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Generation and Evolution of Metabolic Systems: Biophysical Approach to the Origin of Life.

Akira IKEGAMI

Abstract——The most important step for the origin of life from matter is the self-organization of irreversible systems to supply molecules and energy necessary for biological activities and reproduction. The generation mechanism of such systems is proposed based on the physicochemical properties of polypeptides and liposome like structures in the primordial sea. Substrates induced generation of enzymes is more favorable than the complementary duplication mechanism for the initial generation step of metabolic systems. Furthermore, evolution and reproduction of the metabolic systems are enhanced by the assembly of liposomes.

Key words: Metabolic System, Liposome, Stochastic Process, Origin of Life

1. Introduction

Oparin[1] proposed “coacervates” as the initial space for the generation of metabolic systems. Eigen[2] proposed “hypercycle theory” in the origin of life. The cycle includes two-generation processes from polynucleotides to polypeptides and vice versa without any molecular mechanisms to produce such opposite directions. Recently RNA molecules are suggested to be the main macromolecules for the origin of life because they have both enzymatic and self-duplication functions[3].

Life is a complex system composed of several functions, and the origin of life should be investigated on the generation of systems rather than the generation of specific biopolymers. The most essential system of life is the metabolic systems which supply molecules and energy necessary to keep and reproduce the whole life systems. The

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question is how to make such irreversible systems from an almost equilibrium primordial sea. To solve the question, a biophysical approach is proposed based on the physicochemical properties of polypeptides and lipid-like molecules in water without any biological properties or functions.

2. Statistical thermodynamic model of protein structures

Main frames of most proteins, especially enzymes, are composed of several α-helices or β-form structures cooperatively generated by many hydrogen bonds. Furthermore, the overall structures of proteins are supported by several kinds of weak secondary bonds like hydrogen bonds and hydrophobic bonds between side chains.

The stability and fluctuation of α-helical structures of poly-L-glutamic acid and that of copolymers of L-glutamic acid and L-alanine were studied by deuterium-hydrogen exchange experiments. The results indicate that even α-helical structures of polypeptides are not stable but fluctuate randomly at various environmental conditions. Similar fluctuation rates of lysozyme structure were observed by deuterium-hydrogen exchange experiments at various environmental conditions[4].

To discuss the stability and change of these fluctuating structures of proteins in aqueous solution, the statistical thermodynamic model using an equivalent and uniform lattice model was proposed[5] .

In the model, various tertiary structures of a protein were described by bonded or unbonded state of N_0 secondary bonds. All of N_0 secondary bonds in the ideal solution at 0°K correspond to the bonds in crystal. At a certain room temperature, some fraction of N_0 secondary bonds, $N_b \equiv (1+X)N_0/2$ take bonded states, but remaining fraction $N_u \equiv (1-X)N_0/2$ take unbonded states. All the possible secondary bonds N_0 are uniformly arranged on the lattice topologically similar to their distribution in the real protein structure at 0°K. They have the identical number of the nearest neighboring bonds Z and identical bond energy ε irrespective of their positions or species. The equivalent energy loss J is assumed for any two nearest neighbor bonds in the different bond states. The equivalent entropy of chains α is produced when every bond breaks. The probability to take the bonded state is the same for all lattice points at a certain equilibrium condition.

Then, Gibbs free energy of a protein molecule expressed by the molecular field approximation is given by

$$G(T,N_b) = N_u\varepsilon + N_bN_uZJ/N_0 - N_u\alpha T - kT\ln\{N_0!/N_b!N_u!\} \quad (1)$$

By the condition of minimum free energy, the most probable value of X, X_m, is given by

$$\tanh^{-1}X_m = (A + X_m)/BT - C \quad (2)$$

Where $A=\varepsilon/ZJ$, $B=2k/ZJ$, $C=\alpha/2k$ and k is the Boltzmann constant.

