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THE MODULATION OF MEMBRANE FLUIDITY BY HYDROGENATION PROCESSES

II. HOMOGENEOUS CATALYSIS AND MODEL BIOMEMBRANES

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Summary

A homogeneous catalyst, chlorotris (triphenylphosphine) rhodium (I) has been incorporated into model biomembrane structures in the form of lipid bilayer dispersions in water. This enables the hydrogenation of the double bonds of the unsaturated lipids within the bilayers to be accomplished. To decide the optimum conditions for efficient hydrogenation the reaction conditions have been varied. The effect of catalyst concentration, hydrogen gas pressure and lipid composition (with and without cholesterol) have all been studied. The partition of the catalyst into the lipid medium was checked by rhodium analysis. The results show that an increase of catalyst concentration or an increase of hydrogen gas pressure leads to increasing rates of hydrogenation. Successful hydrogenation was accomplished with different types of lipid dispersions (mitochondrial, microsomal and erythrocyte lipids).

A selectivity of the homogeneous hydrogenation process is indicated. The polyunsaturated fatty acyl residues are hydrogenated at an earlier stage and at a faster rate than the monoenoic acids. Furthermore, an increase in the proportion of cholesterol to lipid within the bilayer structures causes a progressive decrease in the rate of hydrogenation. The fluidity of the lipid bilayers can be altered to such an extent by the hydrogenation process that new sharp endotherms corresponding to the order-disorder transition of saturated lipids occur at temperatures as high as 319 K. Some potential uses of hydrogenation for the modulation of cell membrane fluidity are discussed as well as the design of new types of catalyst molecules.

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Introduction

Biological membranes are considered to consist of a lipid bilayer matrix in which proteins are distributed. The lipids of these bilayers consist of a mixture of lipid classes (e.g. phosphatidylcholines, phosphatidylethanolamines) each of which has associated with them a range of fatty acyl residues of different chain lengths and various amounts of unsaturation [1]. The fluidity of the bilayer matrix is determined by the lipid composition [2]. Studies of pure lipid systems have shown that for a given lipid class at a given temperature the fluidity is greater the shorter the chain length and the more unsaturated the fatty acyl residue [3]. This has been shown in a convenient way by measuring the transition temperature of the pure lipid-water systems [4].

The fluidity of cell membranes has been linked to various cellular processes including membrane-bound enzyme activities, transport processes, malignant transformation membrane fusion and protein rotation and diffusion [5]. This has led to the development of various techniques to modulate the fluidity of cell membranes including genetic, nutritional and temperature manipulation [6].

We have recently introduced a new technique for modulating membrane fluidity which consists of hydrogenating the double bonds of the lipid molecules within the bilayer structures using a homogeneous catalyst [7]. In this report we present detailed studies using this techniques of a range of model biomembranes in the form of lipid-water dispersions. We investigate the effect of varying catalyst concentration, hydrogen pressure and lipid composition (including variation of cholesterol content). We also discuss the potential of the method and the design of new catalyst molecules for this technique.

Materials and Methods

Preparation of lipids. Samples of unsaturated phosphatidylcholines were purified from crude preparations of soya and egg-yolk lecithins (Sigma) by column chromatography on alumina [8] followed by precipitation with cold acetone [9]. The purification process was repeated in the case of egg yolk lecithin. The phospholipids were dissolved in benzene and lyophilised to achieve an almost complete removal of solvent. The purity of each preparation was checked by thin-layer chromatography on silicic acid plates developed with a solvent consisting of chloroform/methanol/water (65 : 25 : 5, v/v). Each preparation ran as a single band migrating the same as an authentic sample of pure unsaturated lecithin (Lipid Products, South Nutfield, U.K.). Phosphorus analyses were performed after $HClO_4$ digestion by the Bartlett method [10].

Lipid extraction and analysis. Total lipid extracts of rat liver mitochondria and microsomes and human erythrocyte membranes were obtained by a modification of the method of Sheltawy and Dawson [11]. Briefly, the precipitate resulting from treatment of the membrane preparations with 0.3 M $HClO_4$ was extracted with a solvent consisting of chloroform/methanol/10 N HCl (200 : 100 : 1, v/v). The solvents were partitioned after incubation with stirring for 30 min by adding three volumes 0.1 M HCl and centrifuging. The upper aqueous phase was discarded and the infranatant extracted again with

the acidified organic solvent and the combined lower organic phases were dried under nitrogen, redissolved in benzene and lyophilised.

