Sterol Structure and Function*

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The relationship between chemical structure and biological function, the problem of specificity, can be seen as one of the central issues in chemical and biological research. For a given substrate, modifications that are allowed or forbidden define enzyme specificity, while knowledge of the active site, binding domains and the three-dimensional protein structure delineate the essential elements for the performance of enzyme catalysis. The selectivity of receptors for biologically active ligands can be similarly defined.

A more rarely considered aspect of specificity addressed here relates to the process of natural selection and asks the question: Why have certain bioactive molecules been chosen for a given biological function in preference to others? Why, for example, has glucose become the universal metabolic fuel and the ultimate raw material for biosynthesis rather than one of its 15 configurational isomers? The invariant selection of 20 amino acids and five nitrogenous bases for protein and nucleic acid synthesis also needs an explanation. Is fitness the evolutionary driving force that shaped molecules for optimal function and rationalizes nature's preferences? In some instances, selection of molecules can be rationalized simply by inspection. Thus, in glucose, unique among the 16 aldohexoses, all bulky substituents are equatorially oriented, providing maximum stability and favoring the pyranoside over the aldehyde structure (Fig. 1).

This paper attempts to rationalize the evolution of sterol structure and function in the context of natural selection (1). Specifically, it is of interest to ask why lanosterol, the first cyclic intermediate in sterol biosynthesis, is further metabolized by removal of three methyl substituents attached to the α-face of the tetracyclic

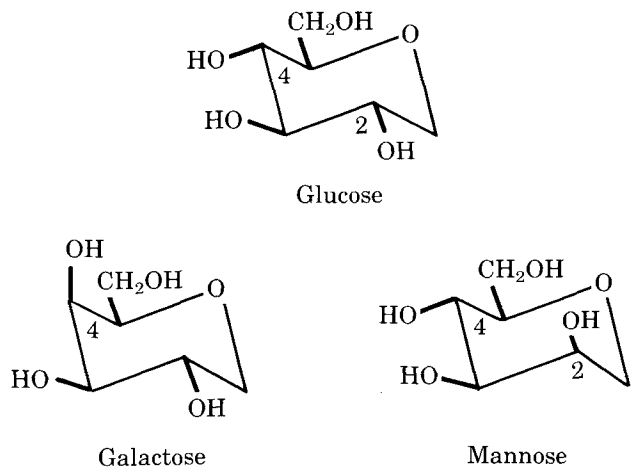

FIG. 1. Glucose is the most stable of the 16 isomeric 6-carbon sugars: all bulky substituents are equatorial.

nucleus (Fig. 2). At the same time this inquiry was begun, it was known that lanosterol is a transient intermediate on the way to cholesterol, but never an end product. Moreover, demethylation of lanosterol was shown to proceed in steps by way of 4,4-dimethylcholest-8,24-diene-3β-ol and 4α-methyl-(8,9)en-3β-ol. Speculating that these modifying events are driven by evolutionary pressures to improve functional competence, we first tested the "fitness" hypothesis by comparing the ability of cholesterol, lanosterol and the partially demethylated intermediates to alter a physical-state parameter (fluidity) in liposomes, conventional model membranes (2). Cholesterol

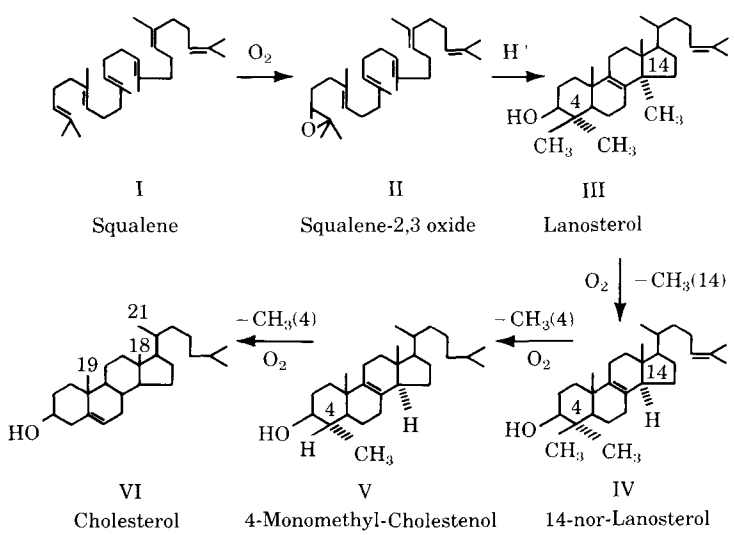

FIG. 2. Aerobic phase of cholesterol biosynthesis.

