Internal actin filament dynamics in the presence of vinculin: a dynamic light scattering study

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Abstract Analyses of dynamic light scattering data by stretched exponential fit show that vinculin has a negligible influence on internal actin filament dynamics and actin bending stiffness which contrasts with our previous observations with talin, another actin and vinculin-binding protein from focal adhesions. The results here agree with kinetic and rheologic measurements.

Key words: Actin; Vinculin; Dynamic light scattering

1. Introduction

Vinculin is a major protein component of focal adhesions and cell–cell attachments [1]. It not only binds to actin in vitro [2,3] but also to other membrane-associated proteins [4,5]. Furthermore, vinculin interacts with phospholipids electrostatically and inserts into the hydrophobic domain of lipid bilayers [6].

In recent steady-state polymerization experiments and transient kinetic binding studies, as well as in rheological measurements, we have shown that the binding of vinculin to actin ($K_d \sim 0.5\,\mu M$) neither interferes with the actin polymerization nor greatly influences the viscoelasticity of actin networks [7,8]. Here, we examine the influence of vinculin on the internal actin filament dynamics by dynamic light scattering, using a stretched exponential fit to the data.

2. Materials and methods

2.1. Reagents

G buffer contained 2 mM Tris-HCl, pH 7.4, 0.2 mM ATP, 0.2 mM DTT, 0.2 mM $CaCl_2$ and 0.05 vol% NaN_3 at 20°C. F buffer contained 2 mM Tris-HCl, pH 7.4, 0.5 mM ATP, 0.2 mM DTT, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 100 mM KCl and 0.05 vol% NaN_3 at 20°C.

2.2. Proteins

Actin was prepared according to Spudich and Watt [9] from acetone powder obtained from rabbit back muscle, followed by a gel-filtration step, as described by MacLean-Fletcher and Pollard [10]. Fractionated G-actin beyond the elution peak (at ~1 mg/ml) was stored in G buffer at 4°C.

Vinculin was isolated from outdated (not older than 10 days) human thrombocytes. After the first ionic exchange column, vinculin was purified by an additional hydroxylapatite column and eluted by a linear gradient of 0.02 to 0.4 M KH_2PO_4 [7].

2.3. Dynamic light scattering

The basic set-up of the light scattering apparatus was as described by Piekenbrock and Sackmann [11] with the following modifications: The 488 nm spectral line of an Innova 70-4 (Coherent) laser with an output of ~1.2 W was used as the light source. Signal detection was achieved by an autocorrelator ALV 3000 supplied by ALV, Langen, Germany. This system allowed the recording of photon autocorrelation functions in real time on a linear time scale of 1024 channels covering a time span from 10 μs up to ~60 ms within a single measurement. A purpose-written computer programme based on the commercially available IGOR Pro software (supplied by WaveMetrics) was used for analysis on an Apple MacIntosh.

Prior to experimentation, cylindrical screw-cap test tubes of ~1.5 cm diameter were cleaned with 0.1 M NaOH and exhaustively rinsed with deionized filtered water. In order to perform measurements in a dust-free environment, G-actin, vinculin and F buffer were passed through a sterile filter (0.2 μm) into the pre-cleaned test tubes which were immediately closed again and left overnight at 4°C. Approx. 1 h before experiments were started, the samples were equilibrated to the measuring temperature of 25 ± 0.1°C in an external water bath.

3. Results

In the present dynamic light scattering studies we have measured the autocorrelation function of the scattered light intensity at different angles which gives information about the internal polymer dynamics of actin filaments after incubation with vinculin. For pure actin, which is known to form long and semiflexible macromolecules of ~7 nm in diameter and up to 40 μm in length, its bending elasticity, κ determines the dynamics in the 10^{-6}–10^{-3} s time regime. Farge and Maggs [12], in a theoretical approach, used the model of semiflexible polymers with the bending elasticity, κ describing the hydrodynamic interaction by an averaged Oseen tensor. It could be shown that this approach is appropriate to explain the properties of semidilute actin solutions. The above authors predict the correct form and the scaling behaviour of the autocorrelation function $g(q,t)$ used in our experiments. The function $g(q,t)$ is a stretched exponential:

$$g(q,t) \approx g(q,0) \exp\left(-\frac{k_B T}{\kappa^{0.25}} c_0\, q^2 t^{0.75}\right)$$

where the scatter vector,

$$q = \frac{4\pi n}{\lambda} \sin \vartheta/2$$

and, c_0 is a constant derived from the hydrodynamic interaction. The amplitude $g(q,0)$ can be normalized to 1 so that the only free parameter is the bending elasticity, κ. A more detailed description of this analysis is given in a subsequent paper [13].