Fatty acid analyses of phospholipids were performed on the corresponding methyl ester derivatives formed by heating the dried phospholipid at 70° C for 2 min with 14% BF_3 in methanol. The methyl esters were extracted into light petroleum, dried over anhydrous Na_2SO_4 and separated by gas chromatography. The instrument used was a Pye series 104 with a column of 10% polyethyleneglycol adipate coated on diatomite C-AN (100—120 mesh) at a temperature of 195° C and a carrier gas of nitrogen. Retention times of authentic fatty acid methyl esters were used to identify fatty acids derived from phospholipid samples. The proportion of each fatty acid was obtained by measuring the relative peak areas from a recorded trace and the percent hydrogenation was calculated from the difference between the relative number of unsaturated bonds in the substrate before hydrogenation and the relative number of unsaturated bond remaining after reaction. The possible loss of fatty acids due to peroxidation was checked in each chromatogram. No changes attributable to peroxidation was observed in the lipids after hydrogenation. The possibility of lipid degradation following hydrogenation was also checked by thin-layer chromatography on silicic acid plates using a solvent of hexane/diethylether/acetic acid (80 : 20 : 1, v/v). No free fatty acids or other degradation products could be detected even after complete saturation of the phospholipids. To determine whether cholesterol itself was hydrogenated in mixed substrate preparations the cholesterol was separated from the phosphatidylcholine by the solvent partition method of Dole [12] and the proton magnetic resonance spectrum compared with that obtained for dihydrocholesterol.

Hydrogenation procedures. Phospholipids (50 mg) were dispersed in 10 ml deoxygenated distilled water by sonicating on ice for 10—20 min with an MSE ultrasonicator. The catalyst used in all experiments was chlorotris(triphenylphosphine)rhodium prepared as described previously [7]. The catalyst was dissolved in a small volume of dry, redistilled tetrahydrofuran and gassed with hydrogen until the colour changed to pale yellow. The dispersed substrate was transferred to a 12-oz high pressure reaction vessel (Fischer and Porter, (Workington, U.K.) and gassed with hydrogen for 30 min. The reaction was initiated by adding the hydrogenated catalyst in 0.3 ml tetrahydrofuran to the reaction vessel and incubating at 37° C with constant stirring.

Other methods. The amount of catalyst associated with lipid dispersions was determined by layering the lipid/catalyst mixtures over sucrose gradients consisting of 51.3 g/100 ml (4 ml), 37 g/100 ml (8 ml) and 23 g/100 ml (4 ml) sucrose and centrifuging at 75 000 $\times g_{av}$ for 1 h. All the lipid remained at the top of the gradient and the rhodium catalyst not associated with the lipid was recovered in the pellet. Rhodium analyses were performed by atomic absorption spectroscopy. Similar results to the sucrose gradient centrifugation were obtained by gel filtration through Biogel A-5m columns in which case the catalyst excluded from the lipid was retained in the gel bed.

A Perkin-Elmer DSC-2 calorimeter with a heating rate of 5° C/min was used to obtain differential scanning calorimeter curves. Samples of purified egg yolk lecithin before and after complete hydrogenation were dispersed in three parts weight of water.

Results

Variation of catalyst concentration

The way in which an increase of catalyst concentration affects the hydrogenation of soya lecithin dispersions is shown in Fig. 1. The amount of hydrogenation of the fatty acyl residues can be seen to depend upon the concentration of catalyst present. At low concentration (less than 1 mol of catalyst per 100 mol of phospholipid) the reaction rate appears to be faster than at higher catalyst concentrations. When present in concentrations of between 1 and 10 mol percent there is a direct relationship between catalyst concentration and the extent of saturation of the substrate. Because the catalyst was added after dispersion of the lipid in water it was of interest to determine the distribution of catalyst between the aqueous medium and the phospholipid bilayers. Light scattering experiments, illustrated in Fig. 2 show that an addition of rhodium catalyst dissolved in water caused an increase in turbidity (curve C, Fig. 2) which is due to the presence of catalyst since solvent alone did not alter the absorbance of either water or lipid dispersions (curve A, Fig. 2). When catalyst in solvent is added to a liposome dispersion, however, (curve B, Fig. 2) there is an initial increase in light scattering. This decreases rapidly over 5 min and eventually the original absorbance is reached after 15—20 min. To demonstrate that the decrease in absorbance represents a partition of the catalyst into the lipid bilayers, the concentration of catalyst in the liposomes was determined directly by atomic absorption spectroscopy (see

