

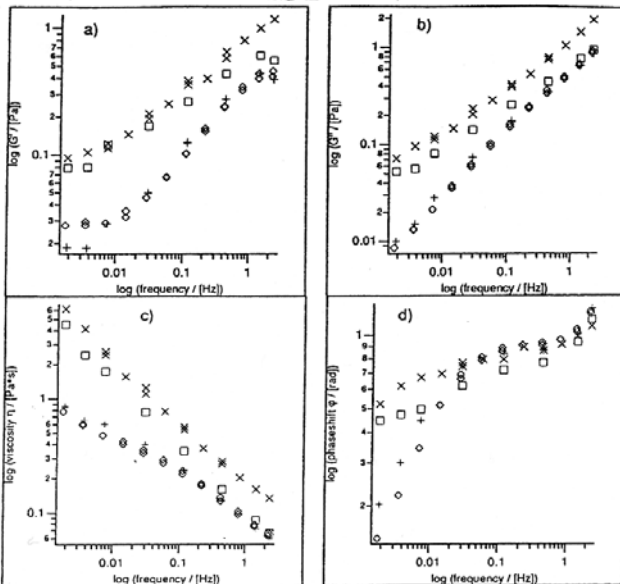
**The viscoelastic moduli of actin/filamin solutions: A micro-rheologic study.**

R. Ruddies, W.H. Goldmann, G. Isenberg & E. Sackmann, Technical University of Munich, Biophysics, E22, D-8046 Garching FRG.

Actin filaments are believed to play an essential role in cell motility, cell structure and in determining the viscoelastic properties of cells. A large number of actin binding proteins, including filamin, have so far been identified to regulate the polymeric structure and viscoelasticity of actin networks as well as its coupling to cell membranes [1]. The aim of this study is to gain further insight into the dynamic properties of actin filament networks in solution when cross-linked by filamin.

Experimentation was performed on an oscillating disc rheometer designed by Müller [2]. The apparatus consists of a glass cuvette with a volume of ~1.5ml. The cuvette base is mounted into an aluminium thermostated holder which is in thermal contact with the solution. A glass disc is placed on the surface of the solution. On top of the disc a small mirror at 45° angle is fixed to deflect light from a laser beam onto a photodiode. Two pairs of magnetic coils surrounding the cuvette serve to orientate the mirror and to apply a shear torque to oscillate the disc. Voltage for coils and signal response are computer controlled and analysed, respectively. Actin was isolated from acetone powder of rabbit back muscle [3]. Filamin was isolated from chicken gizzard [4] and further purified and tested as described in [5]. F-buffer: 100mM imidazole 20mM MgCl<sub>2</sub>, 10mM EGTA, 10mM ATP, pH 7.2.

**Fig.1**



Frequency-dependent measurements at a constant actin concentration of 300µg/ml. Actin (○); actin : filamin at 100 : 1 (×); actin : filamin at 500 : 1 (□); and actin : filamin at 1000 : 1 (+); molar ratios after polymerization in F-buffer.

The viscoelastic properties of protein solutions were measured in a frequency-dependent manner after polymerization. Fig. 1a-d show the storage modulus ( $G'$ ), loss modulus ( $G''$ ), viscosity ( $\eta$ ) and

phase shift ( $\phi$ ) of actin solutions and actin solutions cross-linked with filamin at various concentrations recorded between  $2 \times 10^{-3}$  Hz and 2 Hz. Results of these measurements indicate that polymerized actin and polymerized actin cross-linked with filamin at a molar ratio of 1000 : 1 gave identical curves and values for ( $G'$ ), ( $G''$ ) and ( $\eta$ ). However, decreasing the molar ratio of actin : filamin to 500-100 : 1 results in a significant change in all parameters. The plateau moduli for ( $G'$ ), ( $G''$ ) and ( $\eta$ ) are increased by a factor of 2-2.5 at actin to filamin ratios of 500-100 : 1. Another important aspect is demonstrated by the non-phase shift ( $\phi$ ) between  $2 \times 10^{-3}$  Hz and  $2 \times 10^{-2}$  Hz (at so-called minimum relaxation times) of actin when cross-linked with filamin at molar ratios of 500-100 : 1. This is indicative of permanent, stable cross-linking.

Though only a few measurements of the elastic moduli of cross-linked actin solutions have been carried out, some interesting information can be presented: a) The increase in viscosity up to fivefold at actin to filamin molar ratios of 100 : 1 show that the distance between actin filaments becomes substantially smaller; and as these cannot reach their equilibrium configuration form gels of densely packed filaments. The increase of the elastic portion by a factor of two at high degrees of cross-linking suggests negligible phase separation and actin filament interconnection with filamin. b) For fixed networks of long polymers e.g. actin filaments, the elastic constant has been proposed to be linearly related to the number of cross-links per unit volume which is inversely proportional to the number of monomers between cross-links [6]. The mesh size density determined by quasielastic light scattering is  $\sim 0.5 \mu\text{m}$  for actin concentration  $\leq 0.5 \text{mg/ml}$  [7]. At an actin to cross-linker ratio of 100 : 1 the average calculated contour length between two binding sites ( $L=100 \times 2.7 \text{nm}$ ) is approx  $0.27 \mu\text{m}$ . This result supports the assumption made in (a) that filamin forms tighter actin filament networks. Future work will, therefore, include temperature-dependent measurements of the storage modulus ( $G'$ ) to provide data on entropy elasticity of filamin cross-linked actin networks. This work was supported by the Deutsche Forschungsgesellschaft. We thank Ms H. Kirpal for protein preparations.

1. Pollard, T.D. & Cooper, J. (1986) *Ann. Rev. Biochem.*, 55, 987-1035
2. Müller, O., Gaub, H.E., Bärmann, M. & Sackmann, E. (1991) *Macromolecules*, 24, 3111-3120
3. Spudich, J.A. & Watt, S. (1971) *J. Biol. Chem.*, 246, 4866-4871
4. O'Halloran, T., Molony, L. & Burridge, K. (1986) *Methods in Enzymology*, 134, 69-77
5. Goldmann, W.H. (1992) *Bioch. Soc. Trans.*, 20, 89S
6. Bird, R.B., Curtiss, C.F., Armstrong, R.C. & Hassager, O. (1987) *Kinetic Theory: Dynamics of Polymeric Liquids*, 2nd ed, Vol. 2. Wiley, New York
7. Schmidt, C.F., Bärmann, M., Isenberg, G. & Sackmann, E. (1989) *Macromolecules*, 22, 3638-3649