Thermodynamics of talin and talin-vinculin binding to G-actin.

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It has been shown by various methods that the cytoskeletal proteins, talin and vinculin, bind to actin in vitro [1-3]. The (K_d) determined for the interaction of both proteins with actin was approx 0.3μM at 20°C [1,4]. Here we have examined the influence of temperature on the binding reactions to ascertain thermodynamic parameters in order to gain information of the type of binding and the stability of protein association complexes.

G-actin was prepared as described in [5] and fluorescently labelled with NBD (7-chloro-4-nitro-benzeno-2-oxa-1.3-diazole)[6]. Talin was prepared by the method in [7] and vinculin was purified as described in [8]. The purity of these proteins was determined by gel chromatography and their viability was tested in an F-actin polymerization assay [3]. G-buffer was used in all other experiments: 2mM Tris-HCl, 0.2mM $CaCl_2$, 0.2mM DTT, 0.005% NaN_3, pH 8.0.

Fluorescence titration of talin to NBD-labelled G-actin over a temperature range of 20°C indicate a change in the overall equilibrium constant by a factor of approx 4. Mixing talin in a molar ratio of 1:2 with vinculin [9] under identical conditions affects the affinity to NBD-labelled G-actin only slightly (Fig. 1).

Fig. 1. A van't Hoff plot of the variation of the equilibrium constant with temperature under G-buffer condition

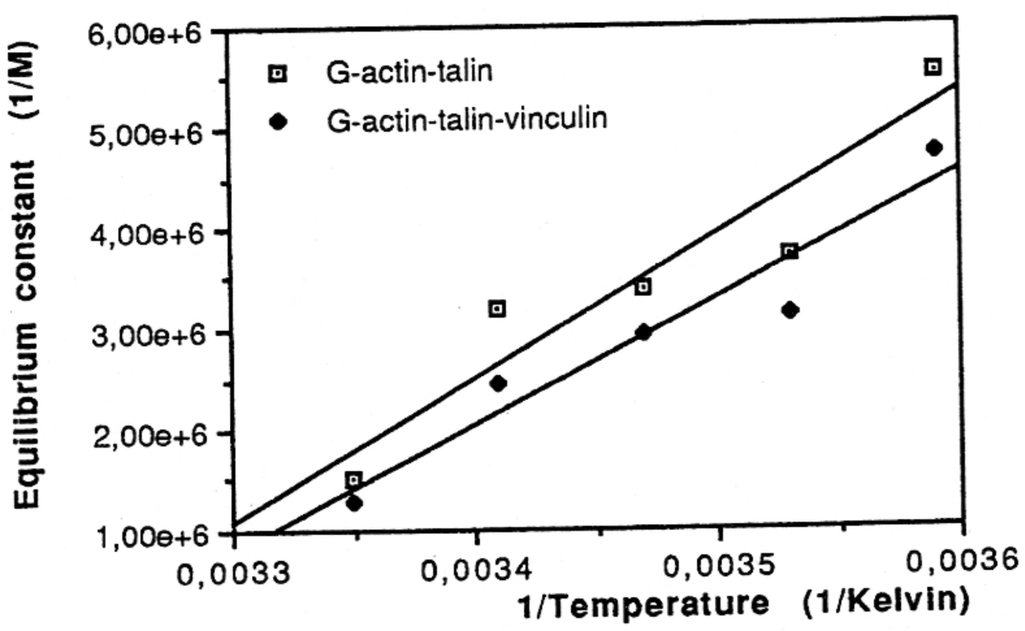

Table 1. Thermodynamic parameters for the binding of talin and talin-vinculin to G-actin calculated from (Fig. 1)

Interaction of G-actin with	ΔH^o (kJ/mol)	ΔG^o (kJ/mol)	ΔS^o (J/mol/K)
talin	-46.2	-35.7	-36
talin-vinculin	-42.5	-34.9	-26

Thermodynamic parameter determined from the van't Hoff plot give indication of the kind of binding and the stability of the protein complex (Table 1). The negative value for enthalpy (ΔH^o) and entropy (ΔS^o) could arise from a non-bonded (van der Waals) interaction, hydrogen bond formation in the low dielectric media and/or protonation accompanying the association [10].

These results suggest the interaction of highly polarizied atoms in these proteins. The source of the monomer stability could reside in the optimization of "van der Waal" contacts between staked molecules. Thus several hydrogen bonds formed in a low dielectric environment such as parts of the contact areas between proteins which are inaccessible to water or ligand binding sites in the interior of a protein could collectively make substantial contribution to (ΔH^o) and (ΔS^o). Further, protein association is often accompanied by the release of protons, but as this is incidental, it makes only minor contribution to the overall energetics [11].

The information presented here allows only a few general statements about the nature of the G-actin-talin and G-actin-talin-vinculin reaction. The negative values for (ΔG^o) favours protein association and because of this process, the system loses heat ($-\Delta H^o$) and forms a stable more ordered complex ($-\Delta S^o$). Comparing the thermodynamic parameters of talin-G-actin and talin-vinculin-G-actin interaction, the small difference in these values could be explained by the low binding stoichiometry of 1:100 (vinculin:actin) [1]. Consequently, the influence of vinculin in this complex on G-actin binding maybe negligible.

To test this assumption the rate of G-actin polymerization in F-buffer in the presence of vinculin-talin and talin at the same total protein concentration were compared, which showed similar values.

The overall conclusion is that vinculin incorporated in the talin-G-actin complex has only little influence on the protein conformation and stability. Future work will involve the reconstitution of these proteins into lipid bilayers to simulate cellular conditions in vitro.

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