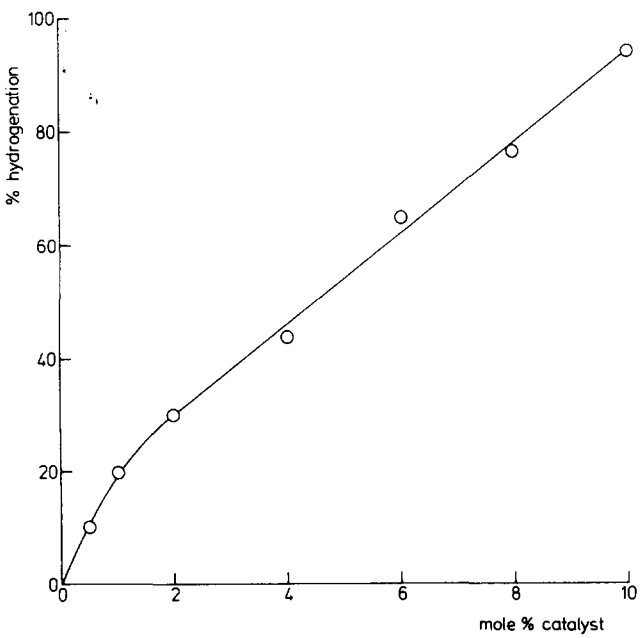


Fig. 1. The effect of varying catalyst concentration on the percentage hydrogenation of soy bean phosphatidylcholine. Phospholipid (50 mg) dispersed in 10 ml water was hydrogenated for 3 h at 37° C under 500 kPa hydrogen pressure in the presence of different concentrations of chlorotris(triphenylphosphine) rhodium (I) catalyst.

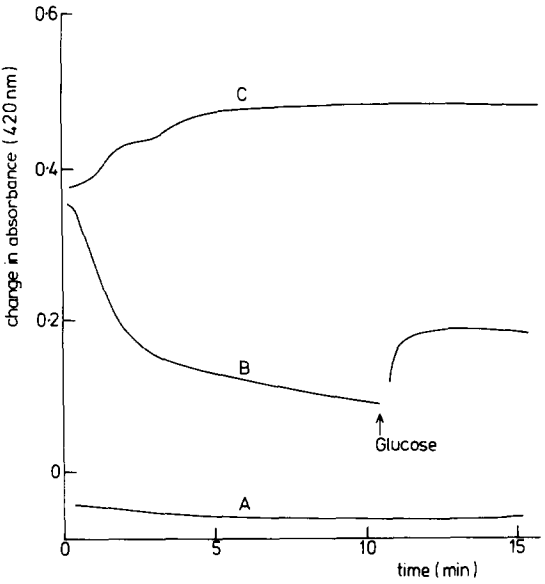


Fig. 2. The changes of absorbance (420 nm) which occur when rhodium catalyst dissolved in tetrahydrofuran is added to aqueous dispersions of egg lecithin. Addition of 20 μl tetrahydrofuran (A) and 20 μl tetrahydrofuran containing 50 μg rhodium catalyst (B) to a dispersion of egg lecithin (2.5 mg) in 2 ml water. Curve C represents addition of catalyst in solvent to water. Glucose was added where indicated to give a final concentration of 15 mM.

Materials and Methods). The catalyst was found to partition almost completely into the lipid phase even at a ratio of 10 mol per 100 mol of phospholipid, the highest concentration examined. The concentration of catalyst in the liposomes after complete hydrogenation of all the unsaturated fatty acyl residues remained unchanged from the initial values suggesting that catalyst does not partition into the water as the reaction proceeds. Addition of glucose (15 mM) to lipid dispersions caused the liposomes to contract to the same extent in the presence or absence of catalyst indicating that the permeativity of the lipid bilayers to glucose was unaffected by the addition of catalyst and tetrahydrofuran.

Variation of hydrogen pressure

Multibilayer structures consisting of purified soya lecithin dispersed in water were hydrogenated in the presence of rhodium catalyst under hydrogen pressures varying between 150 and 900 kPa. The percentage hydrogenation of the phospholipid after 2 h incubation at different pressures is shown in Fig. 3. This shows that increasing the pressure of hydrogen gas up to 900 kPa leads to an increase in the percentage hydrogenation of the fatty acyl residues of the phospholipid. More than 70% of the double bonds initially present are saturated after 2 h under these conditions. The relationship between the reaction rate and hydrogen pressure does not appear to be a direct one because relatively more hydrogenation is obtained at hydrogen pressures up to 300 kPa than are observed to take place at higher pressures.

