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Life and death: the ultimate phase transformation[1]

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Abstract

The order ⇄ disorder phase transformation involved in life and death is discussed. Five bioactive substrate ordering factors are proposed to provide the mechanisms for overcoming the entropic barrier to forming life from a disordered molecular assemblage. Molecular orbital models (AM1 method) are used to analyze the ordering factors. The results are consistent with the genetic activation characteristics and tissue bonding of bioactive silicate glasses.

Keywords: Chiral; Death; Entropic barrier; Life; Molecular orbital calculations; Order factors; Phase transformation

1. Overview

Disorder ⇄ order reactions are one of the most important classes of phase transformation; i.e. gas ⇄ liquid; liquid ⇄ solid; glass ⇄ crystal. The free energy is lowered in each case at the expense of entropy, or vice versa, depending upon the direction of the transformation. An enormous number of such reactions have been studied and detailed physical chemical theories exist, as demonstrated by the many papers in this volume.

Biological systems also fit into the category of order ⇄ disorder reactions. The biological phase transformation has profound consequences; with order comes life, with disorder, death. Living organisms transform a random mixture of organic and inorganic molecules into highly organized macromolecular structures.

Life transforms disorder into order. Death is the onset of the reverse reaction; i.e.

$$\text{Life (Order)} \rightleftarrows \text{Death (Disorder)} \tag{1}$$

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[1] Dedicated to Professor Hiroshi Suga.

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As required by the third law of thermodynamics, life is achieved and maintained at the expense of the entropy of its environment. The mystery of life is the origin of this antientropic transformation; the mystery of death is what stops it.

For nonliving systems the physics of a phase transformation is straightforward; changes in pressure, temperature, or volume modify the interaction of electron wave functions of neighboring atoms which leads to a lower, or higher, total energy for the assembly of atoms. A significant feature of this physical process is its reversibility. As the term implies, invariant points on *T-P-C* diagrams are independent of the direction of heating or cooling. Although kinetic barriers to order ⇄ disorder transformations exist, thereby permitting, for example, glass formation, equilibrium requires that all supercooled or superheated systems will eventually and reversibly transform. A crystal can be cycled through T_m innumerable times and the same space group will always reform upon crystallization.

The phase transformation of a living system is remarkably different. The death of a cell is irreversible. Chemical analysis will show that the same molecules are present in the dead cell; even the supramolecular architecture of the cell wall, nucleus, and organelles is still present. But entropy irreversibly increases following death (Fig. 1). There is no change in temperature, pressure, or chemical environment that will resurrect vitality in a dead cell. The biological transformation from order → disorder is one way.

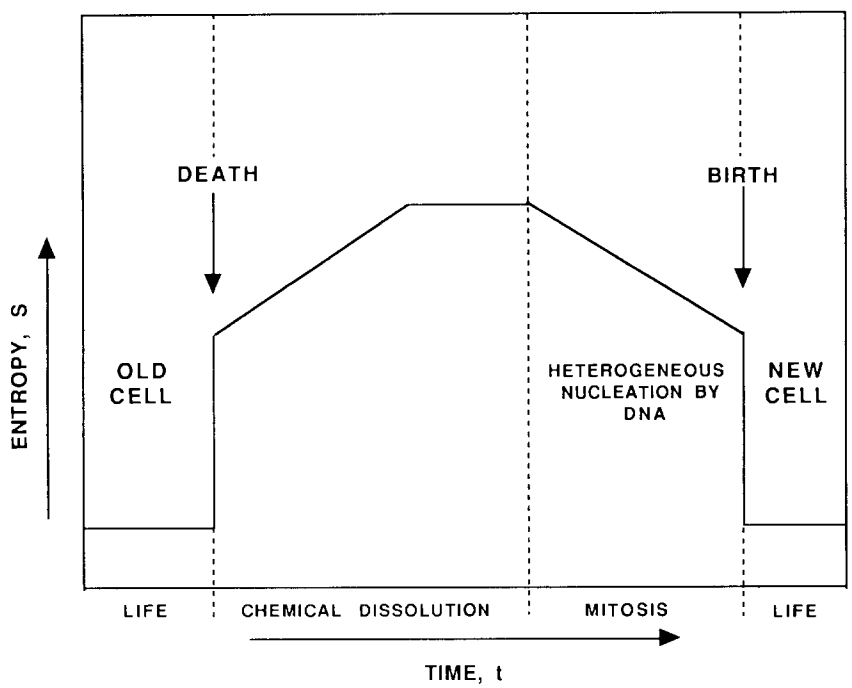

Fig. 1. Schematic of entropy changes involved in life and death transformations.

