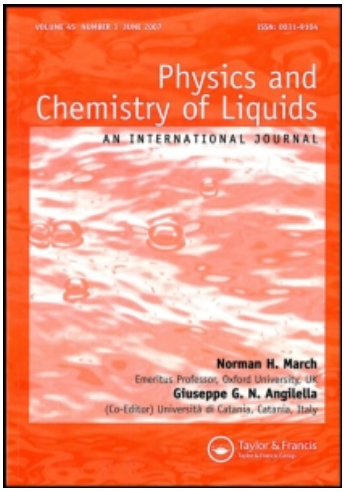

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Interactions Between Cholesterol and 2-Methoxyethanol-the Effect of 2-Methoxyethanol and Other Hydroxylic Compounds on Cholesterol-Proton Acceptor Association

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INTERACTIONS BETWEEN CHOLESTEROL AND 2-METHOXYETHANOL – THE EFFECT OF 2-METHOXYETHANOL AND OTHER HYDROXYLIC COMPOUNDS ON CHOLESTEROL – PROTON ACCEPTOR ASSOCIATION

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Using the 'pure base' calorimetric method, the association enthalpy ($\Delta_f H$) of cholesterol and 2-methoxyethanol (MeOEtOH), triethylamine (TEA) or tri-1-butylamine (TBA) in the presence of MeOEtOH (10% mol) has been determined. The addition of 2-methoxyethanol to the amine brings about the same effect as the addition of other simple alcohols (methanol, butan-1-ol). The increase in the association enthalpy of cholesterol and TBA in the presence of MeOEtOH can be caused by MeOEtOH hydroxyl group involved in the formation of mixed associates. In the case of amine with a weak steric hindrance (TEA), such an effect is not observed. The addition of 10 mol% of water or alcohols to N,N-dimethylformamide (DMF) makes no significant change in the cholesterol-DMF association enthalpy. This would suggest that the hydroxyl groups of added R'OH fail to participate in the formation of cholesterol-DMF associates. A slight increase in $\Delta_f H$, taking place when water is added to DMF, can be attributed to an increased solvation of the associates being formed.

KEY WORDS: Hydrogen bonds, enthalpies of solution, cholesterol, 2-methoxyethanol.

INTRODUCTION

Being one of the cell membrane components, cholesterol interacts with neighbouring lipide molecules in two ways. Its polar hydroxyl group interacts with the polar headgroups of the other molecules through hydrogen bonding, while its hydrocarbon skeleton and its hydrophobic 'tail' interact with non-polar fragments of neighbouring lipide through van der Waals forces. This type of double interaction occurring in membranes causes that other compounds with the same structure (a polar headgroup + a hydrophobic part) e.g. some antibiotics, can be included into the membrane structure. The role of both the mentioned interactions in the formation of phospholipide membranes has been discussed in many papers[1–3], but the direct quantitative determination of energetics of these interactions is still facing numerous problems. It is particularly difficult to experimentally determine the energy of van der Waals interaction. The determination of the energetics of specific

interactions is considerably easier. However, the thermodynamic functions of hydrogen bond formation, e.g. with the participation of cholesterol, can be determined only in isolated and considerably simpler systems that in actual membranes. The experimental techniques (such as spectroscopy or calorimetry) suitable for precise determination of the energetics of interaction require, in principle, the studied system to show a feasibly definable stoichiometry of associating molecules. This is the reason why previous papers[4-8] describing quantitatively the cholesterol interactions concern only the formation of 1:1 associates (complexes) through hydrogen bonds with simple, usually monofunctional proton acceptors. Both in calorimetry and in spectroscopy, the type of solvent used for the experiment is of importance. The solvent should provide a good solubility (calorimetry) and/or transmitance (spectroscopy). Therefore, energetic of interaction has been studied so far either in an inert solvent (mainly CCl_4) or a solvent directly interacting with cholesterol. Due to a very low cholesterol solubility in water, the direct examination of hydrogen bonds in this solvent is impossible. On the other hand, it is well known that water molecules actively participate[1,9-12] in the interactions taking place in the polar membrane layer at the interface. The theoretical studies, carried out recently, on sterol complexation by the polyene antibiotic (Amphotericin B) also point to the role of water molecules in the formation of such complexes[13]. When the direct contact of polar 'heads' of lipide molecule is difficult, hydrogen bonds are formed in the polar layer of membrane through a possible intermediate water molecules.

