where μ is the dipole moment of the protein, a and b are proportional to the net charge on the protein molecule at low ionic strengths, r is a distance of closest approach, taken as an adjustable variable, and c and d are constants described by Edsall and Wyman[27].
Combining Equations 1,2, and 3 we obtain

$$\Delta F = F_o + \frac{b m^{1/2}}{1 + r c m^{1/2}} + d\mu m - \Omega \sigma m \qquad (4)$$

where all terms which are independent of salt concentration (i.e. ΔF_o, a, and const) have been combined into F_o.
According to Equation 4, at low salt concentrations the free energy increases with increase in salt concentration. This is in the region where the second term dominates. In this region, protein binding to Sepharose-lipid is reduced by electrostatic effects of the salt, which in turn decreases the binding affinity as we have observed. Above a critical concentration, m_t, the Debye-Hückel term becomes a constant and the linear terms dominate in Equation 4. And as in hydrophobic chromatography $\Omega\ \sigma$ is larger than d μ for $m > m_t$[22], the free energy decreases with increase in salt concentration. The decrease in free energy corresponds to an increase in protein binding. This is in agreement with our experimental observations.
In conclusion, it may be asserted that intermediate salt concentrations may lower binding affinity of proteins to a gel with hydrophobic properties by increasing the free energy of association.

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2 Shaltiel, S., and Er-el, Z., Proc. natl Acad. Sci. USA *70* (1973) 778.
3 Rimerman, R.A., and Hatfield, G.W., Science *182* (1973) 1268.
4 Shaltiel, S., Ames, F.L.G., and Noel, K.D., Archs Biochem. Biophys. *159* (1973) 174.
5 Hofstee, B.H.J., Analyt. Biochem. *52* (1973) 430.
6 Yon, R.J., Biochem. J. *137* (1974) 127.
7 Doellgast, G.J., and Fishman, W.H., Biochem. J. *141* (1974) 103.
8 Axen, R., Porath, J., and Ernback, S., Nature *214* (1967) 1302.
9 Hofstee, B.H.J., Biochem. biophys. Res. Commun. *50* (1973) 751.
10 Hofstee, B.H.J., Biochem. biophys. Res. Commun. *53* (1973) 1137.
11 Jost, R., Miron, T., and Wilchek, M., Biochim. biophys. Acta *362* (1974) 75.
12 Rosén, S., Biochim. biophys. Acta *523* (1978) 314.
13 Hjertén, S., J. Chromat. *159* (1978) 85.
14 Hjertén, S., J. Chromat. *153* (1978) 239.
15 Stockton, J.M., and Turner, A.J., J. Neurochem. *36* (1981) 1722.
16 Porath, J., Sundberg, L., Fornstendt, N., and Olsson, I., Nature *245* (1973) 465.
17 Hjertén, S., Rosengren, J., and Påhlman, S., J. Chromat. *101* (1974) 281.
18 Låås, T., J. Chromat. *111* (1975) 373.
19 Cashion, P., Javed, A., Harrison, D., Seeley, J., Lentini, V., and Sathe, G., Biotech. Bioengng *24* (1982) 403.
20 Nemat-Gorgani, M., and Karimian, K., Eur. J. Biochem. *123* (1982) 601.
21 Nemat-Gorgani, M., and Karimian, K., Biotech. Bioengng, accepted for publication.
22 Melander, W., and Hováth, C., Archs Biochem, Biophys. *183* (1977) 200.
23 Horváth, C., Melander, W., and Molnár, I., J. Chromat. *125* (1976) 129.
24 Horváth, C., Melander, W., and Molnár, I., Analyt. Chem. *49* (1977) 142.
25 Sinanoğlu, O., in: Molecular Associations in Biology, p.427. Ed. B. Pullman. Academic Press, New York 1968.
26 Tanford, C., in: Physical Chemistry of Macromolecules, p.457. John Wiley, New York 1961.
27 Edsall, J.T., and Wyman, J., in: Biophysical Chemistry, vol.1, p.241. Academic Press, New York 1958.

