Comparison of Lyotropic and Chromatographic Effects of Polar Organic Solvents on Collagen and Cellulose*

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ABSTRACT: Lyotropic and chromatographic effects of aqueous alcohol, glycol, ketone, and nitrile homologs have been compared on the basis of changes in (a) thermal stability of insoluble collagen and (b) catechin mobility in paper chromatography as a function of aqueous solvent composition. Qualitatively similar concentration-dependent activity was observed in the two systems. At low-solvent proportions, the denaturation temperature of collagen decreased while catechin mobility on cellulose increased, activity in both systems increasing with solvent concentration and hydrocarbon chain length in solvent homologs. At intermediate concentrations, activity maxima occurred with a subsequent reversal in both the order and magnitude of the trends at higher solvent proportions, indicative of opposing effects operative at the extremes of binary composition. Branched chain and polyhydric alcohols were less active than their respective linear and monofunctional counterparts. Analysis of solvent lyotropic and chromatographic effects at infinite dilution in terms of the Flory–Peller and Martin–Synge models indicated that the free energy of solvent binding in collagen denaturation and of partition in catechin-cellulose chromatography decreased linearly with increasing hydrocarbon chain length in solvent homologs. At high-solvent proportions, increases in collagen stability and in catechin-cellulose binding followed the order of decreasing dielectric constant in the solvent medium. The chromatographic parallel suggests that collagen denaturation may also be viewed as a partition process involving transfer of residue interactions between the internal structure and the solvent environment. Similar solvent activities in the two systems considered as well as in other unrelated systems indicate that a common interaction mechanism is operative. The role of polar and apolar interactions in accounting for the generality of the solvent effects is considered.

Polar organic solvents such as aliphatic alcohols, glycols, ketones, and nitriles in aqueous solution exhibit characteristic concentration-dependent lyotropic effects on proteins which contrast with those of denaturants such as urea, guanidinium halides, and neutral salts. Activity in the latter compounds which lack hydrocarbon structure, increases monotonically with concentration tending to yield similar denatured states (Von Hippel, 1967; Castellino and Barker, 1968; Tanford, 1968). In contrast, lyotropic effects in dilute aqueous polar solvents increase both with concentration and hydrocarbon content in solvent homologs, show broad activity maxima at intermediate concentrations and stabilization effects at high-solvent proportions, consistent with the formation of alternative structural forms (Martin and Bhatnagar, 1967; Ikai and Noda, 1968; Tanford, 1968; Herskovits *et al.*, 1970a–c). Similar concentration dependence is observed in the lyotropic effects of aqueous solvents on insoluble (Jordan Lloyd and Garrod, 1948; Schnell and Zahn, 1965) and soluble (Schnell, 1968; Herbage *et al.*, 1968; Harrap, 1969; Bianchi *et al.*, 1970; Russell and Cooper, 1969, 1970) collagens. By analogy with globular proteins, increase in lyotropic activity with solvent hydrocarbon content has generally been attributed to disruption of hydrophobic bonding in the collagen structure.

Similar activity–concentration profiles showing chain-length dependence with characteristic maxima and trend reversals at higher solvent proportions have been reported in physical studies on a number of nonprotein systems in which aqueous binary solvent media modify the properties of a third component present (Pittz and Bello, 1969; Gerlsma, 1970; Nakanishi and Ozasa, 1970). The generality of these features of solvent activity and their apparent independence of the detailed physical nature of the systems examined suggests that these effects reflect a common underlying mechanism in which comparative studies might provide further insight.

Accordingly, in the present study, lyotropic effects of aqueous alcohols, glycols, ketones, and nitriles on insoluble collagen have been compared as a function of aqueous solvent composition to similar solvent effects on catechin mobility in paper chromatography. The role of polar and apolar interactions in accounting for the analogous activity patterns in the two systems is considered.

Materials and Methods

Preparation of Collagen. An area of homogeneous, insoluble collagen was obtained by subjecting a sheepskin to commercial type processes of liming, deliming, and acid pickling to remove epidermal and soluble collagenous and noncollagenous protein from the fibrous matrix. The product was neutralized, washed free of salts, and finally acetone dehydrated to give an intact collagen matrix conforming to standard hide powder with a minimum dry protein content of approximately 99.6% by weight (Official Methods of Analysis, Society of Leather Trades' Chemists, Redbourn, 1965). Samples for hydrothermal stability measurements were cut in the form of 2 × 1 cm strips and randomized. Acid-soluble calfskin collagen in 0.15 M potassium acetate buffer (pH 4.8), extracted as described previously (Cooper and Davidson, 1965), was precipitated by slowly adding a 20% w/v solution of potassium chloride in acetate buffer to the protein solution to a final level of 1.5% w/v, prior to measuring thermal stability.

