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Chloroform-Chloroethanol 용매중에서 Poly(*cis*-5-methylproline)의 평형 및 열역학적연구

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Equilibrium Studies and Thermodynamics for the Mutarotation of Poly(*cis*-5-methylproline) in Chloroform-Chloroethanol

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요 약. Chloroform과 chloroethanol 혼합용매속에서 Poly(*cis*-5-methylproline)의 광회전을 측정하였다. 이 혼합용매속에 chloroethanol이 0.5~10부피%일때 형태 A와 B사이에는 평형상태가 이루어졌다. 광회전을 이용하여 5, 25 및 45 °C에서 평형정수를 측정하고 자유엔탈피, 엔탈피 및 엔트로피 변화를 계산하였다. 같은 몰수의 형태 A와 B에서 출발하면 chloroethanol이 3부피% 이상일때는 정방향 변광회전이 일어나고 이하일때는 역방향 회전이 일어났다.

엔탈피와 엔트로피 변화는 정방향 변광회전일때는 양수이고 역방향 변광회전일 때는 음수였다. 반응 추진역은 정방향 회전일때는 엔트로피의 증가이고 역방향 회전일때는 엔탈피의 감소였다. 측정된 열 역학적 자료들은 폴리머와 용매사이의 상호작용 즉 용매중의 chloroethanol이 형태 B분자내의 카보닐기와 선택적으로 수소결합을 형성하며, 형태 A와 B의 형태 에너지 차이에 있다는 것을 설명한다

ABSTRACT. The molar optical rotation of poly(*cis*-5-methylproline) was measured in solvent mixtures of chloroform and chloroethanol. After proper allowance for time-dependent mutarotations, equilibrium states between form A and form B were observed to occur with a solvent composition of 0.5~10 % chloroethanol in chloroform by volume. From the equilibrium constants, which were calculated by optical rotations at equilibrium measured at three different temperatures (5, 25, and 45 °C), the thermodynamic parameters—free enthalpy, enthalpy and entropy changes for the mutarotation—were evaluated. It was found that starting with equimolar concentrations of form A and form B, the forward mutarotation occurred in the solvent compositions of chloroethanol greater than 3 % by volume, whereas the reverse mutarotation resulted in solvent compositions of chloroethanol less than 3 % by volume.

The changes in enthalpy and entropy for the forward mutarotation were found to be positive, while those were for the reverse mutarotation were negative. The driving forces for the forward mutarotation were found to be the increase in entropy, whereas that for the reverse mutarotation was

the negative enthalpy change. The thermodynamic data were explained by the interaction between polymer and solvent, *i.e.*, preferential hydrogen bonding of chloroethanol with the carbonyl group in form B over form A, and by difference in conformational energies between form A and form B.

INTRODUCTION

Numerous studies have been reported which handed helix with all *cis* amide bonds describe the conformations of polyproline (PP) in the solid state and in solution, and its mutarotations in solvent mixtures.[1] It was also known that in appropriate solvent mixtures, equilibrium states between form I and form II were established, which were highly sensitive to small changes in solvent composition.[2] A slight excess of stabilizing energy in favor of one form seemed to be sufficient for a complete shift of the equilibrium. However, there are no reports in the literature of detailed studies of thermodynamics of the solvent-induced mutarotation of PP.

Conformational transitions of biopolymers induced by changes in solvent systems have been investigated in many laboratories. Two general types of explanations for the solvent-induced transitions of proteins, polypeptides and nucleic acids have been proposed:[3] 1) Stabilization and destabilization by direct binding of one or more of the solvent components to the polymer; 2) indirect influences of change in solvent composition, *e. g.*, *via* changes in solvent structure. However, a detailed knowledge of interactions between polymers and solvent molecules has been obtained only in a few cases.[4,5] Complete thermodynamic descriptions of solvent-induced transition of polymer are rarely found.

In the previous paper[6] we reported the synthesis, solution properties of poly(*cis*-5-methylproline) (PCMP), a derivative of PP, and its mutarotation. It was found that the polymer exists in two ordered structures, form A and form B, which have conformations similar to PP form I, a right-handed helix with *cis* amide bonds and form II, a left-handed helix with all *trans* amide bonds, respectively. The transition between the two forms was found to involve *cis-trans* isomerization of amide bond in the polymer and it was induced through changes in solvent compositions. A careful study of mutarotation kinetics showed that the molar optical rotation of the polymer solution was proportional to the concentration of *trans* amide bond in the polymer.[8] This enabled us to evaluate quantitatively the thermodynamic data for the mutarotation. This paper reports the equilibrium and thermodynamic studies of PCMP in chloroform(CF)-chloroethanol (CE).

