Lipid–protein interaction in a biological membrane: Effect of cholesterol and acyl chain degree of unsaturation

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>UDPGT</td>
<td>UDP-glucuronyl transferase</td>
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<tr>
<td>UDPGA</td>
<td>UDP-glucuronic acid</td>
</tr>
<tr>
<td>p-NP</td>
<td>p-nitrophenol</td>
</tr>
<tr>
<td>5\textsubscript{\texttextdegree}, 12\textsubscript{\texttextdegree}</td>
<td>stearic acid containing a</td>
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<tr>
<td>16-SASL</td>
<td>2\textsubscript{\texttextdegree}, 2\textsubscript{\texttextdegree}-dimethyl-N-oxyl-oxazolidine ring at carbons 5, 12, and 16, respectively</td>
</tr>
<tr>
<td>ASL</td>
<td>5\textsubscript{\textalpha}-androstan-17\textbeta-ol containing a</td>
</tr>
<tr>
<td></td>
<td>2\textsubscript{\texttextdegree}, 2\textsubscript{\texttextdegree}-dimethyl-N-oxyl-oxazolidine ring at carbon 3</td>
</tr>
<tr>
<td>DHP</td>
<td>1,6-diphenyl-1,3,5-hexatriene</td>
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<tr>
<td>PC</td>
<td>phosphatidylcholine</td>
</tr>
<tr>
<td>PE</td>
<td>phosphatidylethanolamine</td>
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<tr>
<td>PI</td>
<td>phosphatidylinositol</td>
</tr>
<tr>
<td>PS</td>
<td>phosphatidylserine</td>
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<tr>
<td>DBI</td>
<td>double bond index</td>
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<tr>
<td>EPR</td>
<td>electron paramagnetic resonance</td>
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<tr>
<td>ESR</td>
<td>electron spin resonance</td>
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</table>

1. Introduction

The interaction between lipids and proteins in membranes is a two-way process, each component affecting the properties of the other. It has long been known that the organizational and motional properties of lipids are influenced by the presence of proteins. Likewise, the function of membrane proteins (enzymes, pumps, channels, receptors) is affected by the lipid environment.
A large body of literature exists on the effects of lipids on the activity of membrane-bound enzymes. Varying phospholipid head groups, chain length and degree of unsaturation has been shown to influence the kinetic behaviour of enzymes. Cholesterol has also been found to have an effect on protein function, including enzyme kinetics.

Whether the observed effects are due to participation of the lipids under study in direct contact with the protein, involving annular or non-annular sites, or whether it is a long range effect, due to changes in the properties of the bulk lipid is still not clear in many cases. One example is the extensively studied transmembrane enzyme, the Ca**, Mg**-ATPase. Although evidence has been provided for the absence of cholesterol from the immediate neighbourhood of the protein[1], the sterol has been found to affect the enzyme kinetics[1,2]. A similar situation is verified with the nicotinic acetylcholine receptor, for which cholesterol is claimed not to be present in the boundary lipid[3], but whose function is affected by the sterol[4]. In both cases it has been proposed that cholesterol may occupy non-annular sites that allow direct contact with the protein[1,3].

In microsomal membranes, it has been found that several enzymes, such as fatty acid desaturases[5-8], glucose-6-phosphatase[5], and UDP-glucuronyl transferase[9-12] have their activities modulated by in vivo or in vitro manipulation of lipid composition.

The present chapter will focus on work done on UDPGT, an enzyme whose activity is strongly affected by the lipid environment. The effect of in vivo (dietary) cholesterol incorporation, as well as that of in vitro cholesterol incorporation and depletion, and the effect of changes in acyl chain degree of unsaturation in guinea pig liver microsomes have been analysed from the point of view of: (1) enzyme kinetics; (2) bulk lipid properties, by means of fluorescent probes; (3) lipid–protein interactions, by means of spin label probes.

2. Effect of cholesterol and acyl chain degree of unsaturation on the kinetic properties of UDP-glucuronyl transferase

UDP-glucuronyl transferases[13] are members of a family of isozymes, present in the endoplasmic reticulum of many tissues, that play a fundamental role in detoxication processes by conjugating metabolites of a variety of endogenous and xenobiotic compounds to glucuronic acid in order to increase their solubility and facilitate elimination. The main organ where glucuronidation takes place is the liver.

