Vol. 203, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1994 Pages 338-343

ANALYSIS OF FILAMIN-ACTIN BINDING AND CROSS-LINKING/BUNDLING BY KINETIC METHOD

W.H. Goldmann, R. Senger and G. Isenberg

Technical University of Munich
Biophysics, E22
D-85747 GARCHING. FRG.

Received June 28, 1994

The reaction of smooth muscle filamin and skeletal muscle actin was kinetically examined by double exponential analysis. The overall rate of binding, k_{+1}, is concentration and temperature dependent whilst the overall rate of cross-linking/bundling, k_{+2}, is concentration independent. The activation energy, E_a = 99.5 kJ/mol, was calculated from the Arrhenius Plot.

Filamin is a protein of ~160 nm in length which is found in smooth muscle and non-muscle cells. It is a homodimer of ~500 kDa molecular mass with each sub-unit consisting of an actin binding and self-association site. The flexible structure of filamin allows it to cross-link/bundle microfilaments into networks, for review see [1].

Although the binding of filamin to actin and its cross-linking/bundling activity have been reported [2,3], little is known about the influence of temperature on these parameters and their thermodynamic behaviour. In the present study we have determined the overall association rate constants of the actin-filamin complex and the overall rate of cross-linking/bundling at five different temperatures using double exponential analysis.

MATERIALS AND METHODS

Buffers.

F-buffer: 100 mM KCl, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 2 mM Tris-HCl, pH 7.5, 0.2 mM ATP, 0.2 mM DTT (=dithiothreitol), 0.005% NaN_3 were used in all transient kinetic studies.

Proteins.

Filamin was essentially isolated by the Shizuta et al. method [4]. Low ionic strength extraction of chicken gizzard was followed by Mg^{2+}

0006-291X/94 $5.00

and $(NH_4)_2SO_4$ precipitations and ion exchange and gel filtration column chromatographies. For all experiments, an additional hydroxylapatite column was used to further purify this protein. For calculation, 500,000 molecular mass was used.

Actin was prepared according to Spudich and Watt [5] from acetone powder obtained from rabbit back muscle followed by a gel filtration step as described by MacLean-Fletcher and Pollard [6]. Fractionated G-actin beyond the elution peak at approx 24 µM was stored in G-buffer at 4 °C.

Both, actin and filamin were incubated in F-buffer for 2 h prior to experimentation to ensure fully polymerized actin (=F-actin) and filamin in dimeric form.

Stopped flow experiments.

Light scattering measurements were performed using an SF 61 (supplied by Hi-Tech, Salisbury UK) stopped flow spectrophotometer as described by Goldmann and Isenberg [7]. Protein light scattering was measured at an incident wavelength of 355 nm at 3 nm band path width and at a 90° angle.

All kinetic data were analysed on a commercially available Macintosh LC computer program (IGOR), using a double exponential ($\Delta y/\Delta t = a + b*e^{-k_{+1}*t} + c*e^{-k_{+2}*t}$) fitting routine, and printed on a Macintosh LaserWriter.

RESULTS

All filamin-F-actin studies were carried out above stoichiometric concentrations. Following the rapid mixing in the stopped-flow apparatus, the interaction between filamin with F-actin was accompanied by an increase in light scattering (Fig. 1). The light scattering profile clearly deviated from a single exponential, and we therefore attempted detailed analysis by fitting a double exponential ($k_{+1} >> k_{+2}$) to all traces.

The overall rate, k_{+1} of the filamin-F-actin complex formation over a range of filamin concentrations increased linearly which allowed calculation of the association rate constants. A plot of the rate constants against filamin concentration at various temperatures (Fig. 2) yielded the following rate constants: *0.46 x 10^6 M^{-1} x s^{-1} at 25 °C; 0.33 x 10^6 M^{-1} x s^{-1} at 20 °C; 0.14 x 10^6 M^{-1} x s^{-1} at 15 °C; 0.075 x 10^6 M^{-1} x s^{-1} at 10 °C and 0.027 x 10^6 M^{-1} x s^{-1} at 5 °C.* The temperature-dependent individual rate constants were then plotted in an Arrhenius graph to gain some information regarding the binding thermodynamics (Fig. 3). The high activation energy (E_a) of the association reaction (~99.5 kJ/mol) suggests that the filamin-F-actin interaction involves some rearrangement in protein conformation.