Restoration of life requires total chemical dissolution of the organism and regrowth, by mitosis (cell division) of a new cell (Fig. 1). This disorder → order transformation to form a new cell is not self nucleating, the concept of spontaneous or "homogeneous" nucleation of life is called "a miracle," and is in the domain of metaphysics rather than physics. As is well known, all aspects of cell formation are directed by the base pair sequences in DNA. No laboratory simulations have been able to produce even a small segment of a DNA molecule or even the simplest proteins without first seeding the experiment with DNA [1].

Thus, it is accepted that the disorder → order phase transformation characteristic of life involves the equivalent of "heterogeneous" nucleation by DNA [2].

It is equally well accepted that all DNA is synthesized within a cell, directed by the DNA already present [2]. No DNA is synthesized de novo outside a cell and transported into a cell to serve its replicative function. Even viruses, which can be considered as mostly "naked" segments of DNA, are replicated within a host cell using the biosynthesis pathways of the host.

2. The paradox

The problem of understanding the origin of the disorder → order transformation of biological systems takes the form of a paradox:

(1) DNA synthesis requires DNA. How did DNA biosynthesis begin without DNA?

(2) Likewise, protein synthesis requires enzymes. Enzymes are proteins. Thus, how did protein biosynthesis begin without enzymes?

In other words, how could the reverse transformation reaction in Eq. (1) (Disorder → Order) occur; i.e., "homogeneous" nucleation?

3. Alternative hypotheses

An extensive literature review [1, 3–9] describes four general scientific hypotheses for the origin of order and life.

(1) *Single biological origin*: replication and metabolic processes appear simultaneously within a membrane.

(2) *Double biological origin*: metabolic processes appear first in a pre-biotic organism followed by symbiosis of a replication structure or vice versa.

(3) *Clay-based origin*: metabolic processes evolve on clay-based substrates that provide order and self-replication of proteins; eventually replaced by DNA.

(4) *Outer space origin*: alien life forms adherent to cosmic dust particles enter the earth's atmosphere via comets to seed nutrient pools.

None of these hypotheses, or their subsets, provides a mechanistic solution to the entropic barrier, illustrated in Fig. 1, to forming an ordered assemblage from a disordered one. The "life from space" conjecture [9] simply shifts the question to an

extraterrestrial origin without providing a means of proof; i.e. is not science. The other three hypotheses also offer no means of proof. Stanley Miller's classic experiment in 1955 demonstrated that simple amino acids and sugars can be synthesized from a random mixture of gases in a reducing atmosphere exposed to electrical discharges [5]. However, the macromolecular structures characteristic of life cannot be produced by such nondirected synthesis.

Several years ago, I presented a hypothesis for the origin of ordered biotic structures from a random assemblage of disordered organic molecules [3].

(5) *Bioactive substrates origin*: ordered protein and DNA structures are formed on bioactive inorganic substrates.

The bioactive substrate hypothesis suggests that selective adsorption of biomonomers contributes five ordering factors that eliminate randomness, reduce entropy, and ensure repeatability of the biopolymers formed [3]. The ordering factors are:

(a) substrate steric factors which impose repeatable spatial requirements;
(b) monomeric optic axis orientation which imposes chiral growth;
(c) substrate optic axis orientation which imposes match of monomeric chirality;
(d) irreversible condensation reactions which result in a stable biopolymer; and
(e) polymeric steric factors which limit the selection of additional monomeric units.

