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HEAT OF SOLUTION OF CHOLESTEROL AND ITS INTERACTIONS WITH DIFFERENT SOLVENTS: A CALORIMETRIC STUDY

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ABSTRACT

The values of the standard enthalpy of solution of cholesterol ($\Delta H_s^{\ominus}$) in twelve solvents of various polarities and with different proton-acceptor and proton-donor properties were measured by calorimetry. The enthalpies of the cholesterol interaction and solvation in these media relative to cyclohexane were evaluated. Among the discussed factors affecting the solute–solvent interaction (dispersion, dipole and donor–acceptor interactions), the dispersion forces characterised by the Hildebrand solubility parameter was found to play a considerable role.

INTRODUCTION

Cholesterol (5(6)-cholesten-3-ol) has several extremely important roles in animal organisms. The physico-chemical properties of this lipid, however, have rarely been considered in studies of cholesterol. It is known from spectroscopic data (IR [1,2] and NMR [3]), VPO [4] and dielectric measurements [5,6] that cholesterol shows a high tendency to self-associate despite being an alcohol with a large hydrocarbon radical. The existence of various *n*-mers formed through hydrogen bonds of the hydroxyl group has been postulated. The types of these associates (cyclic or linear) and their size (number of molecules in associate) depend on concentration, temperature and type of solvent. The results obtained by various methods, however, lead to different conclusions concerning the self-association and cholesterol.

Few literature data address the interaction of cholesterol with the simple chemical compounds used as solvents. It is known that the solubility of

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cholesterol increases with the dielectric constant of the solvent [7,8]. Although this relationship is valid within a relatively narrow range (for compounds with limited numbers of carbon atoms), one can assume that a considerable contribution to the solvation of cholesterol is made by non-specific interactions (van der Waals).

Direct information on the energetics of solute–solvent interactions can be obtained from measurements of the dissolution heat. However, there are no reports on the calorimetric measurement of the enthalpy of cholesterol dissolution in commonly used organic solvents.

The present study was to determine the thermochemical data of the dissolution process of cholesterol in several solvents and to discuss them in terms of intermolecular cholesterol–solvent interactions.

The solvents used may be divided into three groups.

(i) Non-polar: cyclohexane (c-C_6H_{12} or Cy), carbon tetrachloride (CCl_4), benzene (Bz), toluene (Tol) and p-xylene (pXy).

(ii) Proton-acceptor: 1,4-dioxane (Diox), tetrahydrofuran (THF), N,N-dimethylacetamide (DMA), ethyl acetate (AcEt) and hexamethylphosphotriamide (HMPA).

(iii) Weak proton-donor: 1,1,2,2-tetrachloroethane ($C_2H_2Cl_4$) and chloroform ($CHCl_3$).

EXPERIMENTAL

The solvents (reagent grade) were purified by methods recommended in the literature [9,10] and distilled directly before measurements. The cholesterol (Sigma, Standard for Chromatography) was dried for several hours at a temperature of 80 °C under a vacuum. The weighing and filling of the calorimeter and ampoules were carried out in a dry-box. The calorimeter used for determining the heat of solution has been described previously [11]. The temperature inside the calorimeter was determined using three thermistors working in a Wheatstone bridge system. The voltage of the unbalanced bridge was measured by means of the Keithley K-148 nanovoltmeter. The temperature in the thermostat was stable to $\pm 1 \times 10^{-5}$ K. The experimental precision was $\pm 0.5\%$.

The enthalpy of solution (ΔH_m) of cholesterol was measured within the concentration range from 2×10^{-4} mol kg^{-1} to 3×10^{-3} mol kg^{-1}. For solvents in which ΔH_m was independent of concentration, the standard enthalpy of solution ($\Delta H_s^{\ominus}$) was taken as an average of 8–12 separate measurements. In the case of systems where a systematic concentration dependence of ΔH_m was observed, the $\Delta H_s^{\ominus}$ values were obtained by graphic extrapolation of the solution enthalpy to infinite dilution.