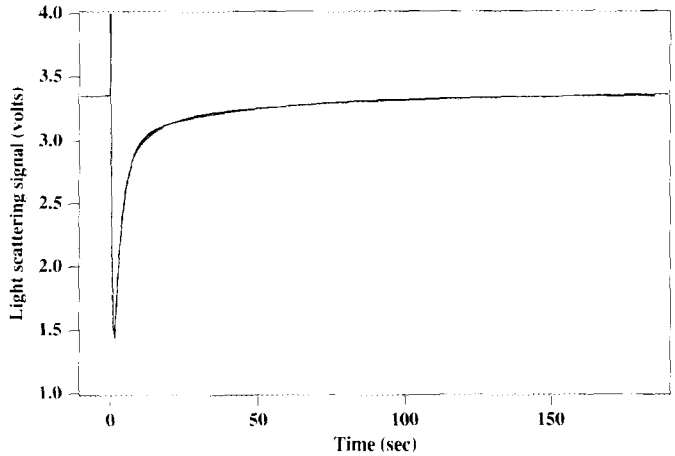

Figure 1. Change in light scattering during the interaction of filamin with F-actin. The interaction of 1 μM filamin with 3 μM F-actin (reaction chamber concentration) at 20 °C produced an increase in light scattering of ~55%. The data were fitted to a double exponential with k_{+1} = 0.3348 (s^{-1}) and k_{+2} = 0.025 (s^{-1}).

The overall rate constant, k_{+2} ($k_{+1} >> k_{+2}$) of the filamin-F-actin species showed negligible filamin concentration dependence, and a plot of the rates between 5 °C and 25 °C (Fig. 4) indicated a rate increase by a factor ~3.5. Since the interaction of actin filaments with filamin produced a large, multiphasic change in light scattering, it is assumed that the filamin concentration dependent overall rate, k_{+1} reflects

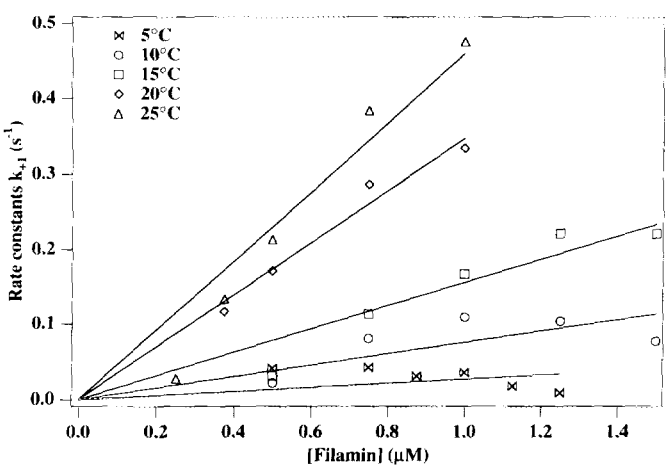

Figure 2. Temperature-dependent overall association rate constants, k_{+1} of filamin and F-actin. The gradient of the lines best fit through zero give the association rate constants at 5°C, 10°C, 15°C, 20°C and 25°C. Buffer: 100 mM KCl, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 2 mM Tris-HCl, pH 7.5, 0.2 mM ATP, 0.2 mM DTT, 0.005% NaN_3.

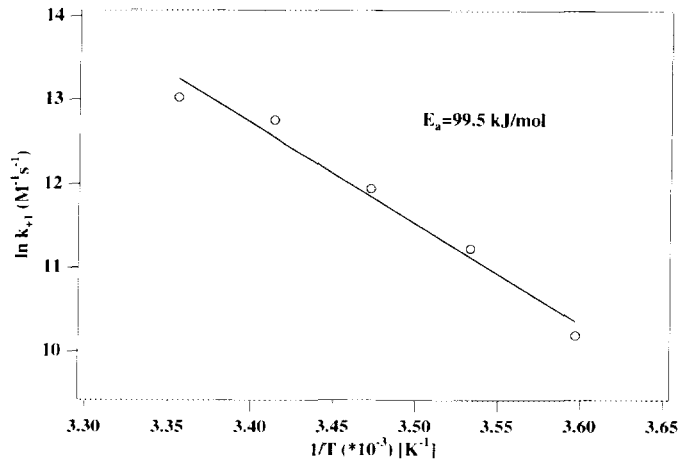

Figure 3. Arrhenius Plot of the temperature-dependent overall association rate constants, k_{+1} (Fig. 2), giving an activation energy (E_a) of ~99.5 kJ/mol.

filamin-F-actin binding and the concentration independent overall rate, k_{+2} subsequent cross-linking/bundling of the actin filaments by filamin. The electron micrograph in Fig. 5 shows actin filament bundles of approx 100 nm spacial distance.