Prior to actin measurements in the presence of vinculin, the accuracy of this assumption was tested by measuring a dilute solution of pure actin at 0.2 mg/ml concentration. The complete set of results is readily explained by applying the above equation: first, the fit is accurate over the entire time range. Second, the dependency on the scatter angle, ϑ follows a strict q^2 law. Third, the bending elasticity derived from these experiments of

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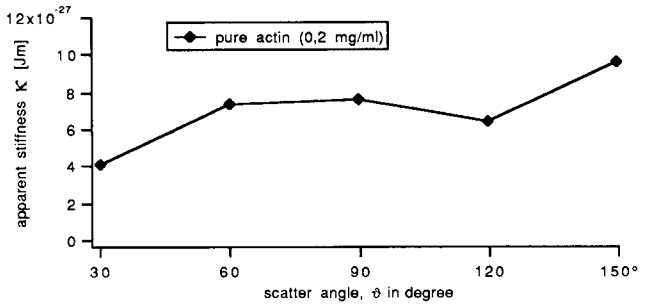

Fig. 1. The apparent stiffness, κ, at various scatter angles, ϑ, of an F-actin solution. c_{actin} = 0.2 mg/ml.

$\sim 1 \cdot 10^{-26}$ Jm is in good agreement with values determined by other methods [14] (Fig. 1). In all subsequent experiments, these measurements were highly reproducible (data not shown).

In order to gain insight into the scatter behaviour of an actin solution incubated with vinculin at a molar ratio of 2:1, three samples were measured at three scatter angles, ϑ. Fig. 2 shows a plot of the normalized dynamic structure factor against decay time in seconds at a scatter angle of 90° for 0.3 mg/ml pure actin (top trace) and actin in the presence of vinculin at a molar ratio of 2:1 (bottom trace).

Fitting those dynamic structure factors by a stretched exponential according to Farge and Maggs [12], the bending elasticity, κ, of polymers in solution can be obtained. Plotting κ against various scatter angles, ϑ, for pure actin and actin in the presence of vinculin at a molar ratio of 2:1 indicates that vinculin has only a negligible influence on the internal dynamic behaviour of actin filaments when compared with pure actin filaments (Fig. 3). The slight difference in apparent stiffness at the various scatter angles for actin in the presence of vinculin can be regarded as insignificant since the values are within the standard deviations. In a similar study, using actin and tropomyosin/troponin at a molar ratio of 7:1:1, the bending stiffness of actin filaments showed an increase of ~50% compared to pure actin filaments [13].

4. Discussion

Actin filaments represent semiflexible polymers up to 40 μm in length, allowing the measurement of the internal dynamics of filaments by dynamic light scattering [15]. Since the theoretical background has been formulated, this method now permits better quantitative interpretation of data (cf. [13]).

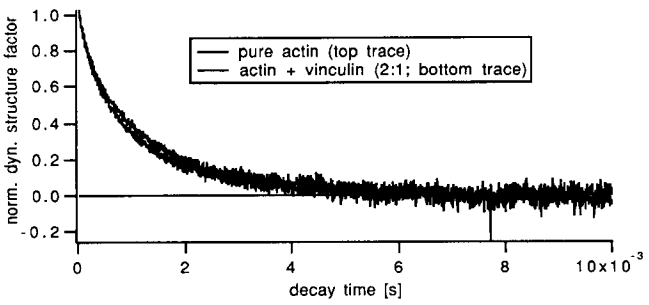

Fig. 2. The normalized dyn. structure factor at various decay times in seconds for pure F-actin solutions (top trace), and for F-actin solutions containing vinculin at a molar ratio of 2:1 (bottom trace). c_{actin} = 0.3 mg/ml.

