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Interactions between cholesterol and triacylglycerols in carbon tetrachloride: calorimetric and spectroscopic studies

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Abstract

The equilibrium constant (K_f) and enthalpy ($\Delta_f H$) of hydrogen bond complex formation (1:1) between cholesterol and triacylglycerols have been determined by means of spectroscopic (IR) and calorimetric methods. The triacylglycerols used (triacetin, tributyrin, trilaurin, tripalmitin and triolein) form weak hydrogen bonds with cholesterol which are shown by the low values of K_f (from 2.4 to 3.3 M^{-1}) and $\Delta_f H$ (from -9.6 to -11.8 kJ mol^{-1}). The basicity of triacylglycerols expressed by pK_{HB} has a predominant effect on the enthalpy of complex formation with cholesterol.

Keywords: Cholesterol; Triacylglycerols; Hydrogen bond complexes; Calorimetry

1. Introduction

One of the most important components of phospholipid membranes is cholesterol (5(6)-cholesten-3-ol). Owing to its polar head group (3β-hydroxyl) and hydrophobic non-polar hydrocarbon group, cholesterol takes part in various interactions within its natural environment. The hydroxyl group of cholesterol is hydrogen-bonded with the polar heads of other molecules (or water), either through acidic hydrogen or the free electron pairs of oxygen. The hydrocarbon side-chain and the planar steroid ring of cholesterol are subject to non-specific van der Waals interactions. The character and strength of cholesterol interactions are undoubtedly of importance to its metabolism, mechanism of arteriosclerosis plaque formation, drug interaction, etc., and from this point of view the qualitative and quantitative characteristics of the interactions of cholesterol in relatively simple, isolated systems seem to be of considerable significance. Recently, physicochemical studies of cholesterol solutions have been frequently under-

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taken, focusing on cholesterol solubility in various solvents [1,2], the density and viscosity of cholesterol solutions [3,4], hydrogen-bond acidity in CCl_4 [5] and phase transitions [6,7]. Thermochemical studies are of primary importance to the quantitative determination of the energy of the specific interactions (hydrogen bonding) of cholesterol with various compounds. From the results obtained in our laboratory it follows that enthalpies of hydrogen bonding of cholesterol are of the same order of magnitude as those of aliphatic alcohols. This concerns both complexes with molecular acceptors [8-10] as well as with pseudohalide anions in CCl_4 [5]. The enthalpy (and to lesser extent the value of $\Delta_f G$) of hydrogen-bond complex formation of cholesterol has been shown to be mainly dependent on the basicity of the proton acceptor used [10].

The present study is aimed at determining and discussing the equilibrium constants and enthalpies of hydrogen-bond complex formation of cholesterol and a series of triacylglycerols such as triacetin, tributyrin, trilaurin, tripalmitin and triolein. The values of association enthalpy in similar systems have already been reported in the literature. The first paper on this subject was published by Parker and Bashar [11]. These authors used IR spectroscopy to determine the enthalpies of hydrogen-bond complex formation ($\Delta_f H$) of cholesterol with triacetin, tributyrin and trilaurin in CCl_4. Costas and Patterson [12] have determined the $\Delta_f H$ value of cholesterol-tripalmitin complexes in CCl_4 by calorimetry, using the Treszczanowicz-Kehiajan model of association. Their result is considerably different from Parker and Bashar's data.

The method used in the present study for the re-examination of the energetics of hydrogen-bond interactions between cholesterol and triacylglycerols differs from those cited above. It combines data obtained by IR spectroscopy and by calorimetry, which, according to many authors, ensures high accuracy in measuring the values of $\Delta_f H$. The parameters describing the hydrogen-bond basicity of the investigated triacylglycerols (triacetin, tributyrin, trilaurin, tripalmitin and triolein) have been also determined for the sake of complete analysis.