4. Evidence for bioactive substrates

The original evidence in support of a bioactive substrate theory for the disorder → order transformation was the epitaxial binding of the levorotatory form of the amino acid alanine (poly-L-alanine) to alpha quartz crystals [10]. Highly specific orientations of poly-L-alanine deposits were formed on $(10\bar{1}0)$ or $(10\bar{1}1)$ crystal planes where repeating silanol groups matched alanine binding sites (Fig. 2a). The epitaxially oriented amino acids were tightly bonded and resisted mechanical abrasion or chemical attack, unlike poly-D-alanine or substrates where spatial distances between bonding sites were not matched. These experiments provided evidence for ordering factors (a), (b), and (c) above and perhaps (d) because the adherent agglomerates could not be removed from the substrate without substantial mechanical force.

5. An inorganic route to biosynthesis

Based upon these empirical findings, Jon West and I have recently completed several calculational studies that indicate a possible mechanistic solution to the order ⇄ disorder paradox of life [11–14]. We have used semi-empirical molecular orbital (MO) calculations (AM1 method) to study the energetics of interaction of hydroxylated SiO_2 clusters and amino acids. Our rationale is that silica, silicates, and water have always been the most abundant compounds of the lithosphere [15] and provide the most probable interfaces for interactions with pre-biotic amino acids.

The MO calculations show that hydrated three-membered silica rings are easily formed during the fracture of silica and silicates [16]. The three-membered silica rings

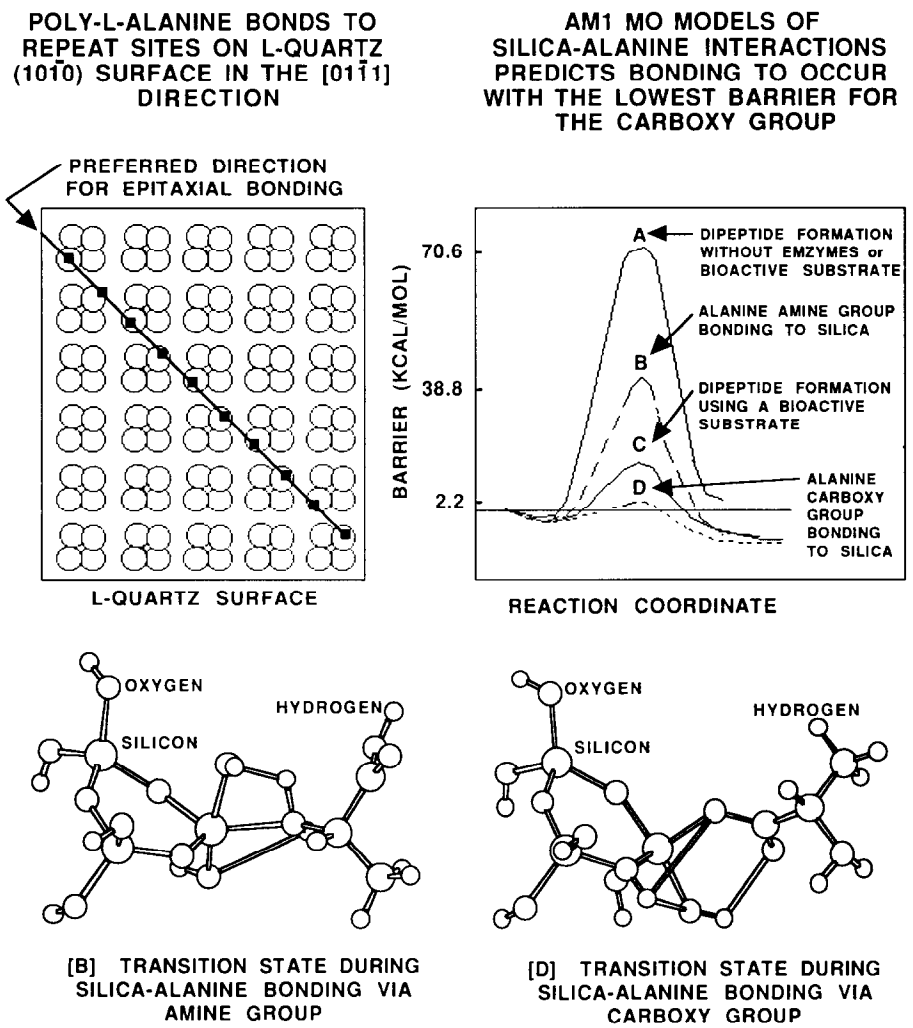

Fig. 2. Ordering and biosynthesis interactions of amino acids with hydrated silica substrates.

