Macromolecular Complexes

Edited by
M. V. EDDS, JR

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Preface

Efforts to analyze and interpret life processes, especially as they relate to development, are ultimately confronted with such questions as: Do the orderly patterns constituting given levels of the organizational hierarchy regularly arise solely as a consequence of the specific interactive properties of the units comprising the next lower levels? Given the proper units and the proper conditions, is the spontaneous emergence of order at a higher level inevitable?

Considerable evidence attests that, at least in some cases, these questions may be answered affirmatively. Examples include the *in vitro* aggregation of certain macromolecules, among which collagen is the prototype, into fibrils of near-crystalline regularity, and the selective aggregation of previously dissociated cells into recognizable tissues. The spontaneous emergence of orderly patterns could also be illustrated by examples from levels higher than the cell or lower than the macromolecule. Between these levels, however, are others about which information is less certain. The time is clearly ripe to begin inquiring into the nature and origin of heterogeneous aggregates of macromolecules, with special emphasis on the interactive properties of their subunits. Actually, the subunits themselves have been identified only in a few cases, and concepts of their structural and functional interrelations are still based largely on inference. But, as F. O. Schmitt has written, we have already learned enough to "glimpse, however imperfectly, the portentous possibilities now opening up before us."

Some of these possibilities are explored in this book embodying the proceedings of a symposium sponsored by the Society of General Physiologists. As the text reveals, exploration of the borderland between the realms of the macromolecular chemist and the functionally oriented electron microscopist has already yielded important information about the organization of living matter; it is thus more than an article of faith that many biological problems can be effectively attacked at this level. Not the least of these prob-

lems are those related to development. It is obvious, but often neglected, that the genesis of form and function occurs, and must be studied, at all levels of the organizational hierarchy. Most of the attempts to relate chemistry and morphogenesis have stressed entities on both sides of the borderland but have been silent on what lies between.

The basic premise of the symposium was that further clarification of the relation between chemistry and morphogenesis will depend heavily on the analysis of aggregates of two or more species of macromolecules. The speakers, who were selected because their main competence lay neither in chemistry nor in morphogenesis, but somewhere in between, were encouraged to discuss the thesis that many aspects of morphogenesis can be attacked in relatively "simple" systems by exploring in detail the properties of the macromolecular complexes which compose them. To this challenge the speakers responded with solid information, both confirming the thesis and raising a host of new morphogenetic problems. Excursions into other areas were inevitable and welcome. Thus, much was said that is related to development only in the sense that it poses the question: How does it develop? But whatever the bias of individual papers, the entire volume is a record of a combined effort to place a point of view before all who may profit from its contemplation.

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M. V. Edds, Jr.

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Molecular Interactions and Structure Formation in Biological Systems

DAVID F. WAUGH [1]

The three-dimensional display of structural complexity in cells and tissues, now so convincingly visible in the evidence from electron microscopy, is at the colloidal level, thus the level where one or two dimensions of the particles (rodlets, lamellae) which result from molecular interaction are near the dimensions of the interacting molecules themselves. We accept the fact that the functional aspects of cells, tissues, organs, etc., must be a natural (thermodynamically acceptable) result of the organization at the level mentioned, and, although we are not in a position to state the type or extent of knowledge which will be necessary to understand the way in which subcellular colloidal aggregates modify each other's activities to produce the net result of "life," several aspects of interaction have been examined in a way which must excite all interested in biological processes. These I have been asked to discuss briefly.

Manipulation and interaction at the molecular level involve selections of the interacting molecules; thus, the interactions are specific. We are familiar with the high degree of specificity exhibited by enzyme-substrate interactions (Wilson, 1959), in energy transfer (Lehninger, 1959), in immunological interactions (Landsteiner, 1945), in the formation of tissues such as collagen and elastin (Schmitt, 1959), in the formation of organs, and during the course of embryonic development, to cite a spectrum of examples. As we shall see, specificity of interaction between molecules coming into close contact may be understood on the basis of short-range interactions between appropriately placed submolecular groups of atoms having different interaction characteristics. Catalyzed chem-

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ical reactions and the development of the more condensed regions of structure (lamellae, fibrils, etc.) may soon be quantitatively accounted for on this basis.

