J. Membrane Biol. 90, 157–161 (1986)

The Journal of
Membrane Biology

Dose-Dependent Nonlinear Response of the Main Phase-Transition Temperature of Phospholipid Membranes to Alcohols

Hiroshi Kamaya,† Shao-mu Ma,† and Sheng H. Lin‡

† Anesthesia Service, Veterans Administration Medical Center, Salt Lake City, Utah 84148 and Department of Anesthesia, University of Utah School of Medicine, Salt Lake City, Utah 84132, and ‡ Department of Chemistry, Arizona State University, Tempe, Arizona 85287

Summary. The effect of 1-alkanols upon the main phase-transition temperature of phospholipid vesicle membranes between gel and liquid-crystalline phases was not a simple monotonic function of alkanol concentration. For instance, 1-decanol decreased the transition temperature at low concentrations, but increased it at high concentrations, displaying a minimal temperature. This concentration-induced biphasic effect cannot be explained by the van't Hoff model on the effect of impurities upon the freezing point. To explain this nonlinear response, a theory is presented which treats the effect of 1-alkanols (or any additives) on the transition temperature of phospholipid membranes in a three-component mixture. By fitting the experimental data to the theory, the enthalpy of the phase transition ΔH^* and the interaction energy, ε^*_{AB} between the additive and phospholipid molecules may be estimated. The theory predicts that when $\varepsilon^*_{AB} > 2$ (where $\varepsilon^*_{AB} = \varepsilon_{AB}/RT_o$, T_o being the transition temperature of phospholipid), both minimum and maximum transition temperatures should exist. When $\varepsilon^*_{AB} = 2$, only one inflection point exists. When $\varepsilon^*_{AB} < 2$, neither maximum nor minimum exists. The alkanol concentration at which the transition temperature is minimum (X_{min}) depends on the ε^*_{AB} value: the larger the ε^*_{AB} values, the smaller the X_{min}. When ε^*_{AB} is large enough, X_{min} values become so small that the plot ΔT *vs.* X shows positive ΔT in almost all alkanol concentrations. The interaction energy between 1-alkanols and phospholipid molecules increased with the increase in the carbon chain-length of 1-alkanols. In the case of the dipalmitoylphosphatidylcholine vesicle membrane, the carbon chain-length of 1-alkanols that caused predominantly positive ΔT was about 12.

Key Words phase transition · phospholipids · alcohols · anesthetics · regular solution theory

Introduction

It has been reported (Eliasz et al., 1976; Lee, 1976; Jain & Wu, 1977; Pringle & Miller, 1979; Kamaya et al., 1984) that shorter alkyl-chain 1-alkanols depressed whereas longer ones elevated the main transition temperature of phospholipid membranes. The crossover from depression to elevation of the transition temperature occurred at about the chain-length of 10 to 12 carbon atoms. It is also known (*see*, for instance, Mullins, 1954) that the anesthetic potency of 1-alkanols suddenly disappears when the chain-length exceeds about 10 to 12 carbon atoms. Lee (1976) proposed that this disappearance of anesthetic potency is caused by the crossover of the transition temperature of phospholipid membranes from depression to elevation.

We found (Kamaya et al., 1984), however, that elevation and depression of the transition temperature were not a simple function of the alkanol alkyl chain-length; the transition temperature was nonlinearly related to the alkanol concentrations and the effect was biphasic. As an example, 1-decanol decreased the transition temperature of dipalmitoylphosphatidylcholine membranes at low concentrations but elevated it when the alkanol/lipid mole ratio exceeded about 0.3. The acyl chain-length of phospholipids also affected the contour of the dose-response curve. Similar biphasic responses in the transition temperature of phospholipid vesicle membranes were reported for short-chain 1-alkanols, i.e., methanol, ethanol, propanol (Jain & Wu, 1977) and ethanol (Rowe, 1983). The transition induced by these highly water-soluble 1-alkanols, however, may not be identical with that induced by sparingly water-soluble long-chain 1-alkanols.

The observed biphasic effect of ligand molecules upon the main transition temperature is interpreted by using the regular solution theory for a three-component mixture. It will be shown that, by analyzing the experimental data of the ligand-induced transition temperature change of phospholipid membranes, one can estimate the heat of phase transition of phospholipid membranes and the interaction energy between ligand molecules and phospholipid molecules.

