjugation of fluorescent label can be estimated from the energy transfer method itself when we compare the transfer efficiency ($E$) determined from measurements of the relative decrease of donor fluorescence with that determined from measurements of the acceptor sensitized fluorescence (Eqs. 1,2) The efficiency of energy transfer determined by both procedures should be the same if the labelling process does not induce conformational changes of the macromolecule which might alter the environment of the donor. Otherwise, the value of $E$ determined from the decrease in donor fluorescence (Eq. 1) will not be accurate [10].

The results obtained for actin are given in Table 1. The transfer efficiency determined from the decrease in donor fluorescence turned out to be much greater than that from the sensitized acceptor fluorescence for both intact and cleaved actin. This means that AEDANS labelling induces structural changes in the environment of tryptophan residues which, in turn, lead to additional quenching of tryptophan fluorescence. These results once again confirm that caution is needed when using modified proteins in studies on structure/function relationship in actin [11]. They also indicate that comparison of the transfer efficiency obtained from the donor and acceptor fluorescence can provide additional structural information.


Fig. 1. SDS-PAGE of ECP-and subtilisin-cleaved G-actins after a cycle of polymerization-depolymerization. Lane 1, intact actin; lanes 2 and 3, ECP- and subtilisin-cleaved G-actins, respectively.

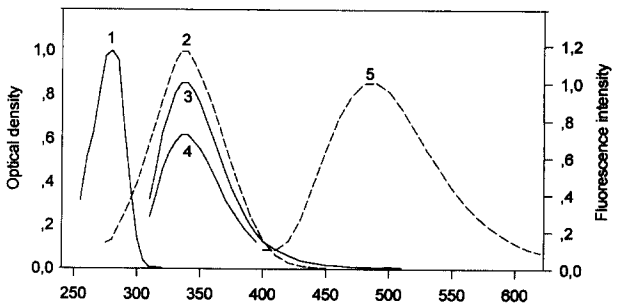


Fig. 2. Absorption and fluorescence spectra of donor (tryptophan residues of actin) and acceptor (AEDANS attached to actin). (1,2) Absorption spectra of tryptophan residues of actin and AEDANS attached to Cys-374; (3,4) fluorescence spectra of unlabelled actin and actin labelled with AEDANS; (5) sensitized fluorescence spectrum of AEDANS attached to Cys-374.

In actin cleaved with ECP 32 or subtilisin, the efficiency of energy transfer determined from measurements of the sensitized acceptor fluorescence is increased compared to that of intact actin (Table 1). The difference corresponds to a change in the donor-acceptor distance from 28.7 to 26.7 Å for intact and cleaved actin, respectively. In contrast, the energy transfer efficiency determined from the donor fluorescence is decreased in the cleaved actin in comparison with that in native actin. Thus, the difference between the values of transfer efficiency determined by the two ways is smaller in cleaved than in intact actin. This means that in the cleaved actin the additional quenching of tryptophan fluorescence by AEDANS is reduced.

The observed structural changes can be localized if we define the contribution of each of four tryptophan residues (Fig. 3) in the processes of the energy transfer. Analysis of the spatial structure of actin [12,13] has revealed that all tryptophan residues are not farther than 20 Å from the C-terminal residue 372 and can contribute to the energy transfer. At the same time, these four tryptophan residues are located in different microenvironments: Trp-340 and Trp-356 are buried while Trp-79 and Trp-86 are partially exposed. This means that the fluorescence spectra of the first two residues must be blue, while that of the others must be more red. In addition, three atoms of serum, which is known to be a strong quencher of tryptophan fluorescence, are in close vicinity to Trp-79 and Trp-86 (Table 2). Therefore, the quantum yield of

Table 1
Efficiency of energy transfer in the intact and proteolytically cleaved actin determined from the decrease in donor fluorescence (D) and from the sensitized acceptor fluorescence (A)

| G-Actin | D | A |
|---|---|---|
| Intact | 0.90 | 0.17 |
| ECP-cleaved | 0.78 | 0.24 |
| Subtilisin-cleaved | 0.80 | 0.24 |