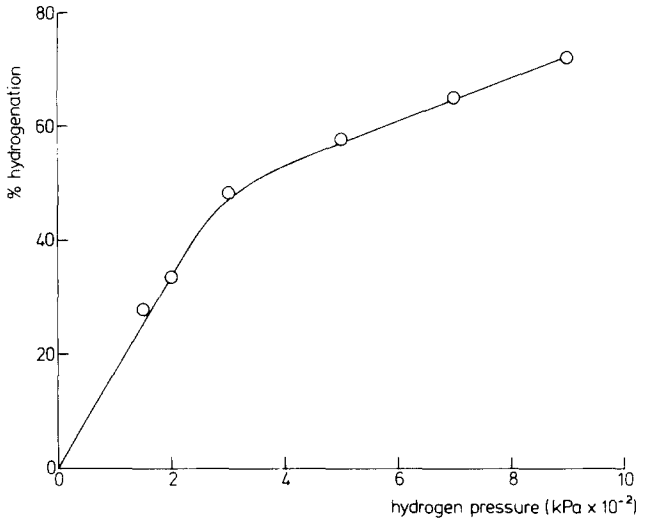


Fig. 3. The relationship between percent hydrogenation of soya phosphatidylcholine and hydrogen pressure. Phospholipid (50 mg) was dispersed in 10 ml water and incubated in the presence of 6 mol rhodium catalyst per 100 mol of phospholipid for 2 h at 37°C under different pressures of hydrogen. Each point represents the mean value of at least two observations.

Variation of lipid composition

Hydrogenation of total lipid extracts of human erythrocyte membranes and rat liver mitochondria and microsomes are presented in Table 1. Dispersions of mitochondrial lipids were hydrogenated to about the same extent as liposomes consisting of soya phosphatidylcholine but less hydrogenation was achieved with lipid dispersions of microsomes and particularly with total erythrocyte lipid extracts. The extent of hydrogenation of these lipid extracts appeared to be related to the amount of cholesterol. A more systematic investigation of the effect of

TABLE I

CHANGES IN THE MAJOR FATTY ACYL RESIDUES OF TOTAL LIPID EXTRACTS OF BIOLOGICAL MEMBRANES DISPERSED IN WATER

Hydrogenations were performed for 2 h under 600 kPa hydrogen pressure in the presence of 4 mol catalyst per 100 mol phospholipid added in tetrahydrofuran (3% final concentration).

Lipid extract	Fatty acid				Hydrogenation (%)
	16 : 0	18 : 0	18 : 1	18 : 2	
Human erythrocyte					
Control	41.0	28.7	18.4	11.7	
Hydrogenated	42.8	28.9	20.9	7.5	14
Rat liver mitochondria					
Control	20.0	45.9	12.3	22.8	
Hydrogenated	23.0	67.7	2.9	5.7	75
Rat liver microsomes					
Control	22.2	46.0	20.2	10.8	
Hydrogenated	24.2	55.8	14.5	2.3	54

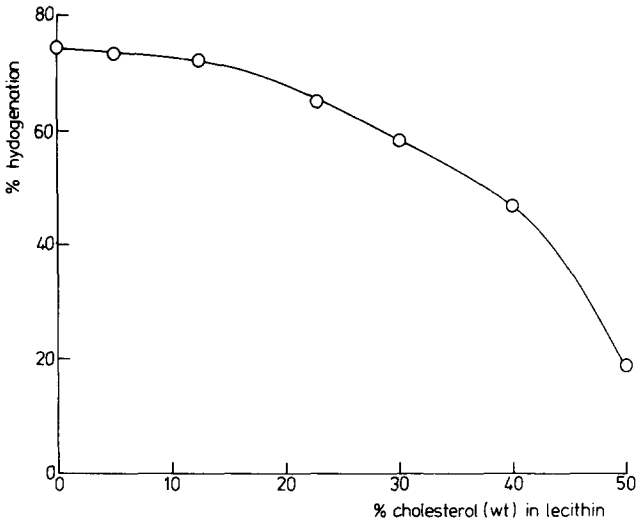


Fig. 4. The effect of increasing the proportion of cholesterol on the percentage hydrogenation of egg yolk lecithin. Phospholipid (50 mg) containing different amounts of cholesterol was dispersed in 10 ml water and hydrogenated for 2 h at 37°C under 600 kPa hydrogen pressure in the presence of 6 mol rhodium catalyst per 100 mol phospholipid. Each point represents the mean value of at least two experiments.