The biological system is clearly one in which most reactions are carried out in the presence of water. There is, however, apparently an abundance of water, and control of the amount and disposition of water is of paramount importance. The more condensed regions, on whose surfaces or within which much of the molecular traffic will take place, must be prevented from increasing in size (or precipitating out) to the point where such interferes with the execution of ordered series of linked reactions by the condensed structures and with a reworking of the molecules of the condensed structures themselves. We suppose that the incorporation or holding of too much water, however, might well interfere with molecular traffic, would certainly reduce mechanical rigidity, and might allow, for example, deformation of the organ or tissue as a whole to produce local (subcellular) deformations in colloidal structure from which the system would not recover. Control of the amount and disposition of water in localities where the condensed structure is organized in the form of a continuous network (gel) is most readily understood. However, deformation of a cross-linked network must necessarily involve both flow of the entrapped fluid and a rearrangement of the relative positions of the elements of the network. A network arrangement is most effective where the network supports some biological function such as the transmission of tension, as with collagen and elastin. In the examples chosen, the fraction of the tissue or organ occupied by water is low, and the tissues are relatively inert.

Many portions of the biological system, however, contain large amounts of water, and interactions of the more condensed structures may be taking place over intervening aqueous regions of considerable size. Here a distortion of the system need not change the relative positions of the elements of the condensed structures; for example, lamellae might slide past one another without altering the interlamellar distance, thus without altering the local distribution of water and solutes.

In considering molecular interaction and the formation of condensed structures, we must first note that the word "molecule" can be given a restricted meaning with respect to small molecules: namely, a group of atoms joined by covalent (infrequently coordination) bonds which are stable with respect to kinetic energy (kT).

The fact that covalent bonds have associated with them preferred or limited distances, directions, and rotations in conjunction with the sizes of the participating atoms (Pauling, 1940) means that the possible conformations of the molecule may be specified. As the number of atoms increases, possibilities arise for various types of interaction between groups of atoms within the same covalently linked assembly. In molecules of large size, such as the proteins, carbohydrates, and nucleic acids, these interactions are numerous and involve all known types of forces. For example, these interactions are involved in determining the structures of the polypeptide chain helices of proteins (Pauling *et al.*, 1951), the interactions of proteins (Waugh, 1954), and the double helix of nucleic acids (Watson and Crick, 1953). Recognition of the importance of the group of short-range forces in determining specificity of interaction, as well as the intramolecular stabilizations just mentioned, is due largely to the work of Pauling and co-workers.

Table 1 gives a general idea of the various types of short-range and long-range interactions. The characteristics of the first of these, the covalent bond, have already been mentioned. The hydrogen bond is a permanent dipole interaction, involving a hydrogen atom covalently bonded to one electronegative atom and the unshared electron pair of another electronegative atom (Pauling, 1940; Orgel, 1959). This bond has a high interaction energy, a result of the small size of the proton which permits the close approach of dipoles. To break a hydrogen bond requires an investment of about 5 kcal per mole. However, if H-bond exchange with water is possible, the investment is much reduced and may be reversed if the groups involved make unusually strong hydrogen bonds with water. Thus the strength of a particular H-bond is dependent on the circumstances permitting a simultaneous interaction with water.

The simple ionic bond, a strong interaction in the absence of water, leads to attraction or repulsion according to Coulomb's law. The interaction energy varies with the dielectric constant of the intervening medium and inversely with the distance between centers. The former varies strongly with distance in an aqueous medium and decreases as the distance between centers decreases (Pressman *et al.*, 1946). Assuming that water hydrates an interacting pair such as an ammonium ion and carboxyl ion, close approach will produce an interaction energy of about 5 kcal per mole (Pauling *et al.*, 1946). We should remember also that charged groups lead