The equation (2) indicates that when molecular parameters of a protein, say PA, satisfy the following condition

$$\alpha ZJ/2\varepsilon k > 1 \quad (3)$$

the first order phase transition of X_m values is expected at a certain transition temperature T_t when a N_0 value of a protein takes infinity. When N_0 values of a protein takes a value between 10^2~10^4, some fraction of the protein molecules takes almost random coil conformation, but remaining fraction of the molecules takes nearly the crystal structure. A typical example of these situations is shown in Fig.4 in Reference 6.

Contrary, above the transition temperature T_t, most of the secondary bond generated at lower temperature decreases and random coil conformations appear.

When $\alpha ZJ/2\varepsilon k>1$, the number of bonded secondary bonds $N_b(T)$ is almost monotonously decreased without any transition.

To estimate the values of molecular parameters (N_0, ε, α, ZJ) in eq.(1), least squares analysis was applied[6] to the sharp heat absorption or specific heat measured by Tsong et al.[7] and Privalov[8] for typical globular proteins or enzymes (molecular weight 13600~25200).

As shown in eq.(1), the free energy of a protein in the lattice model is proportional to N_0 value. Then, we postulated that N_0 values of proteins or enzymes are strictly proportional to their molecular weights of proteins rather than their number of amino-acid residues as follows.

$$N_0 = 0.015MW \equiv \gamma MW \quad (4)$$

That is, N_0 value represents the total secondary bonds in a protein in the ideal solution at 0°K , or in the crystal even it includes extra molecules like heme groups. The

computer analyses of many protein structures in crystal[9] indicate that atomic arrangements of the inside protein structures are very compact like a closed pack state of atomic balls. Furthermore, more than 90% of polar groups in the inside structures are connected by hydrogen bonds. These analyses support our equivalent and uniform lattice model and the assumption (4).

Estimated values of ε, α and ZJ for five proteins are almost the same within 10~15% from their averaged values independent of their MW or pH values (see Fig. 1). Conditions of the first order phase transition (3) are satisfied for all five proteins. These results indicate that the actual values of structural states X, and its fluctuation δX of most proteins should be estimated by eq.(1) as the first approximation. That is, using N0 values obtained from MW by eq. (4), and the averaged values of parameters of ε, α and ZJ.

Furthermore, from the equilibrium probability density $P_e(T,X)$ near the minimum free energy point, the average thermal fluctuations $\delta X/X_m$ of the five proteins are about 10 to 20% depending on the temperature. The corresponding enthalpy fluctuations $N_0 \varepsilon \delta X/X_m$ of these proteins are estimated to be about 15~120 Kcal depending on their molecular weights 10000~40000. The values are almost comparable to the energy to separate many covalent bonds observed by contemporary enzymatic reactions, 40~120 Kcal.

These results suggest strongly that most of the activation energy needed for enzymatic reactions are supplied from the conformational change or fluctuation of the complex between enzymes and substrates.

By the similar “equivalent and uniform assumptions”, structural changes induced by pressure p, or by chemical potential of small molecules μ_i can be reasonably explained in a unified manner[5].

3. Mechanism of enzymatic reactions and substrate induced generation of primitive enzymes

Several important features are revealed for many contemporary enzymes. 1) Most enzymatic reactions are performed near room temperature without any high energy source. 2) The size of most enzymes are larger than their substrates, and binding site of substrates S on E are clefts or pockets originally generated in the structure of enzymes. 3) The reactions are usually reversible and have been well expressed by Michaelis-Menton equation which assumes the intermediate complexes ES or E(P+P’),

$$\begin{array}{ccccccc} & k+1 & k+2 & & k+3 & k+4 & \\ E+S & \Leftrightarrow & ES & \Leftrightarrow & E(P+P') & \Leftrightarrow & EP+P' \Leftrightarrow E+P+P' \\ & k-1 & k-2 & & k-3 & k-4 & \end{array} \qquad (5)$$

Note that the reaction schemes expressed by (5) are composed of two parts. The

second reaction between ES and E(P+P') indicates the reaction only within complexes at the equilibrium condition of temperature T and pressure p. The other three reactions in the scheme (5) depend mainly on the concentrations of small molecules S, P and P' in the solution.