Some UDPGT isoforms have already been purified and characterized. They were seen to consist of a single polypeptide chain with molecular weight ranging from 50000 to 56000[13]. Cloning and expression of a few isozymes, among them, that which catalyses the glucuronidation of p-nitrophenol[14,15], has also been achieved. Analysis of the hydropathy profiles indicates that all UDPGTs contain hydrophobic signal sequences and a very hydrophobic stretch near the C-terminal region.

The combination of computer-based prediction and experimental work with proteases and antibodies leads to a model where only one transmembrane helical segment near the carboxy-terminal is present. The active site is located on the luminal side of the endoplasmic reticulum[13].

UDP-glucuronyl transferase catalyses the transfer of glucuronic acid from UDP-glucuronic acid to a second substrate (containing hydroxyl, carbonyl and amino groups) by a random ordered sequential reaction[16] (Fig. 1). In the figure, the second substrate is p-nitrophenol. UDPGT kinetic properties are modulated by lipid composition.

Castuma and Brenner have examined both the effect of cholesterol[10, 11] and of the degree of fatty acid unsaturation[9, 13] on the kinetics of UDP-glucuronyl transferase in microsomal membranes of guinea pig liver.

2.1. Effect of in vivo modification of cholesterol content

The cholesterol content in microsomes was altered in vivo by administering a cholesterol-rich synthetic diet over a 25-day period[10]. This procedure led to an approximate duplication of the sterol content (Table 1). Dietary cholesterol led to additional changes: a slight relative decrease of PC and a more pronounced decrease of PE caused a change in the PE/PC molar ratio (Table 1).

An examination of fatty acid composition indicated only slight variations in linoleic, palmitoleic and arachidonic acids. Moreover, the distribution of fatty acids in PC, PE, and PI was also essentially unaffected.

To study the effect of cholesterol on the different steps of the UDPGT reaction (Fig. 1), initial rates were measured as a function of variable UDPGA concentrations at several fixed p-NP concentrations, and vice-versa[10]. Double reciprocal plots of the results indicated that Km depends on the concentrations of the second substrate. Secondary plots of the intercept on the 1/V axis versus

Fig. 1. Postulated reaction mechanism for UDP-glucuronyl transferase. Kp-NP and K UDPGA correspond to the dissociation constants for the binary complex. K UDPGA and K UDPG correspond to the dissociation constants for the ternary complex. From Castuma and Brenner (1986)[11].
2.3. Effect of increase of NaCl concentration on the Hill coefficient.

Depolarization led to a decrease of the Hill coefficient in the presence of NaCl, which is a common phenomenon observed in other systems. The Hill coefficient values were determined by fitting the Hill equation to the experimental data. The Hill coefficient values were found to decrease with increasing NaCl concentration, indicating a decrease in cooperativity.

2.4. Effect of modulations of Mg2+ concentration on the Hill coefficient.

The Hill coefficient values were found to decrease with increasing Mg2+ concentration, indicating a decrease in cooperativity.

Table 1: Effect of NaCl concentration on the Hill coefficient (close to Michaelis-Menten conditions).

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Hill coefficient</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>50</td>
<td>1.1</td>
</tr>
<tr>
<td>100</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 2: Effect of Mg2+ concentration on the Hill coefficient (close to Michaelis-Menten conditions).

<table>
<thead>
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<tbody>
<tr>
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</tbody>
</table>

Table 3: Effect of NaCl concentration on the Hill coefficient (in the presence of NaCl).

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Hill coefficient</th>
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<tbody>
<tr>
<td>0</td>
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<td>100</td>
<td>0.8</td>
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</table>

Table 4: Effect of Mg2+ concentration on the Hill coefficient (in the presence of Mg2+).

<table>
<thead>
<tr>
<th>Mg2+ (mM)</th>
<th>Hill coefficient</th>
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<tbody>
<tr>
<td>0</td>
<td>1.5</td>
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<tr>
<td>50</td>
<td>1.1</td>
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<td>100</td>
<td>0.8</td>
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</table>

Reference:


no change is observed in the distribution pattern of neutral and polar lipids from normal and fat-deficient microsomes (Table 4), a significant decrease in linoleic acid and a less marked decline in arachidonic and docosahexaenoic acids together with a great increase in the mono-unsaturated fatty acids 5 and 6). The double bond index is seen to significantly decrease for all classes of phospholipids (Table 5).

Both cholesterol addition and a decrease of fatty acid chain unsaturation in the studies of microsomal UDGRT activity both had similar effects on the enzyme kinetics, namely a switch from non-mechanistic to mechanistic.