DISCUSSION

Determining the rate constants for the interaction between actin cross-linking proteins such as filamin with F-actin is complicated by the formation of actin filament bundles. Using stopped-flow techniques

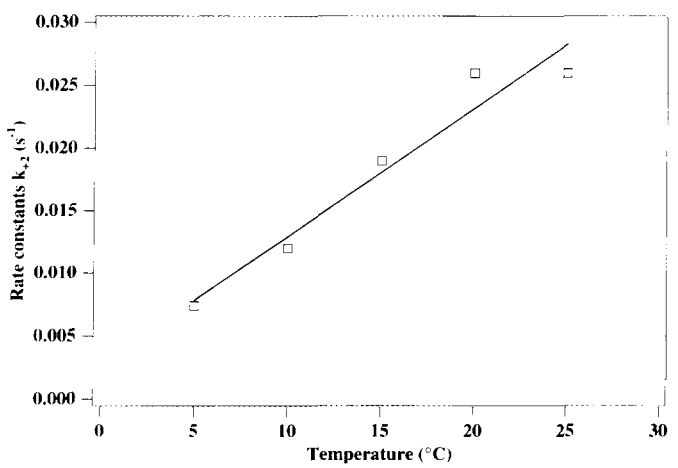

Figure 4. Temperature-dependent overall cross-linking/bundling rate constants, k_{+2}, of a filamin-F-actin complex. Buffer conditions as shown in Fig. 2.

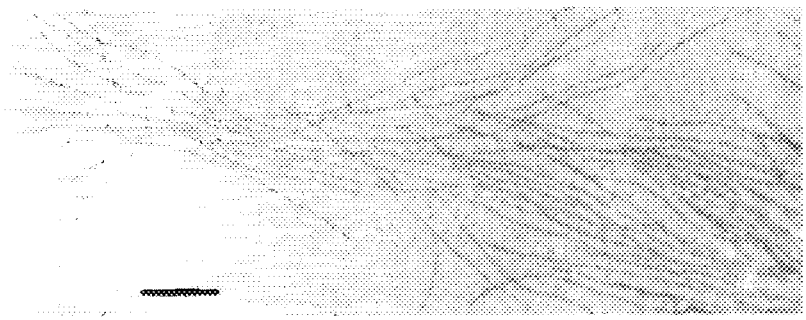

Figure 5. Electron microscopy (negative staining : urinylacetate) of actin filament bundles; c_{actin} = 0.4 mg/ml; $c_{filamin}$ = 0.04 mg/ml. Bar = 400 nm.

described here, the overall rate, k_{+1} of the binding of filamin to F-actin have been measured. The data in Fig. 3 show that the binding reaction has a very high temperature dependence which is indicative of a conformational change for protein-protein interactions. The activation energy of a diffusion-controlled reaction is normally of the order of ~17 kJ/mol, and the activation energy for the binding reaction here is more than 5 times higher. Whilst the temperature dependence of the viscosity of an actin-filamin solution might be expected to yield a larger than normal activation energy, this is unlikely to be >42 kJ/mol [8].

The overall cross-linking/bundling process described by k_{+2} is probably of a more complex nature. Thus, Meyer and Aebi [9] proposed bundle formation of an α-actinin-actin complex in less than 15 mins. This is intriguiging since the light scattering signal in our experiments reached completion after several minutes. Future work will include a more detailed analysis to verify all individual reaction steps.

ACKNOWLEDGMENTS

This work was supported by grants of the Deutsche Forschungsgemeinschaft (SFB 266/C5 and Is 25/7-1). We thank Ms. I. Sprenger for the EM work and Ms. H. Kirpal for protein preparations.

REFERENCES

1. Hartwig, J.H. and Kwiatkowski, D.J. (1991) Curr. Opinion Cell Biol. 3, 87-97.
2. Pollard, T.D. and Cooper, J.A. (1986) Ann. Rev. Biochem. 55, 987-1035.

3. Cortese, J.D. and Frieden, C. (1990) Cell Mot. Cytoskeleton 17, 236-249.
4. Shizuta, Y., Shizuta, H., Gallo, M., Davies, P. Pastan, I. and Lewis, M.C. (1976) J. Biol Chem. 251, 6562-6567.
5. Spudich, J.A. and Watt, S. (1971) J. Biol. Chem. 246, 4866-4871.
6. MacLean-Fletcher, S. and Pollard, T.D. (1980) Biochem. Biophys. Res. Comm. 96, 99-105.
7. Goldmann, W.H. and Isenberg, G. (1993) FEBS Lett. 336, 408-410.
8. Biosca, J.A., Travers, F. and Barman, T.E. (1983) FEBS Lett. 153, 217-220.
9. Meyer, R.K. and Aebi, U. (1990) J. Cell Biol. 110, 2013-2024.