

Thermodynamics of filamin-actin interaction.

W.H. Goldmann

Technical University of Munich
Dept. of Biophysics, E22
D-8046 Garching. FRG.

Since Wang et al. [1] first isolated filamin from chicken gizzard and found evidence for the interaction with actin [2] successive studies have been concerned with its possible role in smooth muscle and non-muscle cells. Hartwig and Stossel [3] e.g., showed that macrophage "actin-binding protein" and filamin had identical properties regarding size, structure, affinity and stoichiometry to actin. In this work the binding of filamin to actin at various temperatures, the stoichiometry by breakpoint titration and the frequency dependence of the viscoelastic modulus by oscillating disk rheometer were examined.

Actin was isolated from acetone powder of rabbit back muscle [4] and fluorescently labelled with NBD (7-chloro-4-nitro-benzo-2-oxa-1,3-diazole) [5]. Filamin was purified from chicken gizzard [6]. After the DEAE-cellulose chromatography (Whatman DE-52) filamin was further purified on a hydroxyapatite column equilibrated with 20mM KH_2PO_4 , pH 7.2, 0.1% mercaptoethanol and eluted with a linear gradient from 100 to 400mM KH_2PO_4 . The purity was analysed on SDS mini slab gel and protein concentration was determined according to Bradford [7]. Filamin activity was routinely checked by falling ball viscosity [8]. G-buffer = 2mM Tris-HCl, pH 8, 0.2mM CaCl_2 , 0.2mM ATP, 0.2mM DTT, 0.005% NaN_3 ; and addition of 100mM KCl and 0.2mM MgCl_2 = F-buffer.

The overall affinity of monomeric actin for filamin in G-buffer over a temperature range of 20°C was determined by continuous titration [9] which changed by a factor of < 2 and showed a value of approx $2 \times 10^6 \text{ M}^{-1}$ at 20°C as also reported in [3]. The thermodynamic parameters calculated from the van't Hoff plot (Table 1) indicate the influence of van der Waals and hydrogen bonding forces ($-\Delta H$; $-\Delta S$). This is similar to previously observed talin -and talin-vinculin to G-actin binding [10].

Table 1 Thermodynamic data

Van't Hoff plot	ΔH^0 (kJ/mol)	ΔG^0 (kJ/mol)	ΔS^0 (J/mol/K)
filamin-G-actin	-11.4	-36.0	-81
Arrhenius plot	ΔH^+ (kJ/mol)	ΔG^+ (kJ/mol)	ΔS^+ (J/mol/K)
filamin-F-actin	+63.0	+78.3	-55

The above data show that the association complex of filamin and actin cannot easily be disrupted by temperature as observed here and in [3] by high ionic strength. This indicates that strong forces contribute to its stability and conformation.

In contrast, the thermodynamic data of the Arrhenius plot deduced from the polymerization rates of actin in the presence of (14 : 1) filamin under F-buffer condition, as described in [11] exhibit aspects of partial immobilization ($+\Delta H$) and charged interaction ($-\Delta S$) (Table 1). Extensive studies still have to be carried out before these results are lucid.

In a specially designed breakpoint titration experiment a detailed description of which is given in [9] the stoichiometry of filamin : G-actin binding was (1 : 12-14). This is consistent with data achieved by sedimentation method [3].

First results using an oscillating disk rheometer [12] show that the frequency dependent viscoelastic modulus changes at a filamin : actin molar ratio of 1 : ≥ 500 . Previous measurements by falling ball indicated the sol/gel transition at a molar ratio of 1 : ~200 [13]. This is an interesting finding, since filamin is regarded as a regulatory protein in the control of cytoplasmic rigidity, cell shape and motility. Further work will, therefore, investigate the physico-chemical and mechanical properties of filamin-actin complexes.

I am grateful to Ms. H. Kirpal for the protein preparations. This work was supported by the Sonderforschungsbereich SFB 266/C5.

- Wang, K., Ash, J.F. & Singer, S.J. (1975) Proc. Natl. Acad. Sci. U.S.A., **72**, 4483-4486
- Wang, K. (1977) Biochemistry, **16**, 1857-1865
- Hartwig, J.H. & Stossel, T.P. (1981), J. Mol. Biol., **145**, 563-581
- Spudich, J.A. & Watt, S. (1971) J. Biol. Chem., **246**, 4866-4871
- Detmers, P., Weber, A., Elzinga, M. & Stephens, R.E. (1981) J. Biol. Chem., **256**, 99-104
- O'Halloran, T., Molony, L. & Burridge, K. Methods in Enzymology, **134**, 69-77
- Bradford, M. (1976) Anal. Biochem., **72**, 248-254
- MacLean-Fletscher, S. D. & Pollard, T.D. (1980) J. Cell Biol., **85**, 414-428
- Goldmann, W.H. & Isenberg, G. (1991) Bioch. Bioph. Res. Com., **178**, 718-723
- Goldmann, W.H., Kaufmann, S. & Isenberg, G. (1991) Biochem. Soc. Trans., **20**, 50S
- Kaufmann, S., Piekenbrock, T., Goldmann, W.H., Bärman, M. & Isenberg, G. (1991) FEBS Lett., **284**, 187-191
- Müller, O., Gaub, H.E., Bärman, M. & Sackmann, E. (1991) Macromolecules, **24**, 3111-3120
- Nunnally, M.H., Powell, L.D. & Craig, S.W. (1981) J. Biol. Chem., **256**, 2083-2086