Proc. Natl. Acad. Sci. USA
Vol. 76, No. 10, pp. 4750–4754, October 1979
Chemistry

Theory of protein–lipid and protein–protein interactions in bilayer membranes

(boundary lipid/cholesterol/liquid crystals/phase transitions)

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Contributed by Harden M. McConnell, June 4, 1979

ABSTRACT **A model for protein–lipid interactions in bilayer membranes where the proteins are very dilute is extended to higher protein concentration, where appreciable lipid-mediated protein–protein interactions occur. It is found that proteins may change the lipid phase transition temperature and that they weaken the phase transition. There exists a critical protein concentration above which the sharp lipid phase transition is abolished. The model also qualitatively reproduces several experimental observations on the physical behavior of bilayers formed from mixtures of cholesterol and phosphatidylcholines.**

The study of the interactions between phospholipids and intrinsic membrane proteins in cell membranes and model biological membranes continues to be an active area of research (1–4). Much of the impetus for these studies comes from the fact that protein–lipid interactions can control some functions of biological membranes. For example, the physical state of the membrane lipids modulates the activity of membrane-bound enzymes (e.g., ref. 5) and affects the lateral distribution of proteins in the membrane surface (6).

In an earlier paper (7), we presented a simple, qualitative model of protein–lipid interactions for the case where the protein lateral concentration was low enough that protein–protein interactions were negligible. Proteins make up a substantial fraction of the surface area of membranes, though, and it is important to take this into account theoretically. Therefore, we now extend the model to allow for large protein concentration. These results were presented at the 1979 Annual Meeting of the Biophysical Society (8). The reader is referred to ref. 7 for a more thorough discussion of the basic theory than that presented below, as well as for more details about other work in the field.

DESCRIPTION OF THEORY

Basic Idea. The following are the basic steps we have taken in developing the theory. (*i*) We adopt the point of view that the protein is a rigid body in the membrane. The perturbation it produces in the structure of the membrane smoothly decays away from the protein. (*ii*) The perturbation is identified with a change in the order of the lipid molecules, and an order parameter is defined as a numerical measure of order. (*iii*) The order parameter is related to the free energy of the system. (*iv*) For given conditions, the spatial dependence of the order parameter about the protein is taken to be that which minimizes the free energy of the system.

Order Parameter. The order parameter is defined in terms of the geometrical properties of the bilayer. When a phosphatidylcholine bilayer is warmed through its phase transition, its thickness decreases by about 20–30% (9). Because volume changes are comparatively small (10), the surface area varies in a reciprocal manner by about the same amount. We define the order parameter u as

$$u \equiv (A_f - A)/(A_f - A_s), \qquad [1]$$

in which A_f and A_s refer to the molecular areas in the "fluid" and "solid" states at the transition temperature in a bilayer without proteins. Thus, u goes from 1 to 0 as the lipid bilayer is warmed through the transition. One can think of u in terms of the membrane thickness; the formal use of the area was a tactical decision made earlier (7) for convenience in dealing with lateral pressure. In this paper we do not discuss the effects of changing the lateral pressure in the bilayer. Note that u is more directly related to the conformational and chain-packing properties of the lipids than to their molecular dynamics.

Boundary Effect of Protein. The protein is assumed to hold the order parameter of the immediately adjacent lipids at some fixed value u_0 which depends only on the nature of the protein and the lipid. For example, u_0 may reflect the adaptation of the thickness of the bilayer to the thickness of the hydrophobic core of the protein.