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was already known to reduce drastically (10-fold) the exit of glucose that had been entrapped in lecithin liposomes (3). Lanosterol, on the other hand, failed to show any significant restraining effect on glucose efflux (4). Later, in more detailed studies, we measured fluorescence depolarization (of diphenylhexatriene) to follow sterol-induced physico-chemical alterations in model membranes. Striking incremental microviscosity changes occurred with progressive demethylation (Fig. 3) (5). Several points are worth noting. The first demethylation, the step that removes the 14 α-methyl group and produces a 4,4 dimethylcholestenol, causes a very substantial increase in $\bar{\eta}$, much larger than the subsequent deletion of one of the two alkyl groups at C_4. The latter reaction affords a further but more modest microviscosity increase. The fitness of a 4α-monomethyl cholestenol approaches but does not equal that of cholesterol itself. In the contemporary biochemical pathway, the orientation of the single methylgroup at C_4 is invariably α(equatorial). By contrast, the 4β(axial)-monomethylderivative, an unnatural sterol, exhibits an effect on $\bar{\eta}$, not superior to that of the 4,4′-dimethyl compound. We regard this result as especially significant in light of the "fitness" hypothesis. It rationalizes the fact that naturally occurring 4β-monomethyl sterol derivatives are unknown so far. It is nature's wisdom to choose only reaction steps which improve function, avoiding those which are functionally neutral. Moreover, it is to be emphasized that lanosterol demethylation is confined to the α-face of the tetracyclic ring system (2). Branched methyl groups elsewhere in the molecule are ordinarily retained, we suggest, because additional demethylations would impair rather than improve functional competence of cholesterol. Supporting this contention, elimination of one of the angular methyl groups (C_{19}) drastically reduces the $\bar{\eta}$ value shown by cholesterol. However, contradicting this generalization, certain corals and gorganians demethylate cholesterol to the 19-nor derivative. An attempt to rationalize this phenomenon is presented elsewhere (2).

Liposomes lack proteins and are composed of arbitrarily chosen phospholipids. For this reason, the studies with sterol-loaded liposomes, while useful for examining lipid-lipid interactions, do not necessarily mirror sterol effects on living cells. For addressing this issue, we chose a sterol-requiring microorganism, *Mycoplasma capricolum*, and determined bacterial growth responses to the sterols in question. In these experiments designed to test biological function, the structure-function relationships observed were the same as those with the artificial membrane system (5). Bacterial growth was optimal with cholesterol, slowest with lanosterol and intermediate with the partially demethylated lanosterol metabolites (Fig. 3). Moreover, the more efficient the sterol as a promoter of growth, the greater the microviscosity of the membrane fractions isolated from the various cultures. The experimental success and validity of the conclusion rests critically on the demonstration that all the sterols examined remained structurally unchanged during bacterial growth. Sterol modifying enzymes of the kind found in higher cells are absent in Mycoplasma.

Taken together, the sterol effects on physico-chemical membrane properties (viscosity or fluidity) and a physiological parameter (cell growth) clearly establish the functional superiority of cholesterol as a molecule shaped by selective modification of a precursor structure. In this instance, perfection seems to have been reached, judging from the inability of any other natural or synthetic sterol structure to equal or surpass the performance of cholesterol. The concept of molecular evolution to functional perfection is likely to prove of general validity. J. Knowles has demonstrated it elegantly for the enzyme triosephosphate isomerase (6).