Theory

To treat the effect of 1-alkanols (or any additives) on the transition temperature of phospholipid vesicle membranes (or other polymers) in water (or other solvents), we consider a three-component regular mixture (or solution) for simplicity. In this case, the Gibbs free energy can be expressed as

$$G = n_A\mu_A^o + n_B\mu_B^o + n_C\mu_C^o + \varepsilon_{AB}\frac{n_An_B}{n} + \varepsilon_{AC}\frac{n_An_C}{n} + \varepsilon_{BC}\frac{n_Bn_C}{n} + RT\left(n_A\log\frac{n_A}{n} + n_B\log\frac{n_B}{n} + n_C\log\frac{n_C}{n}\right) \quad (1)$$

where $n = n_A + n_B + n_C$, and (n_A, n_B and n_C) represent the number of moles of (A, B and C); (μ_A^o, μ_B^o and μ_C^o) denote the chemical potential of pure (A, B and C); and (ε_{AB}, ε_{BC} and ε_{AC}) describe the interactions in A, B and C (i.e., $A-B$, $B-C$ and $A-C$). A brief derivation of Eq. (1) is given in the Appendix.

Suppose we are concerned with the phase transition of A in sovlent C, affected by additive B. In most experimental conditions, the concentrations of A and B are very dilute, typically about 10^{-3} M. In other words, the mole fractions of A and B are in the order of magnitude of 10^{-4}. In this case, it is more convenient to deal with the pseudo two-component (A and B) problem than to treat the original three-component situation. For this purpose we rewrite Eq. (1) as

$$G = n_A\mu_A^* + n_B\mu_B^* + n_C\mu_C^* + \varepsilon_{AB}\frac{n_An_B}{n_A+n_B} + RT\left(n_A\log\frac{n_A}{n_A+n_B} + n_B\log\frac{n_B}{n_A+n_B}\right) \quad (2)$$

where μ_A^* and μ_B^* represent the standard chemical potentials of A and B in the solvent C, i.e.,

$$\mu_A^* = \mu_A^o + \frac{n_C\varepsilon_{AC}}{n_A+n_B+n_C} + RT\log\frac{n_A+n_B}{n_A+n_B+n_C} \quad (3)$$

and

$$\mu_B^* = \mu_B^o + \frac{n_C\varepsilon_{BC}}{n_A+n_B+n_C} + RT\log\frac{n_A+n_B}{n_A+n_B+n_C}. \quad (4)$$

Similarly μ_C^* is given by

$$\mu_C^* = \mu_C^o - \frac{n_An_B\varepsilon_{AB}}{n(n_A+n_B)} + RT\log\frac{n_C}{n}. \quad (5)$$

From Eq. (2), one can find the chemical potential of A as

$$\mu_A = \mu_A^* + \varepsilon_{AB}X_B^2 + RT\log X_A = \mu_A^* + RT\log a_A \quad (6)$$

where $X_A = n_A/(n_A+n_B)$ and $X_B = n_B/(n_A+n_B)$, and a_A denotes the activity of A. In phase transition, one phase of A is in equilibrium with another phase of A, i.e.,

$$\mu_A = \mu_{A'}^*. \quad (7)$$

Here, we assume that the new phase of A does not form a solution with B, but the interaction between the new phase of A and solvent C is taken into account through the use of $\mu_{A'}^*$. The derivation of the expression for the change in transition temperature of A, induced by the presence of B, is well known.

Substituting Eq. (6) into Eq. (7) and carrying out the differentiation of the resulting expression with respect to T yields

$$[(\partial\log a_A)/\partial T] = \Delta H^*/RT^2 \quad (8)$$

where ΔH^* represents the heat of phase transition of A in solvent C. Performing the integration of Eq. (8), we obtain, approximately,

$$[\Delta H^*/(RT_o^2)]\Delta_T = \log X_A + \varepsilon_{AB}^*X_B^2 = \Delta T^* \quad (9)$$

where $\varepsilon_{AB}^* = \varepsilon_{AB}/RT_o$ and T_o denotes the transition temperature of A in solution C in the absence of B.

For practical purposes, it is more convenient to express ΔT^* in terms of the mole ratio $X = n_B/n_A$, i.e.,

$$\Delta T^* = -\log(1+X) + \varepsilon_{AB}^*[X/(1+X)]^2. \quad (10)$$

To find the maximum or minimum of the plot ΔT^* *vs.* X, we set $\partial\Delta T^*/\partial X = 0$ and obtain

$$X_\pm = \varepsilon_{AB}^* - 1 \pm [\varepsilon_{AB}^*(\varepsilon_{AB}^* - 2)]^{1/2} \quad (11)$$

where X_- denotes the value of X at the minimum while X_+ represents the value of X at the maximum.

