Control of lipid microstructures by molecular design and its implications

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Abstract. An overview of the current trends in the lipid design for specific applications has been presented. Lipids with different surface charge and hydrophobic backbone undergo aggregation to produce lamellae or bilayer and multilayer vesicles in aqueous media. Various aspects of present development of chiral superstructures and enzyme-mimics have been discussed. Utility of these molecules for potential applications in immunomodulation and sustained drug-delivery systems is also summarized.

Keywords. Lipids; vesicles; liposomes; chiral superstructures; immunomodulators; sustained-release systems.

Introduction

Bioorganic approaches to the design and construction of synthetic cells have been the focus of much current research (Fendler 1982; Murakami and Kikuchi 1991). The barrier that defines the interior and exterior of a living cell is a cell membrane. Presumably, the primitive forms of life originated from organized assembly of amphiphilic molecules such as lipids which ultimately gave rise to plasma membranes (Fendler 1984; Ringsdorf et al 1988). Biological cell membranes are complex entities. Although lipids are predominant constituents of biological membranes, different biologically active macromolecules such as proteins, carbohydrates, steroids etc. also share the same habitat i.e., the cell membrane. Their important functions generally derive from their propensity to self-organize. Such self-assembly provides internal mesophases (smectic) with controlled sizes, shapes, fluidities, and micro-environments. In this capacity, various bioactive macromolecules carry out important biological functions such as transport, transduction of signals, separation of materials across membrane bilayers, fusion, exo- and endocytosis etc. within the protective matrix of cell membranes (Cevc and Marsh 1987; Slater and Huang 1992).

Molecular aggregate of lipids in aqueous media

Lipids are comparatively unique biomolecules in the sense that they are generally not polymeric in nature. Instead, several molecules of lipids cooperatively organize through *non-covalent* interactions. Lipids and related molecules usually have both a water-soluble and fat-soluble domain and hence they are amphiphilic in nature (Fendler 1980; Shobha and Balasubramanian 1986; Shobha et al 1989; Sundar et al 1991). The self-organization takes place in water because of the fact that they are amphiphilic

in nature. Various kinds of microscopic structures are produced from the self-assembly of lipid molecules. As a consequence of hydrophobic forces in the lipophilic region and electrostatic and hydrogen bonding interactions around aqueous interfaces, these molecules supramolecularly aggregate to produce closed bags or spheres made up of one or more lipid bilayers. These are known as liposomes or vesicles (Brockerhoff 1977). Vesicles composed of synthetic lipids of defined chemical structures are the simplest functional models of membranes (Chapman 1975; Raison 1980). Vesicles contain entrapped water within the enclosures of bilayer mesophases. Generally, vesicles could be unilamellar (one bilayer leaflet) or multilamellar (several bilayers) in organization with apparent spherical or ellipsoidal shapes. Vesicles have been shown to provide effective barrier for transport of water-soluble solutes or ions. However, passage of hydrophilic substances across the bilayer membranes would depend on lipid molecular structure, kind of microstructure produced as a consequence of self-assembly of these lipids and the temperature at which the transport is measured. This is because generally vesicles display a gel to liquid crystalline thermotropic phase transition (Huang and Mason 1986; Small 1986). For a lipid of given chemical structure, there has to be a characteristic critical temperature for such transition which is generally known as melting temperature, Tm (McElhaney 1986; Silvius 1991; Menger *et al* 1993). The basic structural and physical features of synthetic amphiphiles are not only important determinants for the development of new supramolecular assemblies and networks (Fuhrhop 1993) but are also crucial for sustaining biological macromolecules such as membrane proteins in a native folded conformation (Knox *et al*, in preparation). In order to understand complex lipid-protein interactions, it is therefore necessary to replace complex composition of the cell membranes with known quantities of structurally defined amphiphilic detergents or lipid (Schleicher *et al* 1987). Thus, reconstitution of rhodopsin without denaturation could be successfully carried out in partially polymerized vesicular suspensions (Tyminski *et al* 1988).