are energetically metastable due to quantum-mechanical strain of the bridging Si–O–Si bonds (Figs. 3 and 4). The strained three-membered silica rings provide a pathway for binding of alanine [$CH_3CH(NH_2)COOH$] with an energy barrier as low as + 2.2 kcal mol^{-1} (Figs. 2b–2d). The low energy barrier is easily reversible at 25–50 °C and is relatively insensitive to hydrolysis conditions of the molecules. The low energy barrier is due to the formation of a pentacoordinate Si atom in a metastable transition state (Fig. 5). The pentacoordinate silicon state occurs when the –COOH group of an amino acid interacts with a trisiloxane ring. The carboxyl bond polarizes the Si–O–Si bond in the three-membered ring and opens it into a three-membered chain [12]. Water attacks

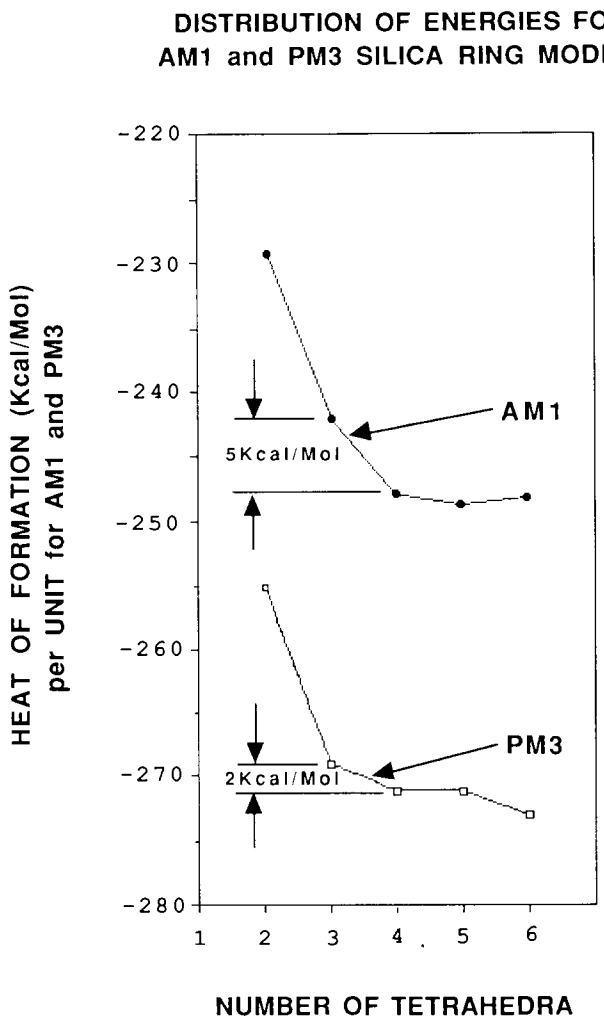

Fig. 3. Effect of number of silica tetrahedra in rings on relative heats of formation. (Note energetic differences between 3-membered trisiloxane rings and the more stable 4- and 5-membered rings.)

three-membered rings in a similar manner, as described experimentally and theoretically using hydrated silica gel systems [17, 18]. (This equivalence of the polar behavior of carboxyl groups of amino acids to H_2O is vital when we examine the bonding of bioactive glasses to living tissues in the next section.)

We have recently completed MO calculations which show that the metastable pentacoordinate Si–OH complex acts like as inorganic enzyme by providing a favorable reaction pathway for polypeptide synthesis [12]. The easily reversible opening and closing of the hydrated silica rings provides a pentacoordinate Si transition state which