Thermodynamic studies on water and alcohol influence on the formation of donor-acceptor associates with the participation of cholesterol have been reported in one of our earlier papers[14]. The presence of hydroxylic compounds (R'OH) brings about an increased energy of cholesterol-amine interactions, but only in the case of sterically hindranced tri-1-butylamine. The present work continues these studies. Beside water and simple alcohols used previously as R'OH, the scope of the present study is widened with systems containing a bifunctional alcohol: 2-methoxyethanol (MeOEtOH). It was also considered worthwhile and of interest to compare the effect of R'OH on the formation of cholesterol associates with amines (nitrogen bases) and with DMF (oxygen base).

EXPERIMENTAL

Cholesterol (Sigma, Standard for Chromatography) was dried for over ten hours at a temperature of about 80 °C over P_2O_5 in vacuum. Cholesteryl methyl ether (ChME) (Sigma) was dried before measurements under vacuum in room temperature over P_2O_5. Solvents (2-methoxyethanol, butan-1-ol, N,N-dimethylformamide) were distilled and dried by means of molecular sieves 4A before use. The preparation of calorimetric ampoules and non-aqueous solutions was carried out in a dry-box. Calorimetric measurements were performed with a calorimeter, as described previously[15]. The accuracy of heat measurements was about $\pm 0.8\%$. No concentration dependence of the enthalpy of solution ($\Delta_{sol}H$) of cholesterol and ChME was

found in the solvents under study. The values of $\Delta_{sol}H$ used in calculation are the averages of about 10 direct measurements of the heat of solution within the concentration range from $5 \cdot 10^{-4}$ mol dm^{-3} to $3 \cdot 10^{-3}$ mol dm^{-3}. The enthalpy of formation of hydrogen bond associates was determined by the 'pure base' method of Arnett *et al.*[16]. It consists of determining the enthalpy of solution of a proton-donor (cholesterol) $\Delta_{sol}H^{(A)}$ and its model (a compound in which the proton is substituted by $-CH_3$ group) $\Delta_{sol}H^{(M)}$ in pure base and in a solvent chosen as an inert reference. The enthalpy of formation of associates $\Delta_f H$ is calculated from the relation:

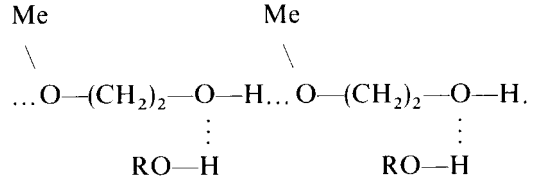
$$\Delta_f H = (\Delta_{sol}H^{(chol)} - \Delta_{sol}H^{(ChME)})_{base} - (\Delta_{sol}H^{(chol)} - \Delta_{sol}H^{(ChME)})_{ref.solvent}$$

Carbon tetrachloride was used as the reference solvent and cholesteryl methyl ether as the cholesterol model compound.

RESULTS AND DISCUSSION

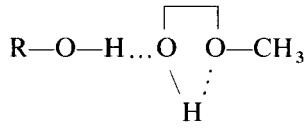
Cholesterol-2-methoxyethanol Associates in 2-methoxyethanol

The 2-methoxyethanol as all alcohols shows proton donor (acidic) properties. Unlike simple alcohols, MeOEtOH molecule is additionally characterised by the presence of a basic centre in the form of methoxy group. Such a structure allows the formation of intermolecular interactions with proton acceptors as well as proton donors. Dilute solutions in inert solvents show only the presence of monomeric MeOEtOH molecules in the form of 5-member rings with intramolecular hydrogen bonds[17,18]. In more concentrated solutions, cyclic dimers[19,20] and linear *n*-mers[17] have also been found. In such self-associates the hydroxyl proton of one molecule is bonded to the methoxy oxygen in the next molecule. In pure MeOEtOH, monomers with intramolecular H-bonds coexist probably with different forms of self-associates, with the latter being predominant. The formation of self-associates and intramolecular H-bonds affects the formation of acceptor-donor complexes and their energetics. The addition of a proton acceptor to MeOEtOH solution in an inert solvent destroys[21] its self-associates and brings about the formation of mixed hydrogen-bond associates. On the other hand, the addition of a compound with strong proton-donor properties (e.g. pentahalophenols) to a concentrated MeOEtOH solution, does not disturb the structure of existing linear self-associates. According to Ginsburg[22] the mixed associates are formed according to the following scheme:



