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Interactions Between Protein Molecules

Tatsuo Ooi*

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Tertiary structures of globular proteins determined by X-ray crystallography indicate configurations of peptide backbones and locations of side chains. The conformation in a native state thus determined may be elucidated by mutual interactions between atoms in the molecule. There are many proteins which exist in aggregated forms *in vivo*, and *in vitro*. These aggregations arise from interactions between unit molecules, and some of proteins show reversible polymerization-depolymerization phenomena with changes in their environments. On a phenomenalogical point of view, these processes are treated by thermodynamics for systems which contain reacting molecules. As a result, the equilibrium constants seem to be factors that control association and dissociation. The forces responsible for intermolecular interactions to bind two or more protein molecules are nothing but those of intramolecular interactions. Discussions are given on such interaction energies between constituent atoms in proteins. Finally, examples are illustrated on the experimental results obtained on polymerization-depolymerization properties of muscle proteins, actin and tropomyosin, and possible mechanisms are discussed.

I. INTRODUCTION

Recent progress in X-ray analysis gives rise to fruitful results on determination of the tertiary structure of globular proteins such as hemoglobin[1], myoglobin[2], lysozyme[3], ribonuclease A[4], ribonuclease S[5], chymotrypsin[6], and caboxypeptidase A[7]. These three dimentional structures obtained so far indicate the compact packing of constituent amino acid residues, side chains of which interact each other to form a stable conformation characteristic for each native protein molecule. These proteins are rather simple and limited ones, because crystallization followed by heavy metal substitution is the minimal condition necessary for X-ray analysis[8].

There are many proteins which can not grow into three dimensional crystals at present stage because of their self-aggregative property. Even though tertiary structures are not yet determined due to difficulty to make crystals, interesting studies are now developing on those proteins. For example, subunit structure of an allosteric enzyme is established by separation of the molecule into catalytic and regulatory subunits[9]. Association and dissociation phenomena also have been elucidated on various bases[10], reconstitution of regular array of protein molecules, and polymerization-depolymerization process of fibrous proteins[11].

When an attention is focused to intermolecular interactions between protein molecules, there is no way to distinguish interaction potentials from those acting

* 大井 龍夫 : Laboratory of Physical Chemistry of Enzyme, Institute for Chemical Research, Kyoto University, Uji, Kyoto.

between intra-molecular atoms, namely, non-bonded potential, electrostatic potential, hydrogen bond, *etc.*[12] The difference is in the magnitude of total energy, since dissociation into isolated monomers can be achieved without breaking of intramolecular interaction. In some cases, reversible association and dissociation process can be obtained only by changing the evironment such as salt concentration[11], pH[13], and temperature[14]. These experimental results show that weaking of intermolecular forces responsible for association is of smaller magnitude compared with the energy for disruption of intramolecular forces which brings about unfolding of the molecule. Of course, some conformational change may be expected during association and dissociation process, and there are some experiments to suggest the conformational change of the molecule on polymerization[15], although the extent of the change is usually small.

In this article, we shall treat systems of reversible association and dissociation on the basis of statistical thermodynamics, and will discuss the nature of intermolecular forces. The meaning of "association" includes irregular aggregation typically illustrated by isoelectric precipitation, but this type of association is excluded here. That is, the system of association has some regularity; one dimensional array corresponds to linear polymerization found in fibrous proteins, two dimensional arrangement to membrane structures, and three dimensional regular association to protein crystals. In other words, a type of crystallization is the process to be dealt with.

II. THERMODYNAMICS OF ASSOCIATION AND DISSOCIATION[16]

Let us consider a system consisting of N_0 macromolecules in a volume V, which are polymerizable into dimer, trimer, and so on at a given solvent condition. That is, the system contains monomer, dimer, trimer, and i-mer molecules in equilibrium. When the numbers of associated molecules denote N_1, $N_2, \cdots$, N_i in the volume V,

$$\sum_{i=1}^{i} i\ N_i = N_0 \quad , \tag{II. 1}$$

or by using a number concentration $C_i = N_i/V$

$$\sum_{i=1}^{i} i\ C_i = C_0 = N_0/V \quad . \tag{II. 2}$$

The mass action law may be applied to the equilibrium reaction between i-mer M_i and monomer M_1,