Molecular design of novel lipids

The natural membrane forming lipids are either anionic, neutral, or zwitterionic in nature and generally contain two fatty acyl chains. More than a decade ago, Kunitake and co-workers demonstrated that upon sonic dispersal in aqueous buffer, dialkyl dimethylammonium ions could form closed structures that are remarkably similar to those of naturally occurring phospholipids (Kunitake and Okahata 1977). Due to structural simplicity and easier amenability to synthetic manipulations, large number of reports have appeared in subsequent literature concerning structure–property relationship in other kinds of new lipids (Murakami *et al* 1980; Sudholter *et al* 1980; Fuhrhop and Matheiu 1984; Rupert *et al* 1986; Kaler *et al* 1989; Fukuda *et al* 1990; Zana and Talmon 1993). Now, several preparations of vesicles from fully cationic, anionic or zwitterionic lipids of completely synthetic, non-biological origin have been characterized. It has been realized that bilayer aggregates can also be generated from amphiphiles that have rigid azobenzene, biphenyl or diphenylazomethine moieties inserted into a single hydrophobic tail (Kunitake *et al* 1981, 1984; Cho and Park 1987; Kunitake 1992).

Chiral membranous assemblies, helical and tubular microstructures

Kunitake and co-workers have successfully employed chiral amino acids as useful modules in the design of bilayer forming molecules (Nakashima *et al* 1989; Ishikawa *et al* 1990). Such synthetic chiral bilayers composed of single hydrocarbon or double hydrocarbon chain amphiphiles display pronounced circular dichroism as a consequence of the formation of higher order supramolecular organizations. In this case, the amphiphiles were first dispersed in vesicular form by ultrasonication and then left standing for several hours. The vesicular structures budded out to form short rodlike structures which eventually got converted into twisted helical ribbons.

Yanagawa and co-workers have developed double chain nucleotide derivatives (Yanagawa *et al* 1989). Upon sonication, dimyristoyl 5'-phosphatidyl-cytidine formed vesicles, which got slowly transformed into circular helical strands with hydrodynamic radii of 25–75 nm in presence of 0·2 M KCl. Interestingly, at lower KCl concentrations (0·01–0·05 M), linear helical strands were seen. On the other hand, at higher KCl concentrations (0·5–1·0 M), the vesicles maintained their morphological integrity.

Ultrathin molecular bilayer ribbons could be generated from solutions of *n*-octyl-D-galactonamide (Fuhrhop *et al* 1988, 1990). In contrast, the corresponding dispersions of diastereomeric amphiphile, *n*-octyl-D-gluconamide produced apparent rodlike structures. The former is about two orders of magnitude less soluble than the latter and consequently a mixture of both straight and twisted ribbons are produced. Interestingly, solubilization of *n*-octyl-D-gluconomide in ortho xylene in which it is much less soluble than in water results in helical ribbons (Fuhrhop *et al* 1990). Long thin tubules could be obtained from amphiphiles, which contain diacetylenic units in their long hydrocarbon chains (Singh *et al* 1988; Merkowitz and Singh 1991). Tubules can be produced either by gradual cooling of resulting vesicular dispersions or by mixing water to ethanolic solution of such lipids. It has also been realized that the specific morphology of the tubules is a critical function of the lipid headgroup chosen. Thus, substitution of a choline headgroup with a smaller hydroxyethanol headgroup results in the formation of tight tubules with a pronounced helical structure (Gaber 1990).

Functional molecular assemblies as enzyme analogues

Studies on the stereoselective hydrolysis of peptide esters in various lipid/surfactant coaggregates, allow one to reveal that considerable stereochemical control is indeed attainable by regulation of the composition of coaggregates, temperature and ionic strength (Lehn 1988; Ueoka *et al* 1988). Appropriate "fine-tuning" of the aggregate microenvironments can produce significant enantioselectivity in such reactions. Such findings have been attributed to the optimization of the enzyme model conformation in the coaggregate ensembles (Ueoka *et al* 1992).

Hamachi *et al* (1991) have shown that a water-soluble heme protein, myoglobin could be embedded within the interbilayer spaces of synthetic amphiphilic assemblies without denaturation. The orientation of protein molecules in such reconstituted systems is believed to be maintained as a consequence of favourable electrostatic interactions between the positively charged side chain residues from the polypeptide chains and the negatively charged phosphate functions of the amphiphiles on the

membrane surface (Kunitake *et al* 1990; Hamachi *et al* 1991). Interestingly, the myoglobin molecules in such a matrix retain their redox capacity also.

Glycolipid analogues as potential immunomodulators

Due to their residence at the cell surfaces, glycolipids can act as ideal systems for binding antibodies, bacteria, lectins, toxins or viruses (Paulson 1985). As a consequence, they play a major role in cell-to-cell communication and interactions with cell adhesion molecules (CAM) (Edleman 1988). Several hundreds of glycolipids are known from natural origin. If provision of a cellular matrix for biological events is the only purpose for their evolution, there is little to justify why nature has spent so much metabolic energy to produce so many of them (Boggs 1986). This points to more important roles which may not be apparent at this point of time. Based on their general structural features, they can be classified into two groups, the glyco-glycerolipids and glycosphingolipids.

