分类号_____密级_____

UPC_____编号_____

中 国 科 学 院

博士学位研究生学位论文

蛋白质“嵌入”磷脂单分子膜的结构与动态学

赵 江

指导教师姓名 李峻柏 研究员

中国科学院感光化学研究所

申请学位级别 博士学位 学科专业名称 物理化学

论文提交日期 2000年5月 论文答辩日期 2000年5月30日

学位授予单位名称 中国科学院感光化学研究所

答辩委员会主席_____

评阅人_____

年 月 日

分类号_____密级_____

UPC_____编号_____

中 国 科 学 院
博士学位研究生学位论文

蛋白质“嵌入”磷脂单分子膜的结构与动态学

赵 江

指导教师姓名 李峻柏 研究员

中国科学院感光化学研究所

申请学位级别 博士学位 学科专业名称 物理化学

论文提交日期 2000年5月 论文答辩日期 2000年6月30日

学位授予单位名称 中国科学院感光化学研究所

江苏工业学院图书馆
藏书章

答辩委员会主席_____

评阅人_____

年 月 日

Protein Penetration into 2D Aggregating Phospholipid Monolayers: Structure, Morphology and Dynamics

Jiang Zhao

A Thesis Submitted for the Degree of

DOCTOR OF PHILOSOPHY IN CHEMISTRY

(Physical Chemistry)

[*] *International Joint Laboratory between Institute of Photographic Chemistry and Max Planck Institute of Colloids and Interfaces, Chinese Academy of Science, P. R. China*

[¥] *Max-Planck-Institute of Colloids and Interfaces, Germany*

Supervisors

Prof. Dr. **Junbai Li**
Chinese Academy of Science, P.R.China

Dr. habil. **Reinhard Miller**
Prof. Dr **Dieter Vollhardt**
Prof. Dr. **Helmuth Möhwald**
Max-Planck-Institute of Colloids and Interfaces
Germany