TABLE 1

Interaction Energies *

Type	Energy (kcal/mole)	Energy and Distance	Range of Action in Aqueous System	Examples
Covalent	60		1–2	Organic compounds
Hydrogen bond	1–6	$1/r^3$	2.4–3.2	Water, acids, urea, proteins, carbohydrates, etc.
Ionic	5–20	$1/r$	2–3	Ions, zwitterions
London–van der Waals (small groups of molecules)	1–2	$1/r^6$	2–3	Atoms, groups of atoms, CH_2, CH_3, NH_2, etc.
London–van der Waals (large molecules)		$1/r^2$	Long	Large planes of atoms
Charge patterns (large molecules)		$1/r$	Long	Large planes, multiple + and − charges near isoionic point

* Suggested from the Introduction by Bernal, 1958.

to an ion-dipole interaction with water molecules and so become hydrated, the interaction energy amounting to about 5 kcal per mole of bound water (Dole and McLaren, 1947).

The last of the forces operating at close approach are the London–van der Waals attractive forces, which result from the polarization of each group of atoms in the fluctuating electrical field arising from the instantaneous configurations of the electrons and nuclei of adjacent groups. For small groups, the energy varies with the inverse 6th power of the distance between centers and directly with the molar refractions of the groups involved (Pauling and Pressman, 1945).

Specificity Effected by Short-Range Interactions

It is of importance to note that both the hydrogen-bond and van der Waals interactions vary, respectively, inversely with the 3d and 6th powers of the distance of separation of the groups. If the maximum interaction energy is released when, for example, two CH_2 groups are 4 Å apart, separating these groups by an additional 2 Å will reduce the interaction energy by a factor of about 11. Likewise for the hydrogen bond, stretching the bond by 1 Å essentially terminates the interaction. We see at once an extraordinary specificity can be accounted for by the requirement that an adequate interaction energy be obtained only when a variety of groups having different interaction characteristics are brought into their proper positions. These groups would include unlike charges, those forming hydrogen bonds, and the bulky groups, interacting through van der Waals forces. The latter, which will essentially define the surface of a large molecule, must fit so that the protuberances on one surface find appropriate cavities on the other surface. Remembering that a stable interaction is produced when the total interaction energy is about 10 to 15 $\times$ kT, and that all local or group interaction energies are summed to obtain the total interaction energy, suggests that the smaller the area of interaction, the more perfect the fit between interacting surfaces or the more numerous the groups giving rise to stronger interactions.

Even the most specific of interactions can now be rationalized on the basis given; for, as is evident, minute alterations in structure may lead to imperfect steric fit, and since energies of inter-

action are so sensitive to distance, a small imperfection in fit produces a large change in interaction energy.

A product of the short-range interaction between two molecules might well be a complex having new properties. A clear case of this type is the dissociation or fragmentation of the protein ribonuclease into two enzymatically inactive portions (Richards and Vithayathil, 1959). Although the fragments have been produced by hydrolyzing a covalent bond, the fragments, on mixing, combine to give a complex which has full enzymatic activity. The ruptured covalent bond is not re-formed on mixing. A variety of exciting possibilities is brought to our attention by this discovery. For example, the interaction of a steroid hormone molecule with an inactive protein might induce enzymatic activity in the latter, this combination controlling a host of subsequent reactions.

Specific interactions are undoubtedly also involved in establishing condensed regions, which usually appear as filaments, lamellae, or helices, at levels of size within an order of magnitude of 100 Å. Currently, the lamellar structure containing lipid and protein appears to be a conspicuous component of cells, the assumption being made that the lipid within the lamella will be present as a double layer or layers. Certainly such an organization of lipid would be expected from the properties of soaps and lipid emulsions (Luzzati *et al.*, 1958; Lawrence, 1958; Palmer and Schmitt, 1941) and the structure of the myelin sheath (Schmitt, 1959). The double-layer arrangement permits the bulky non-polar portions of the lipid molecules to associate, leaving surface layers of polar groups to interact with each other and with water, protein, or other polar substances.