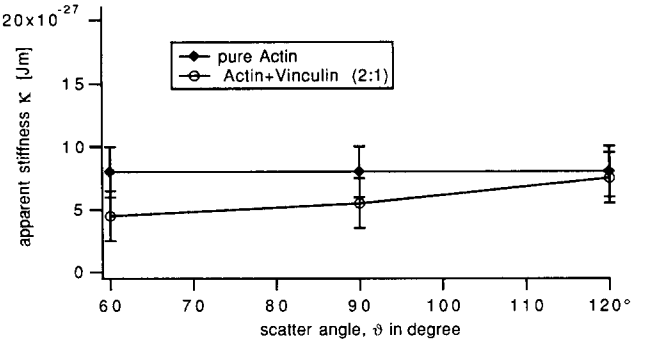

Fig. 3. The apparent stiffness, κ, at various scatter angles, ϑ, of an F-actin solution (top trace) and for an F-actin solution containing vinculin (bottom trace) at a molar ratio of 2:1. c_{actin} = 0.3 mg/ml. The error bars indicate the standard deviation (S.D.) of the reproducibility for three measurements (n = 3) each.

In a previous study, we have demonstrated that talin, a protein closely associated with vinculin in focal adhesions, induces a pronounced increase in filament stiffness when mixed with actin [8,16]. Hence, it was of interest to investigate the internal dynamics of actin filaments in relation to vinculin incubation. Dynamic light scattering data analyzed by a stretched exponential fit show that the influence of vinculin on the flexibility of actin filaments/networks is negligible. This result is in line with previous observations on actin polymerization [7]: in a transient kinetic binding study and in a steady-state actin polymerization assay it could be demonstrated that the binding of vinculin to actin did not significantly affect actin polymerization with respect to its lag phase, polymerization rate and fluorescence amplitude. Further, examination of the effect of vinculin on the viscoelasticity of filamentous actin networks also supports this notion [8]: measuring the storage modulus (G′) as well as the loss modulus (G″) of an actin solution in a frequency-dependent manner in a micro-rheometer showed that the presence of vinculin exhibited insignificant changes of these viscoelastic parameters compared to pure actin solutions.

All in all, the evidence presented by the various methods indicate that the presence of vinculin has little influence, if any, on polymerizing actin and on the viscoelasticity, bending stiffness and internal dynamics of actin filaments. This should be considered when evaluating the function of vinculin during the assembly of actin filaments at focal adhesions and at cell membranes [17].

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References

[1] Otto, J.J. (1990) Cell Motil. Cytoskeleton 16:1–6.
[2] Isenberg, G., Leonard, K. and Jockusch, B.M. (1982) J. Mol. Biol. 158, 231–249.
[3] Ruhnau, K. and Wegner, A. (1988) FEBS Lett. 228, 105–108.
[4] Burridge, K. and Mangeat, P. (1984) Nature 308, 744–746.
[5] Wachsstock, D.H., Wilkins, J.A. and Lin, S. (1987) Biochem. Biophys. Res. Commun. 146, 554–560.

[6] Niggli, V. and Burger, M.M. (1987) J. Membrane Biol. 100, 97–121.
[7] Goldmann, W.H., Niggli, V., Kaufmann, S. and Isenberg, G. (1992) Biochemistry 31, 7665–7671.
[8] Ruddies, R., Goldmann, W.H., Isenberg, G. and Sackmann, E. (1993) Eur. Biophys. J. 22, 309–321.
[9] Spudich, J.A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[10] MacLean-Fletcher, S.D. and Pollard, T.D. (1980) Biochem. Biophys. Res. Commun. 96, 18–27.
[11] Piekenbrock, T. and Sackmann, E. (1992) Biopolymers 32, 1471–1489.
[12] Farge, E. and Maggs, A.C. (1993) Macromolecules 26, 5041–5044.
[13] Götter, R., Kroy, K., Frey, E., Bärmann, M. and Sackmann, E. (1994) Macromolecules (submitted).
[14] Käs, J., Strey, H., Bärmann, M. and Sackmann, E. (1993) Europhys. Lett. 21, 865–870.
[15] Schmidt, C.F., Bärmann, M., Isenberg, G. and Sackmann, E. (1989) Macromolecules 22, 3638–3648.
[16] Goldmann, W.H., Käs, J. and Isenberg, G. (1993) Biochem. Soc. Trans. 22, 46S.
[17] Samuels, M., Ezzell, R.M., Cardozo, T.J., Critchley, D.R., Coll, J.-L., Adamson, E.D. (1993) J. Cell Biol. 12, 909–921.