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Thermal Stability. The temperature of incipient length contraction (or "shrinkage temperature") of the collagen strips exposed to various aqueous organic solvent solutions was measured. The apparatus consisted essentially of a fixed lower and movable upper clamp for sample mounting. Relative movement of the clamps resulting from sample contraction, was magnified by a pulley and pointer attachment. The clamped sample was immersed in a beaker of the solution under examination on a magnetic stirrer hot plate. The temperature was raised at approximately 0.5°/min and the temperature of incipient shrinkage noted (Official Methods of Analysis, Society of Leather Trades' Chemists, Redbourn, 1965).

Thermal stability measurements were also made on the salt-precipitated form of calfskin acid-soluble collagen for comparison with the insoluble collagen system. A suspension of precipitated fibrils in various aqueous organic solvent media containing 1.5% w/v KCl and 0.15 M potassium acetate buffer (pH 4.8) in a test tube was heated at approximately 0.5°/min in a beaker of water. The temperature of incipient dissolution of the particles as gelatin was measured in the test tube. Clearing of the suspension over a narrow temperature range was readily apparent when viewed against a dark background.

Chromatography. To facilitate systematic survey of the effects of a variety of aqueous organic solvent media of varying composition in chromatography, small-scale systems were set up in a temperature-controlled room (23 ± 1°). Ascending chromatography using (±)-catechin as reference compound, was carried out on 2 × 18 cm paper (Whatman No. 1) strips in the machine direction, suspended in 1-l. glass-stoppered reagent bottles. The solvent front was allowed to migrate over a distance of 10 cm prior to removal of the strip and drying at 80°. The strips were then sprayed with 14% w/v ammoniacal silver nitrate and washed in three changes of distilled water with addition of a crystal of sodium thiosulfate to the final wash. Washing was completed in running tap water and the strips were then dried and R_F values calculated.

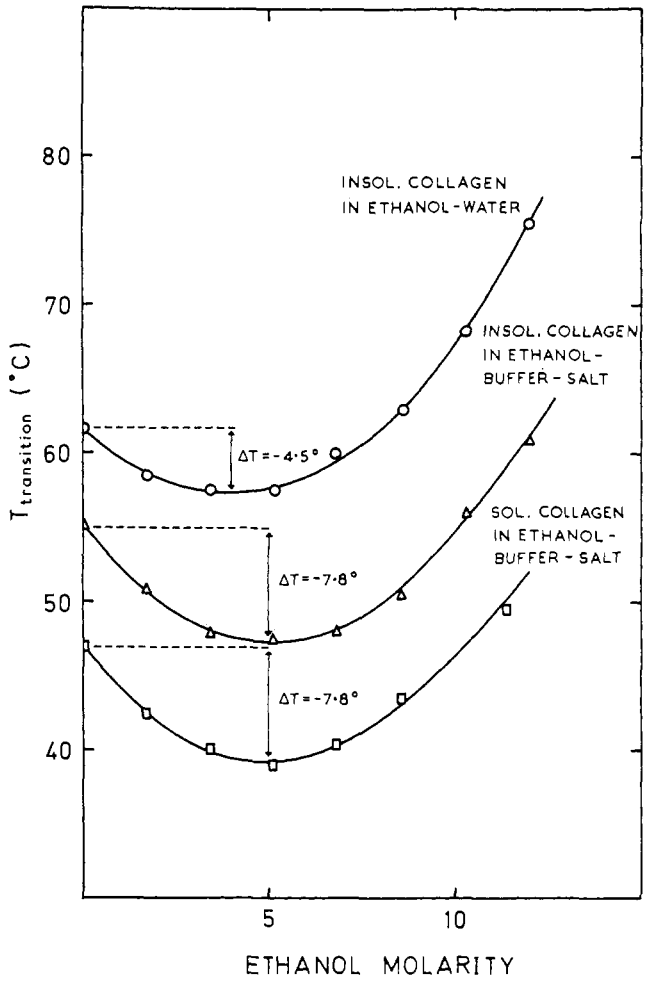

FIGURE 1: Variation in transition temperature ($T_{transition}$) of insoluble collagen in ethanol–water (○) and insoluble (△) and precipitated acid-soluble collagen (□) in ethanol–0.15 M potassium acetate buffer (pH 4.8)–1.7 M potassium chloride with increasing ethanol concentration.