RESULTS AND DISCUSSION

In the weakly and non-polar solvents (c-C_6H_{12}, CCl_4, Bz, Tol and pXy), a systematic concentration dependence of the solution enthalpy of cholesterol is observed. Within the concentration range used, the endothermic values of ΔH_m decrease linearly with the cholesterol concentration, see Table 1 and Fig. 1.

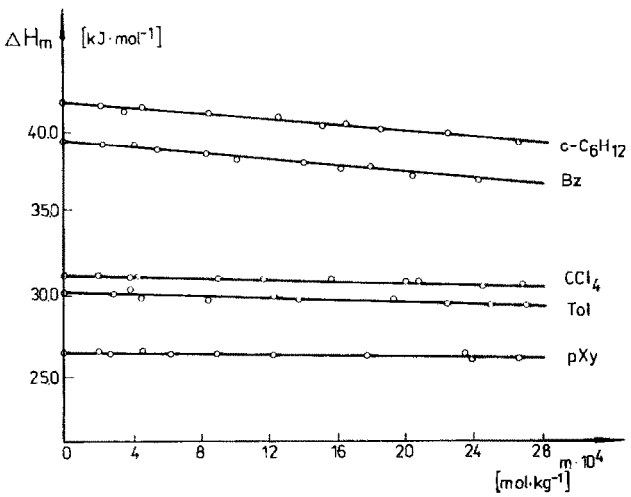

Fig. 1. Concentration dependence of the heat of solution of cholesterol in various solvents at 298.15 K.

TABLE 1

Values of the dissolution enthalpy of cholesterol in various solvents at 298.15 K, m in 10^4 mol kg^{-1} and ΔH_m in kJ mol^{-1}

c-C_6H_{12}		Bz		CCl_4		Tol		pXy	
m	ΔH_m	m	ΔH_m	m	ΔH_m	m	ΔH_m	m	ΔH_m
0	41.9	0	39.6	0	31.2	0	30.3	0	26.4
2.2	41.8	2.3	39.2	2.0	31.3	2.9	30.1	2.0	26.6
3.6	41.4	4.1	39.2	3.9	31.0	3.8	30.5	2.6	26.3
4.5	41.6	5.5	38.8	4.3	31.2	4.5	29.8	4.5	26.6
8.5	41.2	8.3	38.7	9.0	31.0	8.4	29.6	6.2	26.3
12.7	40.9	10.1	38.3	11.8	31.0	12.1	30.1	9.0	26.2
15.3	40.4	14.0	38.2	15.6	30.9	13.7	29.6	12.3	26.3
16.6	40.6	16.2	37.7	20.0	30.7	19.3	29.8	17.7	26.1
18.5	40.2	18.0	37.9	20.8	30.7	22.5	29.4	23.5	26.2
22.5	40.0	20.5	37.3	24.6	30.5	25.0	29.3	23.9	25.9
26.7	39.5	24.4	37.0	26.8	30.5	27.0	29.4	26.6	26.1

The solvents belonging to the first group are characterised by low dielectric constants ($\epsilon = 2.0$–2.4.) and weak polarity ($\mu \approx 0$) i.e. properties facilitating solute self-association. The observed decrease of ΔH_m values with increasing concentration can be interpreted as a result of the dipolar interactions between cholesterol molecules ($\mu = 1.86$ D [12]) or the formation of self-associates through hydrogen bonds.