Landau–de Gennes Free Energy. That part of the free energy density that depends on u is given by the theory of Landau (11) and de Gennes (12):

$$G = Tu^2/2 - u^3 + u^4/2 + |\nabla u|^2/2. \qquad [2]$$

This is a truncated expansion of the free energy about $u = 0$, plus an elastic (gradient) term to account for the energetic cost of spatial variations in u. Energy, temperature, and length are measured in reduced units, which can be related to experimental data (7). For a spatially homogeneous bilayer ($|\nabla u| = 0$), Fig. 1 shows that there are, in general, local minima of G for two values of u. Which of these two phases is observed (has lower G) depends on the temperature. The physical temperature is scaled so that the phase transition occurs at a reduced temperature $T = 1$. Also, the zero of temperature is shifted; in ref. 7 we estimated that $T = 0$ lies ≤ 20 degrees below the transition temperature. The experiments (13) on which this estimate was based have been criticized (14). The effect of this criticism would be to increase the physical temperature interval between $T = 0$ and $T = 1$. The units on the reduced free-energy density are on the order of 1 kcal/mol of lipid and the length unit is probably near molecular size, or ≥ 10 Å.

Variational Treatment. For an arbitrary lateral distribution of proteins, one would like to find u as a function of position in the bilayer so that

$$\iint G[u(r), |\nabla u(r)|]d^2r = \text{minimum} \qquad [3]$$

with the integral taken over the bilayer surface and with the restriction that $u = u_0$ at the protein–lipid interface. To make

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Abbreviation: DSC, differential scanning calorimetry.

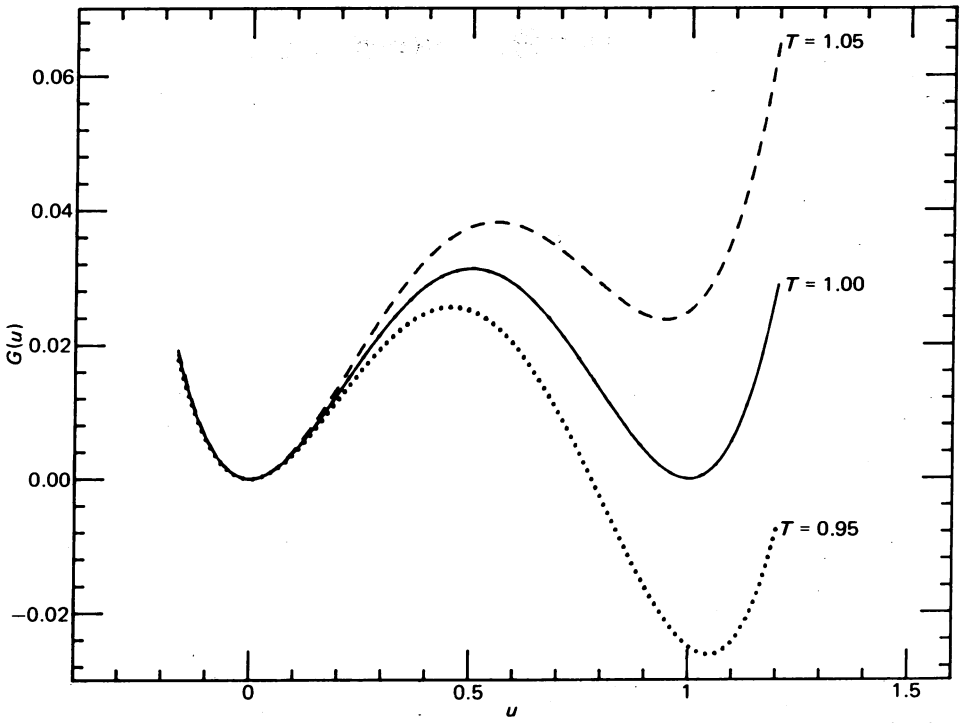

FIG. 1. Dependence of free-energy density (Eq. 2) on order parameter. Plot is for pure lipid bilayer, so $|\nabla u| = 0$ in Eq. 2.

the problem computationally more tractable we make some simplifications. Proteins are represented as cylinders of (reduced) radius r_0, and they are taken to occupy a hexagonal grid as indicated in Fig. 2. This spatial distribution may be viewed as an average one for proteins that do not attract each other strongly or perhaps repel each other due to electrostatic interactions.