2. Experimental

2.1. Materials

Solid triacylglycerols: trilaurin (Sigma, 98%) and tripalmitin (Sigma, 98+%) were crystallized from diethyl ether and dried before measurements, similarly as cholesterol (Sigma, Standard for Chromatography) over P_2O_5 under vacuum. Triacetin (Aldrich, 99%) and tributyrin (Aldrich, 98%) were distilled under reduced pressure. Triolein (Sigma, 99%) was used without further purification. Carbon tetrachloride (POCh, Gliwice, p.a.) was distilled and then dried over 4A sieves.The preparation of the solutions as well as filling of calorimetric and spectroscopic cells was carried out in a dry-box. IR measurements were recorded on a Brucker JFS 85 spectrophotometer within the range 4000-3000 cm^{-1}. A resolution of 1 cm^{-1} was used and 256 scans were accumulated. The calorimetric measurements of the heat of solution in high dilutions were carried out with a calorimeter as described previously [13]. The heat of mixing of cholesterol and triacylglycerol solutions, the heat of dilution and the heat of solution of triacylglycerols with concentrations over 0.06 mol kg^{-1} were measured by means of a Setaram MS80D calorimeter. In determining the enthalpy of solution of pure triacylglycerols, the heat effect of CCl_4 evaporation into the air contained in ampoules or calorimetric cells was taken into account.

2.2. *Method*

A modified version of Arnett's high dilution method was used to determine the enthalpy of hydrogen-bond complex (1 : 1) formation [14]. Its detailed description has been given previously [10] for the systems in which cholesterol was a proton donor. The equilibrium constants (K_f) of complex formation in CCl_4 were determined at 298 K on the basis of the adsorption of free hydroxyl group of cholesterol $\nu_{OH} = 3622\ cm^{-1}$. Both K_f and $\Delta_f H$ values were determined for the solutions with concentrations from 0.06 to 0.15 M of triacylglycerol and from 2×10^{-3} to 5×10^{-3} M of cholesterol.

The hydrogen-bond basicity (pK_{HB}) of proton acceptors is based on the logarithm of the formation constant K_{HB} of the base–4-fluorophenol 1 : 1 complexes in CCl_4 [15].

$$B + 4\text{-}FC_6H_4OH \rightarrow 4\text{-}FC_6H_4OH \cdots B$$

$$K_{HB} = [complex]/[B]\ [4\text{-}FC_6H_4OH] \quad pK_{HB} = \log_{10} K_{HB}$$

The K_{HB} values of triacylglycerols were determined from the adsorption of the free hydroxyl group of 4-fluorophenol $\nu_{OH} = 3614\ cm^{-1}$ in the standard way.

The frequency shift of the ν_{OH} vibration of methanol in CCl_4, $\Delta\nu_{OH}(MeOH) = \nu_{OH}$ (free) – ν_{OH} (H-bonded), which is a spectroscopic measure of hydrogen-bond basicity, was also determined for the triacylglycerols under investigation.

3. Results and discussion

3.1. *Triacylglycerols*–CCl_4

The enthalpies of solution of triacetin and tributyrin in carbon tetrachloride decrease with increasing triacylglycerol concentration (Fig. 1, Table 1). This seems to result from dipole–dipole interactions between the dissolved triacylglycerol molecules in CCl_4. One can assume that the dipole moment of the isolated triacylglycerol molecule is independent of the length of the hydrocarbon substituents since it is derived from the same polar fragment of molecule ($\langle\mu^2\rangle = 8.1 \pm 0.8\ D^2$) [16]. The increase in the chain length can only bring about an increased distance between the interacting molecules and consequently weaken the dipole–dipole interactions. As a result, the strongest concentration-dependence of $\Delta_{sol}H$ is observed in the case of triacetin. For the triacylglycerols with long hydrocarbon chains, trilaurin, tripalmitin and triolein, the values of $\Delta_{sol}H$ were determined only within the range of very low concentrations (from 3×10^{-4} mol kg^{-1} to 1×10^{-3} mol kg^{-1}). The concentration-dependence of $\Delta_{sol}H$ is not evident within this range. Of the liquid triacylglycerols, only triacetin shows the endothermic effect of solution in CCl_4. This would testify to the predominance of