EXPERIMENTAL

Polymerization. The preparation of PCMP was described in the previous communication.[6] Poly(*cis*-5-methyl-D-proline) having a number average molecular weight of 630 and intrinsic viscosity 0.16 in dichloroacetic acid at 25 °C was used.

Preparation of Solutions. The polymer was dissolved in CF. This stock solution was diluted with mixtures of CF and CE to the definite solvent compositions. In order to avoid effects of concentration on the molar optical rotation, the concentration of polymer in different solvent mixtures was held constant (0.4 mg/m*l*). The equilibrium solutions were kept in a thermostated bath.

Measurements of Optical Rotation. Optical rotations were measured with a JASCO ORD, CD, UV-5 spectropolarimeter. A one cm cell equipped with a temperature control jacket was used and the temperature of the equilibrium

solution in the cell was measured with a thermocouple.

RESULTS

Equilibrium Studies. As previously reported,[6,7] the dissolution of PCMP form B in chloroform is followed by the conversion to form A. This mutarotation continues until the conversion from form B to form A is completed. Dilution of the chloroform solution with an excess of alcohol reverses the process and after sufficient time interval form B is recovered. This process represents the completion of a closed thermodynamic cycle. The extent of the forward mutarotation (A→B) is dependent upon both the type and concentration of alcohols used. In the case of chloroethanol, trifluoroethanol and methanol, about 10 volume percent in chloroform solution is enough to complete the forward mutarotation of PCMP, while in the case of *n*-propanol, *n*-butanol and ethanol, higher concentrations are necessary. At volume percentages in the range 0.5~10 of former alcohols, equilibrium states between form A and form B were established.

Both forward and reverse mutarotations have been studied in several solvent systems.[7] In these studies emphasis has been placed on the observation of transient properties of the unstable intermediate between form A and form B, and of the relationship between solvent composition and equilibrium properties of PCMP. Chloroformchloroethanol was chosen as a solvent mixture for the equilibrium and thermodynamics studies on PCMP.

The procedure consists of preparing dilute solutions of PCMP in solvent mixtures ranging in composition from pure chloroform to a mixture of 10 % chloroethanol in chloroform by volume. The concentration of polymer was held constant at 0.4 mg/m*l* in order to avoid any effects of concentration on the optical rotation. At 25 °C the molar optical rotation of these solutions changed for about 30 days, after which time no more change was observed. The values of molar optical rotation at 265 mμ after 30 days in solution are shown in *Fig.* 1. PCMP form A in chloroform gives a molar optical rotation of −580, whereas form B in chloroethanol gives +2500.

By increasing the concentration of chloroethanol the optical rotations are increased, and approach the value form B at 10 % of chloroethanol by volume. A slight deviation was found at the solvent composition of chloroethanol 4~6 %. Since the kinetic studies of forward and reverse mutarotation have not shown any stable intermediate stage, the curvature in *Fig.* 1 does not seem to be a stable intermediate. It might be due to anomalous

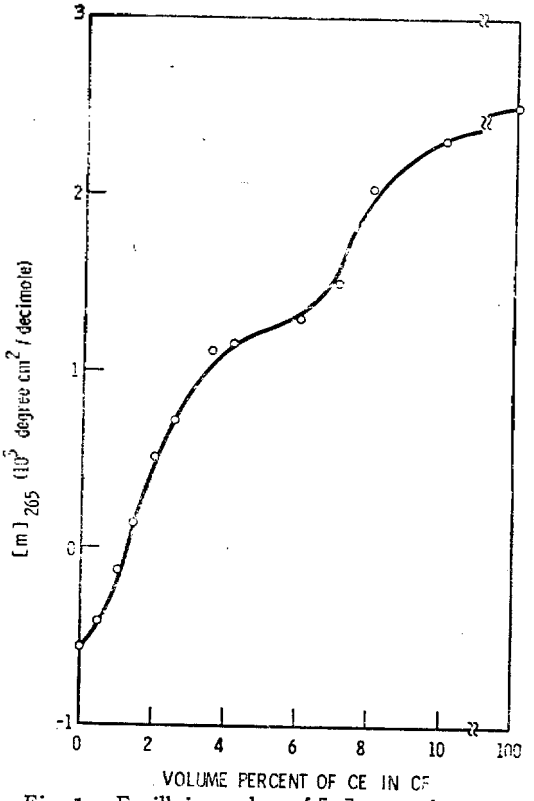


Fig. 1. Equilbrium values of $[m]_{265}$ as a function of solvent composition: chloroethanol (CE)-chloroform (CF).

changes in the physical properties of binary solvent mixtures.