To discuss the molecular mechanism of these enzymatic reactions, the same "equivalent and uniform" lattice model is applied for both E and ES complexes. That is, all the molecular parameters are unchanged, but total lattice points N_0 depend on molecular weights of enzyme E and ES complexes, MW_E and MW_{ES}, expressed by (4). Then, the mechanisms of reaction from S to P and P' and its reverse reaction are expressed as follow.

Suppose a small S molecule (MW_S) interacts with a fluctuating enzyme E and makes ES complex in a cleft or a pocket of the enzyme E, most of the additional lattice points $\Delta N_0=\gamma MW_S$ should be distributed near the contact surface between E and S molecules. Then, Gibbs free energy of the ES complex is given by changing N_0 to $N_{ES}=\gamma MW_{ES}$ by equation (4). The equation (3) indicates that the most probable structural state of ES complex, $X(ES)_m$, is always larger than $X(E)_m$ when equation (3) is satisfied. The excess number of secondary bonds generated by the ES complex formation at the same temperature T is given by $X(ES)_m\gamma MW_{ES}-X(E)_m\gamma MW_E>0$. When most of the excess secondary bonds generated by ES complex formation are concertedly broken by the aids of overall structural fluctuation of the complex, the corresponding excess energy revealed by the secondary bonds formation $(X(ES)_m\gamma MW_{ES}-X(E)_m\gamma MW_E)\varepsilon$ should be available to cut the weak covalent bonds in substrate S. The probability to make such concerted energy transfer should depend on the molecular shape and arrangements in the contact surface area between E and S molecules.

When S is a long molecule, the cleft is better than the pocket for the contact surface area between E and S, because it generates the additional secondary bonds at the surface of S molecule. Then, if the covalent bond energy to cut S molecule into P and P' molecules is comparable to the above value, the reactions from ES to E(P+P') and its inverse reaction are possible by the aids of structural fluctuations of the large E molecule at a certain equilibrium temperature T and pressure p.

The separation of ES complex produces sufficient energy to break a weak covalent bond in the complex. Especially breaking a covalent bond in the small substrate S is more probable than that in the large E molecule, because the separation of smaller products P and P' from larger E molecule is easier than the separation of the original large substrate S molecule.

The inverse reaction, from E+P+P' to E+S, is possible when concentrations of P and P'

molecules are large enough to make much E(P+P') complexes than to make ES complexes.

The stability of the complex should be increased by the entropy decrease near binding sites of the complex. In this case, formation of the complex increases the probability to make a weak covalent bond between P and P', or to dissociate the deformed EP complex without any chemical changes.

Anyway, interaction between larger polypeptides like E molecules and small molecules promotes the generation or degeneration of rather weak chemical bonds in small molecules using the concerted energy transfer of secondary bonds between them. When molecular size of polypeptides are much larger than small molecule, the probability to make concerted energy transfer from polypeptides to small molecules should be increased compared to the opposite direction. Because the conformational changes of many polypeptides are very cooperative as is shown in the denaturation of many proteins.

Similar energy transfers from secondary bonds to a weak covalent bond are expected between almost the same size of polypeptides, though its probability should be smaller than the case of ordinary sizes of E and S molecules. When several aminoacids are associated on a polypeptide, the generation of oligopeptides is possible though the probability is small compared to the case of ordinary enzymatic reactions. Though the generation probability of each oligopeptide should be very small, accumulated oligopeptides accelerate the generation of proper enzymes.

When the concentrations of substrates and aminoacids or oligopeptides in liposomes are large enough to generate new enzymes, the generations of long metabolic systems are possible.

4. The rate of metabolic path generation in a liposome

Suppose a certain local space in primordial sea contains full of prebiologically produced organic molecules. Lipid-like molecules produce bilayer membranes and thermodynamically stable liposomes are generated by the hydrophobic interaction induced by surrounding water molecules. The permeability of small molecules for the liposome membrane depends on their physicochemical characters. Some molecular species are permeable for the membrane almost freely, but most species are impermeable except leak induced by the incomplete structure of the membranes. Therefore, the concentration and composition of small molecules in liposomes become equilibrium with that in the outer space after leak periods.

