The 3-Dimensional Structure of Proteins can be Drawn in a Single Stroke

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1. Fluorescent Protein

Lately GFP is providing a topic for discussion (Fig. 1). The protein absorbs the blue light (about 395 nm) and emits the green fluorescence (about 475 nm). The protein was originally found from jellyfish equoria living in the Caribbean Sea, illuminating at night. And, it is now becoming a strong weapon in the field of biotechnology; from this reason, Prof. Shimomura, the discoverer of GFP, was awarded with the Nobel Prize in 2008, since it enables us to see and trace when and where a particular protein is expressed in the body by fusing GFP with the protein. This is just like the case in which we monitor at any time where in the sea a fish is swimming by attaching a radar device to the fish.

As a matter of fact, a protein is a very precise molecule machine that has its own specific function. In case of mankind, it is presumed that there exist roughly 30,000 proteins of different function. This is just why we can see, hear, run and think!

At present we can clarify the shape of molecular machine experimentally. For any purpose, first, prepare the protein and then subject it to X-ray crystalline structure analysis or NMR (nuclear magnetic resonance).

It is, however, generally difficult to elucidate the actual function clearly. In the field of genetics, a methodology that can correlate a disease with its causative protein has been employed to clarify the function of the relevant protein. Though it is an excellent principle, it was hard to tell where a particular protein was actually working. GFP made it quite possible and easy to specify the function of a protein in detail.

We can easily guess a person on board of a boat off the coast in the early morning to be a fisherman, while a person heading for the firing spot must be a fireman; these facts mean that situations often enable us to guess the role of the subject working there.

There are two ways which conform to the said purpose: luciferase and GFP-based methods. The luciferase, originally obtained from a firefly, can be used to illuminate the cell where a target protein is expressed as a fusion-protein with the luciferase. That is, when a particular protein is expressed, concomitantly the luciferase is co-expressed, leading to the luminescence. Following the same principle, GFP can report the expression of a fused target protein. However, there is a difference between the two methods; as a light energy source, the luciferase method requires chemicals such as luciferin and ATP while the GFP method does not require chemicals but instead needs to be provided with blue light excitation. The GFP method is more convenient in a sense that it does not require the burdensome step of

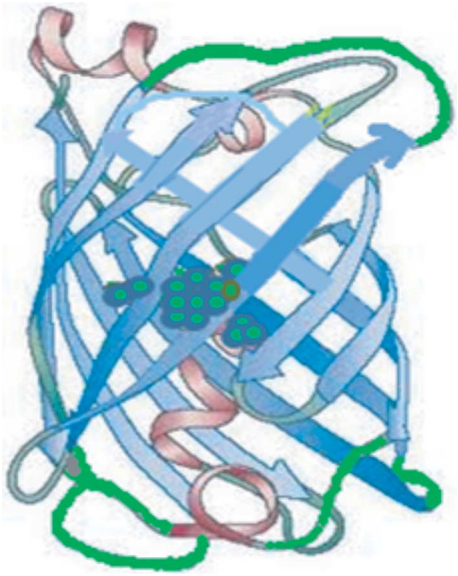

Fig. 1. Structure of green fluorescent protein (GFP). GFP is a protein consisting of 238 amino acids connected in a line and it folds like the shape of basket. Just in the middle of the basket there are the amino acids that receive and emit lights (the constituent atoms are indicated as green small circles). The surface of the basket is surrounded with β sheets that are like 11 narrow panels. In this figure, fundamental/partial structures (secondary structures) of the protein named β sheet (blue) and α helix (faint red) are shown. The coil part (narrow blue and green lines) indicates the amino acids that do not form a secondary structure.