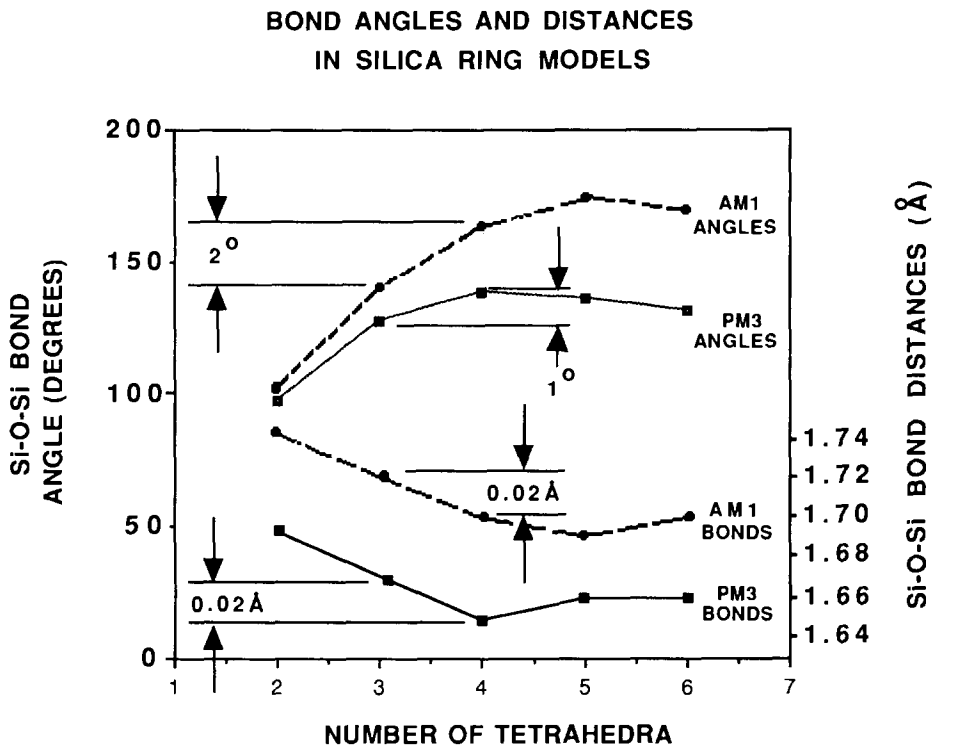

Fig. 4. Effect of number of silica tetrahedra in rings on angles and bond distances. (Note dilational and rotational strains in 3-membered rings.)

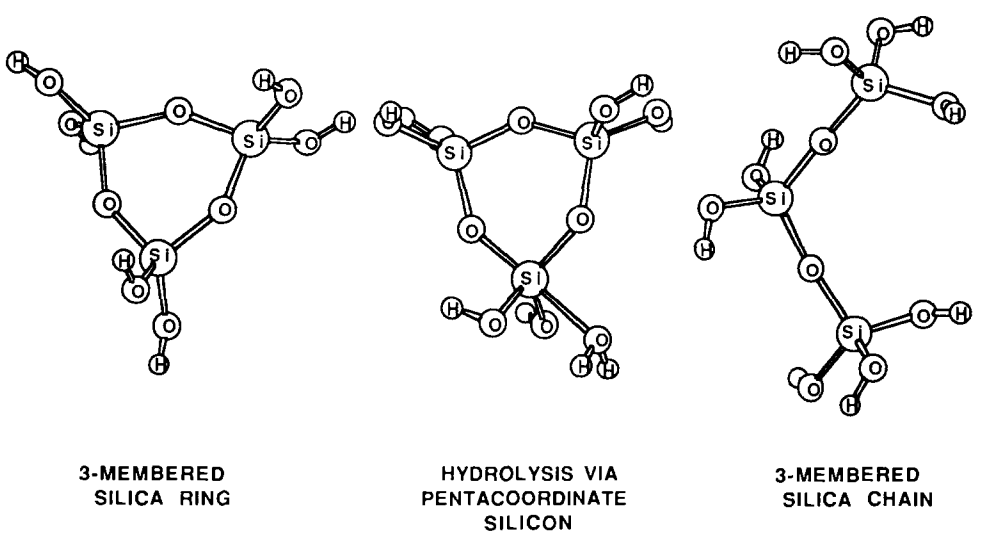

Fig. 5. Molecular reaction in hydrolysis opening of a trisiloxane ring. (Note pentacoordinate silicon.)

serves an enzymatic function providing a low energy pathway to create the dipeptide bond. The MO calculations show that addition of a second amino acid (glycine) to a trisiloxane + alanine cluster results in formation of an alanine–glycine dipeptide and release of a trisiloxane chain. The energy barrier of the saddle point for the biosynthesis reaction of alanine + glycine + trisiloxane is +17.9 kcal mol^{-1}. This barrier for polypeptide formation via inorganic biosynthesis is greater than enzyme-catalyzed peptide synthesis. However, this value is substantially less than the +70.6 kcal mol^{-1} barrier to the formation of peptide bonds without a common intermediate. Fig. 2b shows that the energy barrier for formation of a dipeptide bond without the presence of an enzyme-like structure is very high, +70.6 kcal mol^{-1} calculated with the same MO method.