In these almost equilibrium condition, the generation of primitive enzymes E_1 are possible which catalyze the reaction from substrate S_1 molecules to new S_2 molecules, using S_1 molecules themselves as the molds. When both S_1 and S_2 molecules are permeable for liposome membrane, the liposome creates a unit of irreversible metabolic cycle. When S_2 is impermeable though S_1 is permeable, S_2 molecules accumulate in the liposome, and generate new primitive enzymes E_2, which catalyze the reaction from S_2 to new S_3.

The formation of small space separated from the whole space by liposome membranes is favorable than the free space for the generation of metabolic system using any molds. Even if E_i molecules are generated with the same probability to the inner surface, it could not form any metabolic paths because product S_{i+1} molecules diffuse into whole space. The formation of a small space separated from the whole space by the liposome like membrane is one of the essential factors for the generation of irreversible catalytic processes.

Because the driving force of the metabolic flow is originated in the internal energy difference between substrates S_1 and S_{n+1} molecules revealed by enzymatic reaction, the metabolic flow will continue, until all of S_1 molecules in bulk sea convert into S_{n+1} molecules.

Denote $e_i(r,t)$ and $s_i(r,t)$ as the molar concentrations of pre-enzyme E_i and substrate S_i in a liposome at time t. The generation of pre-enzymes and substrates in a small space of liposomes are shown in Fig. 2 and expressed as follows, when the membrane permeability of S_1 and S_{n+1} molecules are much faster than other substrates and pre-enzymes:

$$de_i/dt = (\kappa_i s_i + \alpha_i e_i) o_{ij} \qquad (1<i<n) \tag{6}$$

$$ds_{i+1}dt = \lambda_i e_i s_i - \lambda_{i+1} e_{i+1} s_{i+1} \tag{7}$$

The first $\kappa_i s_i$ term in equation (6) is the generation rate of E_i molecules induced by its substrate S_i as a mold. The second $\alpha_i e_i$ term is the self-duplication rate of E_i molecules, and o_{ij} are the concentration of constituent monomers or oligomers of E_i molecule. As the rate of E_i generation is very slow, consumed fraction of o_{ij} molecules are supplied through the membrane.

Equation (7) expresses the enzymatic reactions in the metabolic system, where λ_i is the reaction rate from substrate s_i to s_{i+1}, catalyzed by pre-enzyme E_i.

Generation processes of the metabolic system in a liposome are simulated for typical cases. When only the substrate induced generation of pre-enzyme s_i increases rapidly at

the initial stage of E_i generation and reaches to the stationary value $\underline{s_i}$, that is $\alpha_i=0$ then the concentration of E_i molecules in the stationary state $\underline{e_i}$ increases at a constant speed.

The processes under such stationary states are given as follows,

$$d\underline{s_i}/dt = \lambda_{i-1}\underline{e_{i-1}}\underline{s_{i-1}} - \lambda_i\underline{e_i}\underline{s_i} = 0 \quad (8)$$

$$\underline{s_i} = \lambda_{i-1}\underline{e_{i-1}}\underline{s_{i-1}}/\lambda_i\underline{e_i} = \lambda_1\underline{e_1}\underline{s_1}/\lambda_i\underline{e_i} \quad (9)$$

$$d\underline{e_i}/dt = \kappa_i\underline{s_i} \quad (10)$$

$$\underline{e_i} = \kappa_i(\lambda_1\underline{e_1}s_1/\lambda_i\underline{e_i})(t-t_i) \quad (11)$$

where t_i is the lag time required to reach stationary state of E_i generation in the liposome. Then, in the following stationary period of $t > t_i$,

$$\underline{s_i} \cong s_1\sqrt{\lambda_1\kappa_1}/\sqrt{\lambda_i\kappa_i} \quad (12)$$

$$\underline{e_i(t)} \cong \kappa_i\underline{s_i}t = s_1\sqrt{\lambda_1\kappa_1\kappa_i/\lambda_i}\,t \quad (13)$$

The generation rate of a metabolic system (E_1.....E_n) depends only on κ_n/λ_n value in the stationary periods after t_n. To see the effect of self-duplication mechanism, eqs. (6) and (7) are simulated using several α_i values. When $\alpha_i=\kappa_i$, the simulated schemes of s_i generation are nearly the same with the case of $\alpha_i=0$, because the concentration of mold S_i is much higher than that of mold E_i at the initial stage of metabolic path formations. The self-duplication system of pre-enzymes is not the necessary condition for the generation of metabolic systems in a liposome.

