molecular microbiology

edited by

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MOLECULAR MICROBIOLOGY

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CHAPTER

ONE

The Concept and Criteria of Molecular Microbiology

J. B. G. KWAPINSKI

The concept of molecular microbiology is based on the consideration of microorganisms as highly organized, dynamic entities of live matter, the particles and forces of which constantly change and interact with each other and with the environment.

The biological processes occurring in the various forms of microorganisms are governed by the general laws of physical and chemical entities. Thus the synthesis of macromolecules in the various forms of microorganisms and their replication and reproduction depend on the same laws of chemistry that control the behavior of macromolecules outside the established structural entities. Since identical laws apply to the synthesis, function, and composition of macromolecules inside and outside the cells, the composition and function of macromolecules occurring within substructural and structural microbial entities can be studied in the isolated state *in vitro*.

The molecules occurring in microorganisms are probably identical with the molecules existing in other anatomical and nonassembled forms of biocolloids. The constitutional and structural formulas of the molecules ought to be investi-

gated under determinable conditions so that the influence of the environment on the molecular structure and function, as well as the changes occurring within the molecule, may be examined. Subsequently, it may be possible to deduce what the structure and function of the molecules would be like in their biological assembly or environment.

In a more conventional approach to studies on molecular microbiology, the primary, secondary, tertiary, and quaternary structures of selected parts of larger organized systems may be investigated with special attention focused on the chemical bonding, chemical kinetics, and other physical and chemical intra- and intermolecular forces. Although the research thus would be focused on one selected part of a larger, organized microbial system at a time, its ultimate objective would be to elucidate the relationship of selected structural and functional parts to the physicochemical organization of a biological entity or phenomenon. Alternatively, the chemical organization and systematization of macromolecules can often be explained by refe ence to another, similarly organized chemical system, since the structures of small molecules and macromolecules, derived from any source, are fundamentally alike.

An intimate insight into, and understanding of, the specific structures and functions of biomolecules may be acquired by investigating the internuclear and interelectronic relationships displayed at the submolecular level. The biochemical and immunochemical specificity of molecules and biomolecules, their interactions, and their life processes may be explained in terms of quantum biology. By the application of the quantum principle, these phenomena may be considered as specific internuclear and interelectronic reactions that utilize and transform appropriate quanta of static and kinetic energy. Consequently, the study of biomolecules would rely ultimately on investigation of the electron affinities and energy coefficients of the highest filled molecular orbital.

In contrast to the ever-changing kinetic states of energy of biomolecules, the ground state is probably just a longer episode maintained between the electron-promoted, excited state and configurations, the sum of which occupies a much larger time and space in intramolecular reactions than in kinetic states.

The appearance and structure of the dynamic biocolloids, composed of different macromolecules, rest upon the intricate two- and three-dimensional patterns of their molecular constituents. The biopolymers and macromolecular components of different biochemical systems and processes expand and contract during the biochemical interactions, and in a natural environment their structure and form continuously change because of the internal rearrangement of their chain constitutents and the overall molecular shape.

Chemical changes and mechanical functions in the morphologically definable entities are interdependent. Therefore a change in dimension is

followed by a modification in the reactivity of the functional system and the subsequent alteration of the extent of a chemical reaction. The reactivity of macromolecules may be modified by the application of a mechanical force that alters the distances between the interacting groups involved in a chemical transformation. An even greater cooperative change is induced by a reagent interacting with several groups present on a polymeric chain.

Various bioentities, including microorganisms, grow and reproduce by the flow of chemical energy, which usually passes through enzymatic pathways and chemical constituents identical for all live matter. In various live forms, however, certain stereochemical differences and individualization of deoxyribonucleic acid molecules cause the same chemical constituents to link together in different sequences, positions, quantities, and arrangements, thus giving rise to individual and diverse forms of life.

How the biocolloids of microorganisms evolved during the evolution of organic matter is largely a subject of speculation, although substantial conclusions may be drawn from experiments on the activation and assembly of molecules under electric discharge (1) and the polymerization of organic molecules in the presence of heat energy and catalysts (2, 4, 5). From these experiments, it may be surmised that, when methane, ammonia, water, carbon dioxide, and hydrogen, the earliest molecules present on earth, were subjected to ionizing energy or ultraviolet light, the bonds of carbon–carbon, hydrogen–hydrogen, carbon–hydrogen, nitrogen–hydrogen, and hydrogen–oxygen were broken to form free atoms. By reorganization, these atoms yielded more complex molecules, such as formic acid, acetic acid, succinic acid, and glycine. If, subsequently, one of the carbon-bond hydrogen atoms of glycine was exchanged for any of a group of other atoms, about 20 different amino acids would be formed. These organic molecules could be converted, with the participation of heat energy and heavy metals acting as catalysts, to building blocks for polymers and biomolecules, both in the experiments and during the chemical evolution (1–3, 5). Thus it was observed that different, moderately complex organic compounds, such as adenine and ribose, were formed when a mixture of hydrogen, cyanide, and ammonia was heated at 70°C for 25 days. Various bases and nucleotides were synthesized by the action of UV rays on hydrogen cyanide, whereas ribose and deoxyribose were formed by the irradiation of formaldehyde with UV or gamma rays (6, 7). Nucleic acids were synthesized by the interactions between nucleotides and polyphosphate esters at 50 to 60°C.