As was found in the kinetic studies of PCMP mutarotation,[7] the mole fraction of *trans* amide bonds in the polymer can be evaluated according to the relation:

$$X_{trans}=\frac{[m]-[m]_\infty}{[m]_0-[m]_\infty} \qquad (1)$$

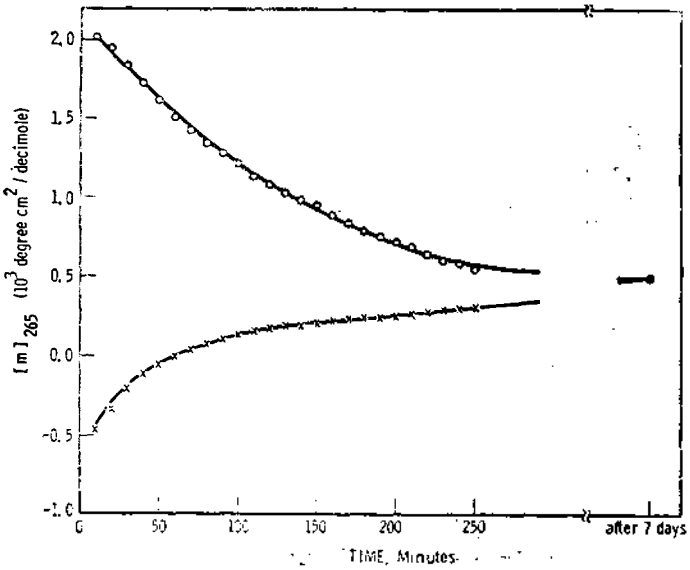


Fig. 2. The time-dependent approach to the equilibrium values of $[m]_{265}$ in chloroethanol-chloform (2 : 100 v/v) at 37.8 °C starting with form A and form B.

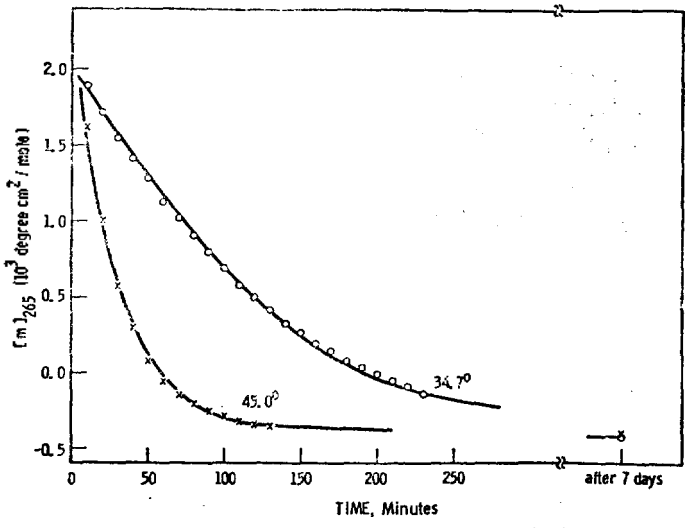


Fig. 3. The acceleration of equilibrium rates at higher temperat ure in chloroethanl-chloroform (1 : 200 v/v).

where X_{trans} : mole fraction of *trans* amide bonds, $[m]$: optical rotation of PCMP solution at $\lambda=265m\mu$, $[m]_0$: optical rotation of PCMP form B(+2500), $[m]_\infty$: optical rotation of PCMP form A (−580).

The derivation of this relation was based on the kinetics studies of the reverse mutarotation of PCMP; the activation energy of the mutarotation was evaluated by two methods. One in the classical Arrhenius method with the apparent rate constants of first order, which covered the whole course of the mutarotation. The other is a new equation, which is independent of reaction orders. Activation energies measured by the two different methods coincided within an experimental error. This resulted to the conclusion that the molaroptical rotation was proportional to the concentraion of *trans* amide bonds in the polymer.

The equilibrium values of high molecular weight of PP in *n*-propanol-acetic acid underwent an abrupt change within a narrow interval of solvent compositions.[2] This suggests and is consistent with the occurrence of a cooperative transition between form I and form II of PP. In our case the change of optical rotation of PCMP are spread over wide range of solvent composition of chloroethanol (0.5~10 % by volume). The concentration of form B is increased with increase in concentration of chloroethanol. The cooperative process became important by increasing the molecular weight of the polymer. The polymer used in this investigation has very low mole

cular weight(Mn=630), the cooperative process was not observed in this system as expected.

As further verification of the establishment of an equilibrium, it was demonstrated that an observed limiting optical rotation can be approached along different kinetic paths. The establishment of equilibrium starting with form A and form B, which were dissolved in the solvent mixture of the same composition (CE: CF=2 : 100 v/v), are shown in *Fig.* 2. The acceleration of the rate of equilibrium with increasing temperature is shown in *Fig.* 3. Since substantial activation energies are necessary for both forward and reverse mutarotations, higher temperatures accelerate the attainment of an equilibrium condition.