By symmetry, we now need consider only the hexagonal area surrounding one protein. The hexagon is further approximated by a circle of equal area, with radius r_1. By imposing circular symmetry, we have reduced the problem to one spatial variable r, the radial distance from the center of the protein. Now $|\nabla u|$ is just $|u'|$, in which $u' \equiv du/dr$. This is taken to vanish at r_1.

Next, applying the Euler–LaGrange equation (15) to the simplified integral [3], we obtain

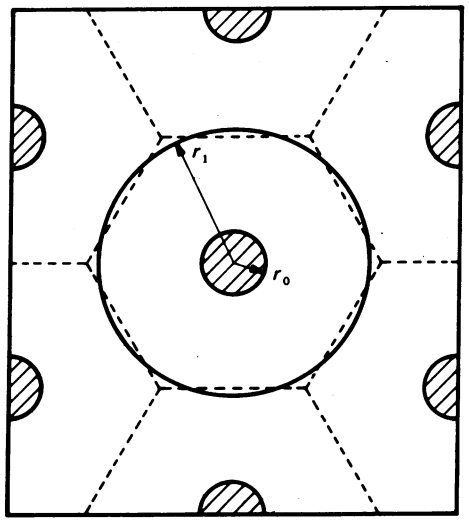

FIG. 2. Crystalline distribution of proteins on surface of membrane adopted to simplify computations. Proteins are hatched circles, and circle of radius r_1 has area equal to that of hexagonal region of lipids belonging to the "unit cell" containing the central protein.

$$u'' + u'/r - Tu + 3u^2 - 2u^3 = 0$$

$$u(r_0) = u_0,\ u'(r_1) = 0 \qquad [4]$$

The solution is computed using a numerical subroutine for boundary-value problems (16). The case of an isolated protein, considered in our earlier paper (7), is regained as $r_1 \to \infty$. Essentially the same free-energy function, but a different geometry, was used to study the isotropic-nematic transition in thin films (17).

RESULTS

The four important theoretical parameters are u_0, r_0, r_1, and T. The value chosen for u_0 is based on experimental information for the specific system under consideration. For the examples in this paper we have set $u_0 = 0.75$, an order between that of "fluid" and of "solid" pure lipid. Other possible choices would have been high order ($u_0 \geq 1$) or low order ($u_0 \leq 0$). We have chosen $r_0 = 1.0$, representing a small protein. Results for larger values of r_0 are not qualitatively different. One might wish to interpret r_0 not as the radius of the protein but as the radius of a complex of protein and a layer of lipids held at fixed order.

Fig. 3 shows the effects of protein–protein separation on the radial decay of the perturbation of the order parameter, at temperatures above ($T = 1.2$) and just below ($T = 0.999$) the pure lipid phase transition. Not surprisingly, increasing the protein concentration decreases the amplitude of the decay.

As in the absence of proteins (Fig 1), there are typically two thermodynamically stable solutions for the order parameter equation, differing in their free energies and amount of order. In general, one is metastable, as in Fig. 4. The existence of two stable phases allows a first-order phase transition in the lipid order, driven either by protein concentration or by temperature.

In Fig. 5 we show the free energies of the solutions as a function of r_1 at fixed $T > 1$, so that $u = 0$ in the pure lipid bilayer. The mean order parameter in the system is likewise