$$M_i + M_1 \rightleftarrows M_{i+1} \quad , \tag{II. 3}$$

then,

$$C_{i+1} = K_i C_i C_1 \quad , \tag{II. 4}$$

where K_i is an equilibrium constant. In general, C_i can be expressed by K_i and C_1 as follows;

$$C_i = \sum_{k=1}^{i-1} K_k C_1^{\,i} \quad . \tag{II. 5}$$

Substituting (II. 5) in (II. 2), we have

$$\sum_{i=1} i \sum_{k=1}^{i-1} K_k C_1^i = C_0 \quad . \tag{II. 6}$$

Therefore, if every K_i is given by the solvent condition, one can obtain the concentration of i-mer C_i at a protein concentration of C_0. The following examples are special ones, which will be found experimentally.

(I)[17] Every K_i is equal to K regardless of i. Since (II. 5) can be written in a simple form

$$C_i = (KC_1)^i K^{-1} \quad , \tag{II. 7}$$

summation in (II. 6) can be easily done,

$$C_1/(1-KC_1)^2 = C_0 \quad . \tag{II. 8}$$

Therefore, C_1 is determined by the total concentration C_0 and the constant K as follows;

$$C_1 = \frac{1}{2K^2C_0}\{1+2KC_0-\sqrt{1+4KC_0}\} \quad , \tag{II. 9}$$

where C_1 is equal to C_0 on infinite dilution $C_0 \to 0$. Once C_1 is determined, C_i can be calculated by (II. 7), substituting C_i in (II. 7).

(II) K_i has a constant value of K_1 for i less than i_0 and K_2 for i greater than i_0. Corresponding to (II. 7), we have the following equations,

$$\left.\begin{aligned} C_i &= K^{-1}(KC_1)^i && i \leq i_0 \\ C_i &= K_1^{-1}(K_1/K_2)^{i_0}(K_2C_1)^i && i > i_0 \end{aligned}\right\} \tag{II. 10}$$

and (II. 6) takes a form

$$C_0 = \frac{C_1}{1-K_1C_1} + K_1^{-1}(K_1C_1)^{i_0}\left[\frac{1}{1-K_2C_1} - \frac{1}{1-K_1C_1}\right] \tag{II. 11}$$

The equation (II. 11) is not easily solved, but the general process of polymerization can be estimated by this equation, since the size of seed, i_0, is assumed to be finite. When C_0 is increased from a very low concentration where K_1C_1 is much less than unity, the second term of the left-hand side of equation (II. 11) can be neglected until K_2C_1 becomes nearly equal to 1. Above the concentration C_1 nearly equal to $1/K_2$, the second term will exceed the first term and then become dominant. Therefore, the number of polymer greater than i_0-mer increases with the concentration C_0 beyond the concentration $1/K_2$.

(III) End effect is taken into accouut. When aggregation occurs so as to form a regular structure such as tubule or helix[18], the molecules located at both ends have different energy from the other molecules situated in the middle. This end effect can be introduced by multiplying a factor A to equation (II. 4), since kT ln A may correspond to the free energy due to the end effect.

Combining (II. 10) and the end effect for large aggregates, one may rewrite equation (II. 10) as follows;

$$\left.\begin{aligned} C_i &= B_i C_1^i \qquad i \leq i_0 \\ C_i &= AD(K_2 C_1)^i \qquad i > i_0 \end{aligned}\right\} \tag{II. 12}$$

where B_i and D are constants in (II. 10). The total concentration C_0 is equal to the sum of amounts of i-mer in the solution;

$$C_0 = \sum_{i \geq 1} i C_i = C_1 + \sum_{1 < i \leq i_0} i B_i C_i^i + ADC_1[1/(1-K_2C_1) - \sum_{1 < i \leq i_0} i (K_2C_1)^{i-1}] \tag{II. 13}$$

This equation shows the similar feature of polymerization to the case (II). That is, polymerization to large molecules does not occur and nearly all the molecules exist in monomer form (the first and second terms) until C_1 approaches $1/K_2$ ($C_1 \approx C_0$), where the third term becomes large, indicating the appearance of polymer molecules. When C_0 is increased over $1/K_2$, the concentration of C_1 or monomer concentration remains almost constant at $1/K_2$, and the amount of polymer increases linearly with C_0. This phenomenon corresponds to a condensation type transition from monomers to polymers.