In another series of experiments, to be discussed later, all of the sample solutions of *Fig.* 1 were subjected to heating (45°C) and cooling cycle (5 °C), and equilibrated at 25 °C. The final values of the optical rotation of the solutions were in agreement with the initial value measured at 25 °C.

These experiments demonstrate that the asymptotic values of molar optical rotation of PCMP are independent on: 1) the initial form of the polymer at the time of dissolving, and 2) the direction from which they mutarotate. They, therefore, can be considered to be true equilibrium values uniquely determined at a given temperature by the solvent composition.

THERMODYNAMICS

Reversibility. The equilibrium data presented in this paper represent true thermodynamic equilibria. Form B, when dissolved in chloroform, mutarotates to form A. Dilution of chloroform solution with excess alcohol reverses the process, yielding form B whose optical rotation is unchanged. Mutarotation by heating or cooling at constant chloroethanol concentration was also found to be completely reversible. The heat precipitation, as observed in denaturation of proteins, does not occur in this mutarotation in the measured range.

Equilibrium Constants. The mole fraction of form B (*trans* amide bonds) were calculated from *Fig.* 1 according to the equation (1). The equilibrium constants for the forward and reverse mutarotations can be obtained as follows:

$$K_f=\frac{X_{trans}}{1-X_{trans}} \quad \text{for } A\rightleftharpoons B$$
$$K_r=\frac{1-X_{trans}}{X_{tras}} \quad \text{for } B\rightleftharpoons A \qquad (2)$$

The evaluated equilibrium constants for the reverse mutarotation at 25 °C were plotted against the solvent composition (*Fig.* 4). At low concentration of chloroethanol the equilibrium constants increase dramatically, indicating that the mole fraction of *trans* amide bonds is approaching one. At high concentrations of chloroethanol, K values approach zero. At chloroethanol concentration of 3 % by volume, equilibrium constant is one, indicating that the concentrations of *cis* and *trans* amide bonds

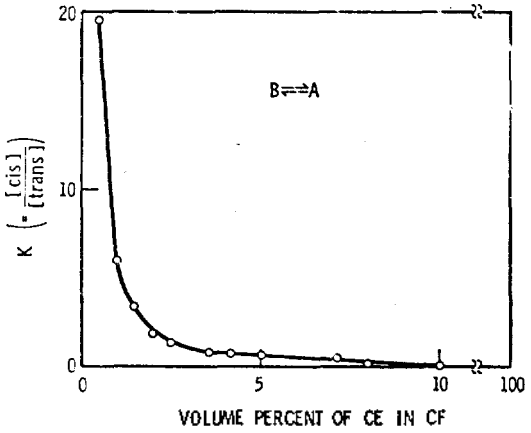


Fig. 4. The equilibrium constants at 25°C as a function of solvent composition: chloroethanol(CE)-chloroform(CF).

are equal.

The equilibrium constants should be calculated from the activities, however, the measurements of activity are nearly impossible in this solvent mixture. Since changes in the concentration of *cis* and *trans* amide bonds is only one order of magnitude in the measured range and the change in the solvent composition is 0.5 to 10 volume percent of chloroethanol, the equilibrium constants evaluated from the concentrations should not deviate significantly from true equilibrium constants.

Standard Free Enthalpy Change. The standard free enthalpy changes at 25°C were evaluated from the equilibrium constants according to the thermodynamic relation:

$$\Delta G^\circ = RT \ln K \qquad (3)$$

The ΔG° values depend upon solvent compositions and temperatures. Only negative ΔG° values are plotted as a function of solvent compositions in *Fig.* 5, which is divided into two zones by the solvent composition of 3 % chloroethanol by volume, at which ΔG° is zero. Starting with equimolar concentrations of form A and form B, the rate of the forward and reverse mutarotation at this solvent composition are equal, so that the concentrations of *cis* and *trans* amide bonds at 25 °C are not changed.

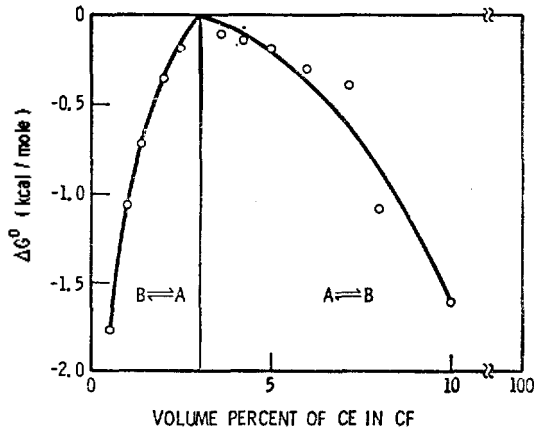


Fig. 5. Standard free enthalpy changes for the forward and reverse mutartoation at 25 °C as a function of solvent composition: cholrethanol (CE)-chloroform (CF).