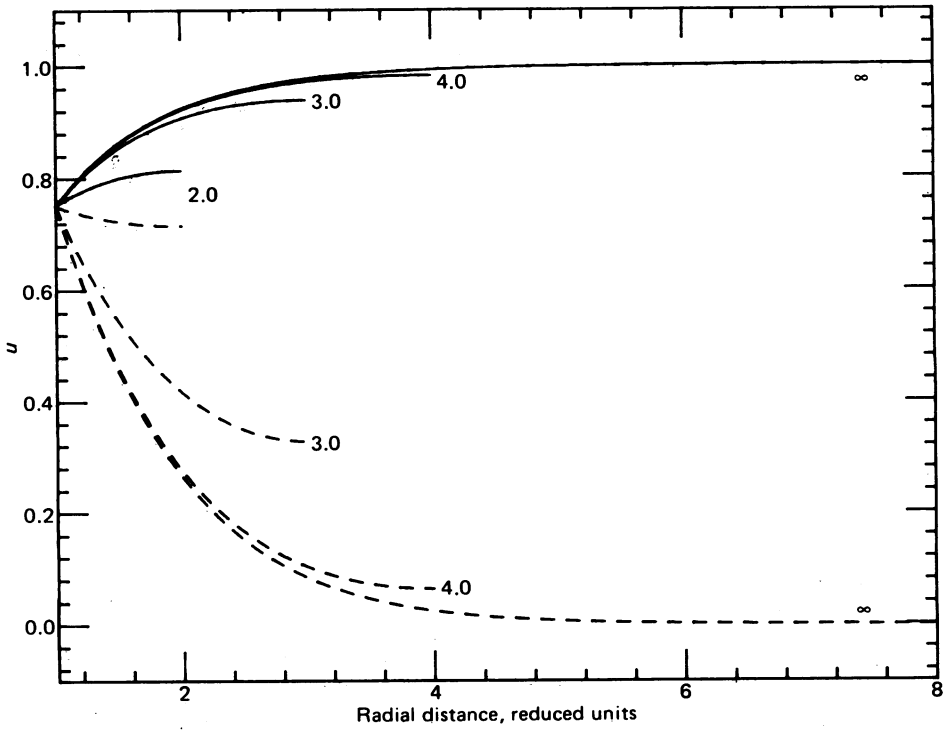

FIG. 3. Profiles of order parameter going radially out from the protein, for various annulus radii (r_1 = 2.0, 3.0, 4.0, and ∞), for temperatures above and below the pure lipid phase transition; r_0 = 1.0, u_0 = 0.75. Solid curves, T = 0.999; broken curves, T = 1.2.

plotted against r_1 in Fig. 6. For dilute protein (large r_1) the disordered phase has lower free energy. At very high protein concentration the situation is reversed. The gradient term in the free-energy expression penalizes large spatial variation in u, and u_0 has been chosen closer to the metastable ordered pure lipid value (0.94 at this T) than to 0. When there are only short distances between proteins, the overall free energy is lower if the lipid order parameter seeks a nearby local minimum in G (see Fig. 1) than if it drops steeply toward the more distant global minimum at u = 0. At some intermediate concentration (r_1 = 4.8 in this case), the free energies of the two phases are equal. *All* lipids simultaneously undergo the phase transition at this protein concentration, though the amplitude of the change in u is 0 for those lipids at $r = r_0$.

At fixed protein concentration, the same type of phase transition can be caused by temperature changes. This is shown in Fig. 7, in which protein concentration is expressed in the more convenient units of fraction of membrane surface covered by protein, $C \equiv (r_0/r_1)^2$. The protein increases the temperature and decreases amplitude of the phase transition, abolishing it above some critical concentration. This concentration is a bifurcation point for Eq. 3. Had u_0 been chosen <0.5, the transition temperature would have been depressed; for u_0 = 0.5 it would have remained at T = 1 for all protein concentrations The protein also induces a broad thermal change in order, which is largest near the critical concentration.