In these three cases, the factor that controls polymerization is the equilibrium constant K, which is equal to exponent of a free energy difference $\Delta F/RT$. The free energy is dependent on a conformation of a given protein molecule, conditions of surrounding media, pH, salts, and thermodynamical parameters, temperature and pressure. When a variable S denotes one of these parameters, dependence of the number average polymerization degree $<i>_n$ on S for case (I) may be expressed by,

$$\frac{\partial <i>_n}{\partial S} = -KC_1 <i>_w \frac{\partial \ln K}{\partial S} = -KC_1 <i>_w \partial(\Delta F/RT)/\partial S \tag{II. 14}$$

where $<i>_w$ is the weight average polymerization degree which is equal to $(1-KC_1)/(1+KC_1)$. The equation (II. 14) shows that the effect of free energy change on $<i>_n$ is significant for a larger aggregate, since $<i>_n/S$ is directly proportional to $<i>_w$.

III. INTRA-AND INTER-MOLECULAR FORCES

The polymerization of protein molecules is influenced through a change in free energies between a polymer and monomers, and the free energy may be determined by a location of side chains exposed outside of protein molecules, and by the surrounding solvents. The problem is what kinds of forces are responsible for interactions between protein molecules, the forces which should be strong enough to bind two or more molecules together but weak enough to dissociate into monomers again. These forces are not covalent, and one can not distinguish intermolecular forces from intra-molecular forces, because there is no specific charactor in the interactions between constituent atoms of molecules.

The interaction forces to be acting may be the following; torsional energy, non-bonded energy, electrostatic energy, hydrogen bond energy, and hydrophobic

bond energy.

a) **Torsinal energy**

The torsional energy comes from the exchange intergral of σ-electrons such as three-fold potential for rotation about *C-C* bond of ethylene. Along the polypeptide main chain, two rotational freedoms, $\phi(N\text{-}C^{\alpha})$, and $\psi(C^{\alpha}\text{-}C)$, have a contribution to torsional energy, the extent of which is not significant, since the barrier height for rotation may be estimated less than 1 Kcal[19]. On the other hand, rotational barrier about *C-C* bonds in side chains may be nearly 3 Kcal[20], so that the position of a carbon atom next to another carbon is restricted approximately at rotational minima, trans and two gauche positions. In intermolecular interaction, this term does not seem to play an essential role.

b) **Non-bonded energy**

Non-bonded energy consists of repulsion between atoms and attraction due to London force, or dispersion force[21]. Although this attractive energy is small in magnitude, an order of a few tenths Kcal, contribution of the energy to intramolecular stability is important because of the presence of many atomic pairs. As for intermolecular interaction, it may play a part of binding especially at a short distance, since the equilibrium distance of minimum energy is approximately 3 Å and farther separation gives rise to marked decrease in attraction as shown in inverse proportionality of six power of the distance. Presumably, at a site of contact between molecules, the non-bonded energy including steric configuration may be of a great importance.

c) **Electrostatic energy**

Groups involved in the electrostatic interaction are divided into two, one permanent dipoles and the other net charges. This energy is essentially Coulombic interaction inversely proportional to the distance between two charges; repulsion between the same signs and attraction between the opposite signs. Properties of the electrostatic interaction to mention are of long range charactor and its strength in magnitude, about 100 Kcal/mole at 3 Å separation of unit charges in vacuum. However the energy diminishes with the increase in dielectric constant *D* and the presence of salts, that is, the energy is easily influenced by the condition of medium surrounding the molecules. The energy between dipoles is similar to that between charges, except for the effective range of the energy, which is shorter than for Coulombic energy, inversely proportional to cube of the distance. The electrostatic energy seems to play an important role in polymerization of molecules, because a change in the pH alters the charge distribution on a surface of a protein, and the addition of salts induces polymerization or depolymerization of proteins as was shown in the case of muscle proteins, actin and tropomyosin.