The heating of concentrated mixtures of amino acids in molten glutamic acid in the presence of polyphosphoric acid, or the heating of amino acids in aqueous ammonia, caused in them intermolecular reactions that yielded polypeptides of considerable size. As described by Fox (1), the hetero-

polyamino acids, or thermal protenoids, were obtained by direct heating of amino acids at such a temperature that water boiled off first and allowed the dry residue to undergo polymerization under the then thermodynamically appropriate condition of a low content of water in the material. It is known that highly polymerized peptides and proteins may acquire a characteristic helical structure because of a specific arrangement of carbon, hydrogen, nitrogen, and oxygen atoms in the amino acid chains. Filamentous proteins are able to aggregate in a characteristic, ordered array, thus generating a highly organized order in a subcellular material. Thus the proteinoids, possessing limited heterogeneity and capable of interacting selectively with certain enzyme substrates, were assembled into organized microsystems that showed many of the characteristics of contemporary cells (3, 4, 8). When the proteinoids were heated in aqueous solution and the clear, hot solution was allowed to cool, numerous microspheres were produced, each containing about 10^{10} molecules. The proteinoid microsystems thus formed communicate with each other through junctions that they form, and are capable of proliferation.

Postulating on the experimental evidence discussed above, one may assume that under natural conditions the molecules synthesized predominantly from methane and containing at least six carbon atoms, as well as other molecules possessing additional SH and NH_2 groups, condensed on the surface of the air–water interface and were cross-linked to form an insoluble product by the effect of electric discharges and heat energy. Consequently, surface films of predominantly hydrocarbon nature might have arisen and acquired multiple SH, NH_2, and O groups on the aqueous site, at a later stage of biomolecular evolution.

The emergence and diversification of organic compounds linked together by electric forces, originating and gradually perfected during the evolution of microsystems, produced molecules of peptides, saccharides, polysaccharides, lipids, and nucleic acids. Interaction between proteins and nucleic acids led to the development of a pattern for protein synthesis that thus was directed by nucleic acid.

The synthesized macromolecules, consisting of different compounds, were gradually linked together and arranged in a specific pattern by the highly specialized informational macromolecules. The evolving microsystems were equipped with the ability to perform complex and interconnected sequences of biochemical reactions, leading to the synthesis of subunits, to be assembled into specific configurations of primitive forms of life, which provided for the most efficient movement of compounds along the pathways. In this way, various forms of biological molecules were exploited and ramified in the ascent of primitive biocolloids to the particulate and unicellular levels of organization as viroids, virions, ribosomes, mitochondria, protoplasts, and protocells. The

assembly or rearrangement of biological structures consisting of many interacting parts has been a cooperative process dependent on bond energy and activation energy.

Not surprisingly, biological differences existing between the fundamentally similar macromolecules occurring in different organisms seem to depend on a very fine chemical bonding or a small and unusual molecule, attached to a larger unit, which is detectable only by highly sophisticated physicochemical and immunochemical procedures. Predictably, very specific biological and live processes occurring in microorganisms will eventually be explained in terms of intimate interactions of hadrons, leptons, gamma quanta, and other families of particles still to be discovered. However, until that stage of scientific progress has been reached, our contemporary experiments in molecular microbiology will have to rely on the utilization of a vital and a rather large part of a functional, living system as a template needed to replicate another or a missing part of the same or a closely related living system.

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CHAPTER

TWO

Molecular Arrangement and Assembly

J. B. G. KWAPINSKI

1. CONFIGURATION AND CONFORMATION OF MOLECULES

In terms of quantum biology, the reactivity of a molecule is described as a group electron problem, assuming a fixed core (52).

The electronic structure of a molecule is expressed by the momentum and position of the electrons in the molecule. The momentum refers to the energy, and the position applies to the distribution of energy or electrons in relation to

the appropriate nucleus. The probability distribution of positions in a molecule is given by the square of a function of the free-space coordinates of the nuclei and electrons, and of the spin coordinate relevant to each particle.