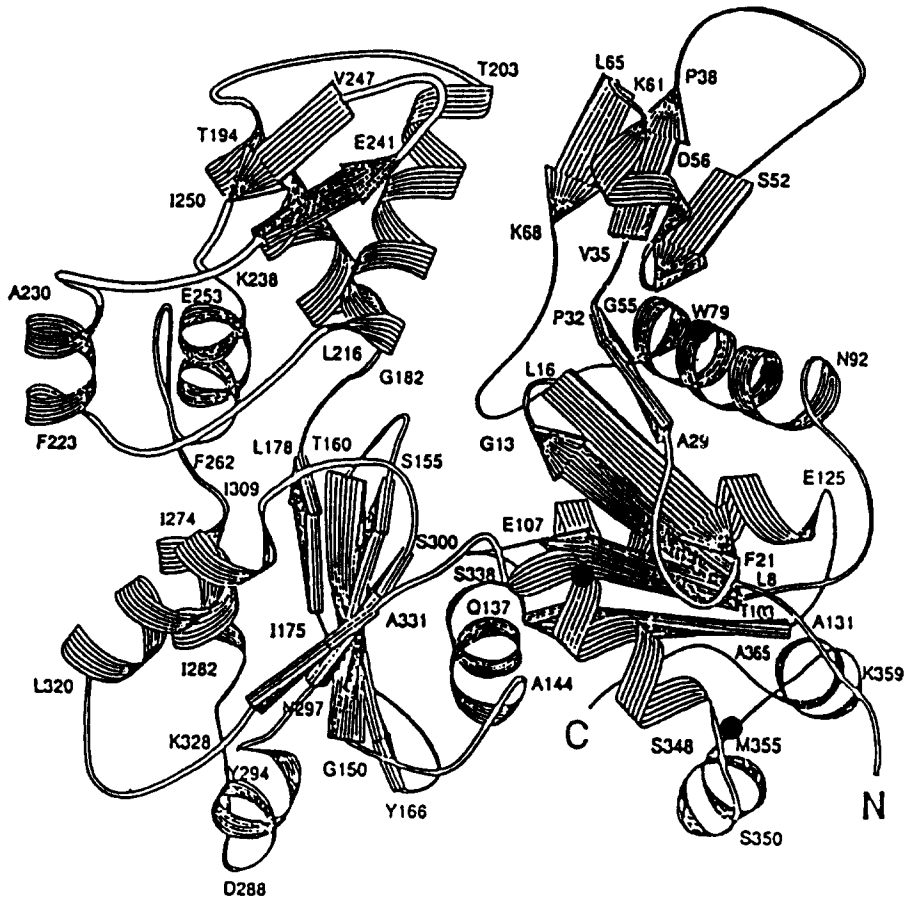


Fig. 3. Localization of tryptophan residues 79, 86, 340 and 356 in the three-dimensional structure of actin [12].

these red spectrum tryptophan residues must be small. Hence, the fluorescence spectrum of actin must mainly be formed by the buried blue spectrum tryptophan residues Trp-340 and Trp-356. This conclusion is consistent with the fluorescence experimental data: the blue position of the actin fluorescence spectrum with a maximum at 320 nm, and the low value of the quenching constant obtained for actin upon acrylamide quenching ($K_{SV}$=1.8 $M^{-1}$) [14], which is typical of the buried tryptophan residues of proteins. If actin fluorescence is determined by Trp-340 and Trp-356, we can conclude that it is these tryptophan residues that participate in the fluorescence energy transfer.

## 4. Discussion

The results presented in this work show that alterations of the actin polypeptide chain induced by proteolysis within the DNase I-binding loop with both the *E. coli* A2 protease (between Gly-42 and Val-43) and subtilisin (between Met-47 and Gly-48) can be transmitted to subdomain I. It is found that cleavage within the DNase loop induces a decrease in the distances between Trp-340/356 and the C-terminal segment. It can be due to a shift of the C-terminal segment or, alternatively, to a perturbation of Trp-340/356 themselves or their microenvironments. Both effects are consistent with the literature data. Thus, the cross-linking experiments have revealed that in ECP-cleaved F-actin Cys-374 cannot be cross-linked with Lys-191 of the adjacent subunit while cross-linking of this cysteine with some other residue within the same and another subunit takes place [2]. At the same time, exposure of the site 359–360 located in the vicinity of Trp-340/Trp-356 was observed upon actin binding with DNase I [11]. If per-

Table 2
Tryptophan residue microenvironments

| Trp | $N$ | Access to solvent | Quenching groups | $R$ (Å) |
|---|---|---|---|---|
| Trp-79 | 59 | partially exposed | $S_\delta$ Met-115 | 6.9 |
| Trp-86 | 63 | partially exposed | $S_\gamma$ Cys-10 | 5.8 |
| | | | $S_\delta$ Met-82 | 4.1 |
| | | | $S_\delta$ Met-119 | 6.9 |
| Trp-340 | 87 | buried | | |
| Trp-356 | 78 | buried | | |

$N$, number of atoms in the sphere with the centre in the centre of the indole ring and radius equal to 7 Å. $R$, distance from the centre of the indole ring (Å).

turbations of the Trp-340/356 environment take place they could also account for the decrease in the effect of AEDANS labeling on the tryptophan residues observed in the cleaved actin.