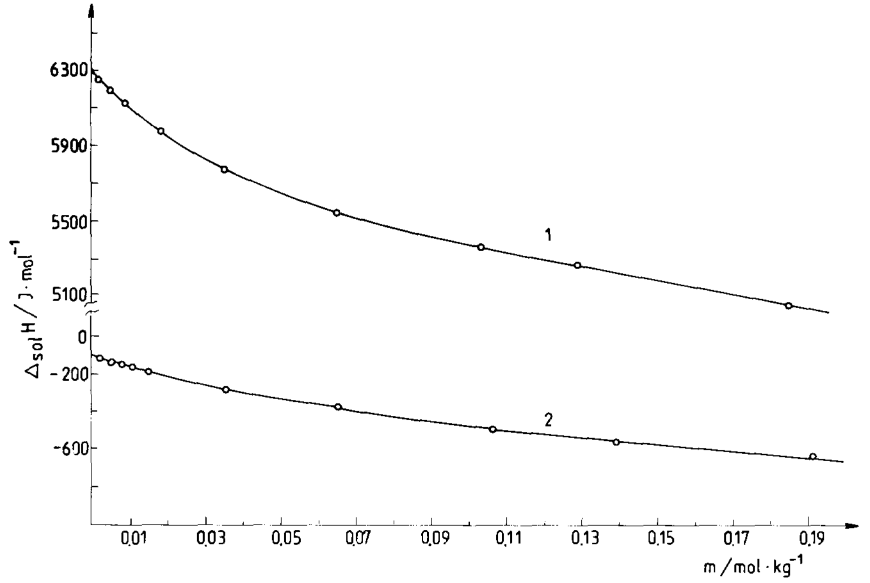

Fig. 1 Concentration-dependence of the enthalpy of solution of triacetin (1) and tributyrin (2) in carbon tetrachloride at 298.15 K.

Table 1
The enthalpy of solution of triacetin and tributyrin in carbon tetrachloride at 298.15 K

Triacetin		Tributyrin	
$m/(\text{mol kg}^{-1})$	$\Delta_{\text{sol}}H/(\text{kJ mol}^{-1})$	$m/(\text{mol kg}^{-1})$	$\Delta_{\text{sol}}H/(\text{kJ mol}^{-1})$
0.0018	6.25	0.0022	−0.12
0.0051	6.19	0.0049	−0.14
0.0092	6.12	0.0075	−0.15
0.0141	6.03	0.0108	−0.17
0.0185	5.98	0.0152	−0.20
0.0350	5.78	0.0355	−0.29
0.0650	5.54	0.0650	−0.38
0.103	5.37	0.106	−0.50
0.128	5.28	0.139	−0.56
0.185	5.05	0.191	−0.65

interactions between triacetin molecules in a pure state over the solute–solvent interactions in solution. On the other hand, the $\Delta_{\text{sol}}H$ values for tributyrin and triolein are negative. In this case, the solvation of solute by CCl_4 molecules seems to be dominant over the solute–solute interactions. The enthalpies of solution of solid triacylglycerols, trilaurin and tripalmitin, (Table 2) have very high values, but they are

Table 2
Enthalpies of solution of triacylglycerols in CCl_4

Compound	Formula	State	$\Delta_{sol}H(CCl_4)$/kJ mol^{-1})	$\Delta_{fus}H$/(kJ mol^{-1})
Triacetin	$(CH_3COO)_3C_3H_5$	Liquid	6.3 ± 0.1 [a]	–
Tributyrin	$(C_3H_7COO)_3C_3H_5$	Liquid	-0.1 ± 0.05 [a]	–
Trilaurin	$(C_{11}H_{23}COO)_3C_3H_5$	Solid	121 ± 2 [b]	124 [c]
Tripalmitin	$(C_{15}H_{31}COO)_3C_3H_5$	Solid	162 ± 2 [b]	166 [c]
Triolein	$(C_{17}H_{33}COO)_3C_3H_5$	Liquid	-2.4 ± 0.2 [b]	–

[a] Values extrapolated to infinite dilution.