Dedicated to my parents

谨以此论文献给我的父母

Index

Abstract 1

1. Introduction 4

1.1 General Introduction 4

1.2 Fundamentals of Physical Chemistry 7

1.2.1 Surface Tension and Surface Pressure 7

1.2.2 Phase Transition and State of Monolayers 8

1.3 Surface Properties of Lipids and Proteins 10

2. Theory 13

2.1 Penetration Thermodynamics 13

2.1.1 Analysis Based on Gibbs' Equation 14

2.1.2 Analysis Based on Pethica's Equation 16

2.1.3 Analysis Based on Butler's Equation 20

2.2 Equation of State for Langmuir Monolayers of Amphiphilic Molecules Capable of 2D Aggregation 22

2.3 Equations of State and Adsorption Isotherms for Proteins 27

2.4 Penetration Thermodynamics for Soluble Homologues 29

2.4.1 Generalised Szyszkowski-Langmuir Equation 30

2.4.2 Generalised Volmer Equation 31

2.5 Thermodynamics of Protein Penetration into Phospholipid Monolayers 35

2.6 Penetration Dynamics 38

3. Materials and Methods 41

3.1 Substances 41

3.1.1 Protein 41

3.1.2 Phospholipid 42

3.1.3 Dodecyl Dimethyl Phosphine Oxide (C_{12}DMPO) 42

3.2 Setup 43

3.2.1 Wilhelmy Plate Method 43

3.2.2 Circular Trough 44

3.2.3 Brewster Angle Microscopy (BAM) 46

3.2.4 PM-IRRAS 47

3.2.5 Grazing Incidence X-ray Diffraction (GIXD) 49

此为试读,需要完整PDF请访问: www.ertongbook.com

4. Experimental Results **51**

4.1 Brewster Angle Microscopy (BAM) Experiments 51

4.1.1 Pure DPPC 51

4.1.2 Pure Protein 53

4.1.3 β-Lactoglobulin Penetration into DPPC Monolayers 55

4.1.3.1 Penetration into Monolayers in the Liquid-Expanded or Gaseous State 55

4.1.3.2 Penetration into Monolayers in the Two Phase Coexistence Region 62

4.1.3.3 Penetration into Monolayers in the Condensed State 63

4.1.4 β-Casein Penetration into DPPC Monolayers 64

4.1.4.1 Penetration into Monolayers in the Liquid-Expanded State 64

4.1.4.2 Penetration into Monolayers in the Two Phase Coexistence Region 67

4.1.4.3 Penetration into Monolayers in the Condensed State 68

4.2 PM-IRRAS Experiments 72

4.3 Thermodynamic Properties of DPPC-Protein Mixed Layer 78

4.4 GIXD Experiments 80

4.5 Penetration of Dodecyl Dimethyl Phosphine Oxide (C_{12}DMPO) into DPPC Monolayers 83

5. Discussion **90**

6. Conclusion **95**

Bibliography **97**

Publication **104**

Acknowledgement **105**

Curriculum Vitae **107**

摘要

气/液界面上组装的单分子膜是典型的二维膜结构体系，已得到广泛的研究。磷脂单分子膜是这类体系的代表。它是生物膜的基本结构单元，对其成膜规律与结构特征的研究已十分清楚。磷脂双层膜与具有特定分子构象和单一分子量的蛋白质结合，构成了生物膜的基本模型。本文将以蛋白质“嵌入”磷脂单分子膜这一模型为基础，探索乳化体系中蛋白质吸附“嵌入”不溶性单分子膜的机理、形态与结构特性。利用不同的实验手段对复合膜的形成过程进行了系统化研究。

利用特殊设计的圆形 LB 槽，结合布鲁斯特角显微镜（BAM），观察到了气/液界面上两种不同结构的蛋白质，β-lactoglobulin 和β-casein，分别吸附并“渗透”进铺展的 DPPC 磷脂单分子膜的动态过程。实验中发现，蛋白质的吸附导致界面上的分子面积增大，尚未聚集的 DPPC 分子在不经过压缩的条件下发生相转变，形成的微畴(domain)结构与压缩纯 DPPC 单分子膜所得到的类似。证实蛋白质已“嵌入”单分子膜。为进一步研究混合膜的形成机理与稳定性，本文使用了切线入射 X－射线衍射仪（GIXD）和傅立叶变换红外光谱仪（FT－IR）对该混合膜进行了一系列研究，并根据实验结果，给出了相应的解释。为了对该机理进行验证，我们选用分子量小于 DPPC 的活性物质 C_{12}DMPO 代替蛋白质进行对比实验，在相同条件下观察到了 C_{12}DMPO 吸附引起 DPPC 相转移的现象。该结果对上述蛋白质吸附诱导的相转移提供了有力的证据。

使用 Fainermann 的理论模型验证此实验结果。理论计算与实验结果相一致。

关键词：磷脂与蛋白质，相转移，布鲁斯特角显微镜，傅立叶变换红外光谱仪

Abstract

As the representative two dimensional model systems at the air-water interface, amphiphilic monolayers have been extensively studied in interfacial science. Proteins, because of their particular importance in nature, are of special importance in many fields. The interest in phospholipid monolayers is prompted by the observation that, as one of the most important components of lecithin, they provide the simplest model systems. Since the phenomenon of penetration was first discovered by Schulman and Hughes, there are a variety of biological and industrial motivations for studying mixed monolayers that consist of insoluble and soluble components, in particular mixed lipid-protein systems. Along with the application of many new optical methods, direct structural and morphological analysis becomes available.

In this work, we performed penetration experiments with β-lactoglobulin and β-casein, proteins with globular and random coil structure, respectively. The aim of our study was to analyze the effect of protein adsorption and penetration into DPPC monolayers at the air-water interface. A circular trough equipped with a Brewster Angle Microscope (BAM) was applied to monitor surface dynamics and the corresponding monolayer morphology. We observed that protein penetration into this monolayer induces the formation of a condensed phase under the condition where no phase transitions occur in the pure DPPC monolayer (low enough surface pressure). The shape of the induced domains is quite similar to that of pure DPPC domains. For a better understanding of the effects of penetrating proteins into a lipid monolayer, Synchrotron Gracing Incidence X-ray Diffraction (GIXD) and Fourier Transform Infrared Spectroscopy (FT-IR) measurements were performed. Using these techniques the mixed lipid-protein layers were further characterized. It is found that the two-dimensional aggregation is certainly caused by the monolayer compression due to increased protein adsorption and the hydrophobic interaction between the lipid molecules. Furthermore, when the penetration starts from a point within the phase transition region, deformation of DPPC domains can be observed.