Under same conditions, the forward mutarotation occurs in solvent compositions of chloroethanol greater than 3 % by volume, whereas the reverse mutarotation occurs in solvent compositions of less than 3 % chloroethanol by volume. The standard free enthalpy changes in both cases are negative. Since the magnitude of ΔG° for a chemical reaction indicates the extent to which it proceeds, the extent of reverse mutarotation was increased by decreasing the chloroethanol concentration, and similarly the extent of the forward mutarotation was increased with the increase of chloroethanol concentration.

Enthalpy Change. In order to evaluate the enthalpy changes, the equilium constants were measured at three different temperatures; 5, 25 and 45°C. The equilibrium solutions were kept at a given temperature for 2 days. The molar optical rotation measured after 1 and 2 days, and the observed molar optical rotation of solutions were found to be constant. The equilibrium states of the solutions at different temperatures were established within a day. The changes in optical rotation caused by different solvent densities at different temperatures were corrected.

The molar optical rotation at equilibrium states at two different temperatures (5 and 45 °C) were plotted against the solvent compositions, shown in *Fig.* 6. In all solvent compositions the molar optical rotations at lower temperature (5 °C) are greater than those at higher temperature (45 °C). The equilibrium at higher temperature was shifted in favor of form A, whereas at lower temperature it was shifted in favor of form B.

The typical van't Hoff plots, log K(K=

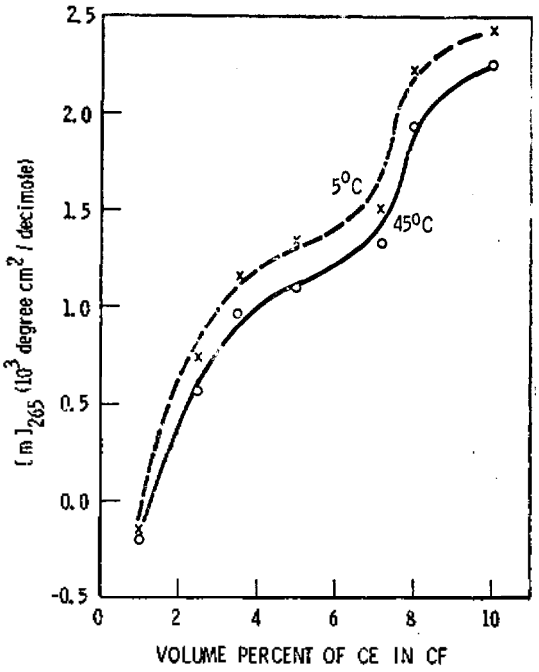


Fig. 6. Equilibrium values of $[m]_{265}$ as a function of solvent composition (CE: chloroethanol, CF: chloroform) at two different temperatures.

[*cis*]/[*trans*]) as a function of reciprocal of absolute temperature, are shown in *Fig.* 7. Though slight deviations at the extreme solvent compositions of 1 and 10 % of chloroethanol by volume were observed, the relationships at three different temperatures are essentially linear. The enthalpy changes of given solvent compositions in measured temperature range were therefore presumed to be constant. Consequently the heat capacity of form A and form B are the same in measured temperature range. In case of protein denaturation, it has been found that enthalpy changes are strongly dependent on the temperature, and that the native and denatured proteins differed in heat capacity by several kcal per residue mole[8]. This was ascribed to the exposure of hydrophobic groups of side chains to aqueous solvent. Since there were no side chains with significant length in PCMP and since the mutarotations were carried out in organic solvent mixtures, differences in heat capacity between forms A and B were not expected.

The enthalpy changes for the reverse mutarotation were evaluated from the slopes in *Fig.* 7. and plotted as a function of solvent compositions in *Fig.* 8. The enthalpy changes for the

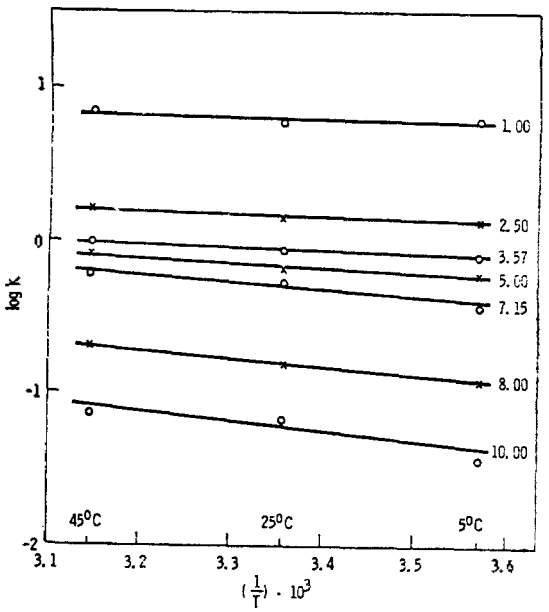


Fig. 7. The van't Hoff plots derived from *Figs.* 1 and 6. The numerals in this figure show the vol% of chloroethanol in chloroform.