[b] Average value for concentrations from 3×10^{-4} to 1×10^{-3} mol kg^{-1} (see text).

[c] Average value (Ref. [17]).

caused by the equally high thermal effect associated with the solid–liquid phase transition. If one takes into account the heat of fusion ($\Delta_{fus}H$), the transfer of triacylglycerol from its pure liquid state to CCl_4 solution is accompanied by an exothermic effect of about -4 ± 2 kJ mol^{-1}. This value is of the same sign and order as the directly measured enthalpy of solution of triolein in CCl_4 (-2.4 kJ mol^{-1}). The exothermic effects of transfer from pure liquid state to CCl_4 solution seem to be brought about by non-specific interactions between solvent molecules and the hydrocarbon chains of the triacylglycerols with long alkyl groups.

3.2. *Cholesterol–triacylglycerol interactions*

Simple esters contain two centres which are potential hydrogen-bond proton acceptors, the oxygens of the alkoxyl and carbonyl groups. The IR data of ν(C=O) and ν(C–O) vibrations indicate that hydrogen-bond interactions (1 : 1) proceed predominantly with the carbonyl group [18]. In the case of triacylglycerols, the situation is somewhat more complex. Each of the three nearly equivalent –COO– groups of those compounds can potentially play the part of proton acceptor. The spectroscopic measurements (IR) performed in CCl_4, and the thermochemic data, fail to indicate the position of a cholesterol molecule bonded in the cholesterol–triacylglycerol 1 : 1 associates. Unfortunately, no information is available concerning the structure of such complexes in existing papers regarding association in analogous systems.

The cholesterol–triacylglycerol complexes in CCl_4 are formed through relatively weak hydrogen-bonds. The equilibrium constants (K_f) as well as the enthalpies ($\Delta_f H$) of complex formation have proved to be low (Table 3). This results from the low basicity of triacylglycerols as shown by pK_{HB} and $\Delta\nu_{OH}$(MeOH). Saturated triacylglycerols with long hydrocarbon chains show a slight increase in basicity, which may be connected with the electrical effect produced by the increase in chain length. The weak and irregular effect of alkyl substituents on the basicity of esters has been recently discussed by Laurence et al. [18]. Slight changes in the basicity of triacylglycerols result in low-grade differences in the enthalpy of cholesterol complex formation. Despite this, there is a noticeable correlation between the basicity (pK_{HB}) and the association

Table 3
Values of equilibrium constant, enthalpy of complex formation of triacylglycerols and cholesterol and the basicity of the triacylglycerols used. Solvent, carbon tetrachloride; temperature, 298.15 K

Compound	K_f/M^{-1}	$\Delta_f H/(kJ\ mol^{-1})$	$\Delta_f H(lit)/(kJ\ mol^{-1})$	pK_{HB}	$\Delta\nu_{OH}(MeOH)/cm^{-1}$
Triacetin	2.4 ± 0.2	-9.7 ± 0.3	-18.0[a]	1.31	65
Tributyrin	2.6 ± 0.2	-9.6 ± 0.3	-14.6[a]	1.30	64
Trilaurin	3.0 ± 0.2	-10.3 ± 0.4	-22.6[a]	1.32	66
Trpalmitin	3.3 ± 0.3	-11.8 ± 0.5	-10[b]	1.34	67
Triolein	2.4 ± 0.2	-9.8 ± 0.3	–	1.32	65

[a] Ref. [11].
[b] Ref. [12].

enthalpy ($\Delta_f H$). Both values change in the following order: tributyrin $\leq$ triacetin $\leq$ triolein $<$ trilaurin $<$ tripalmitin. The values of the equilibrium constant vary in the same way. Tributyrin is an exception and its K_f is somewhat higher than those of triacetin and triolein. It is noticeable that triolein, which is derived from an unsaturated fatty acid, does not show any particular dissimilarity when compared to other triacylglycerols. The hydrocarbon chain length and the presence of double bond in this chain exert a slight influence on the energetics of hydrogen-bond formation with cholesterol.