These calculations demonstrate a possible, energetically feasible pathway for achieving the fourth ordering factor (d); i.e, the irreversible condensation reactions involved in protein synthesis, prior to the existence of enzymes.

The MO calculations also show that the metastable silica–amino transition states have an absorption band in the 220 nm region of the ultraviolet [19]. This is a significant finding because one of the most likely explanations for the chirality of life forms involves circularly polarized sunlight in this region of the spectrum [4]. Living organisms contain only levorotatory amino acids in proteins. When a racemic mixture of amino acids is exposed to right circularly polarized UV photons in this range of energy, the preponderance of amino acids that survive is levorotatory [4]. The L amino acids exposed to the metastable surface transition states of silica and silicates would be preferentially chemisorbed on the substrates due to ordering factor (b) listed above. The UV absorption at 220 nm would provide photocatalysis of peptides on the bioactive substrates thereby stabilizing the L amino acids in the proteins. The chirality of the photocatalyzed reaction would be maintained by selective polymerization of only the monomers which satisfied polymeric steric factors, ordering factor (e).

Thus, before the existence of enzymes or DNA the entropic barrier for disorder → order (non-living → living) illustrated in Fig. 1, would be overcome by the five ordering factors catalyzed by the bioactive substrate, as indicated in Fig. 6.

Lowering the entropic energy barrier (Fig. 6) combined with a low enthalpy of reaction of amino acids with metastable surface states (Fig. 2), and photo catalysis, leads to a plausible low free energy of reaction for protein synthesis and the disorder → order reaction required to initiate self propagating life forms from a nonliving mixture of amino acids.

6. Present day implications

The combination of specificity and variability of inorganic bioactive substrates [3] provides the foundation for what have become highly specific and enormously variable organic structures capable of being replicated over and over again. We can conclude that the inorganic origin of biopolymers is irreversibly and immutably locked into the very beginning of the genetic code.

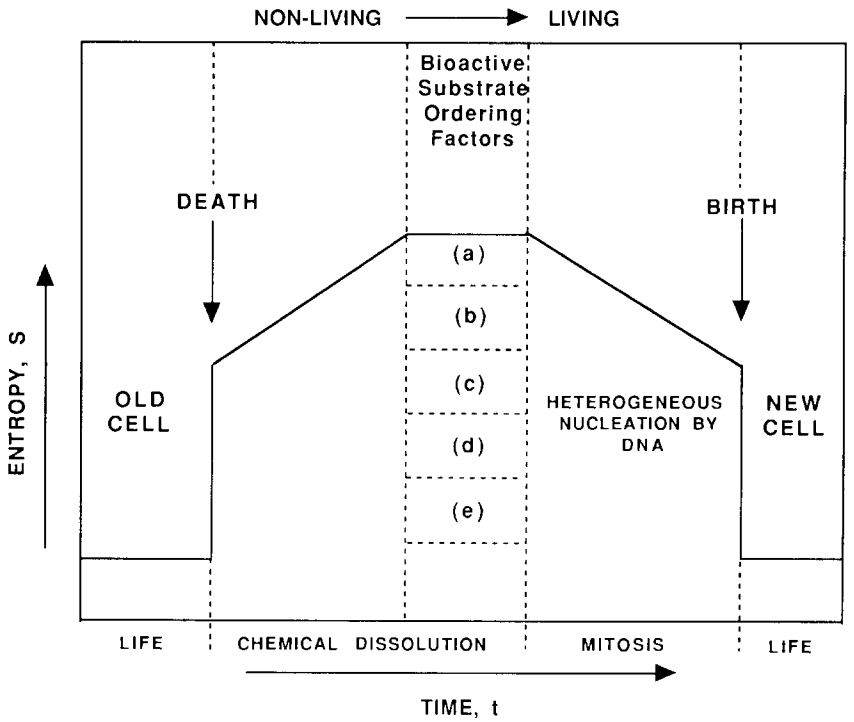

Fig. 6. Hypothesized effect of bioactive ordering factors (a–e) on overcoming entropic barrier in creating life.

