Temperature dependent specific heat capacity (C$_p$) of G-actin and talin or talin-vinculin bound to G-actin.

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Recent studies of the thermal stability of proteins indicate that there is a temperature range, in which stabilizing forces, such as van der Waals interactions and hydrogen bonding, overcome the dissipative forces of the hydration of non-polar residues. The transition energy measured calorimetrically is believed to originate from van der Waals interactions and hydrogen bonding in protein structures [1,2]. Since it has been proposed that the binding of G-actin to talin and to talin complexed at a molar ratio of 1:2 with vinculin could arise from non-bonded (van der Waals) or hydrogen bond formation [3], the differential scanning calorimetry (DSC) method was used as a tool in search for support of this assumption.

G-actin was purified from rabbit back muscle [4] to which the fluorescent marker 7-chloro-4-nitro-benzeno-2-oxa-1,3-diazole (NBD) was attached [5]. Talin and vinculin were isolated from chicken gizzard smooth muscle as described in [6]. The puritiy of all proteins was routinely checked by SDS-polyacrylamide gel electrophoresis of 10% gels. Buffer used for all experiments was 2mM Tris-HCl, pH 8.0; 0.2mM CaCl$_2$, 0.2mM ATP, 0,2mM DTT, 0.005% NaN$_3$ = (G-buffer).

DSC measurements were performed with a high sensitivity MC-2 microcalorimeter (Microcal, Amherst, MA, USA). The data were stored and analysed by an IBM AT computer using the DA-2 software provided by Microcal. Before each experiment proteins were kept at 4°C and then transferred into the calorimeter. The heating scan was started after an equilibration time of 20mins with a scan rate of 90°C/h and a 15sec time increment (filter constant) between each data collection. Temperature dependent specific heat capacities (C$_p$) for all species were estimated after subtraction of the G-buffer baseline.

Scanning G-actin alone (A) and G-actin complexed with talin (B), vinculin (C) and talin-vinculin (D) -all at the same total protein concentration- from 20°C to 90°C, the traces between 20°C and ~40°C show similar behaviour followed by different negative slopes up to 90°C (Fig. 1). As the value of the heat capacity of polar groups is negative it is allowed to assume that the temperature dependence of thermodynamic parameters of protein unfolding is mainly due to the hydration of non-polar groups. It has been shown in many observations of globular proteins that a change of the denaturation enthalpy with a rise in temperature is connected with a decrease of the ordering effect of non-polar groups on water [7].

The slopes of complexed G-actin are less steep compared to G-actin alone, which could be the result of strong intra and/or intermolecular forces in the protein species. In such a state the accessibility of water to defined non-polar groups can be limited due to various reasons (presence of -S-S- bridge, dipole-dipole forces and the emergence of local and/or short-lived contacts between groups).

Estimates of the temperature dependent specific heat capacity (C$_p$) after subtraction of the G-buffer baseline are shown in the inset to Fig. 1. All traces show no sharp transition over this temperature range, but a rather continuous change in intensity with increasing temperature. This kind of behaviour has been reported previously for other low protein concentrations [8]. In most protein-protein bindings, the temperature-induced process is not simple. This becomes especially apparent upon comparison of the heat absorption peak area, with the effective van`t Hoff enthalpy, determined from the sharpness of the peak [9]. Preliminary analysis revealed that most of the peaks are characterized by a calorimetric : van`t Hoff enthalpy ratio substantially greater than unity, which means that the melting process of these binding species are complex. (Fig. 1)
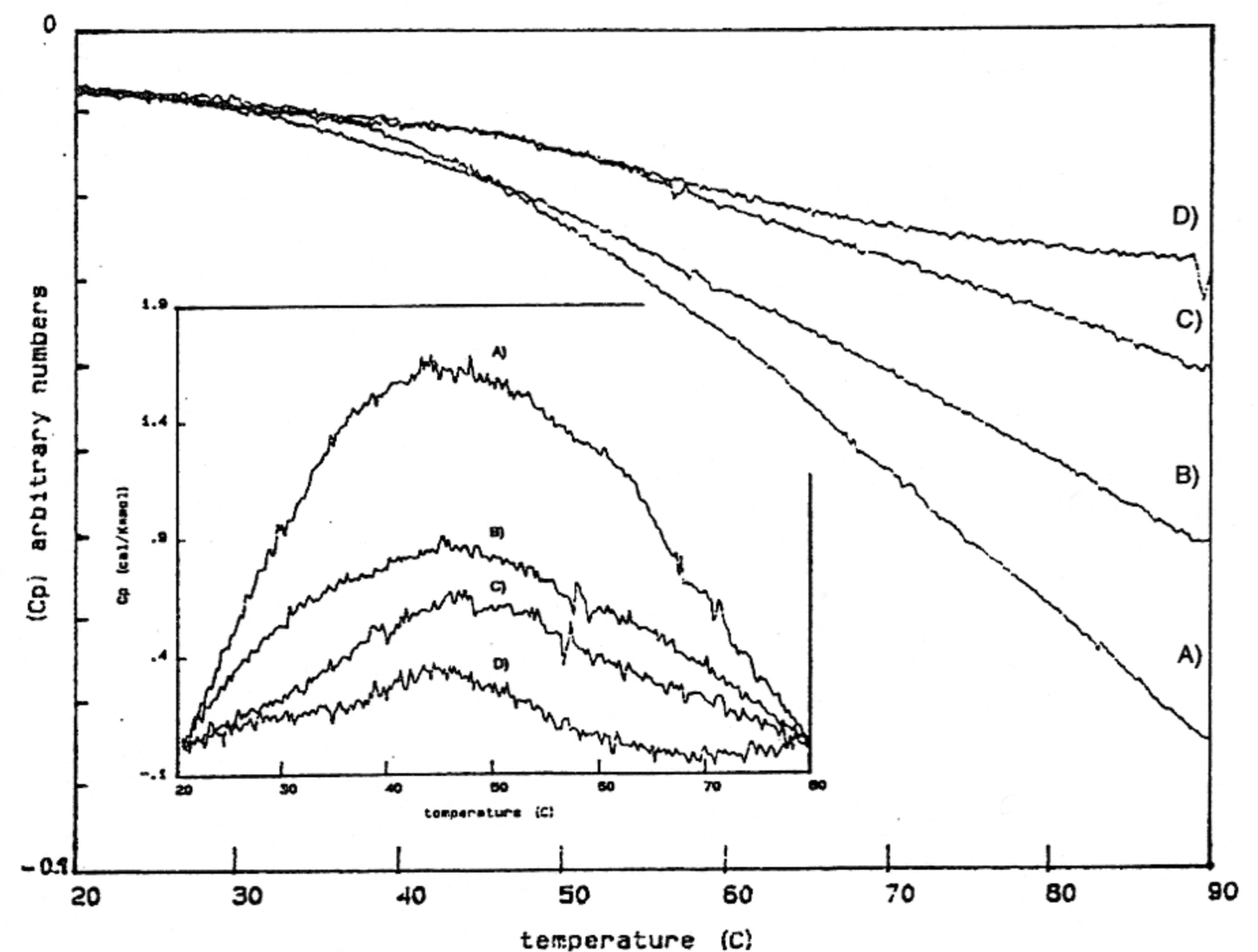
All scans (A-D) were performed in G-buffer at a total protein concentration of 5μM. Molar ratio of actin : talin : vinculin was 3 : 1 : 0.5.

These findings support the assumption that talin, vinculin and talin-vinculin are G-actin binding proteins [10].

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