The high value of the dimerisation enthalpy of cholesterol in $CHCl_3$ ($\Delta H_{dim} = -25.0$ kcal mol^{-1}) reported by Foster [4], is not confirmed by our data. In this solvent, no systematic concentration dependence of ΔH_m was observed although assuming a dimerisation constant, K, of around 10 M^{-1} it should have been apparent in the concentration range used. More reasonable values of the dimerisation enthalpy are those cited by Fehler et al. [3] (-2.5 kcal mol^{-1} in $CHCl_3$), Koval [13] (-2.1 kcal mol^{-1} in CCl_4) and Parker and Bhaskar [14] (-1.8 kcal mol^{-1} in CCl_4). The heat effect of cholesterol dimerisation in $CHCl_3$ would then be within the measurement error range, which could explain the flat ΔH_m versus concentration curve obtained. From the results given in Table 1 and Fig. 1, it can be seen that the slope of $\Delta H_m = f(m)$ varies in the same way as the $\Delta H_s^{\ominus}$ values obtained by extrapolation. The non-polar solvents used form the following sequence: c-C_6H_{12} > Bz > $CCl_4 \approx$ Tol > pXy. It seems to us that the solute–solute interaction of cholesterol molecules decreases in the same direction.

For solvents with proton-acceptor properties (group (ii)) and weak proton-acceptor properties (group (iii)), the enthalpy of solution is constant within the concentration range used (2×10^{-3}–3×10^{-4} mol kg^{-1}). The value of the standard enthalpy of solution of cholesterol in these solvents is a mean of the ΔH_m values found directly (Table 2).

The independence of ΔH_m on concentration for the solvents under investigation is certainly due to the weak solute–solute interaction of the cholesterol molecules in these solvents. One could assume that the interactions among cholesterol molecules in polar basic and proton-donor solvents become increasingly difficult because of the strong solute solvation.

In order to evaluate the energetic effect of the cholesterol–solvent interactions in the solvents used, the heat of solution $\Delta H_s^{\ominus}$ is divided into three components:

$$\Delta H_{s(x)}^{\ominus} = \Delta H^{vap} + \Delta H_{(x)}^{int} + \Delta H_{(x)}^{cav} \qquad (1)$$

where $\Delta H_{s(x)}^{\ominus}$ is the standard enthalpy of solution of cholesterol in solvent "x", ΔH^{vap} is the enthalpy of vaporisation (sublimation) of cholesterol, $\Delta H_{(x)}^{int}$ is the enthalpy of interaction of cholesterol with solvent "x" and $\Delta H_{(x)}^{cav}$ is the enthalpy of cavitation of cholesterol in solvent "x".

The sum of $\Delta H_{(x)}^{int}$ and $\Delta H_{(x)}^{cav}$ is called the enthalpy of solvation, $\Delta H_{(x)}^{solv}$. Subtracting eqn. (1) from an analogous equation for cyclohexane, one can

TABLE 2

Values of the dissolution enthalpy of cholesterol, $\Delta H_s^{\ominus}$, in different solvents (ΔH in kJ mol^{-1})

Solvent	$\Delta H_s^{\ominus}$	δ_s	$\Delta H^{tr}_{Cy \rightarrow x}$	$\Delta H_x^{cav} - \Delta H_{Cy}^{cav}$	$\Delta\Delta H^{int}$	DN [a]
c-C_6H_{12}	41.9±0.5	8.2				0
CCl_4	31.2±0.3	8.6	−10.7	11.0	−21.7	0
Bz	39.6±0.6	9.2	−2.3	28.5	−30.8	0.1
Tol	30.2±0.6	8.9	−11.7	19.6	−31.3	0.1
pXy	26.4±0.4	8.8	−15.5	16.7	−32.2	5
AcEt	28.6±0.4	9.1	−13.3	25.4	−38.7	17.1
THF	27.7±0.4	9.1	−14.2	25.4	−39.6	20.0
Diox	31.5±0.7	10.0	−10.4	53.6	−64.0	14.8
DMA	23.9±0.6	10.8	−18.0	80.8	−98.8	27.8
HMPA	12.1±0.1	10.5	−29.8	70.3	−100.1	38.8
$CHCl_3$	27.6±1.0	9.3	−14.3	31.5	−45.8	4
$C_2H_2Cl_4$	24.2±0.8	9.7	−17.7	43.9	−61.6	