Results

Solvent Effects on Thermal Stability of Insoluble and Precipitated Acid-Soluble Collagens. The effects of a typical aqueous organic solvent solution on insoluble and precipitated acid-soluble collagens were compared initially by examining the variation in thermal stability as a function of aqueous ethanol concentration. The buffer anion (0.15 M in acetate) and precipitant (1.5% w/v potassium chloride) concentrations were held constant throughout. A further comparison was made with ethanol effects on insoluble collagen in the absence of buffer and precipitant. Results are shown in Figure 1.

Similar concentration dependence of the transition temperatures was apparent for the insoluble and precipitated collagens in ethanol–buffer–salt media. Both collagens showed comparable initial molar destabilization to minima (−7.8° in each case) at approximately 5 M ethanol. Transition temperatures for the precipitated form, however, were uniformly lower by approximately 8° throughout the concentration range examined. In the absence of buffer and precipitant, the insoluble collagen showed greater thermal stability generally, but the effects of ethanol addition were qualitatively similar. The stability minimum decreased (−4.5°), however, and shifted to lower concentrations.

Parallel solvent activities on insoluble and precipitated collagens exposed to the same environment is taken as indicative that essentially the same helix–coil transition process is being observed in both cases. In mature, insoluble collagen, however, extensive interchain cross-links prevent the dissolution of the denaturation products as gelatin as for precipitated soluble collagen and also account for greater thermal stability (Veis, 1964; Von Hippel, 1967). In addition, from the parallel effects observed, it is concluded that differences due to the source or mode of preparation of the collagens do not significantly affect the solvent interactions under consideration. Similarly, greater stability generally of insoluble collagen in ethanol–water mixtures alone is attributed to removal of the lyotropic effects of the buffer and precipitant ions. In the present study, the latter system was adopted as a basis for comparison of solvent effects over a range of aqueous composition.

Lyotropic and Chromatographic Effects in Various Aqueous Solvent Media. Lyotropic effects on insoluble collagen and chromatographic effects on catechin-cellulose of the lower aliphatic alcohols are shown in Figure 2. Qualitatively similar shrinkage temperature variations with solvent concentration were apparent for all the homologs, characterized by a region of destabilization at low concentration, a broad destabilization maximum, followed by stabilization at higher solvent proportions. The experimental plots shifted progressively with increasing solvent hydrocarbon chain length as a result of in-

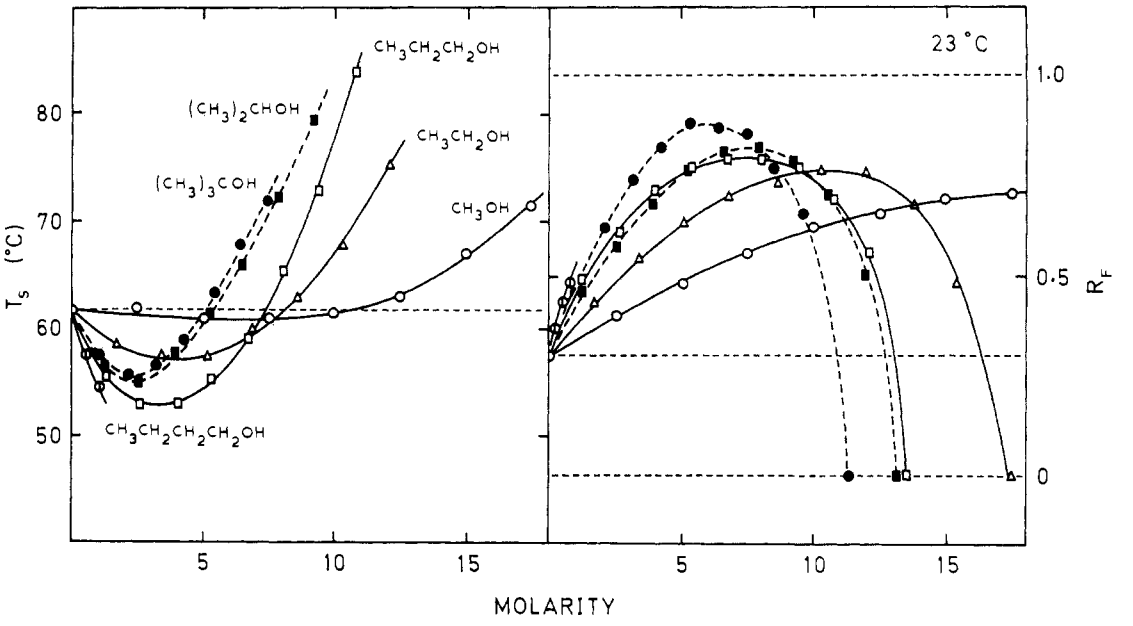

FIGURE 2: Variation in shrinkage temperature (T_s) of insoluble collagen and paper chromatographic mobility (R_F) of catechin with alcohol concentration in aqueous solutions of the lower aliphatic alcohols.