5. The rate of metabolic path generation and evolution in the macroscopic system, "the living system"

Suppose a number of liposomes exist in a macroscopic space of the primordial sea. The average size and lifetime of liposomes are estimated by the dynamic equilibrium between the rate of fission k_f induced by movements of bulk waters, and the rate of fusion k_a induced by encounter of two liposomes. The most reasonable lifetime of liposomes, τ_1 is estimated to be the order of 10 to 10^3 seconds depending on the concentration of lipid-like molecules (Table 1). That is, the molecular components in every liposome are mixed with their neighboring liposomes by their encounters. As the generation rates of each pre-enzyme in a liposome should be very slow compared to the lifetime of liposomes, the overall generation rates of a metabolic system in the macroscopic system is restricted by the diffusion rate of liposomes.

The metabolic path formations in such a macroscopic space[10] are given by the following expressions by expanding L times the space unit of microscopic equations (6) and (7).

$$dE_i/dt = (K_i S_i + A_i E_i)\Pi_{ij} O_{ij} + D_1 \partial^2 E_i/\partial R^2 \quad (14)$$

$$dS_{i+1}/dt = \Lambda_1 E_1 S_1 - \Lambda_i E_{i+1} S_{i+1} + D_1 \partial^2 S_{i+1}/\partial R^2 \quad (15)$$

Here D_1 is the diffusion constant of liposomes. E_i is the concentration of liposomes at (**R**, t) which includes only one E_i molecule irrespective of any other components. Similarly, S_i is the concentration of liposomes at (**R**, t) in which S_i molecules are saturated at the stationary concentration $\underline{s_i}$ irrespective of other components. The concentration of all liposomes is much higher than the concentrations of E_i molecules at **R**, especially at the initial stage of the metabolic path generation. Then, S_1 is equal to the concentration of all liposomes because the concentration of S_1 molecules in all liposomes is equal to that in outside of liposomes c_1^0. These expressions do not affect the final results, because the total numbers of E_i or S_i molecules in a unit volume at **R** are expressed directly by E_i or S_i respectively.

The macroscopic equations (14) and (15) are homologous to the microscopic equations (6) and (7) except the diffusion of liposomes. Suppose the first E_1 molecule is created in a liposome somewhere in primordial sea by the substrate induced mechanism. If the catalytic rate of the E_1 molecule is large enough to reach the equilibrium concentration $\underline{s_2}$ within the lifetime of liposomes τ_1, the E_1 molecule converts much more S_1 molecules to S_2 through the fusion and fission with other liposomes surrounding it. Then, the generation of S_2 molecules in the macroscopic system should not be saturated but regulated by the diffusion of the liposomes which include E_1 molecules. By similar processes, n-step metabolic system E_1, E_2, ...E_n should be expanded in the macroscopic system.

As the diffusion constant of liposomes is much smaller than molecules (see Table 1), the evolution rate of E_i generation induced by substrates in the macroscopic system is given as follows.

$$dE_i/dt = K_i S_i + D_1 \partial^2 E_i/\partial R^2 \quad (16)$$

The difference between macroscopic and microscopic systems is the L^3 times accelerated generation in the macroscopic system. The lag time t_i needed for generation of the first E_i molecule in the macroscopic system can be neglected, because its value is

about L^{-3} times smaller than in a liposome.

As the most “liposomes E_1~E_n” generated from the first living liposome E_1 at R_1=0 are confined within the radius $<R_n^2> = 4D_1t$ (see Table 1) by slow diffusion late, whole living liposomes in such a small space could be assumed as a kind of “living system (LS)”, which generates, grows and evolves the metabolic systems from E_1 to E_n, eating molecules and liposomes in its surroundings.