In order to describe molecules in terms of quantum mechanics, it has been necessary until now to use approximations that do not completely elucidate the intimate structure of molecules. For example, according to Pauli's uncertainty principle, the momentum and the position of a particle or molecule cannot be determined simultaneously. Customarily, therefore, only the momentum of electrons is determined accurately, and, insteadd of position measurement, the probability distribution, which is expressed by the square of the wave function, is calculated. Accordingly, the electronic description of a molecule possessing a given momentum or energy is based on the probability assumption that an electron in a given element of volume is described by the square of its wave function.

The wave function is the solution of the Schrödinger equation, which is the basic equation of quantum mechanics. For a simple case of a single particle of mass m moving in a region in which the force acting on it can be represented by a potential energy function V, the Schrödinger equation is as follows:

$$-\frac{h^2}{8\pi^2 m}\left(\frac{\gamma^2\psi}{\gamma x^2}+\frac{\gamma^2\psi}{\gamma y^2}+\frac{\gamma^2\psi}{\gamma z^2}\right)+V\psi=\frac{\lambda\gamma\psi}{2\pi i\gamma\tau}$$

If it were possible to solve the Schrödinger equation for an assembly of any number of particles, any molecule could be described accurately, and almost complete information about any molecule would be available. The structure of a molecule would primarily be determined by the geometric arrangement, expressed by the space coordinates of each of its atoms, so that the energy of the molecule as a whole is at a minimum. The vibrational force constants would be obtained from the variation of the energy with the variation of the nuclear coordinates (32). However, the Schrödinger equation can be solved only for systems consisting of one to three particles.

Although the exact form of the molecular potential energy is unknown, the total molecular potential energy in the repeating chemical units is represented by harmonic potentials, torsional barriers, the Lennard–Jones potential function, and a compensatory constraint (42). The potential energy may be approximated by the following equation:

$$\mathrm{E}=\underset{\text{All bonds}}{\sum\tfrac{1}{2}K_b(b-b_0)^2}+\underset{\text{All bond angles}}{\sum\tfrac{1}{2}K_r(r-r_0)^2}+\underset{\text{All dihedral angles}}{\sum\tfrac{1}{2}K_\phi\{1+\cos(n\phi^0-\delta)\}}$$

$$+\underset{\text{All nonbonded pairs}}{\sum\mathrm{E}_{ij}\left(\frac{r_{ij}^0}{r_{ij}}\right)^{12}-2\left(\frac{r_{ij}^0}{r_{ij}}\right)^{6}}+\underset{\text{All atomic coordinates}}{\sum\tfrac{1}{2}w(x_i-x_i^0)^2}$$

where K_e is the bond force constant; b, bond length; b_o, equilibrium bond length; K_γ, bond angle bending force constant; γ, bond angle; γ_o, equilibrium bond length; K_o, torsional barrier; θ, dihedral angel; n, periodicity of rotational function; δ, phase; E_{ij}, depth of nonbonded minimum; r_{ij}, distance between atoms i and j; r^0_{ij}, distance of nonbonded minimum; w, constraining force for all atoms; x_i, atomic Cartesian coordinate; and x^0_i, experimental coordinate.

A detailed and precise description of the molecule is attained by determination of its stereochemical formula, which expresses the spatial arrangement of the atoms in a molecule, as determined by accurate measurement of the lengths of the bonds linking these atoms and the angles between the bonds. The positions of the atoms and electrons relative to each other are revealed precisely by the analysis of X-ray, electron, or neutron diffraction patterns. This analysis is used to investigate mutual atomic positions and interatomic distances, as well as the arrangement and combination between atoms, conferred by homopolar, single, or double bonds. From such data, electrondensity maps (Fig. 1) are drawn; these show the deviations of atoms from pure sphericity arising from the usual thermal motions. Stereochemical analysis also provides indications as to the location of the electrons in single or double bonds.

Fig. 1. Electron density map of a molecule (4).

Macromolecules may possess alternative positions of the atoms within the same bond patterns; these are determined by the conformational properties of a molecule, dependent on the interactions of distant parts of the molecule over and above those due to conjugated double bonds. Thus configurational patterns estimated by stereochemical investigations can be supplemented by examination of the conformational properties of the macromolecules. The molecular conformation is determined by the temperature-sensitive van der Waals forces and by mutual interaction of parts of a molecule other than those immediately related by primary valence bonds (4). Similar forces are involved in antigen–antibody reactions (12).

Macromolecules possess a wide range of possible conformations because of their flexibility. The flexibility of a molecule depends on the rotational and torsional degrees of freedom of its bond. The conformation of the isolated molecule is determined by the sum of the effects of the interactions of the more distant parts present in the molecule. The distribution of different conformations is greatly influenced by environmental factors, for example, the presence of similar molecules or solvent molecules. These factors are especially effective in long, flexible macromolecules. The completly stretched-out and the coiled forms of the same molecule represent extreme types of conformation.