(1)

The formation of such mixed associates may be caused by the simultaneous decrease in the electron density of alkoxyl oxygen and its increase for hydroxyl oxygen due to the cooperativity of H-bonds. In diluted CCl_4 solutions 1:1 H-bond complexes[22] are proposed in which the hydroxyl oxygen of MeOEtOH monomer is a proton acceptor according to the following scheme:



(2)

The authors of the cited paper have found no proofs of the existence of mixed associates in which the part of proton acceptor would be played by the atoms of alkoxyl oxygen despite its higher basicity.

The 'pure base' method used in this study made it possible to determine the cholesterol–MeOEtOH association enthalpy ($\Delta_f H$) in pure MeOEtOH. The results are given in Table 1 and compared with analogous data for several other compounds examined previously[6,23]. All the $\Delta_f H$ data concern the cholesterol complexes formed in pure solvent "B". Table 1 includes also the values of proton affinity (PA) which constitutes one of the parameter describing the basicity. As follows from the given data, the basicity of methoxy oxygen in MeOEtOH (PA = 194.3) is lower than that of simple ethers, but is higher than that of hydroxyl oxygen in this compound (PA = 186.7) or in simple alcohols (e.g. for *n*-BuOH, PA = 190.2 and for EtOH, PA = 190.3). Comparing the energetics of cholesterol-MeOEtOH association ($\Delta_f H = -16.6$ kJ/mol) with that of cholesterol-ethers ($\Delta_f H = -12$ kJ/mol), one should point to the fact that despite weaker basic properties the formation of MeOEtOH associates is accompanied by a considerably higher $\Delta_f H$ value. Enthalpy of cholesterol association with MeOEtOH is of the same order of magnitude as that for the association with considerably more basic DMF ($\Delta_f H = -16.5$ kJ/mol). On the other hand, the association of cholesterol with butan-1-ol (weak basic properties) is accompanied by a high change in enthalpy ($\Delta_f H = -19.8$ kJ/mol). The lack of any correlation between $\Delta_f H$ values and basicity for the

Table 1 Enthalpies of H-bond association ($\Delta_f H$) of cholesterol with various solvents and their proton affinities[24,25](PA).

Solvent	$\Delta_f H$/kJ mol^{-1}	PA/kcal mol^{-1}
THF	−11.3	198.5
di-*n*-butylether	−11.9	204.0
MeOEtOH[a]	−16.6	194.3(methoxy) 186.7(hydroxy)
n-BuOH	−19.8	190.2(hydroxy)
DMF	−16.5	211.4

[a]the values of solution enthalpy of cholesterol and cholesteryl methyl ether, required to calculate of $\Delta_f H$ by the 'pure base method' are given in Table 2. The remaining data are from refs[6,23].

In all the cases, the reference solvent is carbon tetrachloride.

discussed series of compounds is due, in our opinion, to different structures of associates formed with cholesterol. In simple ethers or disubstituted amides, the associates are mainly 1:1 complexes with an H-bond between cholesterol hydroxyl group and the free electron pair of oxygen. The $\Delta_f H$ values of such complexes correlate[6] with the basicity expressed by the Donor Number for the series of oxygen proton acceptors. The structure of cholesterol associates with alcohol (in a pure alcohol) is different. The high $\Delta_f H$ value in the cholesterol - butan-1ol system can be interpreted[14] as an effect of the incorporation of cholesterol hydroxyl group into existing linear chain of alcohol associate:

$$\begin{array}{ccccccc} & & \text{Bu} & & \text{Chol} & & \text{Bu} \\ & & / & & / & & / \\ \cdots & \text{H}-\text{O} & \cdots & \text{H}-\text{O} & \cdots & \text{H}-\text{O} & \cdots \end{array} \qquad (3)$$

