Proteins tougher than spider silk

Spider-Man's Web

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Prof. Marek Cieplak leads an interdisciplinary team of scientists studying the physical properties of proteins and nucleic acids.

Researchers working at the laboratory of the PAS Institute of Physics have discovered that a well-known protein may prove to have super-tough mechanical properties, almost twice as strong as spider silk – absolutely perfect for Spider-Man

The mechanical properties of the 1TGF protein will be verified by research centers working closely with the PAS Institute of Physics. If they are confirmed, the results will be pave the way for numerous new applications in surgery as dressings or sutures (not just for Spider-Man style gadgetry).

Large conformational changes

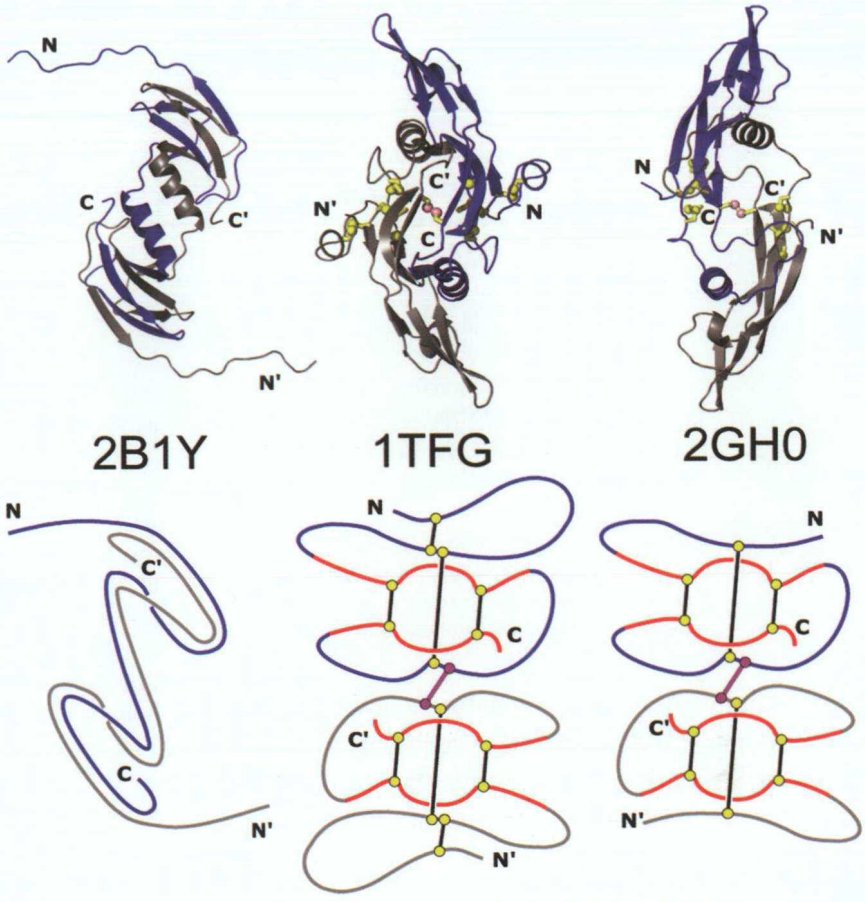
It all began back in 1999, when I became interested in the large conformational changes in proteins that occur during their folding. What happens when proteins are unfolded and the denaturing agent is removed, or when the temperature is changed to room temperature and the unfolded protein becomes globular? In order to describe the changes occurring in proteins, we constructed coarse-grained models. Proteins are formed by ribosomes as long, unfolded chains of amino acids. After they are formed, under the right conditions each chain folds into a globule, creating the native structure with a shape specific to each protein. One major shortcoming of commercially-available theoretical chemistry programs such as Amber, Gromacs, and Charm is that they can only be used for studying short timeframes. Although they obtain excellent results in studies of native processes or those occurring near the native state, this is not the case for the large conformational changes I was interested in. The model needed to be simplified; in the new model, amino acids are represented by single C-alpha carbons. In the simplest version, they are assigned a solid core to stop them from overlapping. Such a polymer containing a core is not yet a protein, therefore it is necessary to introduce an alternative means of interactions between the selected amino acids. This is known as defining a contact map by describing which amino acids interact with one another. If I load such a structure and assign solid cores to all atoms in the amino acids, the amino acid will be represented as a "cluster of grapes." If two clusters overlap a native contact is declared to exist and an optimal potential well is asigned to it. Otherwise there is just a repulsion if the beads representing amino acids come to close. Note that the construction of the model requires knowledge of the native structure.