Our results show that the environment of Trp-340/356 is perturbed upon attachment of AEDANS to Cys-374. These tryptophan residues are in close proximity to residues 137 and 339 which are predicted to be a molecular hinge responsible for opening and closing the nucleotide cleft [15]. This hinge and its surrounding were suggested to transmit conformational changes from the C-terminal segment to the nucleotide cleft [11]. On the other hand, the conformational relationship of the nucleotide cleft with the DNase I-binding loop [4,11,16] as well as with the 18–29 segment [17] has been reported. Reciprocal communication from the C-terminus to the DNase I binding loop also seems possible as in C-terminal truncated actin cleavage with subtilisin slows down [18]. These data, taken together, provide evidence for high conformational coupling within the 'small' domain, which is responsible for interaction with many actin-binding proteins. The position of the intrinsic tryptophan residues in the focus of these transitions could explain the high sensitivity of tryptophan fluorescence to actin polymerization and modifications of the polypeptide chain [19,20].

*Acknowledgements:* We wish to thank A. Morozova for generously providing ECP 32. This work was supported by grants R 31000 and R31300 from the International Science Foundation.

## References

[1] Khaitlina, S.Yu., Collins, J.H., Kuznetsova, I.M., Pershina, V.P., Sinakevich, I.G., Turoverov, K.K. and Usmanova, A.M. (1991) FEBS Lett. 279, 49–51.
[2] Khaitlina, S.Yu., Moraczewska, J. and Strzelecka-Golaszewska, H. (1993) Eur. J. Biochem. 218, 911–920.
[3] Orlova, A. and Egelman, E.H. (1995) J. Mol. Biol. 245, 582–597.
[4] Strzelecka-Golaszewska, H., Moraczewska, J., Khaitlina, S.Yu. and Mossakowska, M. (1993) Eur. J. Biochem. 211, 731–742.
[5] Houk, W.T. and Ue, K. (1974) Anal. Biochem. 62, 66–74.
[6] Frieden, C., Liberman, D. and Gilbert, H.R. (1980) J. Biol. Chem. 255, 8991–8993.
[7] Laemmli, U.K. (1970) Nature 227, 680–685.
[8] Schwyter, D., Phillips, M. and Reisler, E. (1989) Biochemistry 28, 5889–5895.
[9] Turoverov, K.K., Khaitlina, S.Yu. and Pinaev, G.P. (1976) FEBS Lett. 62, 4–7.
[10] Schiller, P.W. (1975) in: Biochemical Fluorescence: Concepts, vol.1 (Chen, R. and Edelhoch, H. eds.) pp. 285–303, Marcel Dekker, New York.
[11] Crosbie, R.H., Miller, C., Cheung, P., Goodnight, T., Muhlrad, A. and Reisler, E. (1994) Biophys. J. 67, 1557–1564.
[12] Kabsch, W., Mannherz, H.G., Suck, D., Pai, E.F. and Holmes, H.C. (1990) Nature 347, 37–44.
[13] McLaughlin, P.J., Gooch, J.T., Mannherz, H.C. and Weeds, A.G. (1993) Nature 364, 685–692.
[14] Kuznetsova, I.M., Khaitlina, S.Yu. and Turoverov, K.K. (1995) J. Muscle Res. Motil. 16, 150.
[15] Tirion, M.M. and Ben-Avraham, D. (1993) J. Mol. Biol. 230, 186–195.
[16] Kim, E., Motoki, M., Seguro, K., Muhlrad, A. and Reisler, E. (1995) Biophys. J. 69, 2024–2032.
[17] Mejean, C., Hue, H.K., Pons, F., Roustan, C. and Benyamin. (1988) Biochem. Biophys. Res. Commun. 152, 368–375.
[18] Strzelecka-Golaszewska, H., Mossakowska, M., Wozniak, Moraczewska, J. and Nakayama, H. (1995) Biochem. J. 307, 527–534.
[19] Lehrer, S.S. and Kerwar, G. (1972) Biochemistry 11, 1211–1217.
[20] Kuznetsova, I.M., Khaitlina, S.Yu., Konditerov, S.N., Surin, A.M. and Turoverov, K.K. (1988) Biophys. Chem. 32, 73–78.