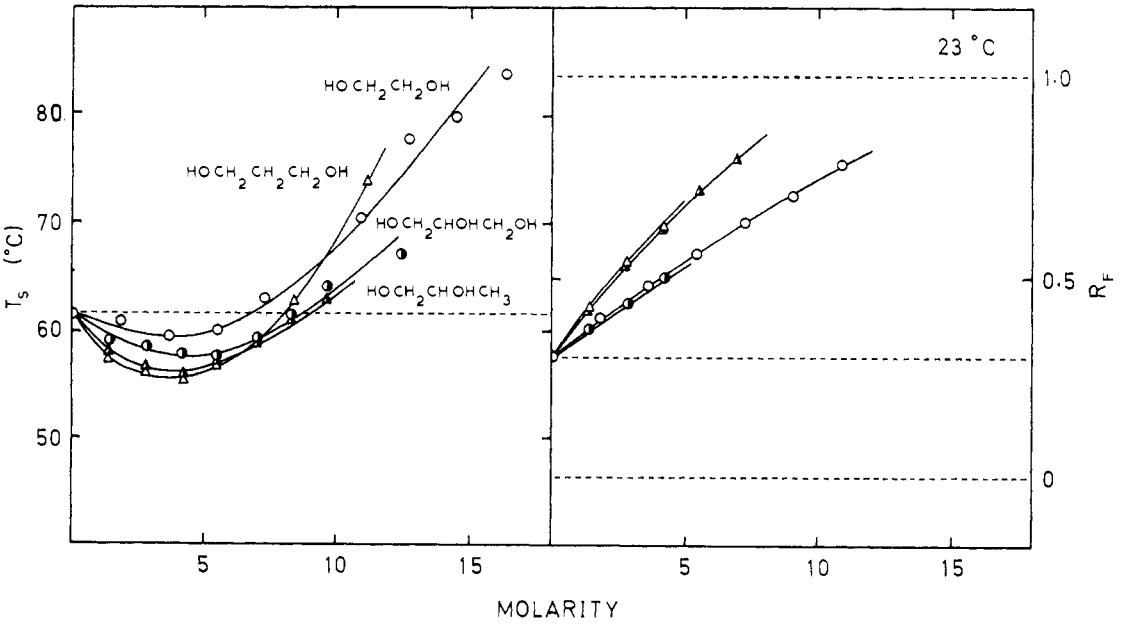

FIGURE 3: Variation in shrinkage temperature (T_s) of insoluble collagen and paper chromatographic mobility (R_F) of catechin with polyhydric alcohol concentration in aqueous solution.

creases in initial molar destabilization and the extent of the destabilization maximum which also shifted to lower concentrations. The order of solvent effects was also reversed at higher concentrations. The branched-chain isomers, isopropyl alcohol and *tert*-butyl alcohol, were less effective than their linear counterparts and gave comparable initial destabilization and maxima intermediate between those of ethanol and *n*-propyl alcohol.

An inverse pattern of variation similar to that noted for solvent lyotropic activity was evident for catechin mobility in paper chromatography as a function of aqueous alcohol concentration. Mobility increased with concentration to a maximum at intermediate concentrations with subsequent reversal in trend. As in the case of lyotropic effects, a progressive shift in the mobility plots with increasing solvent chain length was also apparent. Thus, initial mobilities increased with solvent chain length and mobility maxima shifted to lower concentrations with reversal in the order of effects at high-solvent proportions. Similarly, branched-chain alcohols were less effective than their linear counterparts.

Lyotropic and chromatographic effects of various glycols and glycerol are shown in Figure 3. As for the monohydric alcohols, activity increased with hydrocarbon chain length (comparing ethylene glycol and 1,3-propanediol). In both systems molar activities for ethylene glycol and glycerol were intermediate between those of methanol and ethanol and

those of 1,3-propanediol and the isomeric 1,2-propanediol intermediate between ethanol and *n*-propyl alcohol. Thus, in contrast with methylene group addition, hydroxyl group addition to the hydrocarbon chain of a monofunctional alcohol resulted in a decrease in molar activity (comparing ethanol, ethylene glycol, and *n*-propyl alcohol). A similar negative effect on activity of polar group as opposed to methylene group substitution has been reported in studies on soluble collagen (Harrap, 1969; Hart, 1971) and other proteins (Herskovits *et al.*, 1970a). The effects of the polyhydric alcohols on insoluble collagen in the present study, however, differed somewhat from previous observations on collagen solutions at pH 3 (Harrap, 1969) where ethylene glycol and 1,3-propanediol stabilized the structure monotonically.