Fishing rods and tweezers

The first research into mechanical manipulation of proteins was conducted in 1997, when Hermann Gaub's team in Munich was visited by Julio Fernandez, today a professor at Columbia University. The basic idea involves attaching something to a protein and then grabbing it, usually using atomic microscopes or optical tweezers. The Institute has one such microscope available, equipped with a scanner with extremely sensitive piezoelectric crystals and a probe. The proteins are "fished" using a nano-tip mounted on a miniature lever. The method makes it possible to capture individual protein molecules by the free ends of their chains and subject them to mechanical stretching. We started with the largest known protein: titin, a muscle protein that forms a central part of the sarcomere (together with the better known myosin and actinin). Titin's role is structural; myosin builds up around it, with a part of titin poking out of the myosin, binding to the end of the sarcomere and controlling its elasticity. The natural protein can stretch up to 4 microns. When the protein is stretched using an atomic microscope, the specific force required for the protein structure to break during stretching is important. For titin, this value is 200 piconewtons. The value is around 100 pN for the average protein, while for a DNA molecule the value is just 15 pN.

Done in 20 minutes

The first atomic modeling was performed by the team headed by the renowned researcher Klaus Schulten from the University of Illinois. Although these simulations had faults (since the researchers needed to use a stretching speed that was orders of magnitude larger than in the experiment) the team was nevertheless the first to identify the source of resistance to stretching. For titin, it originates from shearing arising between six hydrogen bonds between near-terminal strands. A peak force is formed and then the tension in the protein goes down when the strands separate. Experiments in stretching pro-



The unprecedented toughness of this group of proteins results from their specific folded form

tein molecules need to be extremely meticulous and require a lot of time, therefore only certain laboratories have the facilities to conduct them. As a result, only around 100 proteins have so far been studied this way. The PAS Institute of Physics conducts computer simulations applying its own theoretical model; the team has used the method to study over 18,000 proteins. The model is simplified and empirical, making the simulations rapid. The results match the actual measurements, and in just 20 minutes the team is able to achieve results similar to those obtained by Schulten's group in six months' work. Our surveys have discovered new mechanisms of resistance to stretching, as well as new proteins whose mechanical stability is over twice that of titin's.

The information we obtain on the basis of the calculations allows us to make numerous comparisons, which can be verified experimentally. Our simulations have generated graphs of characteristic maximum strengths, visualizing the relationship between the applied force and the pulling distance. They are stored in a BSDB database on the PAS Institute of Physics servers. We have been focusing on the 1% of the toughest proteins, demonstrating the existence of proteins with forces reaching 1500 pN. None have so far been subjected to mechanical experiments. The toughest is the transforming growth factor type beta 2 (TGF-beta 2), which performs many functions in the human body, including in wound healing and bone growth.

Loop-the-loop

The unprecedented strength of this group of proteins results from their specific folded form. When the straight protein chain folds creating the native structure, two powerful covalent bonds, known as disulfide bonds, form in certain places along the nearby chain segments. The resulting structure resembles a rope folded in half and sewn together to form a cysteine loop. The loop is threaded with another disulfide bond formed between two other protein fragments: one above and one below the cysteine loop. Pulling the ends of the protein pulls protein fragments through the loop, not unlike trying to get a camel to pass through the eye of a needle. If it does manage to get through, it causes a significant and irreversible change to the shape of the protein. For 1TGF, there is an additional attraction: on one side of the loop, beyond the knot threading bond, there is another loop. This extra loop is longer by two residues than the loop in the cystine knot. If we apply a stretching force to the appropriate ends of the protein, we are faced with a major challenge: the extra loop needs to pass through a much smaller one. This is only possible when the applied force is of sufficient magnitude to deform the loop.

Other types of tough proteins are found in spider silk. Different types of spiders produce different kinds of webs; some use them for catching insects, others for moving around. The webs' mechanical properties have been measured; the type used for catching insects requires around 800-900 pN to be stretched, the other type just 200 pN. We studied monomers in the first instance; once we moved on to testing dimers, we discovered that the results depend on the choice of two amino acids used to implement stretching. Stretching in one direction can require as little force as 100 pN, with the result rising to as much as 1500 pN when stretching in the opposite direction. This research allowed us to discover the toughest protein, which we had previously dismissed. It has a completely different structure to those found in spider webs. Future applications may include using it to absorb large amounts of energy resulting from collisions.

Interviewed by **Patrycja Dołowy**

Further reading:

Sikora M., Cieplak M. Cystine Plug and Other Novel Mechanisms od Large Mechanical Stability in Dimeric Proteins (2012). *Phys. Rev. Letters, vol. 109*, article no. 208101.