Biomolecules originating from various sources or from the same cell may possess similar qualitative and quantitative patterns, yet they differ in atomic detail. Biomolecular structures can be studied in atomic detail by the ap-

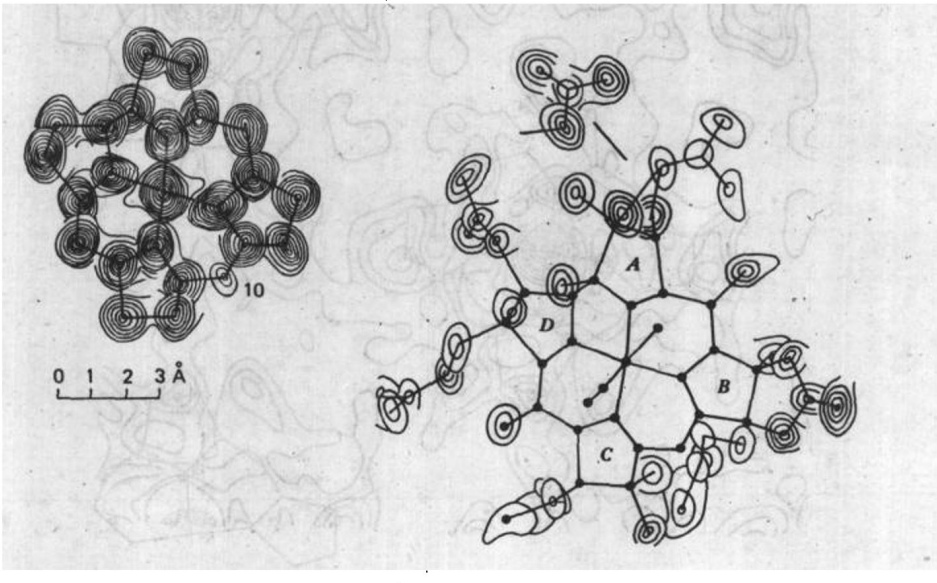

Fig. 2. Electron image of a molecule (4).

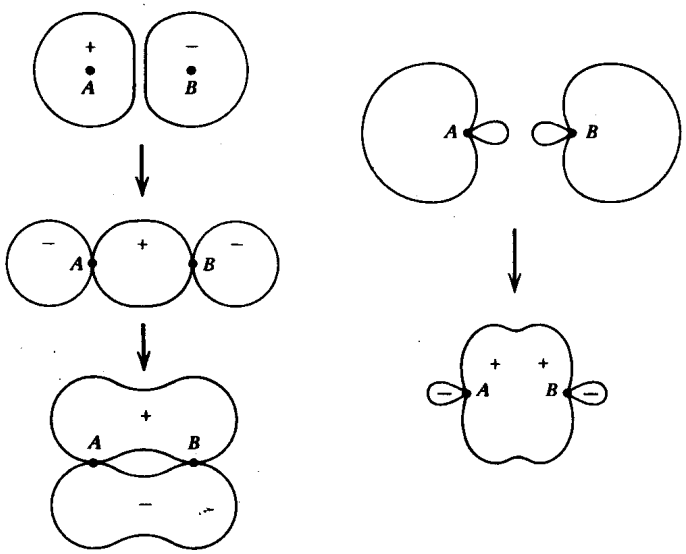

Fig. 3. Emergence of molecules by hybridization of electron orbitals of atoms *A* and *B*; a deformation of molecular symmetry (32).

plication of atomic resolution, which describes the three-dimensional structures. In this method, the positions of heavy atoms in the protein molecule are revealed and measured on an automatic three-circle diffractometer and compared with the positions of heavy atoms in standard crystalline molecules (62). The image of a biomolecule can be obtained by photographing the electron densities representing the arrangement of atoms in a biomolecule (Fig. 2).

Before a molecule is formed by a reaction between atoms, certain orbitals of each atom are combined to form molecular orbitals. Depending on certain symmetry properties, only some orbitals of two atoms mix or hybridize, and individual atomic orbitals are replaced by hybrid orbitals, as shown in Fig. 3. The hybridization of atoms or molecules is associated with a change in energy; for example, the promotion of a fraction of an electron from one atomic orbital to another requires considerable energy. In some cases, the energy can be compensated for by increased binding energy or reduced repulsion of electrons.

2. MOLECULAR ASSEMBLY

The molecular assembly is an orderly association of atoms and interatomic energy bonds, forming a functional molecular structure that bears a conformational stability relative to the environmental forces and the time factor.