These results are summarized in Figs. 8 and 9. The transition temperature is presented in quasi-phase-diagram form in Fig. 8; the sizes of the sharp and broad enthalpy changes are plotted against protein concentration in Fig. 9. The broad ΔH was defined as the enthalpy difference for the system between temperatures just below (T = 0.999) and well above (T = 2.0) the phase transition minus the sharp ΔH from the phase transition. We do not use a temperature lower than 0.999 for the

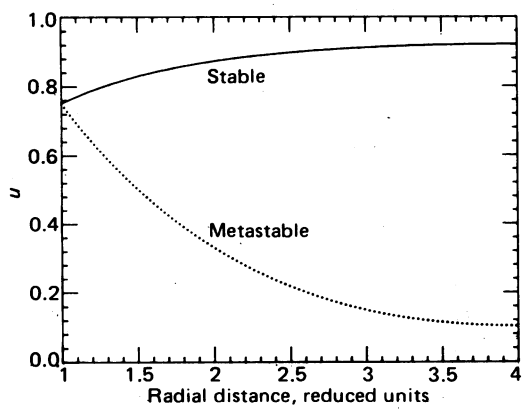

FIG. 4. The two solutions to Eqs. 4 when u_0 = 0.75, r_0 = 1.0, r_1 = 4.0, and T = 1.05. Note that the more ordered solution has the lower free energy even though $T > 1$, the phase-transition temperature in the pure lipid bilayer.

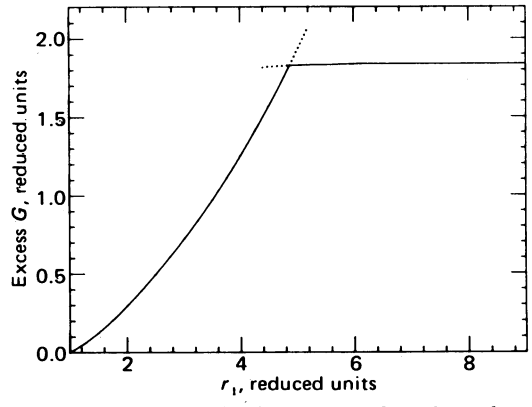

FIG. 5. Free energy of annulus (excess over that of annulus of unperturbed lipids) as function of annulus radius (i.e., protein separation). Phase transition between ordered and disordered solutions to Eqs. 4 occurs near r_1 = 4.8. Dotted lines show metastable extensions of phases near the transition. T = 1.05.

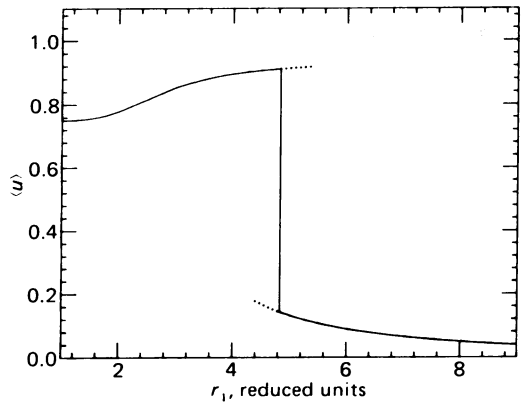

FIG. 6. Mean order parameter in membrane as a function of annulus radius for the same conditions as Fig. 5.

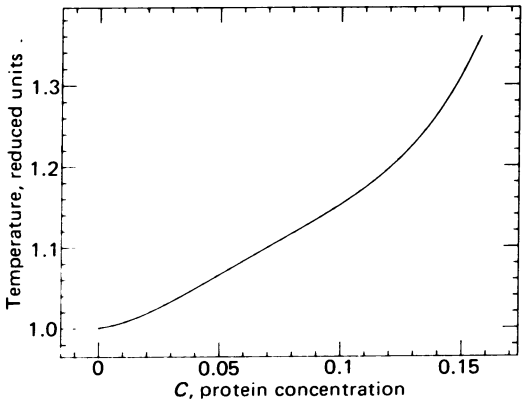

FIG. 8. Phase-transition temperature as a function of protein concentration. Note critical point near $C = 0.16$, $T = 1.36$, beyond which no sharp transition occurs; $r_0 = 1.0$, $u_0 = 0.75$.

broad ΔH calculation because the truncated free-energy expansion [2] produces spuriously large heat capacities under conditions of very low temperature and high order.