In many instances the development of a lamella may not involve any high degree of fit (specificity) of the polar and non-polar portions of the molecules involved. We would expect a low degree of specificity mainly when the interaction leads to the development of a structure which carries out some non-critical physical function, a safe example being that of fat storage—although another might be the essentially two-dimensional expansion of non-functional portions of a plasma or other membrane. Certain functional aspects, however, may require a specific arrangement of non-polar and polar portions which will provide an energy barrier to penetration (permeability). Although the physical conditions establishing the basis for interaction in monomolecular films are probably not duplicated often in biological systems, the effects of short-range interaction in

modifying the properties of films are clearly shown in studies of the evaporation of water through compressed films of stearic acid or cetyl alcohol; the latter present an extraordinary energy barrier to the penetration of water molecules (Langmuir and Schaefer, 1943). This possibility, namely, that non-polar portions of molecules, particularly lipid molecules, may interact to present a high-energy barrier to the penetration of water, is of considerable biological interest; for, in spite of the relative abundance of water in biological systems, local regions at the particulate level may involve boundaries between essentially aqueous environments and environments where the interactions of non-polar portions of molecules are so well integrated that water is virtually excluded. A structure of this type, for example, may be necessary in effecting the key reactions of photosynthesis (Calvin, 1959).

In this introductory discussion, I would like to summarize some of our experiences with two protein systems in which short-range interactions, coupled with particular types of specificity, lead either to extended fibrils or to spherical micelles, namely, the interactions of insulin (Waugh, 1957) and of casein (Waugh, 1958).

Mechanism of Fibril Formation. The insulin fibril forms under conditions where the dimer of $M \sim 11{,}000$ is the prevalent form, i.e., at pH 2. Heating at 80° to 100° C causes a spontaneous transformation into a population of fibrils, the most numerous, and largest, of which are about 200 Å in diameter and many thousands of Ångströms in length. The reaction goes essentially to completion but is reversible in the sense that under alkaline conditions the fibril disaggregates to yield insulin. Evidence which gives a clue to the mechanism of fibril formation, and which suggests that the insulin molecule does not undergo extensive unfolding in the process of forming fibrils, comes from experiments in which fibril segments are seeded into insulin solutions at pH 2. While such solutions alone are stable for long periods of time at temperatures of 20° C or below, the seeded fibrils or fibril segments recruit insulin from solution and in the process grow according to first-order kinetics. Structurally, the new portions of the fibrils obtained after fibril growth at lower temperatures appear to be identical with those which are formed at 80° to 100° C.

When a solution of insulin is heated and the transformation of insulin into fibrils is plotted as a first-order reaction, the resulting curves typically have a lag period which is followed by an essen-

tially linear rise. The extent of the lag period is determined markedly by the initial insulin concentration, as is the slope of the near-linear portion of the curve. A comparison of the reaction kinetics observed with the growth of seeded fibrils and in the absence of seeding suggests that the fibril is first initiated by a nucleation reaction which involves the cooperative effects of three or four interacting units (dimers). Thus the rate of nucleation varies with the 3d or 4th power of the insulin concentration. After initiation, when the fibril has achieved a reasonable size, the fibril grows as a function of its surface area and the 1st power of the free insulin concentration. The cooperative effect established during nucleation is perpetuated, and the surfaces (particularly of the ends) of the fibril present to the entering insulin unit the correct cooperative configuration necessary for bonding.

One particularly interesting consequence of this type of mechanism is that most of the fibrils are initiated during the lag period of the reaction. Those which are initiated during the first few minutes of the lag period dominate the reaction, in the sense that they are responsible for removing most of the insulin. The fibril population at the end of the reaction appears to be relatively

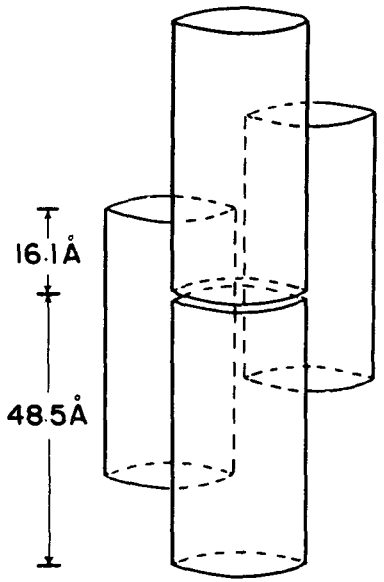

Fig. 1. Diagram of a fibril nucleus, illustrating a cooperative interaction. (From Waugh, 1957.)