In the case of 2-methoxyethanol under study, the structure of cholesterol associates is certainly still different. The higher $\Delta_f H$ value (-16.6 kJ/mol) than that for cholesterol complexes with stronger basic ethers ($\Delta_f H = -12$ kJ/mol) suggests that the cholesterol - MeOEtOH associates are not the 1:1 complexes formed by oxygen of methoxy group. The formation of associates (3) also seems to be doubtful against the reported stability of MeOEtOH self-associate chains (e.g. in presence of pentahalophenols[22]). It follows from our results that in the cholesterol - 2-methoxyethanol system, structures (1) and (2) can be formed, similar to those[22] in the system: substituted phenol-2-methoxyethanol - CCl_4.

The Effect of 2-methoxyethanol on the Cholesterol–Aliphatic Amine Association

The performed measurements of enthalpy of solution of cholesterol and cholesteryl methyl ether in TEA and TBA mixtures with 2-methoxyethanol (10% mol) allowed the determination of $\Delta_f H$ of cholesterol-amine associates in the presence of MeOEtOH. The results of the measurements and calculated $\Delta_f H$ are given in Table 2, and compared with analogous data for amine mixtures with methanol and butan-1-ol. It follows from the results that the addition of 2-methoxyethanol to amines brings about almost the same effect as that caused by the addition of butan-1-ol. The $\Delta_f H$ values obtained in the presence of MeOEtOH and n-BuOH are the same: -23.1 kJ/mol for TEA and -19.4 kJ/mol for TBA. This suggest that the mechanism of cholesterol association with amines in the presence of both alcohols (*n*-BuOH and MeOEtOH) is similar. The effect of alcohol addition on the energetics of cholesterol-amine association is considerably stronger for TBA than for TEA. The addition of 10% mol. of alcohol to TBA can facilitate the interactions with cholesterol through the formation of the following structure:

$$\begin{array}{ccccc} & & \text{R}' & & \text{Chol} \\ & & / & & / \\ \text{Bu}_3\text{N} \ldots & \text{H}-\text{O} & \ldots & \text{H}-\text{O} & \end{array} \qquad (4)$$

Table 2 Enthalpies of solution of cholesterol ($\Delta_{sol}H^{(chol)}$), cholesteryl methyl ether ($\Delta_{sol}H^{(ChME)}$) and enthalpies of association ($\Delta_f H$) of cholesterol in TEA, TBA, BuOH MeOEtOH and in the amine – alcohol (10% mol) mixtures.

Solvent	$\Delta_{sol}H^{(chol)}/kJ\ mol^{-1}$	$\Delta_{sol}H^{(ChME)})/kJ\ mol^{-1}$	$\Delta_f H/kJ\ mol^{-1}$
TEA	8.4 ± 0.2	23.3 ± 0.4	-22.6
TEA-MeOH(10%)	9.6 ± 0.2	25.6 ± 0.3	-23.7
TEA-BuOH(10%)	9.1 ± 0.2	24.5 ± 0.2	-23.1
TEA-MeOEtOH(10%)	9.7 ± 0.2	25.1 ± 0.3	-23.1
TBA	14.0 ± 0.2	22.2 ± 0.4	-15.9
TBA-MeOH(10%)	12.5 ± 0.2	25.3 ± 0.4	-20.5
TBA-BuOH(10%)	11.6 ± 0.2	23.3 ± 0.4	-19.4
TBA-MeOEtOH(10%)	11.3 ± 0.2	23.0 ± 0.4	-19.4
BuOH	13.9 ± 0.1	26.0 ± 0.4	-19.8
MeOEtOH	25.6 ± 0.5	34.5 ± 0.5	-16.6
CCl_4	31.2 ± 0.3	23.5 ± 0.5	–

Which has been previously reported[14] for such systems. This is certainly due to the higher steric hindrance of TBA which impedes direct H-bonds with cholesterol. The participation of 2-methoxyethanol in the cholesterol interaction with TBA is particularly well noticeable when the $\Delta_f H$ values for TBA-MeOEtOH (10% mol) mixtures are compared with those for pure TBA and pure MeOEtOH. The enthalpy of cholesterol association in TBA-MeOEtOH (10% mol) system is considerably higher (-19.4 kJ/mol) than those in pure TBA (-15.9 kJ/mol) and in pure MeOEtOH (-16.6 kJ/mol). The increase of $\Delta_f H$ value by about 3 kJ/mol due to the presence of MeOEtOH is certainly connected with the formation of cholesterol – TBA associates involving the hydroxyl group of MeOEtOH according to structure (4).

