nol. The ions showing m/z 222 and 224 on the mass spectrum corresponded to the 2 ions eluted on the gas chromatogram with identical retention times. Thus peak B was considered to be composed of (*Z*)-11-hexadecenol and $d_2$-(*Z*)-11-hexadecenol. (*Z*)-11-Hexadecenol was found to be biosynthesized from (*Z*)-11-HDA.

Ion m/z 238($M^{+\cdot}$ of bombykol) and m/z 240($M^{+\cdot}$ of $d_2$-bombykol) were observed in the mass spectrum of peak C. The retention time on gas chromatography of peak C was identical with that of synthetic bombykol. Identical results were obtained by mass spectrometry of peak D. Peak D was identified as the (*E, E*) isomer of bombykol. Mass chromatogram using ion m/z 238, 239, 240 and 241 showed that these 4 ions had identical retention times on peaks C and D. The change of ion m/z 238 to 240 was about 15% on both spectra. These data indicate that bombykol and its (*E,E*) isomer are biosynthesized from (*Z*)-11-HDA. The conversion of bombyk acid into bombykol has not been proved yet. Dehydrogenase and reductases in the reduction of bombyk acid to the alcohol, bombykol, are presumed to exist but it remains unknown. Bombykol was produced by applying the $D_2$ precursor to the silkworm pheromone gland. But where biosynthesis occurs in or on the pheromone gland remains unknown.

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## Effect of salt concentration on binding of proteins to a non-ionic adsorbent

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*Summary.* The effect of NaCl concentration on the adsorption of several proteins to palmityl-substituted Sepharose 4B has been investigated. It has been observed that the degree of adsorption first decreases and then increases with increasing salt concentrations, followed by total immobilization. The results are qualitatively explained by the simple theory of Debye-Hückel-Kirkwood, as applied to poly-electrolytes in the presence of salt.

The technique of hydrophobic chromatography[2] has been advanced and proved useful by several laboratories as a method for protein purification[2-7]. Until fairly recently, the most frequently used technique for the preparation of hydrophobic affinity adsorbents with agarose involved coupling of alkyl- or arylamines to CNBr-activated agarose[8]. This provides a matrix with partially ionic character[9]. Desorption of proteins from such gels may therefore be achieved by including salt in the medium[9-11]. More recently, use of neutral adsorbents for protein purification[12-16] and immobilization[9-11] has been demonstrated. It has further been argued that such purely non-ionic adsorbents are preferable to those having mixed ionic and non-ionic characters[17,18].

Recently, we reported on the use of palmityl-substituted Sepharose 4B as a non-ionic matrix for protein adsorption[20,21]. An important property of this gel is that it may bind proteins in the absence of any additional salt. However, some of the proteins adsorbed were found to desorb at intermediate concentrations of NaCl[20].

In the present investigation, binding affinity of the adsorbent for 6 proteins, arbitrarily chosen from those which are not normally immobilized[20], has been examined at different NaCl concentrations.

*Materials and methods.* All resins, chemicals and biochemicals were exactly as described previously[20]. Palmityl-substituted Sepharose 4B was prepared as reported[20], with the difference that dioxane was dried and distilled over sodium. The chromatographic profiles of the proteins tested were obtained using small columns and following our previously reported procedure[20], except in that the original buffer consisted of 5 mM sodium phosphate, pH 7.0, for all proteins.

*Results and discussion.* It is evident from the results presented in the figure and the table that including increasing concentrations of NaCl in the elution buffer frist decreases and then increases the binding affinity of the gel for the proteins examined. An important property of the gel used in this study is that it is essentially uncharged. Also, the ionic strength of the buffer used was such that no electrostatic interactions between these proteins and the few negatively charged groups known to occur in Sepharose[18] could take place. Therefore, desorption of the proteins which are normally immobilized on the matrix[20], and increased elution of the proteins reported here, both occurring at intermediate salt concentrations, may not be due to quenching of electrostatic interactions between the interacting components. On the other hand, the results may be explained in terms of free energy changes occurring in the association process.

The total free energy change $\Delta F$ for association of a protein molecule with Sepharose-lipid matrix may be written as[22-24]

$$\Delta F = \Delta F_o + \Delta F_h + \Delta F_{es} \quad (1)$$

Protein peaks obtained from the elution profiles of proteins at different concentrations of NaCl. Conditions are described in the legend to the figure. (a) no additional salt; (b) 0.05 M NaCl; (c) 0.1 M NaCl; (d) 1 M NaCl; (e) 2 M NaCl and (f) 4 M NaCl.

| Protein | Protein peak* | | | | | |
|---|---|---|---|---|---|---|
| | a | b | c | d | e | f |
| Cytochrome c | 7 | 22.5 | 23.5 | 8.5 | 0 | 0 |
| α-Amylase | 22 | 36 | 33 | 15 | 10 | 0 |
| Peroxidase | 10.5 | 30 | 34 | 27 | 17 | 0 |
| Urease | 14 | 24 | 26 | 14 | 0 | 0 |

* Percent of total applied.

where $\Delta F_o$ is the part of free energy change which is independent of salt concentration and includes the free energy changes by van der Waals interactions in the system. $\Delta F_h$ is the free energy changes due to hydrophobic interactions and $\Delta F_{es}$ is the free energy changes caused by long range electrostatic forces between the charges on the protein molecules and the salt ions present in the aqueous medium.