That nuclear demethylation at the sterol α-face improves and optimizes function is readily rationalized by

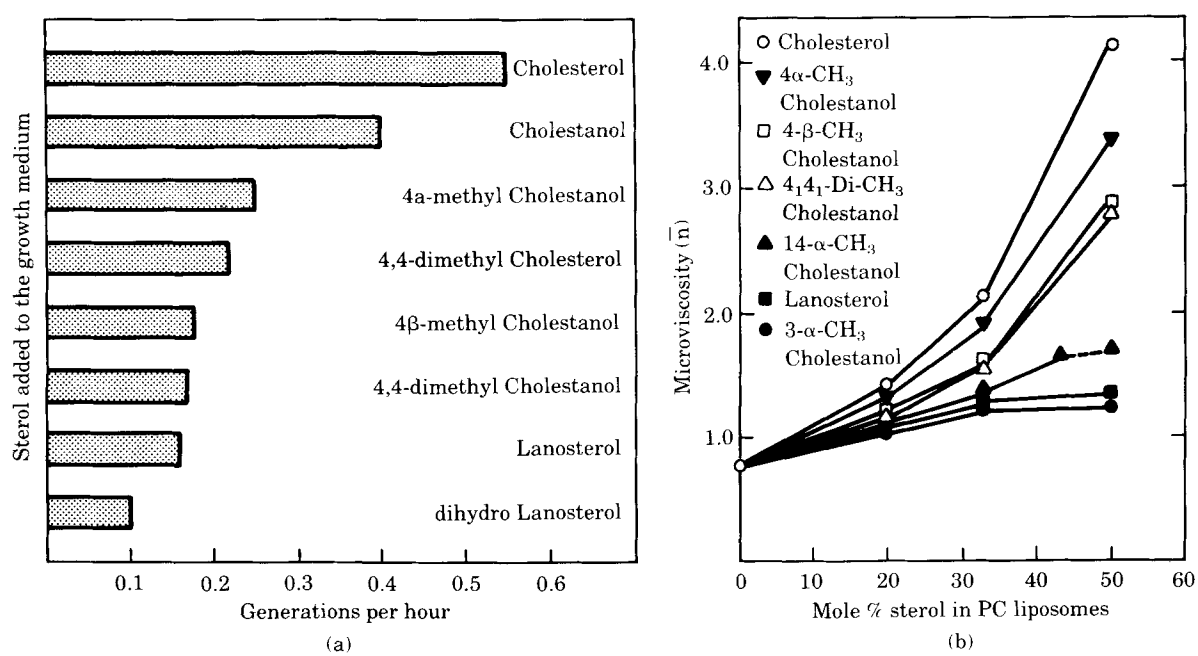

FIG. 3. Sterol effects on living cells.

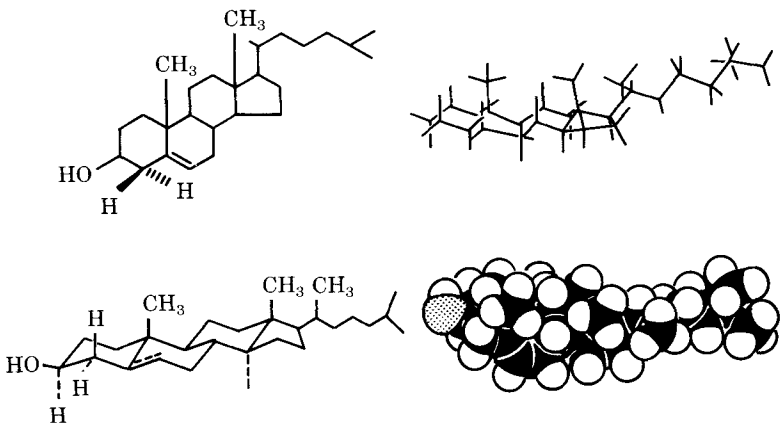

FIG. 4. Cholesterol molecular models.

inspection of molecular models (Fig. 4). These models show, first, that the *trans-trans-antitrans* ring junctions and, second, a set of axial hydrogens at the α-face, as well as the two angular methyl groups at the β-face, create planar surfaces at the top and the bottom, respectively, of the tetracyclic sterol structure. These critical features allow for multiple hydrophobic (van der Waal's) interactions between the rigid sterol nucleus and the paraffinic fatty acyl chains of the phospholipid matrix and, thus, account for the stabilizing effects of cholesterol in the membrane bilayer. The adverse steric effects of α-face methyl groups and the benefit accruing from their metabolic removal are, therefore, readily rationalized.