d) **Hydrogen bond**

The hydrogen bond energy is regarded as one of the most important factors that stabilize the conformation of a protein molecule, an example being demonstrated in the formation of α-helix found by Pauling and Corey[22]. The hydrogen bond seems to be present in the binding sites of polymerized molecules, since there are many hydrophilic groups on the surface of the molecule which are

capable to form this bond. One feature to be noticed on the hydrogen bond is the effect of a solvent, water; that is, the groups at the surface may form hydrogen bonds to water molecules, instead of formation of the bond with another protein molecule. The difference of the energy between those bindings is less than 1 Kcal/mole, so that the hydrogen bond can not be the only energy to combine molecules. However, if the groups are hydrogen-bonded to water molecules and they are involved in polymerization sites, these groups must form hydrogen bonds to some groups of another molecule; otherwise, loss in energy of breaking hydrogen bonds to water (4 Kcal/mole) gives rise to inhibitory effects on the formation of polymers.

e) Hydrophobic bond[23]

The role of hydrophobic bond in aggregation is thought to be essential in some case. The hydrophobic bond energy is different from those described above on the origin of energy. The principal origin of this energy is due to arrangement of environmental water molecules. Therefore, the temperature dependence of this bond is positive[12] contrary to negative dependence of the internal energy due to the energies mentioned previously, suggesting the entropic interaction in nature. When a part of a surface of polymerizable molecules be hydrophobic, the patch of the molecules binds together to form dimer or polymer by hydrophobic bonds. The hydrophobic bond is independent of the pH or presence of salts, unless the structure of water is modified by surface charges or small ions in solutions. Therefore, criterion to identify the hydrophobic bond is to measure temperature dependence and the extent of energy over a wide range of the pH and the salt concentration.

f) Other energies

There might be other interaction energies than those described above, *e. g.*, delocalization energy or a long range force. At present stage, those energies are not so well investigated as to pay a special attention to a role of stabilization for protein aggregation.

IV. INTERACTIONS BETWEEN PROTEIN MOLECULES

The factors which control polymerization or aggregation are pH, salt concentration, temperature, pressure, and chemical reagents. Since the regular aggregation occurs under the change in evironments, the contact of the molecule must show a regular pattern on the surface; that is, the binding site(s) must locate at the specified position(s) of the molecule. Therefore, the first requirement for polymerization is to take such a conformation as to form a localized polymerization site. When the conformation is changed by an environmental condition, depolymerization or irregular aggregation may occur. The second requirement is to interact with each other by aforementioned energies.

a) Electrostatic energy and salt effects

The charge distribution on a surface of a protein molecule is determined by pH. Typical ionizable groups in proteins are basic groups of α-amino of *N*-terminal, ε-amino of lysine, and guanidyl of arginine, and acidic groups of α-carboxyl of *C*-terminal, β-carboxyl of aspartic acid and γ-carboxyl of glutamic acid. In addition,

a few groups can be ionized, imidazol groups of histidine, thiol of cysteine, and phenolic of tyrosine. The pK values of these groups are listed in Table 1[24]. At neutral pH, a common physiological condition, basic and acidic groups are in positively and negatively charged state, so that the net charges of the protein are negative or positive dependent on the excess number in charged groups. Because of repulsive nature between charges of the same sign, and because of a long range charactor of electrostatic interaction, one might expect separation of protein molecules by repulsive force due to net charges, especially at extreme pH where only positive or negative charges are present on the surface. Except some cases, depolymerization occurs in common at alkaline or acidic pH as expected. However, at neutral pH, the molecules are polymerizable, even when the repulsion between net charges is present. There must be some attractive forces to bind molecules each other.

Table 1. pk values of ionizable groups in protein.

ionizable group		pk
α-COOH	(*C*-terminal)	3.6
β-COOH	(Asp)	4.5
γ-COOH	(Glu)	4.6
Imidazol	(His)	6.2
α-NH_2	(*N*-terminal)	7.8
Thiol	(Cys)	9.5
ε-NH_2	(Lys)	10.4
Phenol	(Tyr)	9.7
Guanidyl	(Arg)	ca. 12.0

Among the interaction energy described previously, local electrostatic energy and hydrophobic bond energy in addition to non-bonded energy seem to be important for attraction against repulsion between net charges. Even though the net charges of the molecule are negative or positive, the net charges are resultant of both positive and negative charges of ionizable groups. Therefore, some attraction is expected when the groups are separated in a short distance. For instance, if charges of opposite signs are lined up alternatively on a rigid rodlike molecule, attractive forces between charges of opposite signs of two rods overcome repulsive force between net charges.

