Biophysical Journal Volume 74 April 1998 2138–2139

Book Review

Liposomes in Gene Delivery by Danilo D. Lasic

CRC Press, Boca Raton, FL, 1997. 295 pages. $59.95

Reviewed by Rudolf Podgornik, Laboratory of Physical and Structural Biology, National Institute of Child Health and Human Developement, National Institutes of Health, Bethesda, Maryland

"Gene therapy" is one of those buzzwords that so easily capture public attention. It is emerging as a new and extremely promising technology in medical practice that has the potential of curing diseases on their most fundamental, molecular level. The scientific fundamentals of gene therapy are connected with insertion and expression of functional genes or equivalent macromolecular segments with gene-informational content into a living cell, where they are expected to produce a desired and preengineered therapeutic effect. It stands at the intersection of two broad scientific endeavors: the investigation of different liposome-based drug delivery systems and the molecular biology of DNA. Because of this heterogeneous scientific basis, there are few specialists that are conversant in both of its theoretical underpinnings. The laudable goal of the book by D. D. Lasic is to fill the gap between these two well-established fields in a form and with content appropriate for researchers and workers in the field of gene therapy.

Because lipid-based formulations in the form of (cationic) liposomes appear to be the most promising vehicles for gene transport and delivery into cells, one can give a quick description of the book as liposome-DNA interactions and their role in gene therapy. Thus one of the fundamental questions that emerges is, what is the nature of the liposome-DNA complex and how does it relate to the structure-activity relationship of transfection (i.e., delivery of genes into the cell)? Here the book comes at the right time, just after a major advance in elucidation of the microscopic structure of a certain type of DNA-cationic liposome complex (D. D. Lasic et al. 1997. *J. Am. Chem. Soc.* 119:832; and J. O. Raedler et al. 1997. *Science.* 275:810). X-ray scattering techniques combined with cryomicroscopy have led to a picture of the DNA-cationic liposome complex as a multilamellar lipid bilayer array with partially orientationally ordered DNA molecules intercalated between lipid bilayers. The new, emerging picture of this complex thus seems to be completely consistent with studies of the colloidal structures of DNA and lipid bilayers in the bulk as well as with the nature of DNA adsorption to surface-immobilized cationic lipid bilayers. Loosely speaking, it appears that the DNA-cationic liposome complex is just one of many effective ways to condense DNA, in which cationic lipid bilayers (but not individual lipid molecules themselves!) act like polyvalent condensing cations.

It is far from clear at the moment how elucidation of the structure of the DNA-liposome complex will contribute to a better understanding of the structure-activity relationship or alleviate the problems connected with cationic lipid toxicity in a cellular environment. One thing that appears to be certain is the need for effective but reversible compactification of DNA, just as in the DNA-liposome complex, before successful transfection, as well as robust stability of the complex in the complicated ionic environment of the cell. Unfortunately, there are more questions than answers here, and it is one of the more important features of this book that it constantly stresses the need for a fundamental theoretical understanding of DNA-lipid interactions before some appealing concepts will materialize in medical practice. Although the book ends on a rather pessimistic note, because there are (with the current technology) no gene therapy trials that have shown an unequivocal benefit, it presents a splendid first important step toward systematization of our fundamental knowledge of DNA-lipid interactions in relation to their practical use in gene therapy trials.

Lasic's book, being (as far as I am aware) the first systematic exposition of fundamental ideas behind gene therapy as well as a critical evaluation of practical trials, has the privilege of not addressing any one specific aspect in exhaustive detail, but rather gives a consistent picture of the whole. I also see its importance and beauty in its side-by-side development of theoretical concepts from DNA condensation theory, colloidal forces, liposome and lipid thermodynamics, as well as its practical evaluation of transfection kits, different liposome and lipid formulations, and transfection efficiency.

The first and second chapters start as expositions of the field of gene therapy. In this preamble gene delivery strategies and possible prime candidates for genetic therapy (cancer, cystic fibrosis, infectious diseases, and neurological diseases) are listed and discussed. Two chapters on the molecular biology of DNA follow. This is mostly standard material on the structure and conformation of the DNA molecule, together with an introduction to the stability, different DNA-DNA interactions, and mesophases in concentrated DNA solutions. Theoretical models describing different aspects of DNA behavior are also introduced, with a special emphasis on polyelectrolyte behavior, which is described and discussed within the Poisson-Boltzmann theory. DNA condensation is introduced, and different con-

densed DNA structures are described in fair detail. From here Lasic proceeds to recombinant DNA technology, concentrating on the functional aspects of DNA and its "cut-and-paste" manipulation. This direction is pursued even further in the next chapter, which deals with gene expression in plasmids and their sequential construction. Descriptions of gene regulation, cytoplasmic gene expression, and DNA vectors conclude the part of the book dedicated to the molecular biology of DNA.