We also analyzed adsorption and penetration of dodecyl dimethyl phosphine oxide into a DPPC monolayer. It is found that under certain conditions a phase transition in the lipid monolayer occurs, which is very similar to the effect found with the proteins. Thus, proteins as well as low molecular-weight surfactants induce phase transitions in insoluble lipid

monolayers at a molar area much smaller than the critical value at which a phase transition in such monolayers occurs without the penetrating soluble molecules.

Meanwhile, a new model is proposed to describe the 2D aggregation of the lipid monolayers caused by additional protein adsorption. A set of equation of state for penetrated monolayers was derived by the simultaneous solution of the corresponding equation of state and Pethica's equation. The application of the model to the experimental results shows that the additional presence of β-lactoglobulin leads to an increase in the monolayer coverage, that is, the DPPC aggregation is induced by the second component. The equations of state for mixed monolayers are derived for the cases that DPPC aggregates do not yet occur, and that this aggregation is induced by the penetrated species. The values calculated for the surface pressure, critical surface concentration for aggregation to a condensed phase and the time when the aggregation commences were found to be in a satisfactory agreement with experimental data.

Key words: phase transition, penetration, Brewster Angle Microscopy (BAM), Fourier Transform Infrared Spectroscopy (FT-IR), Synchrotron Gracing Incidence X-ray Diffraction (GIXD),

1. INTRODUCTION

1.1 General Introduction

Amphiphilic monolayers at the air-water interface are representative two dimensional model systems and the object of extensive studies in interfacial science for a long period of time. In principle, two kinds of monolayers can be generalized. In the first case the amphiphiles are dissolved in an aqueous solution and adsorb at the interface. Here, they form adsorption layers, which are also designed as Gibbs monolayers. The well-known Langmuir monolayers are formed by spreading of long chain amphiphiles, which are insoluble in water. The amphiphiles are dissolved in an organic solvent and then spread onto the aqueous surface [1].

Besides the two pure systems mentioned above, mixed monolayers are also frequently used model systems. Mixed monolayers consisting of two surface-active species fall into three categories, (a) those in which both species are soluble, (b) those in which both species are completely insoluble, and (c) those in which one species is soluble and the other insoluble [2].

Monolayers of amphiphilic molecules at the air-water interface receive their increasing interest from several important facts: (a) They serve as well defined model systems to study biological membranes, the interaction of proteins at interfaces, and technically important surfactants. (b) They comprise the structures, which, after controlled formation, are transferred onto solid supports to yield (thin organic) Langmuir-Blodgett layers. After improved preparation the latter may find different technical applications as electronic devices or as matrices for functional organic molecules. (c) They are of intrinsic interest as two-dimensional physical systems offering a variety of independent parameters.

Over a rather long period, due to technical limitations, substantial experimental results on amphiphilic monolayers were only obtained from surface pressure (Π) ~ molecular area (A) isotherms, so that no direct information was available on the orientational and positional order of molecules in the monolayers. In the last decade, rapid progress in the understanding of Langmuir monolayers has been made, particularly as the development of highly sensitive experimental techniques has provided textural and structural information on the condensed monolayer phases.

During the last decade main interest has been focused on phase transitions and ordering in two dimensions [3 -10]. An important aspect for the evaluation of the phase behavior of Langmuir monolayers is their thermodynamic characterization. A prerequisite for the

formation of well developed condensed phase textures is that a plateau region in the surface pressure (Π) ~ area (A) isotherm exists in an accessible temperature region. A plateau region typically represents a two-phase coexistence region of a fluid-like low-density phase and a condensed phase. To optimize the conditions for nucleation and growth of domain textures, the main transition point for this first order phase transition should have a measurable surface pressure.