adding cholesterol to purified phospholipid on the rate of hydrogenation in the presence of rhodium catalyst was undertaken. Egg yolk lecithin was chosen for these experiments because the effect of cholesterol on the physical properties of this phospholipid are clearly understood. Incubations were performed with phospholipid co-sonicated with different amounts of cholesterol to which rhodium catalyst (6 mol per 100 mol phospholipid) in tetrahydrofuran was added. The percentage hydrogenation after 2 h under 600 kP hydrogen is shown in Fig. 4. Increasing proportions of cholesterol in the phospholipid dispersions results in a decrease in the amount of hydrogenation of the fatty acyl residues. Thus, under the conditions employed, 74% of the double bonds initially present were hydrogenated when no cholesterol was included in the dispersion compared with only 24% hydrogenation in dispersions consisting of equimolar proportions of phosphatidylcholine and cholesterol. Nuclear magnetic resonance studies of neutral lipid extracts of hydrogenated mixed

TABLE II

THE PARTITION OF RHODIUM CATALYST INTO LIPID DISPERSIONS

Rhodium catalyst was added in a proportion of 6 mol per 100 mol phospholipid to the dispersed lipid.

Lipid composition	Percent catalyst recovered in liposomes
Egg lecithin	94 ± 2
Egg lecithin : cholesterol	
9 : 1	85
4 : 1	75
1.7 : 1	70
1.25 : 1	57
1 : 1	62

lipid dispersions showed that no detectable amount of dihydrocholesterol was formed under the conditions employed. The effect of cholesterol on hydrogenation of the phospholipid could be due to (a) a decrease in the catalytic rate or (b) a reduction in the amount of catalyst partitioning into the lipid phase. To test these two possiblities the concentration of catalyst in each dispersion was measured. It was found (Table II) that increasing proportions of

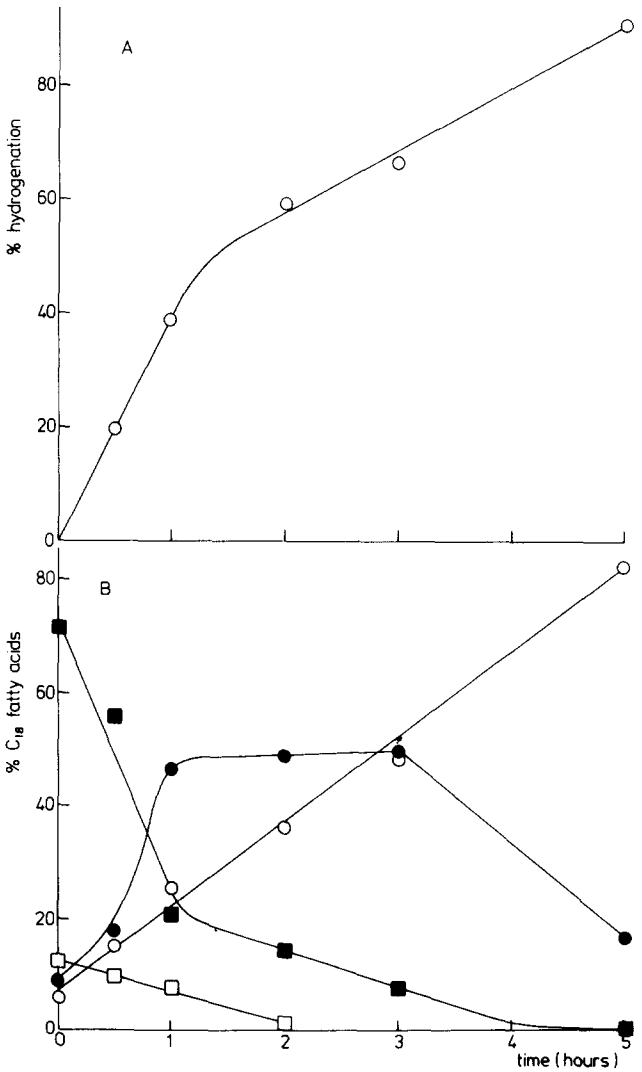


Fig. 5. (a) A plot of the percent hydrogenation of soya phosphatidylcholine dispersions at different times of reaction. Phospholipid (50 mg) dispersed in 10 ml water was incubated for varying times in the presence of 6 mol rhodium catalyst per 100 mol phospholipid at 37°C under a hydrogen pressure of 500 kPa. Each point is a mean of at least three experiments. (b) Changes in the fatty acyl constituents of soya phosphatidylcholine during hydrogenation. The proportions of C_{18} fatty acids were obtained from total fatty acid analyses of samples shown in a. Palmitic acid was present in each sample in a proportion of 23% of total fatty acids. The percentage linolenic (□), linoleic (■), oleic (●) and stearic (○) acids are shown as a function of incubation time.

cholesterol in the dispersion led to a decrease in the amount of catalyst which partitions into the liposomes.