The concept of molecular evolution implies a temporal sequence and, therefore, offers the potential for dating individual steps of the pathway. In organismic evolution this dating, however precarious, is normally based on fossils. Yet fossils are mineral and their imprints devoid of organic substances. Nevertheless, still-existing primitive organisms may give clues to the order of appearnce of some biomolecules. Certainly plausible, if not universally accepted, is the view that the primitive biosphere was anaerobic, in which case the arrival of the sterol structure must have been a late event coinciding with or following the appearance of aerobic cells. Oxygen is essential for the transformation of acyclic (polyprenol) precursors to the tetracyclic ring system. For the same reason it seems likely that the corrins (e.g., Vitamin B_{12}) are more privitive than the contemporary, structurally related porphyrins (7). Indeed sterols, in contrast to pentacyclic triterpenes, have not been found in strictly anaerobic cells. However, the hydrocarbon, squalene, the C_{30} polyisoprenoid precursor of lanosterol and a product of anaerobic processes, is a fairly common lipid constituent of microorganisms including the anaerobic archaebacteria. Three landmarks in the evolution of the sterol molecule can be cited (Fig. 5). Lanosterol has been identified recently in a Cyanobacterium apparently as the sole sterol (8). These bacteria share procaryotic properties and the photosynthetic apparatus (oxygen evolution) characteristic of eukaryotes. Second, the obligate aerobe *Methylococcus capsulatus* produces a mixture of squalene cyclization products, consisting predominantly of 4,4′-dimethylsterols (9). Finally, the dinoflagellates, a transitional group classified both as protozoan and algal, contain principally 4α-methylcholesterol derivatives, the last intermediates on the way to cholesterol. Organisms, therefore,

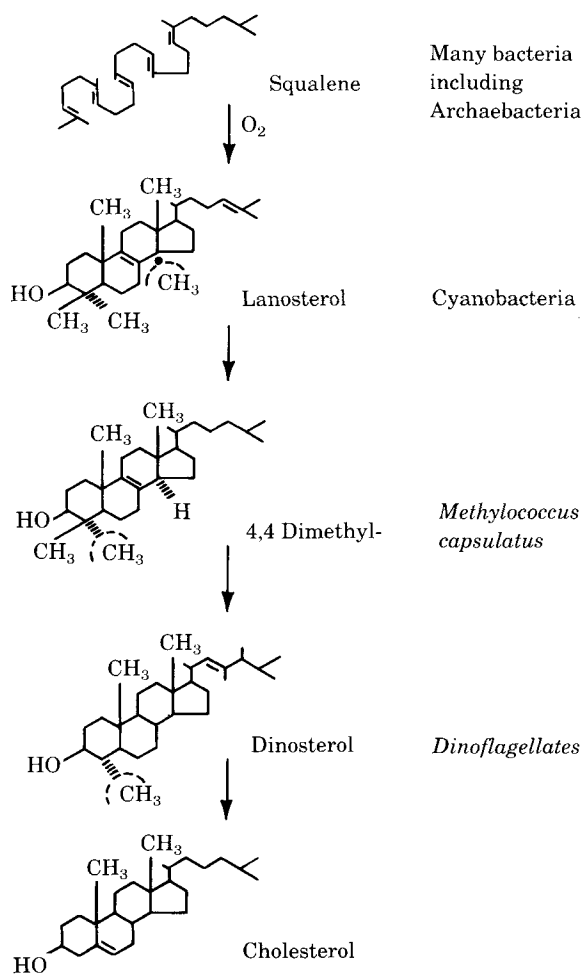

FIG. 5. Sterol "fossils."

exist that have started the biosynthetic pathway, continued it through the first and second demethylation step, but have not taken it to completion. While there is no evidence that these organisms belong to the same evolutionary branch on the way to cholesterol-producing cells, the sterol patterns of these "living fossils" provide strong evidence for the notion of a stepwise historical process directed at molecular improvement.