[a] Gutman's donor number (in kcal mol^{-1} [19,20]).

determine the interaction enthalpy of cholesterol with solvent "x" relative to cyclohexane used as reference

$$\Delta\Delta H^{int} = \Delta H^{tr}_{Cy \rightarrow x} - \left(\Delta H^{cav}_{(x)} - \Delta H^{cav}_{(Cy)}\right) \quad (2)$$

where $\Delta H^{tr}_{Cy \rightarrow x}$ is the enthalpy of transfer of cholesterol from cyclohexane to the given solvent. The $\Delta H^{tr}_{Cy \rightarrow x}$ value is also the difference between enthalpies of cholesterol solvation in solvent "x" and cyclohexane ($\Delta\Delta H^{solv}$).

The enthalpy of cavitation used in eqn. (2) can be found from a simple relationship resulting from the regular solutions theory of Hildebrand and Scott [15]

$$\Delta H^{cav} = V_y \delta_s^2 \quad (3)$$

where V_y is a molar volume of cholesterol in solution [16] (390.8 cm^3 mol^{-1}). All the components of eqn. (2) and the solubility parameters of the solvents used are given in Table 2.

The procedure used to evaluate the relative enthalpy of the solvent–cholesterol interactions ($\Delta\Delta H^{int}$) provides rather approximate results, mainly due to the error in the ΔH^{cav} determination used. It seems that neither SPT theory [17] nor the Sinanoglu method [18] can give the precise ΔH^{cav} values for a molecule as big as cholesterol. However, the $\Delta\Delta H^{int}$ and $\Delta\Delta H^{solv}$ (i.e. $\Delta H^{tr}_{Cy \rightarrow x}$) values presented in Table 2 allow us to state that the interactions and solvation of the large, weakly acidic cholesterol molecule are affected by several factors.

Considering the basic solvents (group (ii)), one can state that $\Delta\Delta H^{int}$ is not a simple function of the basicity expressed by DN. For instance, 1,4-dioxane, a considerably weaker proton-acceptor than AcEt and THF,

seems to interact with cholesterol to a greater extent than either of these. Taking also into account the fact that dioxane does not show a stable dipole moment, one may assume that these solute–solvents interactions are due to the stronger dispersion forces in dioxane as compared to THF and AcEt. The solubility parameter can be a measure of the dispersion interactions [15] because $\Delta H^{disp} = -V_y \delta_s \delta_y$. In the series of systems where substance "y" is always the same (cholesterol), ΔH^{disp} is proportional to the δ_s of the solvent. The dioxane solvent parameter is greater than that of THF and AcEt. Among the solvents used HMPA shows the highest basicity; its DN is higher by 11 units than that of DMA. Despite this, the interaction of cholesterol with both solvents is similar: $\Delta\Delta H^{int}_{(HMPA)} \approx \Delta\Delta H^{int}_{(DMA)}$. This may be explained by the strong compensation effect of the dispersion interaction in DMA as the solubility parameter for DMA is somewhat higher than that of HMPA.

The solvents of the third group ($CHCl_3$ and $C_2H_2Cl_4$) which are weakly acid are characterised by relatively high values of $\Delta\Delta H^{int}$, higher than those for basic THF and AcEt. The solubility parameters of $CHCl_3$ and $C_2H_2Cl_4$ are also higher than those of THF and AcEt. This also suggests that in the solvent–cholesterol interactions, the dispersion forces are more important than the hydrogen bonds through which the solute molecules act as proton donors. In the case of $CHCl_3$ and $C_2H_2Cl_4$, the existence of hydrogen bonding interactions between the oxygen of cholesterol and the weakly acidic protons of the solvent cannot be excluded. Undoubtedly the two kinds of solute–solvent interactions discussed (i.e. dispersion interactions and hydrogen bonding) are also accompanied by dipole interactions in all the solvents.

The nature of the interactions in cholesterol solvation and their effect on the enthalpy of its solutions require further thermochemical studies.

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