The application of new sensitive optical methods, such as synchrotron X-ray diffraction, fluorescence microscopy, and Brewster Angle Microscopy (BAM), have revealed a world of rich and intriguing phenomena in the structural and morphological study [11]. The molecular organization of condensed monolayer phases affects their optical properties. In particular, Brewster angle microscopy offers a powerful method to visualize the morphology of amphiphilic monolayers on a microscopic scale without requiring probe molecules [12-14]. The optical anisotropy resulting from differences in the molecular tilt is the basis of the contrast mechanism. Large differences in size and shape have been found. The application of BAM has provided experimental evidence that a large number of condensed phase domains in Langmuir monolayers have a well-developed inner texture, which is due to the orientational order of the tilted amphiphilic molecules [15-22].

The introduction of synchrotron X-ray diffraction at grazing incidence (GIXD) has provided access to the lattice structure and the positional order of condensed monolayer phases on the molecular scale [23-25]. In recent papers, correlations between the microscopic crystal structure and macroscopic textural features have been demonstrated by coupling GIXD and BAM results. Recent systematic studies have shown that textural and structural properties of the condensed phase of Langmuir monolayers are strongly affected by the chemical structure of the amphiphiles [26-30].

In a series of papers beginning in 1985, Dluhy and his coworkers demonstrated the feasibility of acquiring IRRAS spectra from monolayers of single and double-chain amphiphiles at the A-W interface [31-35]. This initial success has triggered a recent expansion of activities in this area. Though many problems were encountered, IR spectroscopy still provides several well-known advantages for the molecular characterization of lipids and proteins. This technique monitors molecular vibrations that produce dipole moment oscillations. The observed frequencies are dependent on molecular conformations and configurations. Many biophysical issues such as lipid acyl chain conformation, head group structure and

此为试读,需要完整PDF请访问: www.ertongbook.com

interactions with ions, protein secondary structure, and the orientation of ordered regions can thus be addressed [36-42].

Proteins, because of their particular importance in nature, are of increasing interest in many fields. The interest in phospholipid monolayers is prompted by the observation that these lipids, as one of the most important components of lecithin, are the key components in many biological systems. Because of their ideal amphiphilic properties at the air-water interface, phospholipid monolayers provide the simplest model for studying penetration systems [43 - 45].

The phenomenon of penetration of insoluble monolayers of cholesterol, hexadecyl alcohol, etc., by long-chain water-soluble compounds such as sodium salts of long-chain alkyl sulfates was first discovered by Schulman and Hughes [46 - 48]. Since then, much interest arose from biological implications of penetration experiments involving biologically significant material, excited particularly by analogies between the monolayer and biological membrane structures [49 - 56]. The kinetic and equilibrium properties of many penetration systems have been extensively investigated [57 - 86].

Along with the experiments, a series of theoretical work was published aiming at a systematic theoretical analysis [87 - 109]. In spite of wide interest there have been serious limitations in the theoretical and experimental treatment even of the simplest penetration systems. In most cases, the theories did not provide analytical expressions for equations of state of penetrated monolayers. Consequently a theoretical analysis of the fundamental properties of insoluble monolayers, such as the main phase transition of first order and the corresponding two-phase coexistence region, has not been performed. It has been shown that the application of some known theoretical approaches on the experimental data of a simple model system was limited in quite a narrow region of the molecular area or the surface pressure [110].

Recently a set of equations of state for penetrated monolayers was derived by the simultaneous solution of the corresponding equation of state and Pethica's equation [110,111]. The theoretical treatment of penetrated monolayers, which is able to condense two-dimensionally, is based on the generalized Volmer equation of state and the quasi-chemical model of the aggregation equilibrium. It has been shown that this theoretical model can be used to describe the equilibrium protein penetration into Langmuir phospholipid monolayers in the whole two-phase coexistence region [112]. This protein isotherm takes into account a

two-dimensional aggregation of the lipid, which was experimentally corroborated by the lysozyme penetration into DPPC monolayers.