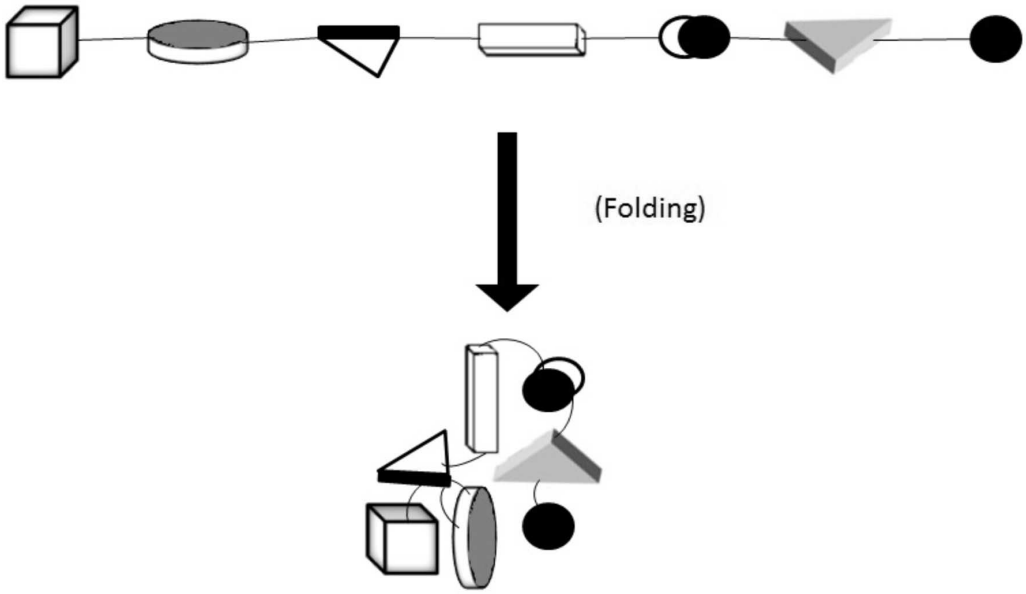

Fig. 2. Structure formation of proteins (folding). Proteins can be compared to a work of building blocks composed of differently shaped ones linearly piled up in a various order. The order of various blocks makes a unique protein. If a mass of blocks linked linearly are shaken in a box, they will gather in a clump and will be stabilized. Similarly, proteins are shaped.

introducing chemicals into the cell where the target protein is expressed, meaning that the GFP method can detect or measure the expression of a protein in living cells.

These methods, especially the GFP one, are extraordinarily convenient because these can be performed automatically (by fusion protein) without attaching the sensor device manually to each target molecule, which is inevitable for fish-monitoring devices.

2. Protein is Formed "by Itself"

It's really miraculous, however, that the fine and elaborate device of protein can be formed "by itself". In the latter half of 1960's Anfinsen discovered the principle that a protein molecule made in a linear form can change by itself into a 3 dimensional molecule of a functional structure. In short, Anfinsen's principle is based on the same theory as "Water flows from a higher place to a lower place".

Thermodynamics tells us that the structure of protein shifts in the direction of reducing the system's free energy and is finally stabilized in the global minimum. The protein can be compared to building blocks consisting of various shapes such as ●, ■ and ▲ (actually the protein consists of 20 kinds of molecules called amino acids and three blocks are linked together to one string like a sweet-potato runner in an arbitrary order. (Fig. 2).

When the linked blocks are thrown into a box and then shaken, the blocks inside will pile up and gather together and finally freeze. This process is called "folding", in Protein Science, which leads to a stable and functional structure. In reality, the final protein structure is always its specific shape.

Though this process is qualitatively explained, it is still a hard nut to crack to interpret the whole process quantitatively. Its success enables us to design an arbitrary structure of protein, deserving Nobel Prize!

3. 3D-Structure Drawn with a Single Stroke

Now, a really interesting fact is that the protein 3D-structure is generated as if drawing with a single stroke of a pen. This may seem natural considering the linear polymer structure of protein but deliberate consideration will make us realize that this is exceptionally ingenious molecular design and a way of construction. In other words, if we ignore the string which connects the blocks (amino acid side chains) and look at the part of the blocks only, we will find initially separate blocks gather in a definite shape without the intervention of human power or else. Although a challenge to shape up a molecular structure artificially using AFM (atomic force microscopy) has started, it demands a lot of time (hours or more) to fabricate a single molecule of a relatively simple structure. The nature fabricates Avogadro's number of molecules (the order of 10^{23}) within millisecond. So the protein folding is a sort of divine work!

Nowadays, this seemingly divine work can be interpreted to be the fruit of long-termed molecular evolution. The protein, such a miraculous molecule, is a kind of masterpiece of the natural selection just as a potter fabricates and selects a favorite one out of many works. Based on this idea, a novel engineering field of evolutionary molecular engineering has emerged since 1990's and has supported the authenticity experimentally.

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