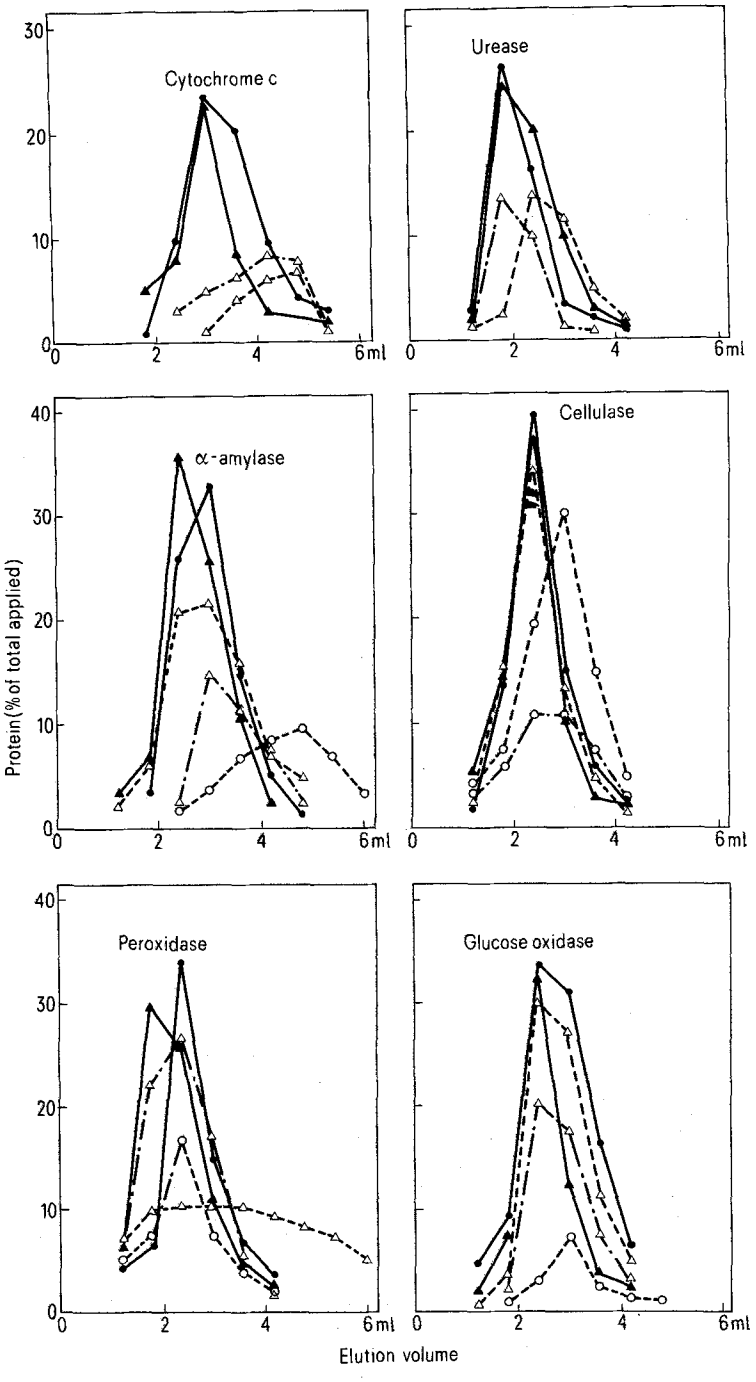
The free energy of hydrophobic interactions has been studied by Sinanoğlu[25], and in our system it is the free energy associated with the formation of a cavity in the solvent for accomodation of a protein molecule and the lipid chains. This may be written as

$$\Delta F_h = \text{const} - \Omega\sigma m \qquad (2)$$

where m is salt molarity, $\sigma$ is a constant called the molal surface tension increment[22], and the magnitude of $\Omega$ is determined by the contact area between the protein molecule and the ligands in the complex.

To obtain $\Delta F_{es}$, the protein is assumed to behave as a single macroion and therefore at sufficiently low ionic strengths the Debye-Hückel theory prevails[26]. At higher salt concentrations, the macromolecule is assumed to act as a neutral dipole, for which the Kirkwood model is applicable[27]. Thus, the net electrostatic free energy change for such an association process may be described by

$$\Delta F_{es} = a + \frac{bm^{1/2}}{1 + crm^{1/2}} + d\mu m \qquad (3)$$




Typical chromatographic profiles of proteins tested at different NaCl concentrations. 0.3 mg protein was applied to the column (0.47 × 7 cm) and 0.6 ml fractions were collected at room temperature and read at 408 nm for cytochrome c and 280 nm for other proteins. The flow rate was 0.45 ml/min and decreased for salt concentrations above 1 M. NaCl concentrations utilized were: 0.05 M (▲——▲), 0.1 M (●——●), 1 M (△—·—△), 2 M (○ - - - ○), 4 M (○ · · · ○) and no additional salt (△ - - - - △).

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. $\Delta F_o$, 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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## 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