Comparison of the data we obtained with that cited in the literature is given in Table 3. The enthalpy of cholesterol association with tripalmitin in CCl_4 determined in this study is similar to the result obtained by Costas and Patterson [12] by means of the calorimetric method (-10 kJ mol^{-1}). On the other hand, the enthalpies of cholesterol complex formation with triacetin, tributyrin, and trilaurin cited by Parker and Bhaskar [11] seem to be overestimated. The values of $\Delta_f H$ within the range from -18 to -23 kJ mol^{-1} would be rather expected for the proton acceptors of clearly basic character, e.g. such as substituted amides or DMSO [10]. This discrepancy is due to the use of spectroscopy [11], which can be deceptive in determining $\Delta_f H$, especially in systems with low equilibrium constants.

The low values of the enthalpies of complex formation observed for cholesterol and triacylglycerols are not an exception. Similarly weak H-bonds with cholesterol are formed by the carbonyl group of diethyl carbonate or the oxygen of ethers (simple and cyclic) [10]. The enthalpies of complex formation between cholesterol and compounds of this type range from -10 to -12 kJ mol^{-1}, their equilibrium constant being about 1.0 ± 0.2 M^{-1}. As can be seen from the data given in Table 3, the cholesterol – triacylglycerol complexes have values of $\Delta_f H$ from -9.6 to -11.8 kJ mol^{-1}, showing at the same time higher equilibrium constants: 2.4 M^{-1} to 3.3 M^{-1}. An association constant with a similar value is shown by DMF, which is characterised by considerably higher basicity and $\Delta_f H$ values (-17.6 kJ mol^{-1}). In order to compare the K_f values (as well as pK_{HB}) of triacylglycerols with those of monodentate proton acceptors, the higher probability of complex formation with tridentate triacylglycerols (statistically three times higher) should be taken into account. This is a result of the presence of three

Table 4
Equilibrium constants K_f, enthalpies of formation of cholesterol complexes $\Delta_f H$ with various proton acceptors and their hydrogen–bond basicity

Compound	K_f	$(K_f(\mathrm{corr})/\mathrm{M}^{-1}$	$\Delta_f H/(\mathrm{kJ\,mol}^{-1})$	$\mathrm{p}K_{\mathrm{HB}}$	$\mathrm{p}K_{\mathrm{HB}}(\mathrm{corr})$	$\Delta\nu_{\mathrm{OH}}(\mathrm{MeOH})/\mathrm{cm}^{-1}$
Triacylglycerols[a]	2.8 ± 0.5	(0.9 ± 0.2)	-10 ± 1	1.32	(0.83 ± 0.02)	65 ± 2
DEC		1.1	−10.4		0.88[b]	76
Ethyl acetate		1.5	−13.1		1.07[b]	83
DMF		3.4	−17.6		2.10[b]	150

[a] Average values for the examined triacylglycerols.
[b] Ref. [19].

almost equivalent –COO– groups capable of complexing cholesterol. The statistically corrected values of equilibrium constants ($K_f(\mathrm{corr}) = K_f/3$) change in the same manner as $\Delta_f H$ for triacylglycerols as well as for simple proton acceptors (Table 4). The same mode of change occurs in the basicity expressed by corrected $\mathrm{p}K_{\mathrm{HB}}$ values ($\mathrm{p}K_{\mathrm{HB}}(\mathrm{corr}) = \mathrm{p}K_{\mathrm{HB}} - \log 3$) and $\Delta\nu_{\mathrm{MeOH}}$. For selected compounds, all the values increase in the following order: triacylglycerols < diethyl carbonate (DEC) < ethyl acetate < DMF. In conclusion, it follows from the results of this study that the energetics of the hydrogen-bond complexes between cholesterol and fatty compounds are associated to a large extent with its proton acceptor basicity.

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