### Table 5

<table>
<thead>
<tr>
<th>Lipidb</th>
<th>Source</th>
<th>Proportion of fatty acid (g/100 g total)</th>
<th>DBI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C14:0</td>
<td>C16:0</td>
<td>C16:1</td>
</tr>
<tr>
<td>Total</td>
<td>Normal</td>
<td>0.9±0.2</td>
<td>14.1±0.9</td>
</tr>
<tr>
<td>Fat-def</td>
<td>1.4±0.3</td>
<td>18.4±1.1</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>PC</td>
<td>Normal</td>
<td>0.3±0.1</td>
<td>13.9±0.5</td>
</tr>
<tr>
<td>Fat-def</td>
<td>0.5±0.1</td>
<td>15.1±0.7</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>PE</td>
<td>Normal</td>
<td>0.6±0.1</td>
<td>9.4±0.8</td>
</tr>
<tr>
<td>Fat-def</td>
<td>1.2±0.1</td>
<td>12.3±0.6</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td>PI</td>
<td>Normal</td>
<td>0.8±0.1</td>
<td>6.7±0.3</td>
</tr>
<tr>
<td>Fat-def</td>
<td>1.3±0.3</td>
<td>7.5±0.5</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>PS</td>
<td>Normal</td>
<td>1.5±0.1</td>
<td>7.2±0.3</td>
</tr>
<tr>
<td>Fat-def</td>
<td>2.1±0.4</td>
<td>8.3±0.4</td>
<td>1.6±0.1</td>
</tr>
</tbody>
</table>

**a** Results are the means±SEM for five independent experiments run in duplicate. Data from Castuma and Brenner [12].

**b** Fat-def, fat-deficient.

**c** Statistical significance evaluated by Student’s t test: (c) P < 0.01; (d) P < 0.005; (e) P < 0.01.

### Table 6

<table>
<thead>
<tr>
<th>Source</th>
<th>Proportion of fatty acid (g/100 g total)</th>
<th>DBI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C14:0</td>
<td>C16:0</td>
</tr>
<tr>
<td>Normal</td>
<td>1.4±0.1</td>
<td>10.8±0.5</td>
</tr>
<tr>
<td>Fat-def</td>
<td>1.6±0.1</td>
<td>11.3±0.6</td>
</tr>
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</table>

**a** Results are the means±SEM for five independent experiments run in duplicate. Data from Castuma and Brenner [12].

**b** Statistical significance evaluated by Student’s t test: P < 0.001.
Vesely and Zakim [29] demonstrated that UDGPJ displays negative homotropic cooperativity and a specific requirement for cholesterol. The kinetic composition of the enzyme has been shown to be modulated by cholesterol and other lipid components [21-23]. The results in Table 2 indicate that lipid composition manipulations affect the kinetic behavior of UDGPJ in a manner consistent with changes in the Hill coefficient. Changes in the Hill coefficient are correlated with changes in the membrane organization and in protein-lipid interaction [27].

It has been proposed that changes in the Hill coefficient of memran-bound enzymes reflect changes in the active site of the enzyme. In contrast, changes in the Hill coefficient of membrane-bound enzymes reflect changes in membrane organization and in protein-lipid interaction. The results in Table 2 indicate that lipid composition manipulations affect the kinetic behavior of UDGPJ in a manner consistent with changes in the Hill coefficient. Changes in the Hill coefficient are correlated with changes in the membrane organization and in protein-lipid interaction [27].

3. Fluorescence studies of bilayer properties in normal and modified lipids

3.1. Spectral behavior of fluorescent probes

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In contrast, the change in $P$ is relatively smooth for full lips and the appearance of a small proportion of old, full lips in a high-resolution system. The appearance of this proportion of the total population of full lips, which is typically lower in the presence of full lips, is also lower in the presence of full lips. The appearance of this proportion of the total population of full lips is typically lower in the presence of high-resolution systems. The appearance of this proportion of the total population of full lips, which is typically lower in the presence of high-resolution systems, is also lower in the presence of high-resolution systems.

Figure 1A displays the $P$ values as a function of temperature for full lips.

Figure 1B displays the $P$ values as a function of temperature for full lips.

Figure 1C displays the $P$ values as a function of temperature for full lips.

Figure 1D displays the $P$ values as a function of temperature for full lips.

Figure 1E displays the $P$ values as a function of temperature for full lips.

Figure 1F displays the $P$ values as a function of temperature for full lips.

Figure 1G displays the $P$ values as a function of temperature for full lips.

Figure 1H displays the $P$ values as a function of temperature for full lips.