Hildebrand et al. [20] have used modern genetic engineering techniques to demonstrate that certain genes are activated by hydrated silicon. Volcani reports that more than 60 genes are Si-sensitive [21]. Keeting et al. [22, 23] have shown that hydrated soluble silicon will enhance the proliferation of bone cells (osteoblasts) and active cellular production of transforming growth factors. These findings help explain the unique characteristics of certain compositional ranges of silicate glasses (termed bioactive glasses) which form a bond with living tissues [24–30].

Bioactive glasses have very narrow compositional boundaries to their ability to form bonds between living and non-living matter. Fig. 7 shows a ternary compositional cross section of the Na_2O–CaO–SiO_2–P_2O_5 glass system. There is a constant 6 wt% P_2O_5 in all the compositions. The limit of the bone-bonding boundary is at 60% SiO_2. Glasses with more than 60% SiO_2 are biologically inert. Glasses with compositions from 42 to 52% bond to both soft connective tissues as well as to bone [30]. These compositions are designated as having Class A bioactivity [26]. An important aspect of this compositional range is the osteogenic properties of the glasses. Class A bioactive glasses release concentrations of soluble hydrated silicon which activate bone cells to produce growth factors. The hydrated silica gel layer that forms on the glass surface [28] adsorbs and desorbs the cell growth factors which enhance bone growth many times faster than nonosteogenic Class B bioactive materials, and even more rapidly

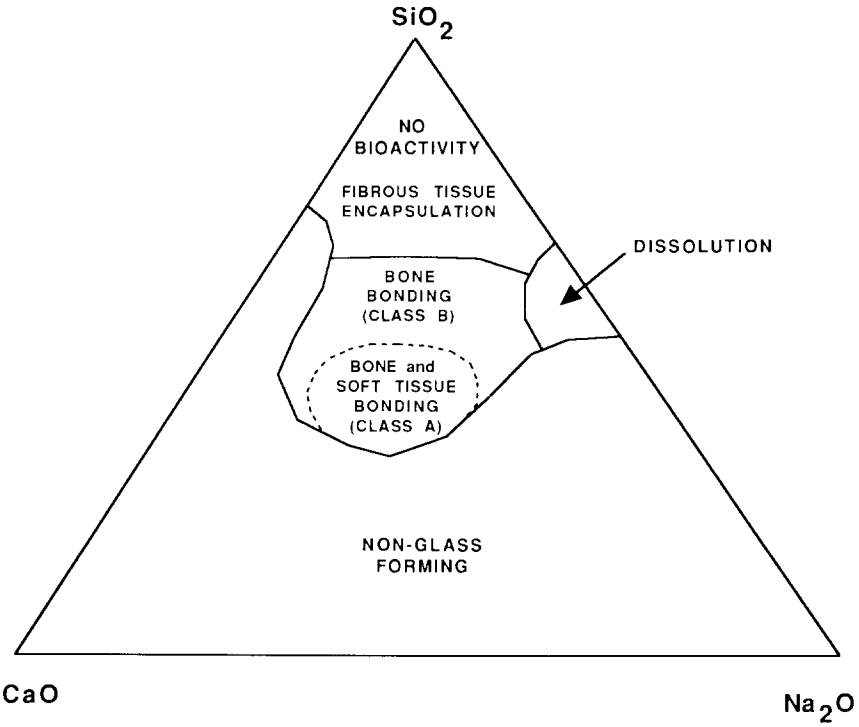

Fig. 7. Bioactive glass bonding boundaries for bone and soft tissues. All compositions contain 6 wt% P_2O_5.

than autogenous bone by itself [26, 31]. Details of the consequences of the sequence of the inorganic–organic reactions involved in growth of living matter to the nonliving substrate are given elsewhere [26].