Parallel studies of lyotropic and chromatographic effects were extended to include purely proton-acceptor solvents such as the lower aliphatic ketones (acetone, methyl ethyl ketone, and diethyl ketone) and nitriles (acetonitrile, propionitrile, and butyronitrile). Activity patterns (not shown) were similar to those reported for the alcohols and glycols. Activity maxima were only observed in the case of acetone and acetonitrile, however, where the range of aqueous miscibility was not restricted. The characteristic activity variation with aqueous composition was thus independent of the presence or otherwise of potential donor hydrogen atoms in the organic solvent examined.

Lyotropic and chromatographic effects at the low-solvent concentration extreme were analyzed quantitatively in terms of the Flory (1957), Peller (1959), and Martin–Synge (1941) models advanced to account for these phenomena, respectively. Initial gradients of the activity–concentration plots were obtained by fitting least-squares parabolas to the initial data points (using a Hewlett-Packard Calculator Model 9100B) and the molar effect at infinite dilution for each solvent was thereby determined. In the chromatographic system, R_M' values for each solvent were calculated from

$$R_M' = \ln (1/R_F^0 - 1) \quad (1)$$

where R_F^0 is the extrapolated molar R_F at infinite dilution. The free energy of partition, ΔF_{part}, is then related to R_M as follows (Martin, 1949; Bate-Smith and Westall, 1950)

$$\Delta F_{part} = RT \ln A_M/A_S (1/R_F - 1) \quad (2)$$

$$= RT \ln A_M/A_S + RTR_M \quad (3)$$

where A_M and A_S are the cross-sectional areas of the mobile and stationary phases, respectively. Plots of R_M' (proportional to the free energy of catechin partition at low solvent concentration) *vs.* solvent methylene group content decreased linearly with gradients varying for each solvent series (Figure 4).

In the protein system, constants (k_b) for the preferential binding of solvent molecules at exposed sites in the denatured state were calculated from (Peller, 1959; Mandelkern and Stewart, 1964)

$$k_b = \frac{\Delta H_{res}}{nR(T_M^0)^2} \frac{T_M - T_M^0}{C} \quad (4)$$

where T_M and T_M^0 are the denaturation temperatures of the protein in the presence and absence of the solvent at concentration C, ΔH_{res} is the enthalpy of denaturation per repeat unit or residue, and n is the mole fraction of binding sites