Selectivity of hydrogenation

The time-course of hydrogenation of soya lecithin in aqueous dispersions is shown in Fig. 5a. The curve obtained appears to consist of two regions. There is in the first region an initially fast rate of hydrogenation which is sustained until about 50% of the double bonds are saturated. This is followed by a second region where the remaining unsaturated bonds are hydrogenated but at a slower rate. In these experiments the overall reaction was half complete after slightly more than 1 h incubation and after 5 h 94% of the double bonds had been saturated. The distribution of C_{18} fatty acids of the phospholipid molecules after various times of incubation are shown in Fig. 5b. It can be seen that hydrogenation in the presence of rhodium catalyst is markedly selective in that saturation of the polyenoic fatty acids, linoleic and linolenic acids, takes place predominantly during the initial stages of hydrogenation whereas conversion of oleate to stearate remains constant throughout the incubation. In quantitative terms most of the polyenoic fatty acids are hydrogenated at a faster rate than oleic acid although a proportion of these acids are hydrogenated at a slower rate during the latter half of the reaction. Examination of the fatty acids present at the end of incubations performed under different pressures of hydrogen (Fig. 3) showed a similar pattern suggesting that the biphasic nature of the curve was related to differences in the extent of hydro-

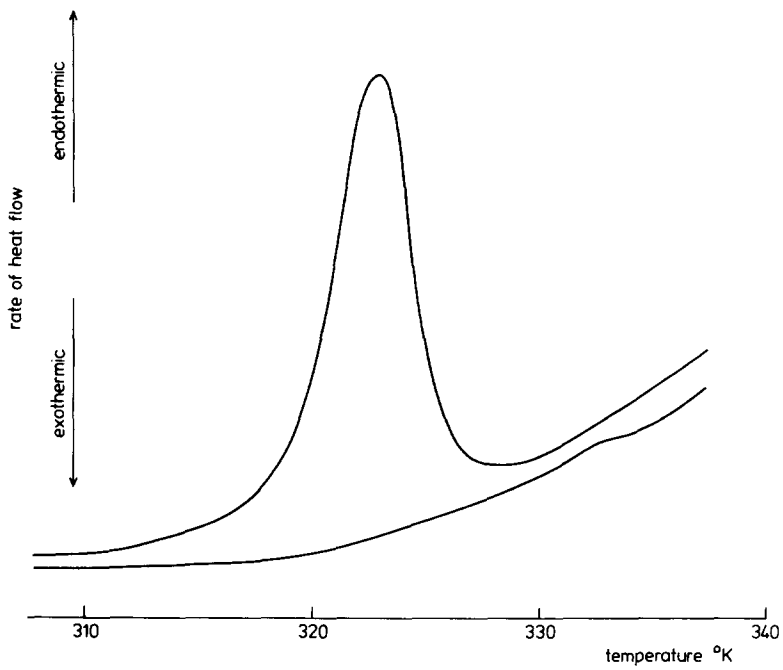


Fig. 6. Differential scanning calorimetric heating curves of completely saturated egg yolk phosphatidylcholine (upper trace) and the unsaturated native phospholipid (lower trace). Each preparation contains 6 mol % rhodium catalyst.

genation of the lipid.

Fluidity and phase transitions

To show how hydrogenation can alter the fluidity of phospholipid bilayers, a dispersion of egg yolk lecithin was incubated for 3 h under the conditions used in Fig. 4. A fatty acid analysis of this preparation showed that the phospholipid was almost completely saturated. A sample of hydrogenated phosphatidylcholine was then examined by differential scanning calorimetry and the curve obtained, together with an unhydrogenated preparation containing the same amount of catalyst and tetrahydrofuran, is shown in Fig. 6. No lipid endothermic phase transitions were observed with the unsaturated, control sample over the temperature range 275—344 K but the completely hydrogenated dispersion exhibited a single sharp phase transition at a temperature of 319 K. The calculated enthalpy for the transition was 27.3 kJ/mol.