Lockhoff and co-workers developed a series of structurally defined, simple glycolipid analogs in view of their fundamental importance as cell-wall amphiphiles by joining lipophilic hydrocarbon chains with carbohydrate headgroups (Paulson *et al* 1980). While natural glycolipids produce in general an immunosuppressive effect, these novel glycolipid analogs elicit significant immunostimulation (Lockhoff 1991). As a result these systems are excellent candidates for treating patients suffering from immunoprophylaxis (Lockhoff 1991).

Although, the molecular mechanism that are responsible for such immunological activities of glycolipid analogs are still unknown, there is evidence that even subtle structural changes in the polar headgroups of glycolipids and phospholipids have strong effects on mutual miscibility in solid and liquid crystalline mesophases. Since these factors also control the membrane fluidity, the conformation of receptor proteins in such membrane matrix can also get altered (Koynova *et al* 1988).

Reactivity control and regulated delivery systems

The molecular structures of the amphiphiles and lipids and the physical states of their microstructures can determine various properties. Thus, liposomes of one molecular structure can effectively retain hydrophilic solutes in a way better than the liposomes produced from another lipid. Such abilities to control and regulate the reactivities of two species or sustaining a material inside its aqueous millieu have important implications (Surolia and Bachhawat 1977; Curatolo *et al* 1985; Smith *et al* 1994). In particular, this aspect is crucial for utilization of vesicles as entities for encapsulation of bioactive substances and drugs and to obtain stabilized drug carriers. Uptake of liposomes by the reticuloendothelial system (RES), mainly the fixed macrophages of liver and spleen, actually proved to be beneficial for those anxious to deliver drugs to macrophages. For instance, this is useful in the treatment of certain microbial diseases, enzyme deficiencies or metal-storage diseases, the activation of tumoricidal macrophages and immuno-potentiation of vaccines (Gregoriadis 1988; Bachhawat 1991). As expected the composition and other properties of liposomes (vesicle size and charge, and the extent of hydration of the headgroup, for example) are likely to play important roles in their interactions with and possible penetration into epidermis. Agents incorporated in the vesicle bilayer presumably reduce the energy barrier of their interaction with its epidermal lipidic environment and thus

promote coalescence. Evidence obtained so far (Ghyczy and Niemann 1992) indicates that appropriately tailored liposomes usually with diameters of 200 nm or less do indeed enter the epidermis. These are to be seen as deformed spherical structures between the corneocytes of the stratum corneum (Junginger *et al* 1991). The list of vesicle entrapped molecules which are claimed to assist the epidermis to function (especially for ageing women) is long. These include peptides, carbohydrates, proteins and special lipids, among others. Some of these are believed to mitigate harmful effects of free radicals and also inhibit their generation as a result of the combined action of oxygen and UV radiation etc. (Gregoriadis 1994). Many new cosmetic industries are now interested in developing receptor-mediated targeted drug delivery. These new liposomes are equipped with a homing device (rhamnose) specific for keratinocytes expressing the rhamnose receptor. The idea is that such liposomes would interact with the target cell more effectively and deliver their active agent more efficiently. It however, appears that exploitation of liposomal vesicles, is only in its infancy. Consequently, there is a lot of scope for newer opportunities (Gregoriadis 1994).

Conclusions

Clearly, there are two distinct facets which are becoming conspicuous in such research initiatives. One involves fundamental approaches involving bioorganic and biological investigations of defined lipid assemblies and their interactions with proteins and other lipid interactable macromolecules. The second approach attempts to exploit new systems for developing novel materials for nanotechnology, biotechnological purposes, pharmaceutical formulations and drug delivery.

   The purpose of this article is to review current trends in the molecular design of lipids and their potential from a bioorganic point of view using literature descriptions and explanations that strive for brevity, clarity and simplicity rather than for completeness and rigour. Obviously, it is beyond the scope of this article to encompass many other important aspects. For a specific research subject, one needs to go to the primary literature for substantive details. Nevertheless, it appears clear from foregoing discussions that the stage is ready for exciting new findings. Near future will witness multifarious applications involving designed lipids and amphiphiles both in biotechnology and in materials science, two most emerging disciplines of the present time (Schnur 1993).

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