Other structural details which bear on the issue of functional perfection include the length of the isooctyl side chain, the stereochemistry of its attachment to the ring system and the location of the nuclear double bond.

The carbon skeleton of the isooctyl side chain remains invariably intact after squalene cyclization, throughout the demethylation sequence, except for the reduction of the 24,25 double bond. Synthetic analogs bearing straight rather than terminally branched chains of various lengths (from n = 2 to n = 9) have been prepared (10). All of these analogs are inferior to cholesterol in raising the microviscosity of phospholipid vesicles (11). The native cholesterol side chain causes maximum ordering eliminating the need for evolutionary pressures to modify it. Whether the normal (isooctyl) chain is also optimal for sterol functions in natural membranes is not yet known. When supplied to a sterol requiring yeast mutant (GL7), cholesterol again is the growth factor superior to all side chain-lengthened or shortened analogs (11). However, the validity of these results is clouded by the possibility that efficiency of cellular uptake from the growth medium may be rate-limiting when the sterol side chain is lengthened or shortened. Even if this were the case, however, the observed pattern would still point to the superiority of the natural side chain in effecting a biological event, provided cellular uptake or transport is an active mechanism.

The side chain conformation is equally crucial for sterol fitness. Only the thermodynamically favored natural conformer 17 (20) "right-handed" (R) supports the growth of sterol-requiring microorganisms (12). This selectivity is consistent with the accepted model for sterol-membrane interactions that specifies the extended *trans*-orientation of the side chain with respect to the ring system.

The above considerations apply primarily, though perhaps not exclusively, to cholesterol, the ubiquitous sterol of animal cells. More difficult to rationalize are the secondary side chain alkylations characteristic for sterols of fungi and plants and in extraordinary variety in marine invertebrates (13). For yeast, the extra methyl group at C_{24} is clearly beneficial. Yeast sterol auxotrophs grow faster on ergosterol than on cholesterol. It seems certain that the added bulk the side chain alkyl groups provide, influences membrane fluidity in the more interior regions of the membrane bilayer, but the functional consequences of these changes are unknown at the molecular level.

Introduction of the 5,6 double bond by reduction of 5,7-cholesta-dien-3β-ol terminates cholesterol biosynthesis. In animal tissues, only a small fraction of the diene escapes reduction, notably in the skin, to form Vitamin D on exposure to ultraviolet light. Lathosterol, the Δ^7 isomer, must be viewed as an intermediate, not an end product of sterol biosynthesis. Investigating the membrane effects of a wide variety of cholestenols, Ranadive and Lala have found cholesterol to be superior to all other double bond isomers in controlling solute transport in phospholipid vesicles (14). While there are no clues why this should be so, two speculations may be offered. Cholesterol melts (148 C) at a substantially higher temperature (by >15 C) than the Δ^1, Δ^4, Δ^7, $\Delta^{8(14)}$ cholestanols. Second, a Δ^5 double bond greatly facilitates oxidative formation of the 4,5-en,3-one system, a structural feature characteristic of various steroid hormones. Alternatively, shifting the double bond from the natural location in the ring system may have subtle conformational effects and, hence, alter adversely the sterol-phospholipid interactions in the membrane bilayer. In early biological tests, growth of anaerobic yeast also was faster on cholesterol than on the above-mentioned isomers (15).

So far "sterol competence" has been discussed with respect to two parameters, effects on the physical state of membrane bilayers and promotion of microbial growth. However, in the last few years, a much greater functional diversity has been recognized for the sterol molecule per se. There is evidence from our laboratory and the observations of L. Parks for multiple roles of sterols in biomembranes, especially in yeast. Such phenomena referred to as "metabolic effects" (16,17) or "sparking effects" (18) appear to be performed only by species-specific sterols (ergosterol in yeast or cholesterol in the sterol auxotroph *M. capricolum*), at levels corresponding to a minor fraction of the total organismic sterol requirements. Sterol structure-function relationships will, therefore, have to be reexamined and extended from responses of artificial membranes or what is a composite biological response (growth) to defined events at the molecular level.

ACKNOWLEDGMENTS

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