The addition of salts is also effective on electrostatic interaction as shown by Debye-Hückel theory of strong electrolytes. Small ions in solutions form ion atomospheres around charged groups on a molecule, giving rise to a shielding effect of electrostatic field produced by the charge. As a result, the repulsive force between net charges diminishes with increasing salt concentrations or ionic strength. Therefore, molecules separated by repulsion between net charges in salt free solutions may become polymerizable on the addition of salts, because attractive forces such as hydrophobic bond initially weak to combine molecules may become effective when the repulsion is weakened by small ions added.

Since the cohesive forces are non-covalent in nature, aggregated molecules

should be depolymerized into unit monomers on dilution as discussed before. In this case, salts may inhibit the dissociation. An example is shown in Hemoglobin subunits, which are separated from $\alpha_2\beta_2$ to $2\,\alpha\beta$ on dilution and the dissociation occurs easier with a lower salt concentration[25].

b) Effect of temperature and hydrophobic bond

The role of a thermodynamical parameter, temperature is considered to have two effects; one, induction of conformatinal changes necessary for polymerization or depolymerization, and the other, modification of interaction energies. When no or little conformational change is confirmed experimentally by physico-chemical methods, one may conclude that the effect of temperature is strengthening or weakening of interaction energies, and if some change in conformation is detected, the effect might be destruction of the structure necessary for polymerization in addition to alteration of energies. As mentioned before, the hydrophobic bond is the only energy that has positive temperature dependence; that is, the higher the temperature, the stronger the bond until 60−70°C. Therefore, one may suggest the presence of hydrophobic bonds in polymerization sites by the experiment which shows an increase in energy with raising temperature.

The hydrophobic bond acts between hydrophobic groups, aliphatic and aromatic side chains, which are located normally inside of the molecule[26], but when some groups are forced to expose on the surface and the molecules are still soluble in water, intermolecular hydrophobic bonds can be formed.

c) Effects of pressure[27]

The effect of pressure, another thermodynamical parameter, on polymerization is based on a thermodynamical law, or Le Chatelier's law; that is, a change in a state occurs in the direction to decrease a volume of the system. When depolymerization causes a decrease in volume, polymerized molecules dissociate into monomers on applying pressure. In other words, desruption of intermolecular bonds produces the decrease in volume. Such forces are hydrophobic bond and salt bridge between charged groups. Under a high pressure above 5000 Kg/cm^2, breaking of interamolecular bonds may occur to enhaunce denaturation of the protein. Care should be taken for change in pH under pressure, because undissociated salt molecules in a solution tend to dissociate into ions which have smaller

Table 2. Interaction energies R_{ij}: Distance between i th and j th atoms.

Energy	Dependence of distance R_{ij}	Effect of Temps. for internal energy	Volume change on breaking bonds
non-bond	$E_{non}=\frac{A}{R^{12}{}_{ij}}-\frac{B}{R^{6}{}_{ij}}$ *	(negative)	—
electrostatic	$E_{ch}=\frac{e_i e_j}{DR_{ij}}$	(negative)	positive
dipole	$E_{dipole}=\frac{1}{DR^{3}{}_{ij}}\left\{(\mu_i\mu_j)-3\frac{(\mu_i a_j)(\mu_j a_i)}{R^{2}{}_{ij}}\right\}$	(negative)	
H-bond	various form (covalent in part)	negative	no or slightly positive
$H\phi$-bond	long range	positive	positive

* Lennard-Jones as an example

volume due to strong hydration, and the pH in solutions may be altered. In addition, a transition in the structure of water seems to take place at high pressure where interaction energies may be modified; hydrophobic bond through the change in arrangement of surrounding water molecules, and electrostatic energy through dielectric constant of water. These effects are not precisely known experimentally, and it is hoped to clarify the pressure effect on solvent structures and on the behavior of solutes in solutions.

The properties of interaction energies are listed in Table 2.

d) Chemical reagents

The use of chemical reagent reveals two aspects of aggregation of proteins. One is to identify the amino acid residues responsible for polymerization, or polymerization sites, by attaching some chemical groups to side chains, the well known procedure of chemical modification. The other is to modify intermolecular interactions, such as urea and organic solvents; in this case, no chemical bond is formed.