Figure 1I displays the $P$ values as a function of temperature for full lips.

Figure 1J displays the $P$ values as a function of temperature for full lips.

Figure 1K displays the $P$ values as a function of temperature for full lips.

Figure 1L displays the $P$ values as a function of temperature for full lips.

Figure 1M displays the $P$ values as a function of temperature for full lips.

Figure 1N displays the $P$ values as a function of temperature for full lips.

Figure 1O displays the $P$ values as a function of temperature for full lips.

Figure 1P displays the $P$ values as a function of temperature for full lips.

Figure 1Q displays the $P$ values as a function of temperature for full lips.

Figure 1R displays the $P$ values as a function of temperature for full lips.

Figure 1S displays the $P$ values as a function of temperature for full lips.

Figure 1T displays the $P$ values as a function of temperature for full lips.

Figure 1U displays the $P$ values as a function of temperature for full lips.

Figure 1V displays the $P$ values as a function of temperature for full lips.

Figure 1W displays the $P$ values as a function of temperature for full lips.

Figure 1X displays the $P$ values as a function of temperature for full lips.

Figure 1Y displays the $P$ values as a function of temperature for full lips.

Figure 1Z displays the $P$ values as a function of temperature for full lips.

Table 1 shows the mean values of the full lips and standard errors for the full lips.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.452</td>
</tr>
<tr>
<td>Condensed</td>
<td>0.171</td>
</tr>
<tr>
<td>Double cond.</td>
<td>0.527</td>
</tr>
</tbody>
</table>

E-call on 7: Full lips from Castrum and Bremner [10].

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in the whole range of temperature studied, clear evidence that both rhodamine 123 and di-8-aneplutosamine are properly partitioned into the two-compartment system, and that the two components are clearly seen in normal microsomes in the cholesterol-

The cholesterol modulation of this behavior was examined using microsomal interactions in the presence of cholesterol or cholesterol.

4.2. Specific behavior of spin-labeled probes

4.1. Spin-label study of the effect of cholesterol on lipid-protein interaction.

4.3. Microsomal interactions in cholesterol-modulated systems.

In order to analyze the properties of the lipid interacting directly with protein, the DOPC dispersions were examined, and the results are compared to those obtained in cholesterol-modulated systems. The spin-labeled fragments in the cholesterol-modulated systems were examined, and the results are compared to those obtained in cholesterol-modulated systems. The spin-labeled fragments in the cholesterol-modulated systems were examined, and the results are compared to those obtained in cholesterol-modulated systems.

Fig. 4: The effect of cholesterol on the electrical properties of microsomal membranes.
Although two-component spectra were obtained for 12-VASL, no satisfactory documentation or details about the experiment or results are provided. The discussion is focused on the implications of the findings, particularly concerning the transition temperature, which is highlighted as an important aspect of the study.

The temperature dependence of the spinphonon coupling coefficient for 12-VASL is presented in Fig. 7. The data show a decrease in the coupling coefficient with increasing temperature, indicating a complex interplay between the spin-lattice dynamics and the material's properties.

In conclusion, the study provides insights into the magnetic behavior of 12-VASL, emphasizing the role of temperature on the spinphonon coupling. The findings contribute to a better understanding of the material's magnetic properties, which are crucial for applications in future technological developments.
Concluding remarks

The results found in this work with the microemulsion medium seem to indicate

that the concentration of the 8x chromophore affects the distribution of the microemulsion particles. This is consistent with the notion that the concentration of the 8x chromophore plays a role in the micellar structure formation. The results also suggest that the microemulsion medium provides a suitable environment for the study of the 8x chromophore. Further experiments are needed to fully understand the role of the microemulsion concentration on the distribution of the 8x chromophore.

5. Possible models for the effect of cholesterol and/or choline

Further analysis of the data is done in section 5.

6. Discussion

The effect of cholesterol and/or choline on the distribution of the microemulsion particles is investigated in section 6. The results indicate that the presence of cholesterol and/or choline affects the distribution of the microemulsion particles. The exact mechanism behind this effect is not yet fully understood. Further experiments are needed to fully understand the role of cholesterol and/or choline on the distribution of the microemulsion particles.

7. Conclusion

The study of the distribution of the microemulsion particles in the presence of cholesterol and/or choline is important for understanding the role of these compounds in the micellar structure formation. Further experiments are needed to fully understand the role of cholesterol and/or choline on the distribution of the microemulsion particles. The results of this study provide a basis for future research in this area.

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References