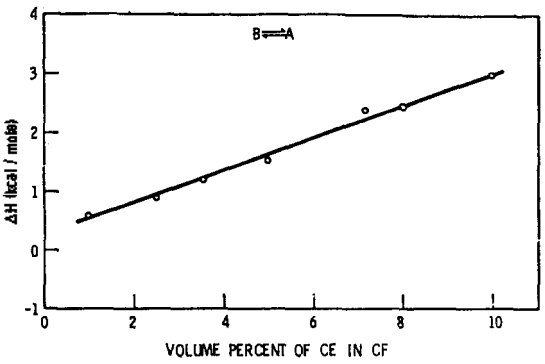


Fig. 8. Enthalpy change for the reverse mutarotation as a function of solvent composition: chloroethanol (CE) -chloroform (CF).

reverse mutarotation are also dependent on the solvent compositions and are positive in all solvent compositions. In the solvent mixture, 1 % chloroethanol by volume, in which form A is more stable than form B, the enthalpy for the reverse mutarotation is increased. This result leads to the conclusion that the enthalpy of form A is originally higher than that of form B. In the kinetics studies of the mutarotation of PCMP, it was found that the activation energy for the forward mutarotation was 2.5 kcal per residue mole lower than the activation energy of the reverse mutarotation in a similar solvent mixture[7]. Although difference in activation energies caused by different solvation could not be excluded, the main reason for the difference seems to be the difference in enthalpy between form A and form B.

Entropy Changes. The entropy changes for the mutarotation of PCMP were evaluated and plotted against solvent compositions in *Fig.* 9. The entropy changes are positive for the forward mutarotation and negative for the reverse mutarotation, and are also dependent on the solvent composition. A plot of $\Delta G°$ values as a function of temperature are shown *Fig.* 10. According to the relation:

$$\left(\frac{\partial \Delta G}{\partial T}\right)_p = -\Delta S \qquad (4)$$

the slope of tangents to curves in *Fig.* 10 are $-\Delta S°$. The values of $\Delta S°$ calculated from slopes of the tangents to the curves at 25 °C correspond within 10 % error with the values (*Fig.* 9) evaluated from $\Delta G°$ and ΔH. In 3.57 % of chloroethanol, $\Delta G°$ increases with temperature. Extension of the curve to 47 °C gives $\Delta G°=0$, and above this temperature the standard free enthalpy change becomes positive. The value of $\Delta G°$ is very small in this solvent composition and hence the difference between the values of entropy and enthalpy terms is minute; the direction of the mutarotation (sign of $\Delta G°$) is controlled by the temperature.

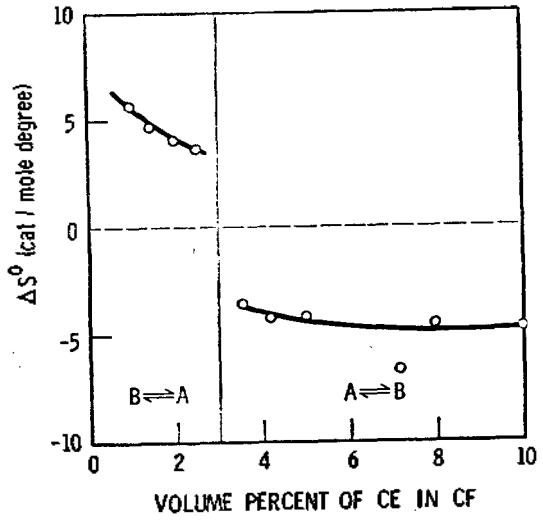


Fig. 9. Entropy change for the forward and reverse mutarotation *vs.* solvent composition: chloroethanol (CE)-chloroform (CF).