The chemical modification is performed for the purpose to search reactive groups on the surface, modification of which results in loss of polymerizability and dissociation into monomers or subunits. The reactive groups here are usually ionizable groups and many reagents have been tried to obtain such reactions; FDNB (fluoro-dinitro-benzen)[28], DNS (5-dimethyl-amino-1-naphtalene-sulfonate), for amino groups, Iodo- and Bromoacetate for imidazol groups[29], Iodine for tyrosine[30], NEM (N-ethyl maleimide) for cysteine[31], and so on. There is no way to modify hydrophobic side groups chemically, because the groups are constituted of saturated stable chemical bonds, and an attempt to identify a location of hydrophobic groups on the surface has been done by using fluorescent dyes which combine to hydrophobic groups. In identification of polymerization sites and their location on the surface, the careful interpretation is required, because the modification might lead to a conformational change from the original conformation necessary for polymerization.

Another effect of chemical reagents, modification of intermolecular interaction, is shown, as an example, by splitting of a protein molecule into subunits, *e. g.*, in a high concentration of guanidine-HCl or urea. Urea and guanidine salts had been initially thought to be hydrogen-bond-breaking reagents, but current interpretation favors the belief that these action on proteins is hydrophobic bond breaking[11], the interpretation which is supported by several experimental facts. Although the use of these reagents succeeds in separation of subunits, not only intermolecular bonds but intramolecular bonds are destroyed. This is why the reagents are called denaturing reagents. Detergents show the similar effects on dissociation. The molecular mechanism of the action on protein molecules is not well established, and thermodynamical treatments are found in the article by Tanford[32]. Other reagents, organic solvents, *e. g.*, alcohol, show different behaviors from the desruptive reagents described above. The decrease in dielectric constant by the addition of organic solvents results in the increase in electrostatic interactions, but on the other hand, ionization of ionizable groups of the protein surface may be depressed by the same reason. In addition, hydrophobic groups of the solvent molecule such as a methyl group of methyl alcohol may play a role in

breaking hydrophobic bonds. Therefore, the effects of organic solvents on proteins are complicate and it is hoped to obtain more experimental facts to elucidate the mechanism.

e) Hydrogen bond and other interactions

The importance of hydrogen bonds in cohesion of molecules is illustrated by the β-structure which leads to formation of a *sheet* connected by hydrogen bonds between CO and NH of peptide backbone[33]. The characteristic feature of this bond is a directionality of the bond[34] in contrast to arbitrary directionality of the hydrophobic bonds. However, as mentioned before, a single hydrogen bond in water is not strong enough to bind two or more large molecules, so that several hydrogen bonds, as in the case of β-structure sheet, or a few bonds coupled with other interaction energies may be necessary for binding the molecules together.

Since hydrogen bond contains covalent nature, a state of π-electrons involved in this bond will be altered so as to arise different energy levels, creating an additional delocalization energy to produce a more stable state when hydrogen bonds are formed[35]. The energy due to electronic structure in protein molecules might have an essential role in intramolecular interaction as well as intermolecular interaction, but at present stage no established experiment to indicate the importance is reported.

f) Conformation

The forces responsible for polymerization of molecules to one, two, and three dimensional aggregation, and for subunit structures of some proteins, become effective, when sites of the contact are located regularly on the surface; That is, according to a proper steric arrangement of side chains at the sites several interactions described above may occur simultaneously, stabilize the structure and produce directionality of polymerization. The necessary condition to conserve such a conformation is some rigidity of the conformation to hold the configuration of a binding site. Otherwise, an entropic effect of intrachains may disturb the formation of the site. Therefore, little conformational change of the monomer is expected on polymerization, and on the contrary the desruption of the native conformation by chemical reagents may lead to depolymerization or splitting into subunits.

In some cases, relatively large conformational changes are observed. The change may be interpreted in two ways; one, the change occurs so as to transform the conformation of a monomer which is unfavorable one in itself to bind each other, to a given conformation necessary for polymerization, and the other, the conformation of an attached monomer is forced to be altered by a strong binding energy to polymers. In the both cases, the conformation of isolated monomers and that in polymer may be representative two stable states of the monomer, because the molecules would have a definite structure in solutions as discussed before.