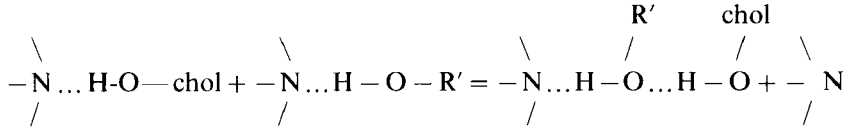
Assuming that the pure amine and the amine containing 10% mol of R'OH are different solvents, one can calculate the transfer enthalpy of cholesterol – amine associate ($\Delta_{tr}H^{(AB)}$) from pure amine to the two-component system. The calculation of $\Delta_{tr}H^{(AB)}$ is possible owing to the use of the following cycle:

$$\begin{array}{ccccc} \mathbf{A}(S_1) & + & \mathbf{B}(S_1) & \xrightarrow{\Delta_f H^{(S_1)}} & \mathbf{AB}(S_1) \\ \downarrow \Delta_{tr}H^{(A)} & & \downarrow \Delta_{tr}H^{(B)} & & \downarrow \Delta_{tr}H^{(AB)} \\ \mathbf{A}(S_2) & + & \mathbf{B}(S_2) & \xrightarrow{\Delta_f H^{(S_2)}} & \mathbf{AB}(S_2) \end{array}$$

where: **A** is cholesterol, **B** is amine, S_1 is pure amine as solvent and S_2 is amine-R'OH (10% mol) mixed solvent. The transfer enthalpy of the cholesterol-amine associates is calculated from the equation:

$$\Delta_{tr}H^{(AB)} = \Delta_{tr}H^{(A)} + \Delta_{tr}H^{(B)} - \Delta_f H^{(S1)} + \Delta_f H^{(S2)} \qquad (5)$$

and would correspond to the enthalpy change for the following process:



(6)

The calculated $\Delta_{tr}H^{(AB)}$ value would show the extend of energetic advantage of the formation of cholesterol-amine associate with the participation of hydroxyl group of R′OH over that without this participation. The values of transfer enthalpy of cholesterol (A) and amine (B) as well as $\Delta_{tr}H^{(AB)}$of associate (AB) calculated from Eqn. (5) are given in Table 3. The obtained results show that, in the case of tri-1-butylamine, the changes in enthalpy resulting from reaction (6) are exothermic and amout to about -6 kJ/mol regardless of the alcohol used. Probably due to steric hindrance, a 'free' molecule of TBA is a worse proton acceptor than the oxygen electron pair in TBA-R′OH complex. In the case of triethylamine the calculated values of $\Delta_{tr}H^{(AB)}$ constitute low positive values. This would suggest that the cholesterol-TEA associates show the character of direct hydrogen bonds also in the presence of added alcohols. The oxygen free electron pairs in TEA-R′OH associate constitutes no competition for the strongly basic free electron pair of TEA, and reaction (6) does not take place.

The Effect of Compounds Containing Hydroxyl Groups (Water, Alcohols) on the Cholesterol – DMF Associates

This part of the present study was aimed at the examination whether the addition of hydroxylic compounds affects the association enthalpy of cholesterol with a typical oxygen proton acceptor such as DMF. The good miscibility of DMF with water made it also possible to perform the measurements in the system: DMF-H_2O (10% mol). The experimental $\Delta_{sol}H$ data for cholesterol and cholesteryl methyl ether as well as calculated Δ_fH values of the cholesterol – DMF associates in pure DMF and in DMF-R′OH (10% mol) mixtures are given in Table 4.

As can be seen, 10% mol of hydroxylic compounds added to DMF does not affect the Δ_fH value considerably. The differences in Δ_fH values are ± 1.6 kJ/mol. Low

Table 3 Enthalpies of transfer of cholesterol ($\Delta_{tr}H^{(A)}$), amine ($\Delta_{tr}H^{(B)}$) and associates ($\Delta_{tr}H^{(AB)}$) from pure amine to amine R′OH mixtures.