A plot of the rate of surface reactions of silicate glasses as a function of SiO_2 content is shown in Fig. 8. The ratio of Na_2O/CaO in the glasses is maintained constant for the compositions plotted, thus Fig. 8 is a cross section of Fig. 7 which passes through the three regions of no bioactivity, Class B bioactivity, and Class A bioactivity. Fig. 8 looks remarkably similar to compositional plots for other types of phase transformation; i.e., paraelectric → ferroelectric; paramagnetic → ferromagnetic, etc, where small changes in composition affect the sensitivity of a structure to a change in symmetry and physical properties.

In the case of bioactive glasses the boundaries shown reflect the sensitivity of the nonliving material to inducing, or perhaps even controlling, the disorder → order transformations involved in forming the macromolecular structures of living tissues.

7. Implications for the future

The discovery that man-made materials may activate genes that produce growth factors has profound implications. Class A bioactive glasses are already being used

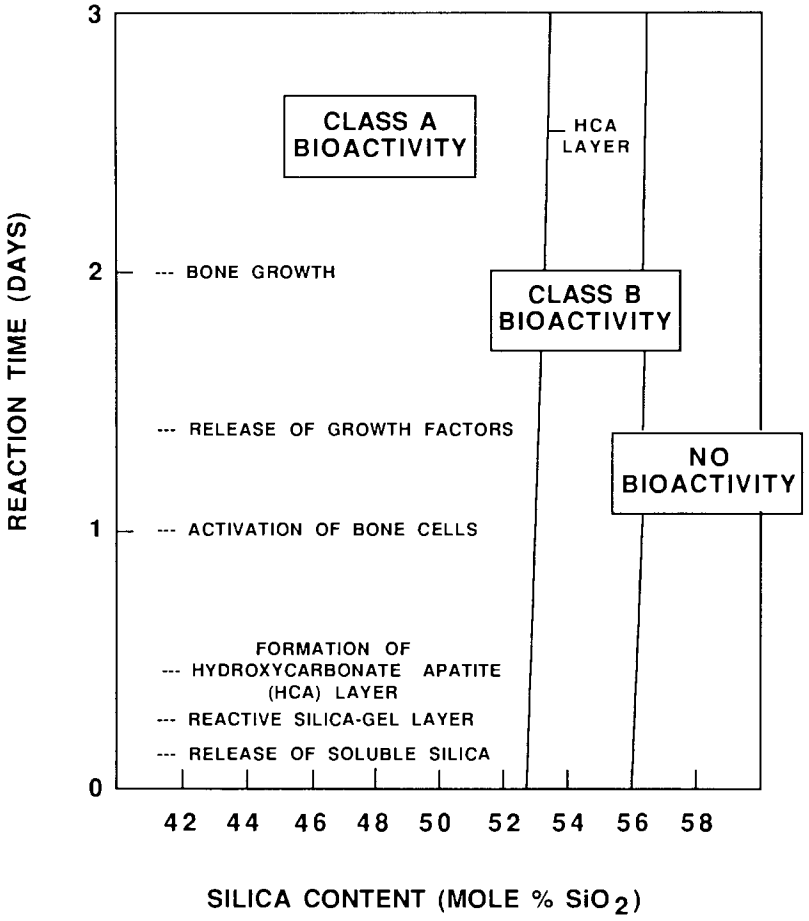

Fig. 8. Biological phase boundaries for Na_2O–CaO–P_2O_5–SiO_2 bioactive glasses as a function of SiO_2 content. (Note large differences in rate of biological reactions as the phase boundaries are crossed.)

clinically for replacement of the ossicles of the middle ear [32], as tooth root implants to maintain the stability of the alveolar ridge for edentulous patients [33], as a particulate to stimulate bone repair around teeth with previous periodontal disease [34], and as a particulate to augment bone repair around failed orthopedic devices. It should be possible in the future to use this concept of bioactive substrate activation of cells to reverse such devastating diseases as osteoporosis which is caused by a "slowing down" of the growth of osteoblasts. The understanding of inorganic activation of organic, macro-molecular ordering reactions may also lead to the design of therapeutic treatments for diseases of the skeletal system or perhaps even dietary supplements which will inhibit onset of the diseases. Thus, understanding the mechanisms underlying the mystery of the disorder → order transformation from nonliving to living offers hope for prolonging the quality of life, one of the most important goals of science today.

Acknowledgment

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