From Eq. (11), we can see that for $\varepsilon_{AB}^* < 0$, neither maximum nor minimum exists; for $\varepsilon_{AB}^* > 2$, both maximum and minimum exist; for $\varepsilon_{AB}^* = 2$ only one solution exists with $X_- = 1$ (an inflection

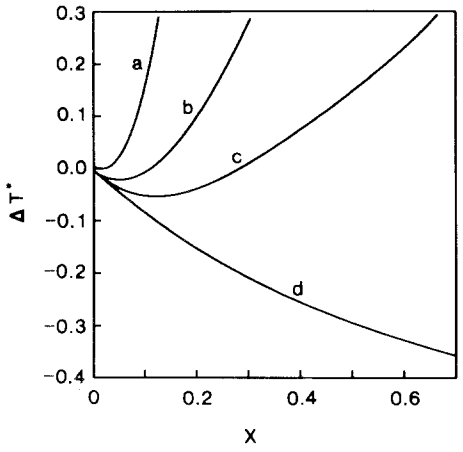

Fig. 1. Plot of ΔT^* *vs.* X for various ε_{AB}^* values. The ε_{AB}^* values are: $a = 30$, $b = 10$, $c = 5$, and $d = 2$, where X is the mole ratio, $\Delta T^* = \Delta T[\Delta H^*/(RT_0^2)]$

point); and for $\varepsilon_{AB}^* < 2$ again neither maximum nor minimum exists. It should be noted that Eq. (10) provides a universal plot of ΔT^* *vs.* X; this type of curve is shown in Fig. 1. A main feature of this type of plot is that from the minimum point alone one can determine both ΔH^* and ε_{AB}^*. For example, if one experimentally finds that the plot of ΔT *vs.* X has a minimum point at $X = 0.125$, then from Fig. 1, one can obtain $\varepsilon_{AB}^* = 5$ or $\varepsilon_{AB} = 5RT_o$, and $\Delta T^* = -0.0550$ or $\Delta H^* = RT_o^2(\Delta T^*/\Delta T)$. In this way, one can determine both ε_{AB} and ΔH^*, the heat of phase transition of A in solvent C.

For convenience, a table of ε_{AB}^* and $X_{\min}$ (or X_-) is shown in Table 1. From Table 1 and Fig. 1, we can see that the larger the ε_{AB}^* values, the smaller the $X_{\min}$ values. For example, for the case of $\varepsilon_{AB}^* = 30$ (*see* Fig. 1), the minimum point takes place at such a small X value that the plot ΔT *vs.* X shows positive ΔT for practically all X values.

Materials and Methods

Synthetic dipalmitoylphosphatidylcholine was obtained from Sigma. The preparation showed a single spot by thin-layer chromatography. Reagent grade 1-alkanols (1-decanol, 1-undecanol, 1-dodecanol, 1-tridecanol and 1-tetradecanol) were obtained from Eastman Organic Chemicals. The purity was checked by gas-chromatography (Shimadzu, Columbia, Ohio) and single peaks were confirmed. Water was purified by triple distillation, once from alkaline potassium permanganate solution. The specific conductivity of obtained water was $1.1 \times 10^{-6}\ \Omega^{-1}\ \mathrm{cm}^{-1}$. Absence of surface-active contaminants was checked by dynamic surface tension measurement as previously described (Shibata et al., 1981).

The vesicles were prepared by ultrasonic irradiation of aqueous suspension of 1.0 mM phospholipid in the cuphorn of a Branson Ultrasonic Disruptor (Danbury, Conn.) at a temperature several degrees above the phase transition, as previously reported (Kamaya et al., 1981). The preparation was aged at 4°C for two weeks to obtain homogeneous and relatively large vesicle suspension (Wong et al., 1982). Long-chain alkanols were mixed with the vesicle suspension by sonication under the flow of nitrogen gas.

Table 1. $X_{\min}$ and ε_{AB}^*

$X_{\min}$	ε_{AB}^*
0.05	11
0.10	6.05
0.20	3.60
0.30	2.82
0.40	2.45
0.50	2.25

The phase transition temperature was measured by the light absorbance at 350 nm, using a Perkin-Elmer 554 uv-visible spectrophotometer equipped with a programmable electronic temperature-controller, and a micro-stirrer. The cuvette temperature was raised (heating scan) at a rate of 0.5°C per min, and monitored by a filament thermistor inserted into the cuvette and a Digitec amplifier (United Systems, Dayton, Ohio). The photometer output was recorded together with the temperature signal on an X-Y recorder.