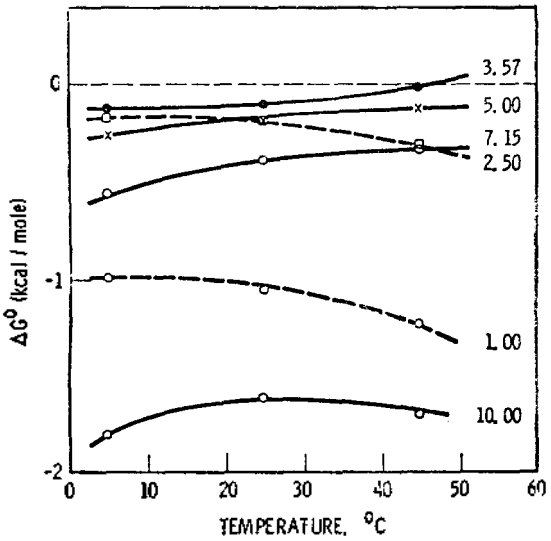


Fig. 10. Standard free enthalpy changes as a function of temperature. The numerals in this figure show the vol% of chloroethanol in chloroform.

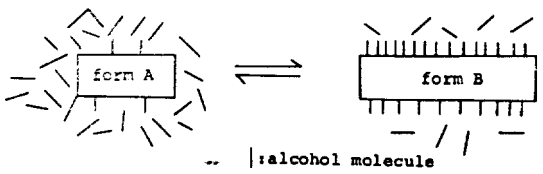
DISCUSSION

Kinetics, equilibrium and ir-spectroscopic studies of PCMP in various solvent mixtures

revealed that hydrogen bonding was responsible for the mutarotation of PCMP[7]. Carbonyl groups in PCMP form B participate more in hydrogen bonding with alcohols as compared to those in PCMP form A. Accordingly, it is felt that the mutarotation of PCMP is a reversible reaction of hydrogen bonding between carbonyl groups in the polymer and the solvent. In the case of PP, Engel *et al.*[9~11] found that most of carbonyl groups in both forms I and II of PP formed hydrogen bonds with alcohols in solvent mixtures containing alcohols greater than 50 mole percent. If the alcohol concentration is less than 50 mole pecent, form II showed a greater tendency to hydrogen bond than form I. Form II formed more hydrogen bond by a factor 2~5 depending both concentrations and types of alcohol.

This preferential binding of alcohol with the polymer was explained by the fact that the carbonyl groups in form II of PP are more exposed on the surface of the helical structure as compared with those in form I and hence they are in a favorable conformation for formation of hydrogen bonds. Since the methyl groups in form A of PCMP stay in the vicinity of the carbonyl groups, it was expected that the preferential formation of hydrogen bonds in PCMP might be more pronounced than in PP.

The mutarotation of PCMP can be visualized in a solvent mixture of low alcohol concentration as shown in *Scheme* 1: hydrogen bonds between polymer and alcohol molecules were formed more in form B than in form A.



Scheme 1.

This reversible formation of hydrogen bond between carbonyl groups in PCMP and solvent is ascertained:

1) The forward mutarotation occurs in the solvents capable of forming hydrogen bonds such as alcohols and aliphatic acids,

2) the concentration of form B at equilibrium increases with increasing alcohol concentration and with increasing acidity of the alcohols,

3) ir-spectra shows that form B forms more hydrogen bonds than form A in a solvent mixture of chloroethanol-chloroform containing 1 % chloroethanol by volume[7], and

4) the rate of the forward mutarotation increases with alcohol concentration, whereas the rate of the reverse mutarotation decreases with the alohol concentration[7].

The solvent interactions with polymer in this study can be divided into two terms; interaction between polymer and chloroethanol, and between polymer and chloroform. Due to the formation of hydrogen bonds and the high polarity of chloroethanol, the former interaction should be the dominant factor.

The forward mutarotation is accompanied by the formation of hydrogen bonds, whereas the reverse mutarotation results in their cleavage. The hydrogen bonding energy will be released during the forward mutarotation and hence the enthalpy change must be negative and *vice versa*. These observations are consistent with the measured enthalpy change for the mutarotation (*Fig.* 8).

The measurements of activation energies for the forward and reverse mutarotations[7] and of enthalpy changes in the solvent mixtures revealed that the conformational energy of form A is about 2 kcal per residue mole higher than the energy of PCMP form B. This difference in energies can be ascribed to either one or both of two factors: one is the tortional po-

tential of form A due to the distortion of ϕ and ω angles, which might result from the introduction of methyl groups on the 5-position of prolyl ring.[7] The other is that prolyl rings have different self-energies[12] depending on the geometry of the rings (Traub and Shmueli,[13] Leung and Marsh[14]). The differences in geometry of prolyl rings of form A and form B result in different conformational energies for both forms.