Previously, the penetration experiments were mostly limited to the thermodynamic description. Essential progress in this work has been achieved not only in the theoretical description of the penetration process, but also in the direct experimental characterisation of the condensed phase which is formed during the penetration of soluble components into a Langmuir monolayer. Using Brewster angle microscopy, which allows direct characterisation of the long-range orientational order, owing to the optical anisotropy induced by tilted aliphatic chains, an interesting phenomenon was observed, namely that protein penetration can induce first-order phase transitions in fluid (gaseous) Langmuir monolayers of dipalmitoylphosphatidylcholine (DPPC). Further experiments illustrated that not only the adsorption of protein, but also of soluble amphiphilic molecules can induce the same aggregation due only to an increase in the surface pressure beyond a specific value. The use of Brewster angle microscopy made it possible to observe directly the development or change of the condensed phase domain morphology during the penetration process. Coupling with Grazing incidence X-ray diffraction interesting results on the lattice structure and thus on the composition of the condensed phase domain were provided [112-114].

In this work, the theory of penetration, including dynamics of soluble surfactant and protein adsorption and penetration, and also the dynamics of 2D aggregation in Langmuir monolayers induced by the penetration of soluble components is systematically reviewed (chapter 2). In the experimental part (chapter 4), the results of penetration into DPPC monolayers by proteins, β-lactoglobulin and β-casein, and soluble surfactants, Dodecyl Dimethyl Phosphine Oxide (C_{12}DMPO) are reported in detail, obtained by means of a specially designed circular penetration trough. In order to interpret the mechanisms of observed interesting phenomena and to obtain structural information, FT-IR and Grazing incidence X-ray diffraction are carried out. Based on experimental results and theoretical analysis, discussion and conclusions are given in chapter 5 and 6 respectively.

1.2 Fundamentals of Physical Chemistry

1.2.1 Surface Tension and Surface Pressure

The boundary region between two bulk phases, especially the air-liquid interface, is not a simple mathematical two-dimensional plane without thickness. Indeed, it can be regarded as a

region of finite thickness across which the internal energy, density, or any other thermodynamic property changes gradually. The free energy change of the area region can be written as:

$$dG = -SdT + VdP + \gamma dA + \sum \mu dN \tag{1-1}$$

Hence, the surface tension can be obtained:

$$\gamma = \left(\frac{\partial G}{\partial A}\right)_{T,P,N} \tag{1-2}$$

For a surface covered by an insoluble monolayer, the surface pressure Π, the two-dimensional analogue of the hydrostatic pressure, is defined as the difference between the surface tension of pure solvent and the film-covered surface, i.e.,

$$\Pi = \gamma_0 - \gamma \tag{1-3}$$

where γ_0 and γ are the surface tension of the pure solvent and the film-covered surface [115].

1.2.2 Phase Transition and State of Monolayers

The isothermal measurement of surface pressure of amphiphilic monolayers upon changing the area per molecule is called $\Pi - A$ isotherm. The area of the monolayer is varied by moving a barrier across the surface. When the monolayer is compressed, the pressure becomes measurable in a well-defined molecular area (A) by using a film balance.

There are some gross differences found in the behavior of various types of films, which is in analogy to bulk matter, allow them to be characterized as gaslike, liquidlike, and solidlike. Fig. 1-1 shows the characteristic shape of a generalized $\Pi - A$ isotherm. Neglecting details,