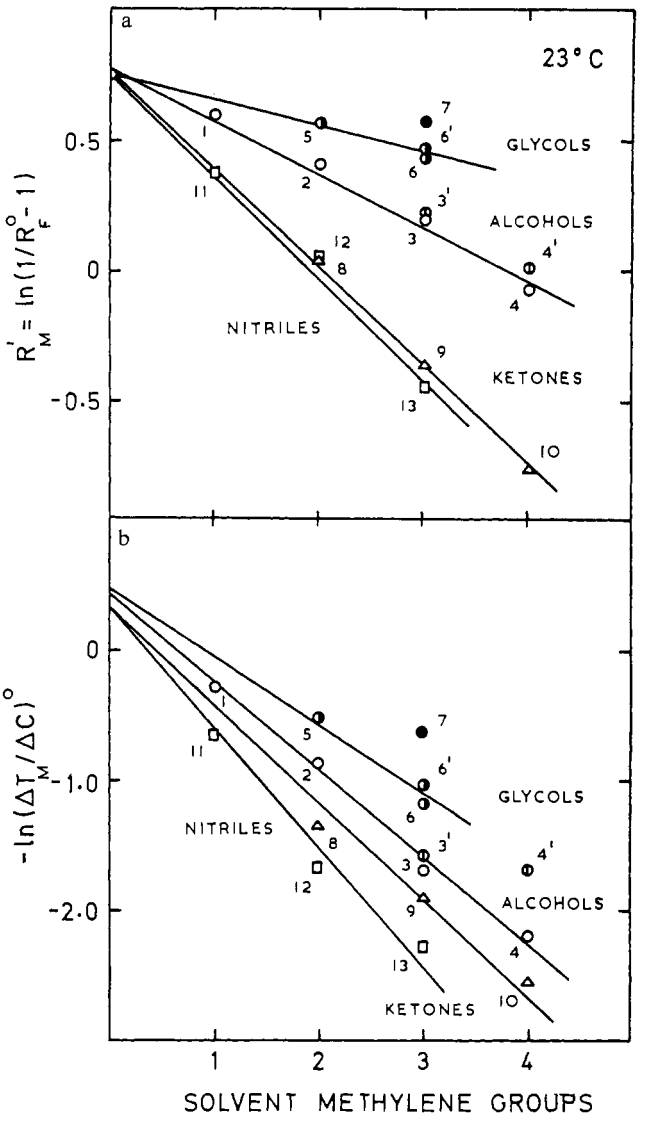

FIGURE 4: Linear relationship between (a) partition free energy (ΔF_{part}, proportional to $\ln(1/R_F - 1)$, eq 3) in chromatography and (b) binding free energy (ΔF_b, proportional to $\ln(\Delta T_M/\Delta C)$, eq 7) on collagen, respectively, and solvent methylene group content in aqueous solution at infinite dilution.

(1) methyl alcohol
(2) ethyl alcohol
(3) *n*-propyl alcohol
(3′) isopropyl alcohol
(4) *n*-butyl alcohol
(4′) *tert*-butyl alcohol
(5) ethylene glycol
(6) 1,3-propanediol
(6′) 1,2-propanediol
(7) glycerol
(8) acetonitrile
(9) propionitrile
(10) butyronitrile
(11) acetone
(12) methyl ethyl ketone
(13) diethyl ketone

available for interaction with the solvent in the denatured state. At infinite dilution

$$k_b = \frac{\Delta H_{res}}{nR(T_M^0)^2}\left(\frac{\Delta T_M}{\Delta C}\right)^0 \quad (5)$$

where $(\Delta T_M/\Delta C)^0$ is the initial gradient of the T_M *vs.* solvent concentration plot. The free energy of binding, ΔF_b, is given by

$$\Delta F_b = -RT \ln k_b \quad (6)$$

$$= -RT \ln \left(\frac{\Delta H_{res}}{nR(T_M^0)^2}\right) - RT \ln \left(\frac{\Delta T_M}{\Delta C}\right)^0 \quad (7)$$

TABLE I: Molar Chromatographic and Lyotropic Activities of Solvent Homologs and Corresponding Free-Energy Changes with Chain Length at Infinite Dilution.

	R_F^0 [a]	$R_M' = \ln(1/R_F^0 - 1)$	$\Delta(\Delta F_{part})$ $(CH_2)_{mean}$ [b] $(=RT\Delta R_M')$ (cal/mole)	$-\left(\frac{\Delta T_M}{\Delta C}\right)^0$ [c] (deg/mole l^{-1})	$-\ln\left(\frac{\Delta T_M}{\Delta C}\right)^0$	$k_b \times 10^2$ (mole $l.^{-1}$)	$\Delta(\Delta F_b)$ $(CH_2)_{mean}$ [d] $(=RT\Delta \ln k_b)$ (cal/mole)
Water	0.32	0.75					
Methyl alcohol	0.36	0.58		1.3	−0.26	1.17	
Ethyl alcohol	0.40	0.41	−120	2.2	−0.79	1.97	−395
n-Propyl alcohol	0.45	0.20		5.4	−1.69	4.84	
n-Butyl alcohol	0.52	−0.08		9.0	−2.20	8.07	
Isobutyl alcohol	0.45	0.20		4.8	−1.59	4.31	
tert-Butyl alcohol	0.50	0		5.1	−1.63	4.57	
Ethylene glycol	0.36	0.58		1.6	−0.47	1.44	
1,2-Propanediol	0.40	0.41	−57	2.8	−1.03	2.51	−308
1,3-Propanediol	0.39	0.45		3.2	−1.16	2.87	
Glycerol	0.36	0.58		1.9	−0.64	1.70	
Acetone	0.49	0.04		3.8	−1.34	3.40	
Methyl ethyl ketone	0.59	−0.36	−221	6.7	−1.90	6.01	−440
Diethyl ketone	0.68	−0.75		12.8	−2.55	11.50	
Acetonitrile	0.41	0.36		1.9	−0.64	1.70	
Propionitrile	0.49	0.04	−231	5.3	−1.67	4.75	−542
Butyronitrile	0.61	−0.45		9.8	−2.28	8.79	