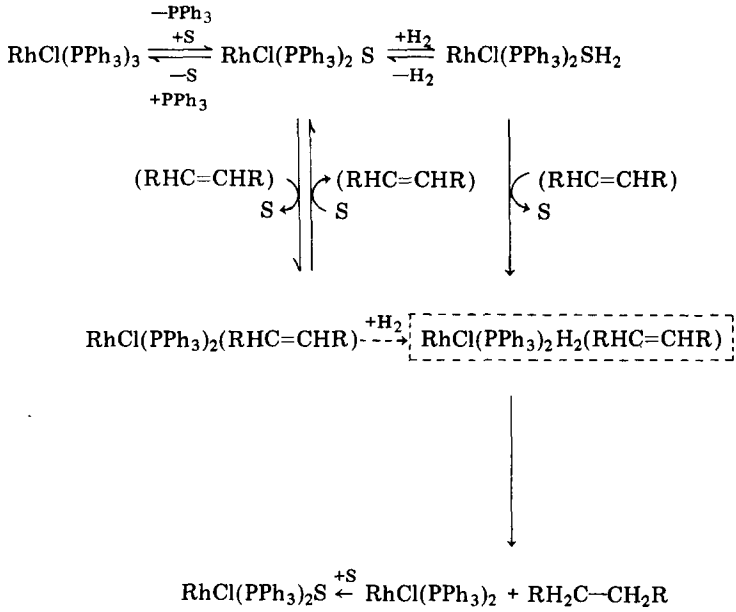
Discussion

The fluidity of the lipid matrix of cell membranes is important for providing the environment for protein organisation, movement and function. Various cellular processes have been linked with this fluidity characteristic including protein rotation, transport processess, protein aggregation and malignant transformation, etc. [5]. The modulation of membrane fluidity has been considered and discussed with reference to molecules such as cholesterol, polypeptides and proteins. Their effects on membrane fluidity have been related to the inhibition of lipid chain mobility and lipid diffusion characteristics.

It is now well known that the presence of a *cis* double bond in the hydrocarbon chain of phospholipid molecules can considerably affect the lipid phase transition temperature of bilayer structures (an increase in the number of double bonds in the chain causes a lowering of the lipid transition temperature). The transition temperature itself provides a reference for the fluidity characteristics since the lipid undergoes a change from a rigid relatively immobile condition to a fluid structure when heated above this temperature [4]. Our approach to the modulation of membrane fluidity is to reduce the unsaturated double bonds of the fatty acyl residues associated with membrane lipids in situ by means of homogeneous catalytic hydrogenation [6].

The present experiments extend our original observations [7]. Increasing hydrogen pressure leads to appreciably faster rates of hydrogenation and the rate of hydrogenation is also related to the catalyst concentration in the bilayer. The mechanism of hydrogenation by chlorotris(triphenylphosphine)-rhodium catalyst has been studied by Wilkinson and his colleagues [13—15]. The reactions involved are summarised in the scheme on next page.

The association of the catalyst with solvent ligands (S) is known to affect the rate of hydrogenation [13] as does the ratio of triphenylphosphine to rhodium [15]. The increased rate of hydrogenation in the presence of low catalyst concentrations seen in Fig. 1 is believed to be due to the release of a second triphenylphosphine ligand to yield the more reactive species $RhCl(PPh_3)$ [16]. The deep red colour of $RhCl(PPh_3)_3$ in tetrahydrofuran turns to pale yellow-orange when hydrogen is bubbled through the solution due to the formation of



the hydride species of the catalyst. There is some evidence that hydrogen activation can proceed after complex formation between the substrate and catalyst [17] although, in our hands, prior hydrogenation of the catalyst before addition to the dispersed substrate often resulted in greater rates of hydrogenation. The orientation and binding of the substrate to the catalyst is considered to be the rate-limiting step in the overall hydrogenation process [15]; transfer of hydrogen to the alkene is a relatively fast reaction.

In our experiments the rhodium catalyst appears to distribute rapidly throughout the entire substrate when added in a solvent of tetrahydrofuran to the phospholipid dispersions. The light scattering experiments indicate that addition of rhodium catalyst in tetrahydrofuran to liposomes of phosphatidylcholine causes a transient increase in light scattering which is eventually restored to original levels whilst addition of solvent alone has no effect. When glucose is added to the suspension of liposomes containing rhodium catalyst the liposomes contract suggesting that the permeability properties of the bilayers are retained in the presence of catalyst and solvent. The use of tetrahydrofuran as a solvent results in complete accessibility of the catalyst to all of the substrate. Consistent with this, all of the available lipid becomes hydrogenated and all of the linolenic acid is reduced at an early stage of the reaction.

The results of our studies of various lipid compositions from different membranes show that in all cases a satisfactory hydrogenation of their double bonds is accomplished. The variations in the amount of hydrogenation which occurs in a given time with these lipid extracts can be related to the relative amount of cholesterol associated with the lipid system. This reduction in the amount of hydrogenation is due to the fact (as shown by atomic absorption spectroscopy) that the amount of catalyst which partitions into the lipid dispersion decreases as the amount of cholesterol increases. This can be linked with the fact that the presence of cholesterol in the lipid bilayer modulates the lipid

fluidity, increasing its viscosity thereby causing a reduction of solubility of the catalyst into the lipid environment. Many other studies have shown that the permeability of model membrane systems to a variety of molecules is considerably reduced by the presence of appreciable amounts of cholesterol in the lipid bilayer [18,19].