Results

For the present binary system of lipid and long-chain 1-alkanol, the onset point where the absorbance suddenly increased in the heating scan was taken as the transition temperature. This is because the ligand tends to become a membrane component. In this case, the endpoint of the change in the absorbance scan loses its theoretical meaning, and the midpoint becomes smeared. Agreement of the data obtained by the spectrophotometric method to those obtained by differential scanning microcalorimetry was excellent as reported previously (Kamaya et al., 1981). Figure 2 shows experimental results on the onset temperature of the main transition temperature of the dipalmitoylphosphatidylcholine vesicle membrane in the endothermic scan. The melting temperature of the dipalmitoylphosphatidylcholine membrane without alkanols was 314.1 K, and the changes in the transition temperatures by anesthetics are plotted as a difference from this value. The alkanol concentrations are expressed by the mole ratio between the total amount of alkanol versus the total amount of phospholipid because the amounts of phospholipid and long-chain 1-alkanol, dispersed as monomer in the aqueous phase, are small. Although the actions of 1-tetradecanol and 1-tridecanol appear to be a simple elevation of the transition temperature, extrapolation of the plot to zero alkanol concentration by the polynomial least-squares method intersected the temperature axis below the control, indicating the existence of the temperature minima.

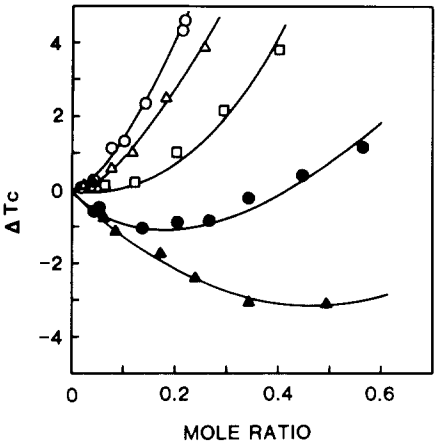

Fig. 2. Effect of 1-alkanols on the temperature of gel-to-liquid-crystalline phase transition of dipalmitoylphosphatidylcholine vesicles. ○, 1-tetradecanol; △, 1-tridecanol; □, 1-dodecanol; ●, 1-undecanol; and ▲, 1-decanol. The alkanol concentration is expressed by mole ratio to the phospholipid

Discussion

In this section we shall apply the theory to analysis of our experimental results. In Fig. 2, the comparison between experimental and calculated results is shown; the agreement is reasonably good. The whole system consists of three components: 1-alkanol, phospholipid and water. In the dilute solution approximation, water is considered to be the dominant component. The concentration of solvent water is assumed to be unchanged during phase transition. Thus, the three-component system can be treated as a pseudo two-component system. The mole ratio described in the figure applies to the total alkanol concentration versus the total phospholipid concentration. Although the value approaches 0.5, the total concentration of solutes is still very small when compared to the solvent water concentration.

In Table 2, the interaction energies ε_{AB}^* between the phospholipid membrane vesicle and 1-alkanols are presented. As is to be expected, ε_{AB}^* (1-decanol) $< \varepsilon_{AB}^*$ (1-undecanol) $< \varepsilon_{AB}^*$ (1-dodecanol) $< \varepsilon_{AB}^*$ (1-tridecanol) $< \varepsilon_{AB}^*$ (1-tetradecanol). The values for apparent ΔH^* for the alkanol-mixed dipalmitoylphosphatidylcholine membranes scatter between 39.5 kJ mol^{-1} for decanol to 330 kJ mol^{-1} for 1-tetradecanol. The ΔH^* values presented here are a theoretical prediction of the calorimetric data, and are comparable to the van't Hoff enthalpy of phase transition. The difference between theoretical values and measured values is often attributed to the cooperativity of the phase transition (Hinz & Sturtevant, 1972; Mabrey & Sturtevant, 1976).