The next chapter is, in a way, a bridge between the molecular biology of DNA and the drug delivery systems discussed in the second part of the book. It deals with the delivery of plasmids into cells where they are supposed to be expressed. It introduces naked DNA, and viral and nonviral delivery systems. The latter in particular is dealt with in exhaustive detail. Only viral and colloidal delivery systems are argued to be important for gene therapy, and of these two, viral vehicles are inferior with respect to safety. This leaves us with colloidal delivery systems, of which lipid- and liposome-based methods are the most promising.

Liposomes are introduced in the following chapter. The author has written profusely on this subject before; Chapter 6 is a tutorial on liposome properties that are of relevance specifically for interaction with DNA or more generaly for drug delivery systems. There is a thorough discussion of cationic lipids and cationic liposomes, which are of the utmost importance for complexation with DNA. This chapter again contains a broad overview of theoretical models of colloid stability and liposome formation, as well as practical methods of liposome preparation and characterization. Liposome interactions with cells and the toxicity and safety of cationic liposomes, especially in relation to in vivo applications, are dealt with in detail. Genosomes (DNA-lipid complexes) are introduced in the next chapter, which effectively connects the physical properties of DNA and cationic lipids. A thorough discussion of the structure of the DNA-cationic lipid complex connects the properties of DNA when it is in bulk, as well as when it is adsorbed to (surface) immobilized cationic lipid bilayers with the microscopic structure of a genosome. Structural x-ray scattering data, cryomicroscopy pictures, and atomic force microscopy results are presented to support the interpretation of the ordered, intercalated structure of DNA between cationic lipid bilayers in a genosome. The interesting features of the strong ordering of DNA as well as lipid bilayers in a genosome are explained in terms of colloidal forces and strong DNA-lipid interactions. In relation to in vivo studies, the pharmacokinetics, biodistribution, and toxicity of genosomes are examined, and different liposome formulations are compared with regard to their safety in in vivo administration.

The next two chapters deal with the gene expression of genosomes in vivo and in vitro. The efficiency of different delivery methods is compared, and existing human studies of genosome administration are analyzed. The author pleads for a more systematic approach and better characterization of genosome formulations before a "very cautious optimism" can bear more tangible fruits. There is as yet no general understanding of the connection between genosome activity and its structure. Data are presented to support the hypothesis that condensed and/or tightly packed DNA is a prerequisite for efficient gene expression upon systemic administration. Large, loose, amorphous complexes do not protect DNA sufficiently and cannot achieve reasonable biodistribution. We know even less about the mechanism of transfection, and the author presents a plausible but highly hypothetical model of genosome transfection. Much more work is needed to understand this part of the gene delivery process.

The book concludes with a discussion of alternative gene carrier mechanisms (polyelectrolytes and artificial viruses) and with an introduction to other gene therapy apporaches, such as antisense nucleotides and ribozymes, which could involve cationic liposomes.

This book was written for a very heterogeneous audience, as the field itself is just a conglomerate of different subfields with their own scientific practices and concepts. It is going to be a dissappointment for anyone looking for a collection of fast cookbook recipes; it is not easy reading for any specialist who is not willing to broaden his or her knowledge, as gene delivery systems are a paramount example of interdisciplinary science. It is intended for researchers, physicians, or students who want to have a deeper grasp of the intricacies of the fundamental biophysical properties of DNA and cationic liposomes and their interaction in gene delivery. It contains as much theoretical foundation as is possible to give at present, because our understanding of the structure and workings of genosomes is still incomplete. It is hoped that the repeated pleas for a more solid theoretical understanding of the structure and properties of genosomes will not go unnoticed.

In summary, this book by D. D. Lasic is a well thought out and in-depth introduction to the field of gene therapy and liposome gene delivery. It comes from the biophysics community and is primarily intended for it. I recommend it strongly to research workers interested in the fundamental problems of this field. It should also make interesting reading for physicians concerned with more practical aspects of genosome applications, providing a proper perspective on gene delivery and manipulation.