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Calorimetric studies on monomeric and polymeric actin[1]

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Summary. Differential scanning calorimetry of polymeric F-actin at pH 8.0 showed that the polymer had a concentration-independent thermal profile with a single transition temperature of 81 °C. In contrast, the thermal profile of G-actin was concentration-dependent, and although it resembled the F-actin profile at lower concentrations, it was found to have a more complex profile at higher protein concentrations.

The technique of differential scanning calorimetry (DSC), in which the heat capacity of a sample is measured as a function of temperature, has recently emerged as a useful approach for the study of macromolecular structure and conformational change, and this technique has been applied to a variety of proteins[4]. We have therefore been interested in using DSC to investigate the properties of actin, a critically-important contractile protein[5], and in this report, the thermal characteristics of monomeric and polymeric actins are described.
Materials and methods. G-actin was prepared from rabbit skeletal muscle by the method of Spudich and Watt[6] and was found to be homogeneous by electrophoretic analysis[7] (results not shown). F-actin was then made by the addition of 0.1 M KCl to the G-actin preparation, Ca^{2+}-free polymeric actin was prepared by extensive dialysis at 4 °C of G-actin against 0.2 mM ATP, 0.2 mM dithiothreitol, 5 mM phosphate, pH 7.0[8], and nonpolymeric actin was prepared from G-actin by amination and subsequent dansylation of Tyr-69 by the procedure of Chantler and Gratzer[9].
DSC studies were performed on a Perkin-Elmer DSC-2 instrument using 75-μl sample containers. Studies were performed at pH 8.0 and at a scan rate of 10 °C per min with a range setting of 0.2 mcal/sec. Temperature scans were routinely performed from 10 °C to 90 °C, and in studies on the reversibility of thermal transitions, samples which had been raised to a given temperature were immediately cooled to 10 °C at a nominal rate of 320 °C per min.
Results and discussion. When G-actin preparations were examined by DSC, it was found that the thermal profile obtained was concentration-dependent (fig., A, B). Below 6 mg/ml, the protein showed a single major endothermic

transition with a transition temperature (T_m) of 81 °C, whereas at and above 6 mg/ml, 2 transitions were observed, with transition temperatures of 77 °C and 81 °C. This unusual concentration-dependent behavior, which has not been reported for any other protein, suggests that at higher concentrations, elevated temperature may induce the formation of oligomeric actin which has different denaturation characteristics than either G-actin or, as shown later, F-actin.

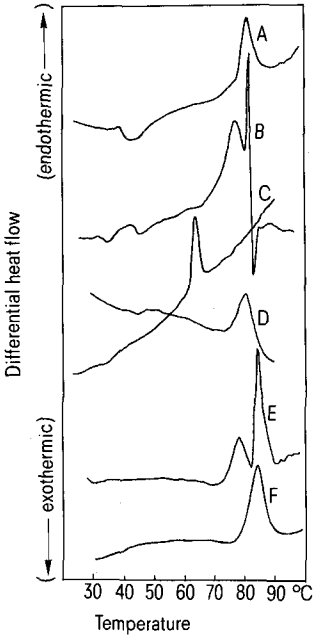
This idea was supported from studies on nonpolymerizable G-actin (fig. C) in which it was found that the dual transition at elevated protein concentration did not occur, indicating that when G-actin-G-actin interactions are inhibited, only a single transition is observed. The single transition of the modified actin had a lower T_m (64 °C) than that of G-actin, suggesting that the amination and dansylation of Tyr-69 significantly alters the conformation of the actin monomer in addition to inhibiting polymerizability.

In contrast to the concentration-dependent behavior of G-actin, the DSC profile of F-actin (fig. D) did not show this effect between 3 mg/ml and the concentration of F-actin in the pellet obtained by centrifugation at 100,000 × g for 3 h. The single transition of F-actin (T_m = 81 °C) was very similar if not identical to that seen in dilute G-actin samples (fig. A). This suggests that the polymerization of actin does not dramatically affect the conformation of the monomer, in agreement with earlier circular dichroism studies[10], and that actin-actin interactions in the F-actin polymer do not significantly contribute to the thermal profile.