As a pre-enzyme E_i is generated by spontaneous condensation reactions of oligomers associated on the substrate S_i as a mold, the generations of several kinds of E_i molecules with different structures and activities are expected.

To discuss the evolution of the macroscopic metabolic systems, let us suppose two such pre-enzymes, E_i and E_i’, are generated in the stationary state of a metabolic system. Then the generation rates of proenzymes E_i and E_i’ are expressed by

$$d\underline{S_i}/dt = \Lambda_{i-1}\underline{E_{i-1}}\underline{S_{i-1}} - \Lambda_i\underline{E_i}\underline{S_i} - \Lambda_i'\underline{E_i'}\underline{S_i} \quad (17)$$

$$d\underline{E_i}/dt = K_i\underline{S_i} = K_i\Lambda_1\underline{E_1}\underline{S_1}/(\Lambda_i\underline{E_i} + \Lambda_i'\underline{E_i}) \quad (18)$$

$$d\underline{E_i}'/dt = K_i'\underline{S_i} = K_i'\Lambda_1\underline{E_1}\underline{S_1}/(\Lambda_i\underline{E_i} + \Lambda_i'\underline{E_i}) \quad (19)$$

If K_i’>K_i, then $d\underline{E_i}'/dt = d\underline{E_i}/dt$, in other words, the generation speed of E_i’ molecules exceeds that of E_i molecules in the living system irrespective of Λ_i or Λ_i’ values. By the same mechanism, the pre-enzymes with larger K_i values are selected for every metabolic step and become predominant in the living system.

Since the rates of growth, and then evolution of living systems are restricted by the small diffusion constant of liposomes, much more accelerated encounters between living systems are expected, when we take into account the effect of waves or tidal currents. Apparent diffusion constants estimated from these macroscopic movements of bulk water, suggest very fast evolution and reproduction of metabolic systems[10].

6. Discussion

Since the proposed generation mechanisms of metabolic systems are based on the physicochemical properties of polypeptides and lipid-like molecules in water, essentially the same types of generations and evolution mechanisms are expected for primordial age of the earth.

In substrate induced generation of protein enzymes, complexes between a substrate and several peptides become unstable after their catalytic activity works. That is, when a pre-enzyme gets a higher enzymatic activity, it promotes the further generation of

proper enzymes. Namely, natural selection in the functional level is useful in the initial generation processes of metabolic systems. Mutational selection at information level should be useful only after the generation of fundamental metabolic systems.

When pre-enzymes are ribozymes like in “the RNA world”, several difficulties are expected compared to “the protein world”. One of the important difficulties is how to get energy to separate chemical bonds in the substrate of “ribozyme” . In the proposed mechanism of protein enzymes, the energy to separate a chemical bond in a substrate is supplied from the concerted generation of several secondary bonds between the substrate and the enzyme.

On the contrary, structures of various t-RNA molecules are composed of typical base pairs which are almost stable at room temperature and difficult to make many three-dimensional structures. Micro-calorimetric recording of heat absorption observed for various transfer RNA molecules in aqueous solution, several absorption peaks were reported by Privalov[11]. These results indicate that several steps are necessary for overall structural change of t-RNA tertiary structure. A number of different conformational steps are unfavorable for concerted energy production necessary for enzymatic reactions.

The other important difficulty to generate ribozymes from monomer or oligo-nucleotides is the lack of information. Even if a liposome can supply sufficient amount of monomers through membranes, the probability to find a unique sequence for E_i molecules composed of 100 monomers of 4 different species is $1/4^{100}$. The value is too small to find a specific sequence in the whole space and in lifetime of the earth. Furthermore, it is difficult to duplicate the specific ribozyme E_i without any mechanism or information to select the specific sequence from a number of random copolymers.