[a] R_F^0 values are molar R_F values at infinite dilution calculated from initial gradients of least-squares parabolas fitting the R_F–concentration plots. [b] ΔR_M (mean) calculated from gradients of linear least-squares fits to the R_M' *vs.* methylene group plots (Figure 4). [c] $(\Delta T_M/\Delta C)^0$ values are molar values at infinite dilution calculated from initial gradients of least-squares parabolas fitting the T_M–concentration plots. [d] $\Delta \ln k_b = \Delta \ln (\Delta T_M/\Delta C)^0$ obtained from gradients of linear least-squares fits to the $-\ln (\Delta T_M/\Delta C)^0$ *vs.* methylene group data (Figure 4).

at infinite dilution. Plots of $-\ln (\Delta T_M/\Delta C)^0$ (proportional to the free energy of solvent binding) against solvent methylene group content (Figure 4) decreased linearly while the gradients varied for each solvent series as in the chromatographic systems. The substantially linear relationships obtained for both the chromatographic and protein systems suggested that the $\ln (A_M/A_S)$ (eq 3) and $\ln \Delta H_{res}/nR(T_M{}^0)^2$ (eq 7) terms remained constant under the conditions of comparison.

In Table I the relative effectiveness of the various solvents are compared in terms of their molar chromatographic and lyotropic activities at the low-solvent extreme determined from initial gradients of the experimental plots. Corresponding R_M', k_b, and mean $\Delta(\Delta F)$ per methylene group for each solvent series (from gradients of linear plots for straight-chain homologs) are also tabulated.

Discussion

The increase in lyotropic effect on collagen with solvent chain length at low concentration has been attributed by previous investigators to disruption of hydrophobic bonds in collagen (Schnell and Zahn, 1965; Schnell, 1968; Harrap, 1969) as in other proteins (Schrier and Scheraga, 1962; Von Hippel and Wong, 1965; Herskovitz *et al.*, 1970a–c; Gerlsma, 1970). While a degree of intermolecular hydrophobic bonding between apolar side chains in adjacent molecules in insoluble collagen cannot be discounted, similar solvent effects are also observed on isolated molecules in dilute collagen solutions. In the latter case, the surface disposition of the apolar side chains in the rodlike collagen structure (reviewed by Ramachandran, 1967) precludes intramolecular hydrophobic stabilization comparable to that in globular proteins. Moreover, only a limited contribution to structural stability can be expected from hydrophobic interactions between contiguous side chains on the molecular surface. These considerations suggest that an alternative nonspecific mechanism of solvent interaction is operative.

As distinct from bulk medium effects, a direct binding mechanism for solvent activity is supported by recent studies of alcohol binding to cross-linked collagen membranes in aqueous solution (Bianchi *et al.*, 1970). While the method of measurement did not differentiate between polar and apolar interaction, alcohol binding at low concentration also increased with hydrocarbon chain length in homologs. In addition, the order and magnitude of preferential alcohol binding was found to reverse at alcohol concentrations above 30% v/v, similar to the solvent effects in collagen denaturation reported here.

As an alternative to apolar solvent binding, direct hydrogen bonding through the donor–acceptor properties of the solvent functional group to polar groups, particularly the peptide bonds, in collagen also requires consideration. In terms of current concepts, such binding can be expected to give rise to lyotropic effects as a result of (a) electronic shifts at peptide bonds leading to increased rotational freedom (Schleich and Von Hippel, 1969; Von Hippel and Schleich, 1969a) as well as (b) disruption of intramolecular hydrogen bonding by competitive solvent binding (Peller, 1959; Mandelkern and Stewart, 1964).

The nonspecific character of polar interactions would also serve to account for the qualitatively similar variation in solvent effects in the catechin-cellulose chromatographic systems examined in which direct hydrophobic interactions appear to be largely excluded. The parallel affinity of polyphenolic compounds for cellulose and collagen (Roux, 1958) is generally attributed to their capacity for multipoint hydrogen bonding through the polar groups, a factor also responsible for the stabilization of collagen in vegetable tannage (Shuttleworth and Cunningham, 1948; Lollar, 1958). On this basis, catechin mobility increases with solvent concentration in paper chromatography reflect polar catechin–solvent and cellulose-solvent interactions occurring at the expense of catechin-cellulose binding. Similarly, the chromatographic mobilities of various ions on polyacrylamide taken as an indicator of relative binding affinities for peptide groups have been shown to correlate closely with relative lyotropic effects on proteins (Von Hippel and Schleich, 1969b).