DISCUSSION

Connection to Experiments. There is currently a great deal of discussion about whether membrane proteins order or immobilize nearby lipids (1, 3, 4, 13, 14). The present theory models either situation, depending on the value chosen for u_0. Comparison between theory and magnetic resonance experiments requires one to make a (plausible) connection between u and the dynamics of the lipids.

Regardless of the choice of u_0, as the protein concentration increases, the amplitude of the lipid phase transition decreases theoretically. A broad transition is present and behaves as indicated in Fig. 9. Such behavior is found experimentally—e.g, in differential scanning calorimetry (DSC) data on bilayers containing the apolipoprotein of myelin (18). The hypothesis that this protein holds adjacent lipids at an intermediate order ($u_0 \cong 0.5$) is supported by Raman spectroscopic evidence (19).

Lateral Phase Separation. Although we are, in principle, dealing with a binary mixture, so far we have fixed the positions of the proteins. Restoring these translational degrees of freedom would lead to a phase diagram in which the coexistence *line* in Fig. 8 is replaced by a *region* of lateral phase separation. It is clear (Fig. 5) that the order perturbation tends to attract proteins to each other, but the extent of the two-phase region depends on a balance between this factor and many others not considered in this work. These include, e.g , possible disruption of the solid lipid lattice by protein (20, 21), direct (electrostatic) interactions between proteins, entropy of mixing, and attachment of proteins to cytoskeleton. If the two phases have similar protein concentrations and the driving force for macroscopic phase separation is not large (low boundary free energy between the phases), then the phase separation may be difficult to detect experimentally.

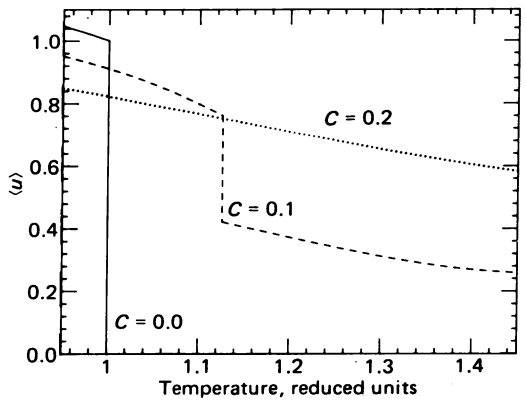

FIG. 7. Dependence of mean value of order parameter in membrane on temperature for three protein concentrations. C is fraction of surface area occupied by protein; $r_0 = 1.0$, $u_0 = 0.75$.

Relevance to Cholesterol-Containing Bilayers. Many important questions remain about the biological and physical effects of cholesterol in cell and model cell membranes. For example, the phase diagram of bilayers formed from binary mixtures of cholesterol and phosphatidylcholines has not been satisfactorily established. Here we discuss whether it is profitable to think of cholesterol as a very small protein in such a system. This question has been examined previously from other viewpoints (e.g., refs. 2, 4, and 22 and refs. therein).

In studies on cholesterol–dipalmitoyl phosphatidylcholine bilayers by DSC the heat absorption curves appear to be decomposible into sharp and broad components (23, 24). As a function of cholesterol mol % (X_{ch}), the enthalpy change for the sharp peak decreases fairly linearly, starting from the value for the pure lipid phase transition at $X_{ch} = 0$ and disappearing around $X_{ch} = 20$–25. The enthalpy for the broad peak rises from zero ($X_{ch} = 0$) to a maximum (near $X_{ch} = 20$–25) and then decreases. The temperature of maximum heat absorption for the sharp peak drops only 1–2 degrees below the pure lipid value when $X_{ch} \cong 20$.