The total enthalpy change for a conformational change of PCMP in a solvent mixture, without change in cocentration and temperature, can be written as:[15]

$$\Delta H=\Delta H_{conf}+\Sigma\Delta h_{int}+\Sigma\Delta h_s+\Delta H_{el} \qquad (5)$$

The conformational enthalpy change (ΔH_{conf}) is about 2 kcal per residue mole as described above. Interaction between adjacent peptide groups (Δh_{int}) and electrostactic interation (ΔH_{el}) could not be predicted precisely, however the ΔH_{el} term might be negligible, since PCMP has no charged groups. The enthalpy changes caused by interaction between polymer and solvent (Δh_s) are pronounced, since the total enthalpy change is strongly dependent on the solvent composition.

It should be noted that, in the case of PP, formation of hydrogen bonds lowers the energy of form II more than that of form I. A similar result is also expected in PCMP, since both polymers have similar conformations. In the case of PCMP in solution of high chloroform content, the energy of form A should be lowered more than that of form B, because form A is stable in chloroform. These factors, together with the preferential formation of hydrogen bonds with form B, affect Δh_s, resulting in the total enthalpy change for the reverse mutarotion increasing linearly the chloroethanol concentration. Since the hydrogen bonding energy is 4~7 kcal per mole, the main factor in the enthalpy change during the mutarotation should be the formation of hydrogen bonds.

The entropy changes for the forward and reverse mutarotation were found to be negative and positive, respectively. When we consider the orderedness of the solvent molecules (chloroethanol) in *Scheme* 1, the forward mutarotation is accompanied by a change from a disordered to an ordered state and hence by the negative entropy changes. For the reverse mutarotation, ordered solvent molecules are converted to a more disordered state and hence the entropy change is positive.

In the same manner, the total entropy changes can be divided according to eq. (5). The entropy change caused by conformational changes should not be consideriable, since both forms A and B are ordered structures. There-

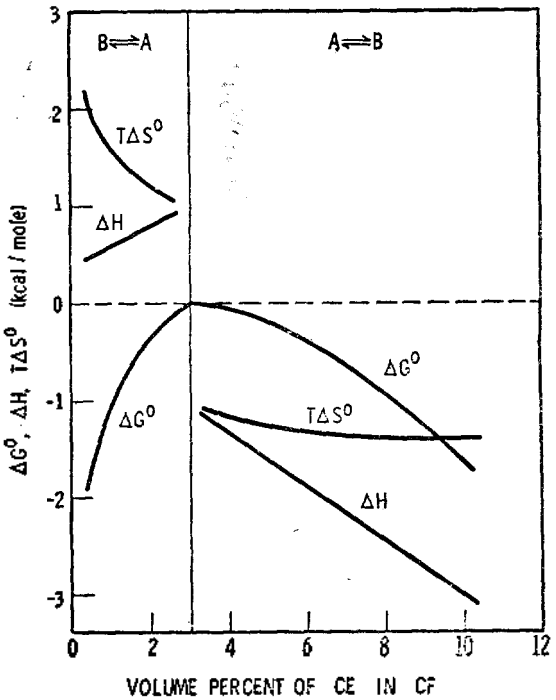


Fig. 11. Thermodynamic data as a function of solvent composition at 25 °C: CE: chloroethanol, CF: chloroforoform.

fore, entropy change caused by the interaction between polymer and solvents must be dominant in the total entropy term.

All of the thermodynamic data are once again summarized in *Fig.* 11. The enthalpy and entropy terms for the forward mutarotation are both negative. The absolute value of enthalpy change is higher than that of entropy term, resulting in negative free enthalpy changes. Consequently the driving force for the forward mutarotation is the negative enthalpy change resulting from the formation of hydrogen bonds between polymer and chloroethanol, and from the difference in energy between form A and form B. For the reverse mutarotation, enthalpy and entropy terms are both positive. Since the entropy term is higher than the enthalpy term, negative free enthalpies are obtained. Therefore, the driving force for the reverse mutarotation is the positive entropy changes resulted from the cleavage of hydrogen bondings during the reverse mutarotation.

If there were only the interactions between polymer and alcoholic solvents in this system, *i.e.*, formation of hydrogen bonds, it would be expected that the changes in enthalpy and entropy should be parallel along the solvent compositions. However, considerable deviations are found at the solvent compositions of low and high chloroethanol concentrations. This might be attributed to the interaction between polymer and chloroform or some other factors such as interactions between adjacent amide groups.

The magnitudes of the measured changes in entropy and in enthalpy were very small in comparison with those of chemical reactions. All ΔH values were less than 3 kcal, and most of ΔS° were less than 6 cal per degree residue mole in the measured range of solvent composition. These results showed clearly that *cis-trans* isomerization of amide bonds in the polymer is being induced by the interaction between polymer and solvent molecules.

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