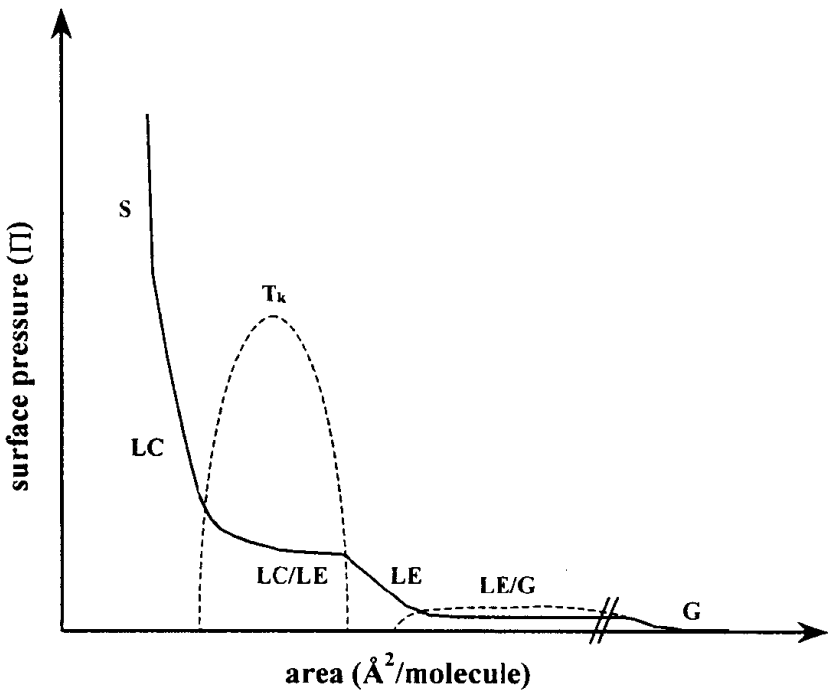

Fig. 1-1. Monolayer phase going along an isotherm

the single-phase regions are designated as gaseous (G), liquid (L) and solid (S) state. There are characteristic differences in the three types of state. The lower the temperature, the lower is the surface pressure of the plateau, but the more extended is the area range for the plateau. The dotted line with an apex forms the border of the two-phase coexistence region for the main transition LC – LE characteristic for this type of isotherm. The apex represents the critical temperature T_k, above which a condensed phase cannot be formed. For the type of isotherms at temperatures higher than T_k, a continuous increase of surface pressure with decreasing area per molecule suggests that over the whole range no phase transition to the condensed state exists. The complexity of the behavior undoubtedly arises from the fact that film-forming molecules are all polar-nonpolar in type. The polar end (or head) interacts strongly with the water substrate, and this may be in a structured way with much hydrogen bonding, ion atmosphere, while the nonpolar sections (or tails) also interact, but with each other and in some different way.

1). Gaseous films (G). The gaseous film is considered to be a diluted surface solution of surfactant in a pure solvent. The film obeys the equation of state of a more or less perfect gas; the area per molecule is large compared to the actual molecular area, and the film may be expanded indefinitely without phase changes. Its surface pressure can be put into the form

$$\Pi = -\frac{RT}{A_0} \ln f_1^s N_1^s \qquad (1\text{-}4)$$

where A_0 is the molar area of the solvent and f_1^s is the activity coefficient of the solute in the surface phase. As with bulk matter, this state should always be reached at sufficiently large molecular areas.

2). Liquid films (L). Such films are coherent in that some degree of cooperative interaction is present; they appear to be fluid (as opposed to rigid or showing a yield point) and their Π - A plots extrapolate to zero at areas larger (up to several times larger) than that corresponding to a molecular cross section so that some looseness or disorganization in the structure is indicated. There are at least two distinguishable types of L films.

(a) Liquid expanded films (LE). On compression of a gaseous film, a first-order transition to the LE state may occur. One may apply the two-dimensional analogue of the Clapeyron equation,

$$\frac{d\Pi}{dt} = \frac{\Lambda H}{T\Delta A} \tag{1-5}$$

where ΔH is the latent heat of vaporization of the LE state. The LE state may generally be observed with long-chain compounds having highly polar groups, such as acids, alcohols, amides, and nitriles. The Π - A plots tend to extrapolate to a value in the range of 40 ~ 70 $Å^2$ at zero Π, depending on the compound. Such monolayers are fluid and coherent (and thus liquid-like), yet the average intermolecular distance is much greater than for bulk liquids. A typical bulk liquid is perhaps 10% less dense than its corresponding solid state, yet a liquid expanded monolayer may exist at molecular areas twice that for the solid-state monolayer. This type of film has a rather high compressibility if compared to bulk liquids but appears to be surface potential probing. Characteristically, LE films may show a first-order transition to gaseous films at low pressures.

(b) Liquid condensed films (LC). The LC type film is of relatively low compressibility. It can be viewed as a semisolid film having more or less solvent molecules between the polar heads. On compression, the solvent molecules are squeezed out until a solid film is obtained, or in some cases where the polar heads are large they may gradually assume a staggered arrangement.