Another aspect of a conformation is symmetry. The regular aggregation occurs in a similar way to a crystallization process, so that the symmetry of the molecule must be held in the location of binding sites. Suppose a site A binds to

a site B, arrangement of $ABAB\ldots$ gives rise to a linear aggregation if the sites lie on a line, but to another helical structure if not. When the polymer forms a ring structure of a finite size, the locations of A and B should satisfy a geometrical condition to form the ring.

V. EXAMPLE OF POLYMERIZATION

The regular aggregation occurs for proteins which are not easily extracted by water from organs. Typical examples are found in muscle proteins.

a) Actin

Actin, which constitutes thin filaments in myofibril, is extracted by water from acetone-treated dried muscle obtained after removal of myosin[36]. This protein exists in a globular form in salt free solution, and a linear polymerization occurs when monovalent salts are added. The optimal salt condition is 0.06 M for KCl, and the polymer tends to depolymerize on the addition of a large excess of salts. The molecular weight of a monomer (G-actin) is 57,000[37] and that of the polymer (F-actin) is over 10^6.[38] By electron microscopic observations[39], F-action is compose of two-stranded superhelix of a length of a few microns with a repeating period of 370 Å, in which about 13 monomers are connected.

The polymerization is dependent on the protein concentration in the presence of medium amounts of salts, say 0.03 M[40]. The polymerization of molecules occurs above a certain critical protein concentration which is dependent on the salt concentration. The polymerization reaction is expressed by equation (II. 10) or (II. 12), where $C_1=1/K_2$ corresponds to a critical protein concentration C_{cri}. The temperature dependence on C_{cri} has been found to be negative[41], that is, a decrease in temperature causes depolymerization of F-actin, indicating some role of hydrophobic bonds in polymerization. The measurements of difference spectrum on polymerization show involvement of tryptophan and possibly tyrosine residues at the polymerization site[41].

According to the discussions describe proviously, G-F transformation of actin may be elucidated as follows; first, the fact that the addition of salts induces the polymerization suggests the repulsive force to be electrostatic between net charges of monomers. This is supported by experiments of the pH dependence of polymerization[42], the experiments which have shown the increase in polymerization with lowering the pH and complete depolymerization above pH 10, and second, negative temperature dependence of critical concentration suggests the cohesive force to be hydrophobic. Presumably, G-actin molecules separated by the electrostatic repulsion bind together by reduction of energy due to the addition of salts, and by attractive forces including hydrophobic bonds at a specified site on the surface where tryptophan and tyrosine residues are located. Application of pressure makes F-actin depolymerized and a decrease in volume of 80 ml per mole is obtained[43]. The results also support the above interpretation.

b) Tropomyosin

Another structural protein of muscle fiber, tropomyosin, was discovered by Bailey[44], and has recently been paid attentions to a role in interaction with

action and troponin. The monomer molecule has a rod-like shape and is 400 A in length. Contrary to actin, this protein exists in fibrous forms at a low salt concentration, and the addition of salts gives rise to depolymerization. This effect suggests the attractive force to be electrostatic in nature. Apparently, a local charge distribution on the surface seems to be heterogeneous, resulting in attractive forces between local charges of different molecules against the repulsion between net charges. Depolymerization occurs with an increase in the pH above 9, although no significant conformational charge is observed, the consistent observation with the above interpretation

Tropomyosin is a typical protein which contains almost 100 % of helix, as is expected by its rod-shape. On heating the solution, depolymerization takes place also above about 40°C, where the helical content decreases to 70-60 %[11]. The result sugests that the rod-shape is necessary for polymerization to bind molecules tightly. To keep a long fibrous structure, the site of contact must be side-by-side to some extent, *i. e.*, some overlap may be present to make the polymerized molecule straight.

The polymerization of tropomyosin is, therefore, interpreted by the simplest equation (II. 7). According to the equation, polymerization should be dependent on protein concentrations, and the experiments showed that depolymerization did occur with a decrease in the protein concentration[45].

c) Other proteins which have subunit structures

Large molecules over a molecular weight of several hundreds thousand initially thought to be single molecules have been dissociated into subunits by the use of various kinds of reagents or the choice of the solvent condition. TMV proteins, coat proteins of phages, metabolic enzymes, *etc.*, consist of subunits, molecular weights of which are of an order of 10^4. These subunits can be reconstituted to original aggregations after isolation of subunits[46]. By examination of the conditions for splitting and recombining subunits, one may deduce the interaction energy responsible for the intermolecular association.

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