The reported values of the transition enthalpy for dipalmitoylphosphatidylcholine membranes scatter from the measured value of 36.4 kJ mol^{-1} by differential scanning calorimetry (Lippert & Peticolas, 1971; Mabrey & Sturtevant, 1976; Blume, 1979; Correa-Freire et al., 1979; Stümpel et al., 1981) to the theoretical value of 7280 kJ mol^{-1}, estimated according to the vant' Hoff model (Hinz & Sturtevant, 1972). Because longer chain alkanols appear to stabilize the solid-gel phase of the membrane, the present data, which showed larger ΔH^* with the increase in the chain-length of the alkanols, may be reasonable. The concentration dependence of ΔH^* and ε_{AB}^* will be analyzed in a forthcoming paper.

Table 2. Results of ε_{AB}^* and ΔH^*

	ε_{AB}^*	ΔH^*
Decanol	2.20	39.5 kJ
Undecanol	3.94	53.0
Dodecanol	9.36	110
Tridecanol	26.01	210
Tetradecanol	51.01	330

From Fig. 1 and Eq. (11), for the case of $\varepsilon_{AB}^* = 2$, $X_- = X_+ = 2$, and the $X = 2$ concentration is an inflection point. This point has been borne out by the experiment of Eliasz et al. (1976) on the effect of 3-phenyl propanol on the transition of dipalmitoylphosphatidylcholine.

In this paper, we have shown how the experimental data of phase-transition temperatures of phospholipid membranes in the presence of binding additives can be analyzed to obtain the apparent heat of phase transition of phospholipid membranes and the apparent interaction energy between the membrane and the additive. In concluding this paper, we would like to point out the approximations introduced in the theory. It should be noted that the regular solution theory is used, that the pseudo two-component approximation for a three-component mixture has been introduced, and that the new phase appearing in the phase transition is assumed to be pure. These approximations can be improved. As discussed earlier, the regular solution theory deals with random mixing of isotropic liquids; anisotropy of the bilayer is ignored. Also the regular solution theory assumes that the potential energy depends only upon nearest-neighbor contacts.

The present model only concerns a transition occurring at a critical temperature and does not specify the nature of the transition. Even if the transition is the change from the liquid-crystalline to, say, the hexagonal phase (Cullis & DeKruijff, 1979) or to the interdigitated state (McIntosh et al., 1983; Simon & McIntosh, 1984), the model still holds. Although the model is crude, we have shown

that useful information can be obtained even with a simple regular solution model. To improve the present model, interactions beyond the nearest-neighbor pair should be considered and the relative size of ligand and lipid should be taken into account, i.e., by replacing N_b with nN_b and including a surface energy term.

This study was supported by NIH grants GM25716, GM26950 and GM27670, and by the Medical Research Service of the Veterans Administration. The authors thank Mr. H. L. Blue for his assistance in preparing the manuscript.

References

Blume, A. 1979. A comparative study of the phase transitions of phospholipid bilayers and monolayers. *Biochim. Biophys. Acta* **557:**32–44

Correa-Freire, M.C., Freire, E., Barenholz, Y., Biltonen, R.L., Thompson, T.E. 1979. Thermotropic behavior of monoglucocerebroside-dipalmitoylphosphatidylcholine multilamellar liposomes. *Biochemistry* **18:**442–445

Cullis, P.R., DeKruijff, B. 1979. Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochim. Biophys. Acta* **559:**399–420

Eliasz, A.W., Chapman, D., Ewing, D.F. 1976. Phospholipid phase transitions. Effects of *n*-alcohols, *n*-monocarboxylic acids, phenylalkyl alcohols and quaternary compounds. *Biochim. Biophys. Acta* **448:**220–230

Hinz. H.J., Sturtevant, J.M. 1972. Calorimetric studies of dilute aqueous suspensions of bilayers formed from synthetic L-alpha-lecithins. *J. Biol. Chem.* **247:**6071–6075

Jain, M.K., Wu, N.M. 1977. Effect of small molecules on the dipalmitoyl lecithin liposomal bilayer: III. Phase transition in lipid bilayer. *J. Membrane Biol.* **34:**157–201

Kamaya, H., Kaneshina, S., Ueda, I. 1981. Partition equilibrium of inhalation anesthetics and alcohols between water and membranes of phospholipids with varying acyl chain-lengths. *Biochim. Biophys. Acta* **646:**135–142

Kamaya, H., Matubayasi, N., Ueda, I. 1984. Biphasic effect of long-chain *n*-alkanols on the main phase transition of phospholipid vesicle membranes. *J. Phys. Chem.* **88:**797–800

Lee, A.G. 1976. Interactions between anesthetics and lipid mixtures. Normal alcohols. *Biochemistry* **15:**2448–2454