In our treatment, cholesterol is approximated by setting $r_0 \approx 0.3$ (≈ 30 Å^2/cholesterol molecule) and setting $u_0 \approx 0.5$. The results thus obtained are qualitatively the same as those in Fig. 9 and in the DSC studies. Although the DSC results can be interpreted in terms of lateral phase separation for $0 < X_{ch} <$ 20–25 below the pure lipid transition temperature, it was noted that the relationship between the relative sizes of the broad and sharp ΔH values as a function of X_{ch} does not bear out the obvious interpretation of pure lipid and cholesterol–lipid complex phases (23). Our results suggest that it is not necessary to invoke lateral phase separation [or interfacial phenomena (24)] to explain such two-component DSC behavior.

Other evidence that the region $T < 1$, $0 < X_{ch} <$ 20–25, is a single phase recently has been obtained in this laboratory. The lateral diffusion coefficient of fluorescently labeled phospholipids (25) and the spectral characteristics of spin-labeled phospholipids (ref. 25, and unpublished work) undergo sharp changes when the border of this region of the phase diagram is crossed. This is hard to reconcile with the presence of two phases in relative amounts that vary with T and X_{ch}. The sharp changes in the dynamics of the probes are more consistent with

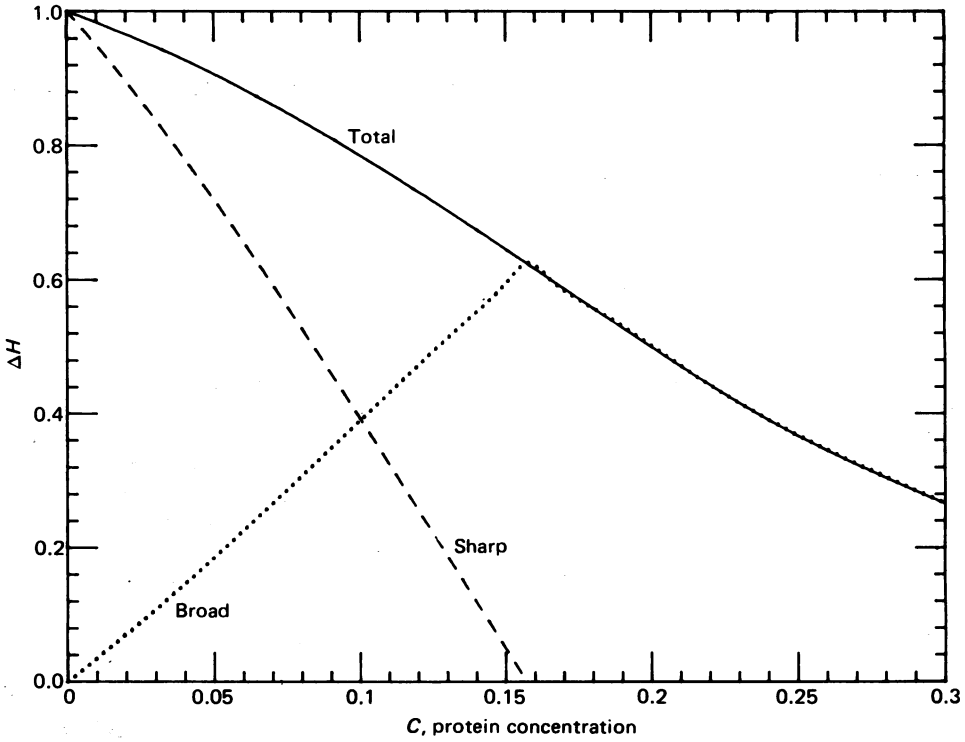

FIG. 9. Effect of protein concentration on enthalpy changes. Total enthalpy change is $H(T = 2.0) - H(T = 0.999)$. The sharp change is that occurring at the phase transition, and the broad change is (total − sharp).

the abrupt change in the number of *gauche* bonds (i.e., in u) that occurs at the phase transition in our theory.

This research was supported by National Science Foundation Grant PCM 77-23586. J.C.O. is the recipient of National Institutes of Health Fellowship 1 F32 AI05912-01.

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