3). Solid films (S). The term solid is really a rheological one; a solid film should exhibit a static shear modulus. The general appearance of S-type films is that of a high density and either rigid or plastic phase. Most fatty acids and alcohols exhibit this type of film at sufficiently low temperatures or with sufficiently long chain lengths. This area is probably that of close-packed hydrocarbon chains. Alternatively, there may be a break at lower pressures to a LC type behavior [1,3,5,11,108].

1.3 Surface Properties of Lipids and Proteins

In many body organs, there is a large surface area relative to the volume of tissues. Major representatives among the biological surface-active molecules being self-assembled at this interface are lipids and proteins. It is obvious that lipids and proteins provide a variety of functions at bio-interfaces. From a biological viewpoint their role as surfactants, i.e., as molecules able to reduce the surface tension at interfaces, is especially important. On the other hand, their surface activity is displayed in the bilayer of biological membranes. It is the lipids in biological membranes, which primarily provide the structural framework of a

membrane, while the proteins carry out the chemical processes that are special to membranes. Biological membranes are the substrate for adsorption and desorption processes of lipids and proteins from the liquid medium surrounding the membrane and give the most prominent example of liquid interfaces, where proteins, whether membrane bound or extracellular, interact with lipids (Fig. 1-2). The surface properties of these substances at interfaces have a fundamental physiological role; some examples are the interface of plasma and organelle membranes, the alveolar surface, stomach wall, etc.

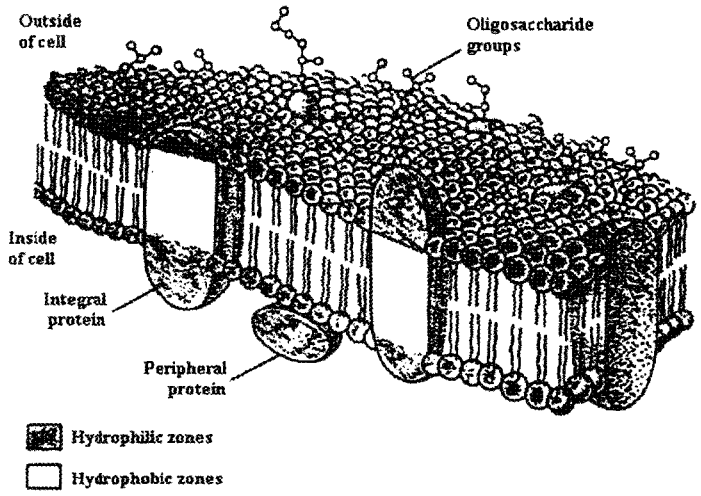

Fig. 1-2. Fluid mosaic model

Interactions of lipid monolayers with proteins have been an active research topic for a number of years. Various combinations of lipids and proteins were used in these studies aiming to determine the adhesion and degree of penetration of proteins into the lipid monolayers, and to model some reactions that take place at the membrane surface – for instance, enzyme-substrate and antigen-antibody reactions. The influence of various factors on these interactions – lipid structure, lipid phase state at the interface, initial surface pressure of the film, bulk and surface protein concentrations, lipid and protein charge, the presence of ions and anesthetics in membrane interactions, pH, etc. – was established [116].

Generally, the mechanisms of incorporation of protein into lipid monolayers can be grouped into three categories: (a) free penetration, typical of lecithin; (b) binding-mediated penetration, typical of cholesterol and some glycosphingolipids; (c) binding-inhibited penetration, typical of the albumin-ganglioside system and a specific lipid hapten-antibody system [104].

Due to their amphiphilic character, most lipids and proteins readily form stable monomolecular films at the air-water interface. Spread monolayers of pure (phospho)lipids have been widely used for a long time in studying the kinetics of spreading, determination of the occupied area per molecule, type of the surface pressure-area isotherms, lateral and normal molecular interactions, lateral diffusion of molecules in the plane of the surface, etc.

此为试读,需要完整PDF请访问: www.ertongbook.com