Solvent	$\Delta_{tr}H^{(A)}$/kJ mol^{-1}	$\Delta_{tr}H^{(B)}$/kJ mol^{-1}	$\Delta_{tr}H^{(AB)}$/kJ mol^{-1}
TEA-MeOH(10%)	1.2	−0.02	0.1
TEA-BuOH(10%)	0.7	−0.04	0.2
TEA-MeOEtOH(10%)	1.3	−0.01	0.8
TBA-MeOH(10%)	−1.5	0.03	−6.1
TBA-BuOH(10%)	−2.4	0.06	−5.8
TBA-MeOEtOH(10%)	−2.7	0.02	−6.2

*The transfer enthalpy of pure components (A) and (B) is the difference between their enthalpies if solutions in solvents S_1 and S_2.

Table 4 Enthalpies of solution of cholesterol ($\Delta_{sol}H^{(chol)}$), cholesteryl methyl ether ($\Delta_{sol}H^{(ChME)}$) and formation enthalpy ($\Delta_f H$) of cholesterol – DMF associates in pure DMF and DMF-R'OH mixtures. Enthalpies of transfer of cholesterol ($\Delta_{tr}H^{(A)}$), DMF ($\Delta_{tr}H^{(B)}$) and ($\Delta_{tr}H^{(AB)}$) from pure DMF to DMF–R'OH mixtures.

Solvent	$\Delta_{sol}H^{(chol)}/kJ\ mol^{-1}$	$\Delta_{sol}H^{(ChME)}/kJ\ mol^{-1}$	$\Delta_f H/kJ\ mol^{-1}$
DMF	27.5 ± 0.6	36.3 ± 0.3	-16.5
DMF-H_2O(10%)	28.1 ± 0.3	38.5 ± 0.5	-18.1
DMF-MeOH(10%)	27.8 ± 0.4	35.0 ± 0.4	-14.9
DMF-BuOH(10%)	26.5 ± 0.5	33.7 ± 0.4	-14.9
DMF-MeOEtOH(10%)	27.7 ± 0.2	36.3 ± 0.3	-16.3
Solvent	$\Delta_{tr}H^{(A)}/kJ\ mol^{-1}$	$\Delta_{tr}H^{(B)}/kJ\ mol^{-1}$	$\Delta_{tr}H^{(AB)}/kJ\ mol^{-1}$
DMF-H_2O(10%)	0.6	0.02	-1.0
DMF-MeOH(10%)	0.3	-0.01	1.9
DMF-BuOH(10%)	-1.0	0.02	0.6
DMF-MeOEtOH(10%)	0.2	-0.02	0.4

positive $\Delta_{tr}H^{(AB)}$ values of the cholesterol-DMF associates in alcohol containing systems suggest that the hydroxyl group of alcohol does not participate in the formation of cholesterol-DMF associates. The addition of water brings about a weak increase in the absolute values of $\Delta_f H$ and an exothermic value of the complex transfer enthalpy $\Delta_{tr}H^{(AB)}$ from pure DMF to the DMF-H_2O mixture. A similar slight increase in $\Delta_f H$ and $\Delta_{tr}H^{(AB)}$ (by about -2.5 kJ/mol) due to added water has been observed previously[14] for the systems: di-1-butylamine (DBA)-water (10% mol) and TEA-water (3% mol). It can be assumed that this low exothermic effect is brought about rather by the stronger solvation of cholesterol-DMF complexes (or cholesterol-DBA, cholesterol-TEA) in the water-organic system due to the high polarity of water.

The steric hindrance impeding direct intermolecular interactions of cholesterol seems to be an indispensable factor for the participation of R'OH compounds in association of cholesterol with proton acceptors. The type or R'OH compound which provides hydroxyl groups (water, alcohol or alkoxyalcohol) has no effect on the observed phenomenon. Of the system examined so far, the increase in energy of cholesterol interactions caused by the participation of hydroxyl group of R'OH in associates was observed only in the case of tri-1-butylamine.

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