Review of 'Protein folding guides disulfide bond formation'

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Abstract

The mechanism of how protein folding drives disulfide bond formation is poor understood and the methods implemented on molecular dynamics software usually do not cover the problem of disulfide bond. Meng, using coarse-grained molecular simulation with disulfide bonds stabilized a harmonic potential, created a novel way to mimic the formation and rupture of disulfide bonds. By implementing his method to the bovine pancreatic trypsin inhibitor, the folding mechanism of this protein are resolved and as anticipated, the disulfide bonds do have important effect in folding process.

THE information to fold a protein is fully contain in the primary amino acid sequence, which were based on oxidative refolding experiments on disulfide bond formation in ribonuclease [1] [2]. The method using in chemistry experiments to monitor the formation and rupture of disulfide bonds is acid quench technique and a superior way of separating the intermidiates [3]. But the experiments still could not resolve the nontrival relationship of the protein folding and the formation of disulfide bonds. Whether disulfide bond formation drives protein folding or vice versa? To solve this, we have to reach out to molecular dynamics.

Energy function of Coarse-Grained Model for protein with disulfide bonds: Meng used a C_α representation of a polypeptide chain. For a given conformation,with 0 denoting the corresponding coordinates in the native structure, the energy function is given by

$$\begin{aligned} E_p(\Gamma,\Gamma_0) = \sum_{i=1}^{N-1} K_r(r_{i,i+1}-r_0)^2 + \lambda \sum_{i=1}^{N-2} K_\theta(\theta_i-\theta_{0i})^2 \\ + \lambda \sum_{i=1}^{N-3} [K_1(1-\cos(\varphi_i-\varphi_{0i})) \\ + K_2(1-\cos(3(\varphi_i-\varphi_{0i})))] + E_{NON} + E_{SS} \end{aligned} \quad (1)$$

where $r_{i,i+1}$ is the distance between two consecutive residues, θ_i and φ_i are the bond angle formed by three consecutive residues, and dihedral angle formed

by four consecutive residues respectively. As default, $K_r = 100\varepsilon/\mathring{A}, K_\theta = 20\varepsilon/rad^2, K_1 = \varepsilon and K_2 = 0.5\varepsilon$. The term E_{NON} in Eq. S1 contains both the native and nonnative contact interactions, which is approximated by Lennard-Jones(LJ) potential

$$E_{NON} = \lambda \sum_{i<j-3} \varepsilon_1(i,j)[5(\sigma_{ij}/r_{ij})^{12} - (\sigma_{ij}/r_{ij})^{10}] + \varepsilon_2(i,j)(\sigma_{ij}^{nn}/r_{ij})^{12} \quad (2)$$

Meng defined two residues are in native contact if the distance between them is within 5.0 $\mathring{A}$ and if they are in native contact, Meng set $\varepsilon_1(i,j) = \varepsilon$ and $\varepsilon_2(i,j) = 0$, otherwise Meng set $\varepsilon_1(i,j) = 0$ and $\varepsilon_2(i,j) = \varepsilon$.
The disulfide bond are *formed* if it satisfy four criteria:(i)Proximity:two reactive thiol groups must be in proximity $d_\alpha < \bar{d}_\alpha + \delta_\alpha$ where $\bar{d}_\alpha$ is averige distance, and δ_α is the dispersion of a disulfide bond, estimated from distribution of distance of two Cys residues that form S-S bond. The $\bar{d}_\alpha$ and dispersion was calculated from generating folding trajectories at $k_BT = 0.9\varepsilon$, in which the protein's folded state is stable. (ii)Orientation: the Cys residues must have right orientation. (iii)Solvent exposure: S-S bond formation requires the thiol groups accessible to oxidizing agents. To mimic this, Meng calculate the number density, which represents the solvent accessible surface area, $\rho = 3n/(4\pi R_s^3)$, where n is the number of residues within a spherical shell with radius,R_s, drawn from the center of a given disulfide bond. If i^{th} conformation satisfies both the proximity and the orientation criteria and if $n_\alpha^i < \bar{n}_\alpha - \beta_O\delta_\alpha$, then Meng assume that the S-S bond are formed. And $\bar{n}_\alpha$ is averige number density calculated in the same way as $\bar{d}_\alpha$. β_O is a parameter that controls the probability of S-S bond formation directly, representing the oxidizing condition of environment.
After the disulfide bond formed, the two Cys are stabilized by the harmonic potential as same as *Eq.1.*
Rupture condition: (i) $n_\alpha^i < \bar{n}_\alpha - \beta_R\delta_\alpha$ (ii)

$$R_\alpha^i(t) = \frac{1}{\Delta t}\int_t^{t+\Delta t} \frac{2}{n(n-1)} \sum |r_{ij}|(s)ds \quad (3)$$

where $|r_{ij}|$ is the distance between beads i and j, and n is the number of beads within R_0. If (i) is satisfied and $R_\alpha^i < R_0$, then a disulfide bond is reduced. By altering β_R we can control the probablity of rupture.

THE results: By performing thousands of simulations, very valid results were obtained. The β-hairpin is fully form long before the formation of 14-38 disulfide bond which is formed first amout the three disulfide bonds, indicated by the fact that 84% of the trajectries formed it first. Then [14-38] rearranged to form [5-55] and [30-51], which are more kinetically stable. Based on the cumulative analysis of all of the oxidizing events, [5-55] and [30-51] rearranged to form the intermediates [5-55,14-38](N') and [30-51,14-38](N*) mostly,respectively. The intermediate [5-55,30-51](N_{SH}^{SH}) was considerably less formed from the state of [5-55] and [30-51]. But N' and N* usually rearranged

to the state of N_{SH}^{SH}, after which would directly transform to complete folded state N with probablity of 84%.
To verify the β-hairpin importance that is always formed before first disulfide bond, Meng stabilized the β-hairpin by increasing λ(Eqs.1 and 2), only the part of residues in the β-hairpin. The time for forming [14-38] decreases as λ increases, and this result is in quantitative accord with experiments [4]

This research proved that Go model has great advantage in study of protein folding, and this easy-understanding method of simulation of disulfide bonds gave out well-fitted results with experiments, which indicates this probably is a general method that could be implemented in many kinds of simulations which involves formation and ruption of disulfide bonds.

References

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