The results show that, by incorporating a homogeneous rhodium triphenylphosphine catalyst into the lipid bilayers, the hydrogenation of the double bonds of the lipids in bilayer structures in water can be accomplished. The studies also show that there is selectivity for this process, so that for example the linolenic fatty acid residues are all hydrogenated before the linoleic residues. There is also a selectivity between lipid bilayers which contain little or no cholesterol compared with bilayers containing appreciable amounts of cholesterol. This indicates that the technique may be selective for particular fatty acid residues and for membranes which have little cholesterol present.

This satisfactory hydrogenation of the model biomembrane lipid-water structures raises the question as to the advantages and potential which this technique has over other techniques used for altering membrane fluidity in natural biomembranes and cell systems. These include genetic manipulation, drug treatment, nitritional supplements and in some systems changes of environmental temperature. Thus lipid auxotrophs of bacteria and yeast which are genetically deficient in enzymes responsible for lipid synthesis have been isolated and studied [20]. Certain drugs are known to alter phospholipid [21] and fatty acid [22] composition of the membranes of microorganisms and lead to marked changes in fluidity and it has been shown that some control over changes in membrane fluidity can be achieved by combining drug treatment with appropriate nutritional supplementation [23]. Indeed, nutritional supplementation alone provides considerable scope for altering membrane fluidity of microorganisms [24,25], cells in tissue culture [26,27] and to a limited extent the membranes of whole animals [28]. Changes in environmental temperature are also known to lead to changes in the fatty acid composition of some cell membranes. The present method using homogeneous catalysis has the advantage of being able to change membrane fluidity at a constant temperature without altering the individual genotype. It also provides the selectivity for studying the function of particular fatty acids associated with the membrane lipids, e.g. the polyunsaturated acids such as arachidonic, linolenic and linoleic acids present in many cell membranes and the highly unsaturated long chain acids such as docosohexenoic acid found to occur in certain membranes like axons and retinal thylakoid membranes.

We can instance two potential applications. (a) Studies of platelet adhesiveness. When blood platelets are stimulated by a variety of agents the polyunsaturated fatty acids are released from the membrane and converted into prostaglandin endoperoxides. Some of the endoperoxide derived from arachidonic acid is used to synthesise thromboxane which has recently been shown to act as a potent platelet aggregating factor [29]. The selective hydrogenation of the arachidonic acid present in the membranes could enable studies to be made of this aggregation process.

(b) Studies of lymphocytes. The microviscosity of lipid layers of normal lymphocytes is almost twice that of malignant transformed lymphoma cells

from rats, mice and humans [30,31]. An analogous difference has also been observed in human lymphocytes from normal and chronic lymphatic leukaemic patients where it was shown that by increasing the cholesterol content of the lymphoma cell and hence decreasing the membrane fluidity the replication rate of these cells is reduced. The hydrogenation of malignant transformed lymphocytes provide an alternative method for affecting the membrane fluidity and relating this to cell replication processes.

The successful application of the homogeneous catalyst method for the modulation and selective hydrogenation and deuteration of natural cell membranes (as distinct from model biomembranes) where competition from membrane proteins and serum proteins occur may be more difficult. Our preliminary experiments show that nevertheless satisfactory hydrogenation of certain biomembrane structures can be accomplished. We can already envisage a number of improvements that can be made to catalyst design for future applications. These consist of (a) avoiding the use of solvents such as tetrahydrofuran as a carrier for the catalyst molecule. (b) The design of simpler and smaller catalyst molecules which have low toxicity to living organisms. (c) The use of alternatives to hydrogen gas (certain hydrogen donor molecules are known to act as a source of hydrogen [33] in many catalytic hydrogenation processes). (d) To achieve by suitable catalyst design an even greater selectivity for hydrogenation of specific double bonds including the hydrogenation of one half of a bilayer leaving the other half unaltered. We have already performed preliminary studies with new catalysts which have some of these improved properties, e.g. water solubility. These studies will be presented in forthcoming papers.

We consider that the method of manipulating the double bonds in membranes and thereby modulating membrane fluidity by using homogeneous catalyst may have considerable use in cell biology. The possibility of incorporating catalysts into model and natural membranes so as to carry out reactions other than hydrogenation may also have considerable value.

Acknowledgments

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