The T_m common to both G-actin and F-actin has a high value compared to the denaturation temperatures reported for these proteins from spectrophotometric[11] and viscometric[12] studies. It is possible that this apparent discrepancy is caused by the fact that in the earlier studies, as samples were incubated at elevated temperatures during the assay procedures rather than being rapidly heated as in the DSC studies, a time-dependent thermal denaturation of the actin occurred which therefore caused the denaturation point to be lowered. The DSC studies also show that in comparison to the transition temperatures of a variety of other proteins[4], actin has a relatively high T_m value which suggests that the actin monomer is a moderately heat-stable protein by virtue of its compact globular structure[13].

It was also found that thermal denaturation of both G-actin and F-actin is an irreversible event when the actin is heated to 80 °C (results not shown). This contrasts with the observation of Contaxis et al.[11] that bovine cardiac actin denaturation up to 80 °C is reversible and time-independent. It is possible that this difference may be explained by sequence differences in the 2 actins, as previous studies have shown that minor sequence alterations can dramatically affect the thermal properties of proteins[14].

In addition to polymeric F-actin, polymeric Ca^{2+}-free actin forms have recently been found to exist at low ionic strength[8,15,16]. When the thermal profile of the Ca^{2+}-free polymeric actin which contains bound phosphate[8] was recorded at low ionic strength (0.2 mM ATP, 0.2 mM DTT, 5 mM phosphate, pH 7.0), it showed similar characteristics (fig. E) to G-actin in that a concentration-dependent dual transition was observed (T_m values of 78 °C and 84 °C). In contrast, the addition of 0.1 M KCl to the Ca^{2+}-free polymeric actin abolished this effect, and the actin gave a single transition (T_m = 84 °C) over a wide concentration range (fig. F). These results indicate that despite the F-actin-like appearance of Ca^{2+}-free actin at low ionic strength, there must be significant differences between the actin-actin interactions in this system and those that occur at higher ionic strength in the presence and absence of Ca^{2+}. This conclusion therefore supports the results of previous studies[8,15,16] in which other methods have indicated that Ca^{2+}-free polymers are assembled differently from the F-actin polymer.



DSC profiles of various actin preparations. Details of sample preparation and calorimetric analysis are given in the methods section. Sample identities: *A* G-actin at 4 mg/ml in 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM β-mercaptoethanol, 3 mM Tris HCl pH 8.0; *B* G-actin at 7 mg/ml (same buffer); *C* nonpolymerizable actin at 6 mg/ml (same buffer); *D* F-actin at 9.2 mg/ml (same buffer plus 0.1 M KCl); *E* Ca^{2+}-free polymeric actin at 9.2 mg/ml in 0.2 mM ATP, 0.2 mM dithiothreitol, 5 mM phosphate pH 8.0; *F* Ca^{2+}-free polymeric actin at 9.2 mg/ml (same buffer plus 0.1 M KCl).

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2 Present address: Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907, USA.
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4 Privalov, P.L., Adv. Protein Chem. *33* (1979) 167.
5 Perry, S.V., Biochem. Soc. Trans. *7* (1977) 593.
6 Spudich, J.A., and Watt, S., J. biol. Chem. *246* (1971) 4866.
7 Weber, K., and Osborn, M., J. biol. Chem. *244* (1969) 4406.
8 El-Saleh, S.C., and Johnson, P., Int. J. Biol. macromolec. *4* (1982) 430.
9 Chantler, P.D., and Gratzer, W.B., Eur. J. Biochem. *60* (1975) 67.
10 Hegyi, G., and Venyaminov, S.Y., FEBS Lett. *109* (1980) 134.
11 Contaxis, C.C., Bigelow, C.C., and Zarkadas, C.G., Can. J. Biochem. *55* (1977) 325.
12 Asai, H., and Tawada, K., J. molec. Biol. *20* (1966) 403.
13 Suck, D., Kabsch, W., and Mannherz, H.G., Proc. natl Acad. Sci. USA *78* (1981) 4319.
14 Matthews, C.R., Crisanti, M.M., Gepner, G.L., Velicelebi, G., and Sturtevant, J.M., Biochemistry *19* (1980) 1290.
15 Avissar, N., Kaminsky, E., Leibovich, S.J., and Oplatka, A., Biochim. biophys. Acta *577* (1979) 267.
16 Maruyama, K., Biochim. biophys. Acta *667* (1981) 139.

0014-4754/84/010083-02$1.50 + 0.20/0