Lippert, J., Peticolas, W.L. 1971. Laser Raman investigation of the effect of cholesterol on conformational changes in dipalmitoyl lecithin multilayers. *Proc. Natl. Acad. Sci. USA* **68:**1572–1576

Mabrey, S., Sturtevant, J.M. 1976. Investigation of phase transitions of lipids and lipid mixtures by high sensitivity differential scanning calorimetry. *Proc. Natl. Acad. Sci. USA* **73:**3862–3866

McIntosh, T.J., McDaniel, R.V., Simon, S.A. 1983. Induction of an interdigitated gel phase in fully hydrated phosphatidylcholine bilayers. *Biochim. Biophys. Acta* **731:**109–114

Mullins, L.J. 1954. Some physical mechanism in narcosis. *Chem. Rev.* **54:**289–323

Pringle, M.J., Miller, K.W. 1979. Differential effects on phospholipids phase transitions produced by structurally related long chain alcohols. *Biochemistry* **16:**3314–3320

Rowe, E.S. 1983. Lipid chain length and temperature dependence of ethanol-phosphatidylcholine interactions. *Biochemistry* **22:**3299–3305

Shibata, A., Suezaki, Y., Kamaya, H., Ueda, I. 1981. Adsorption of inhalation anesthetics on the air/water interface and the effect of water structure. *Biochim. Biophys. Acta* **646:**126–134

Simon, S.A., McIntosh, T.J. 1984. Interdigitated hydrocarbon chain packing causes the biphasic behavior in lipid/alcohol suspensions. *Biochim. Biophys. Acta* **773:**169–172

Stümpel, J., Nicksch, A., Eible, H. 1981. Calorimetric studies on saturated mixed-chain lecithin-water systems. Nonequivalence of acyl chains in the thermotropic phase transition. *Biochemistry* **20:**662–665

Wong, M., Anthony, F.H., Tillack, T.W., Thompson, T.E. 1982. Fusion of dipalmitoylphosphatidylcholine vesicles at 4°C. *Biochemistry* **23:**4126–4132

Received 19 August 1985; revised 1 November 1985

Appendix

A brief derivation of the expression for the Gibbs free energy of a three-component regular solution is presented below. In the Bragg-Williams approximation, the canonical partition function can be written as

$$Q(T) = \frac{(n_A + n_B + n_C)!}{n_A! n_B! n_C!} \cdot q_A^{n_A} \cdot q_B^{n_B} \cdot q_C^{n_C} \cdot \exp\left[-\frac{1}{kT} (\varepsilon'_{AB} n_{AB} + \varepsilon'_{AC} n_{AC} + \varepsilon'_{BC} n_{BC}) \right] \quad \text{(A-1)}$$

where (n_A, n_B, n_C) represent the number of molecules of (A, B, C). For example, $q_A^{n_A}$ denotes the partition function of pure A. (n_{AB}, n_{AC}, n_{BC}) represent the average number of nearest neighbor pairs (A-B, A-C, B-C). Note that

$$\varepsilon'_{AB} = \varepsilon''_{AB} - (\varepsilon''_{AA} + \varepsilon''_{BB})/2 \quad \text{(A-2)}$$

where, for example, ε''_{AA} denotes the interaction energy of a nearest neighbor pair A-A.

In the Bragg-Williams approximation, we have, for example,

$$n_{AB} = z n_A n_B / (n_A + n_B + n_C) \quad \text{(A-3)}$$

where z is the coordination number.

The Helmholtz free energy A is related to $Q(T)$ by

$$A = -kT \log Q(T). \quad \text{(A-4)}$$

Substituting Eq. (A-1) into Eq. (A-4) yields

$$A = n_A \mu_A^o + n_B \mu_B^o + n_C \mu_C^o + \varepsilon_{AB} \frac{n_A n_B}{n} + \varepsilon_{AC} \frac{n_A n_C}{n} + \varepsilon_{BC} \frac{n_B n_C}{n} + kT \left(n_A \log \frac{n_A}{n} + n_B \log \frac{n_B}{n} + n_C \log \frac{n_C}{n} \right) \quad \text{(A-5)}$$

where $n = n_B + n_A + n_C$, $\varepsilon_{AB} = z'_{AB}$ and $\varepsilon''_A = -kT \log q_A$, etc. Ignoring the pressure-volume PV term for a condensed system, $A = G$.