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Table 1. Physicochemical properties of liposomes. To estimate the average size and life time of liposomes, suppose two sizes of liposomes, L_1 and L_2, are present in the primordial sea, where L_2 is the liposome composed twice of lipid molecules in a L_1 liposome. Then the concentration of $[L_1]$ and $[L_2]$ at equilibrium condition are given by $d[L_2]/dt=4R_1T/3\eta[L_1]^2-k_f[L_2]=0$. The first term represents the diffusion controlled fusion of two L_1 liposomes, and the second term represents the fission of L_2 liposomes induced by the water movements. To estimate the equilibrium size distribution of liposomes, we put, $4R_1T/3\eta=k_f$. When liposomes are spherical form, $2m[L_2]+m[L_1]=[M_0]$, where $[M_o]$ is the total concentration of lipid molecules in the solution, and m is the number of lipid molecules in a L_1 liposome.

For the estimation of numerical values shown in Table 1, let us assume tentatively that a cylinder shape of radius 3Å and length 40Å and molecular weight 800 for the lipid-like molecules.

Wt fraction of lipids (gr/l)	10^{-1}	10^{-1}	10^{-3}	10^{-3}
Diameter of liposome R (m)	10^{-7}	10^{-6}	10^{-7}	10^{-6}
Diffusion constant D at 300°K	$2.5*10^{-12}$	$2.5*10^{-13}$	$2.5*10^{-12}$	$2.5*10^{-13}$
No. of liposomes/l	$7.5*10^{13}$	$7.5*10^{11}$	$7.5*10^{11}$	$7.5*10^{9}$
Life time of liposomes (sec)	1.2	120	120	12000
$\langle x\rangle = (2Dt)^{1/2}$				
after 1hr (m)	$1.3*10^{-4}$	$4.2*10^{-5}$	$1.3*10^{-4}$	$4.2*10^{-5}$
after 1 year (m)	$1.2*10^{-2}$	$4.0*10^{-3}$	$1.2*10^{-2}$	$4.0*10^{-3}$
after 10^4 year (m)	1.2	1.2^{-1}	1.2	1.2^{-1}

Fig. 1. Estimated molecular parameters for five globular proteins or enzymes.
Abbreviations: Cyr ○ : cytochrome c; Rna □ : ribonuclease A; Lys Δ: lysozyme; Mgl ● : myoglobin; Chm ■ : α-chymotrypsin;　The energy is expressed in kcal/mole and the entropy is in eu. Vertical bars indicate the range of estimated parameter values at various pH range shown in the upper space.

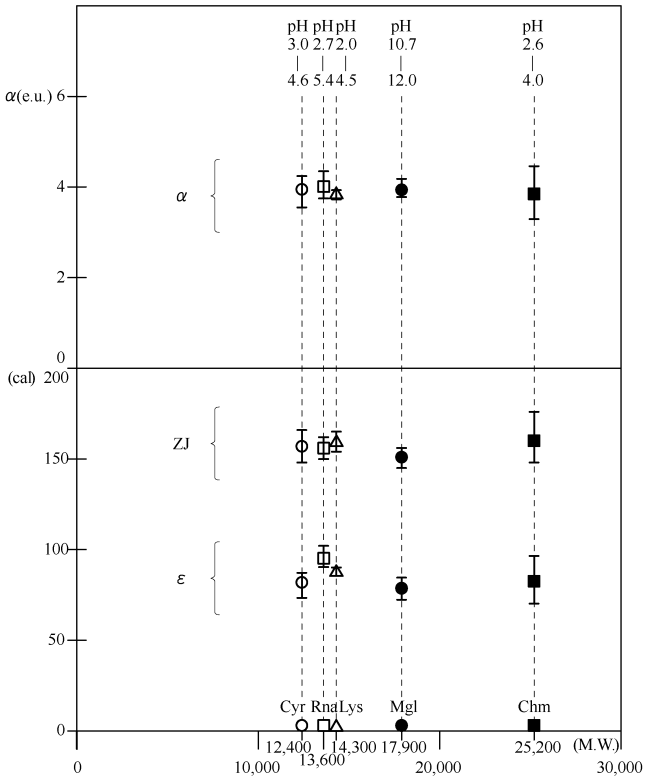


Fig. 2. Reaction schemes in a liposome.