In order to reconcile the effects of solvent hydrocarbon structure on lyotropic and chromatographic activity with a predominantly polar interaction mechanism, it has been suggested that solvent apolar groups influence such interactions indirectly through their effects on local water structure (Russell *et al.*, 1968; Russell and Cooper, 1969, 1970). Hydrocarbon structure is generally considered to enhance the structure of water in its vicinity, an effect often referred to as "hydration of the second kind" (Frank and Evans, 1945). Such ordering also increases with linear chain length, but is less marked for branched-chain isomers. Thus, hydrogen bonding interactions involving the functional group of a polar solvent and a particular substrate might be expected to be enhanced in a predominantly aqueous environment due to increased local interaction between surrounding water molecules induced by the pendent hydrocarbon structure. A similar proposal that vicinal hydrocarbon structure enhances interactions at a polar group has been put forward on the basis of ultrasonic absorbance studies in aqueous dioxane and polyethylene glycol solutions (reviewed by Hammes, 1968). In terms of this mechanism, the converse effect of hydroxyl group addition to a hydrocarbon chain (comparing mono- and polyhydric alcohols) in reducing molar activity is due to the opposing "structure-breaking" effect of the polar group which more than offsets the increase in hydrogen bonding potential. Cancellation of methylene group effects in the vicinity of a polar group has been reported previously (Von Hippel and Wong, 1965).

The proposal that solvent hydrocarbon structure effects polar interactions indirectly has been criticized by Herskovits *et al.* (1970a) on thermodynamic grounds. In particular, while the association of carboxylic acid homologs in aqueous solution increases with hydrocarbon chain length, enthalpies of dimerization are similar, consistent with an entropy-driven process as for hydrophobic bonding (Schrier *et al.*, 1964). As pointed out by these authors, however, quantitative evaluation is complicated by the fact that association in aqueous media involves simultaneous changes in solute–solute, solute–solvent, and solvent–solvent interactions which may result in considerable internal compensation.

The parallel between solvent lyotropic and chromatographic activities at low concentration is further emphasized by analysis of data at infinite dilution in terms of the Flory–Peller and Martin–Synge models. In both systems, a substantially linear decrease in interaction free energy with increasing solvent chain length in the order of effectiveness, glycols < alcohols < ketones < nitriles, was found. Comparison at infinite dilution eliminates the possibility that these effects are due to changes in medium properties such as dielectric constant. The chromatographic parallel suggests that collagen denaturation may also be viewed as a partitioning process involving transfer of residue interactions between the internal structure and the solvent environment.

At high-solvent proportions, collagen stabilization observed in the present and previous studies (Schnell and Zahn, 1965) and in soluble collagen (Harrap, 1969; Bianchi *et al.*, 1970) appeared analogous to solvent stabilization effects on α-helical polypeptides and proteins (Davidson and Fasman, 1967; Martin and Bhatnagar, 1967; Ikai and Noda, 1968; Tanford, 1968). This stabilization has been attributed to preferential strengthening of cooperative hydrogen bonding in protein structures in media of reduced dielectric constant. Similarly, in the present study stabilization effects at high-solvent proportions increased with solvent hydrocarbon content and hence followed the order of decreasing dielectric constant in the media. An opposing effect of this type at high-solvent proportions qualitatively accounts for the maxima in lyotropic activity observed at intermediate solvent compositions. Progressive reduction in catechin mobility at high solvent proportions with increasing solvent hydrocarbon content is also consistent with enhanced catechin-cellulose hydrogen bonding in media of reduced dielectric constant.

Parallel activity profiles showing characteristic maxima and chain-length dependence have been reported in other non-protein systems in which aqueous binary solvents effect the properties of a third component which lacks hydrophobic bonding potential. In particular, remarkably similar variations are observed in starch gelation temperatures in aqueous alcohols and glycols (Gerlsma, 1970), in aqueous alcohol effects on the thermal perturbation spectra of aromatic compounds (Pittz and Bello, 1969), and in diffusion studies on iodine in ethanol–water and *tert*-butyl alcohol–water mixtures (Nakanishi and Ozasa, 1970). Physical and thermodynamic properties of alcohol–water mixtures also exhibit chain-length dependence with characteristic inflections at intermediate compositions (Franks and Ives, 1966). In addition, similar variations occur in the solvent extraction of vegetable tannin constituents bound to collagen over a range of aqueous solvent compositions (Merill *et al.*, 1947; Russell *et al.*, 1967). The generality of these features of solvent activity strongly suggests that a common interaction mechanism is operative governed only by the intrinsic properties of the aqueous binary solvent mixtures under consideration. A mechanism involving direct polar binding between solvent and substrate in which solvent hydrocarbon structure plays an indirect role would alone appear to possess the required nonspecificity to account for the generality of the solvent effects observed in a variety of systems.

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