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Chemical Oscillations in a Closed Sequence of Protein Folding Equilibria

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Abstract. In this paper we prove the existence of sustained oscillations in a deterministic model for a cycle of three sequential equilibria in protein folding. Our system takes into account the interconversion between three of the habitual secondary structures of proteins: α-helix, β-strands and random coil and assumes nonlinear reaction rates. The model can be extended to more general chemical reactions of closed sequences of equilibria.

1 Introduction

The cooperativity parameter characterizing the state-transitions in protein folding, as well as the modelling of the transformations between these states as first order kinetic reactions, have been the subject of intensive study within the last few decades and continue to awaken an increasing interest [1, 2].

Most of the theoretical approaches deal with the cooperativity parameter derived from the statistical mechanical model of the helix-coil transitions developed in the 60's [3, 4]. While these models roughly approximate the thermodynamical parameters of such systems, there is a lack of deterministic approaches that capture dynamic details of the protein collective motions, in particular protein folding oscillations reported in experiments [5, 6].

The sequential transitions between protein secondary structures at equilibrium has been associated with the cooperativity character of these transitions [1, 2]. In a recent article Fersht suggests that even short peptides, which at first glance display much simpler phase transitions, could follow higher order reaction rates, due to the cooperativity exhibited by these short polypeptide chains [7]. If this is the case, then we should clearly expect reaction orders different from the ones for large proteins with complex secondary structures, characterized by marked cooperativity.

We propose in this paper a deterministic reversible and cooperative dynamic model of a cyclic three state protein folding system, similar to the three-state folding experiment described in [5]. The cooperativity is taken into account by defining reaction rates as functions of the time dependent reaction product concentrations, multiplied by reaction rate constants. The dynamical system we obtain generalizes the dynamic model for irreversible, first order, autocatalytic reactions among different states of a polyfunctional macromolecule subjected to mass conservation derived in [8], and can be extended to more general chemical

reactions of three equilibria. We prove the existence of sustained, permanent oscillations in the system. The possibility of time dependent reaction rates, leading to dumped oscillations, is also discussed.

2 Three equilibria model

We consider a three states chemical system formed by the chemical species α-helix, β-sheet and random coils, representing three secondary structures coexisting in a single protein molecule (see Figure 1). α-helix and β-sheet are forms of regular secondary structures. In α-helix, which resembles a spring, the α-amino group of i-*th* residue is linked by a hydrogen bond to the carbonylic oxygen atom of residue $i+4$. β-sheet consists of β-strands connected laterally by three or more hydrogen bonds forming a twisted sheet. Random coil is the ensemble of random protein conformations characterized by the absence of hydrogen bonds.

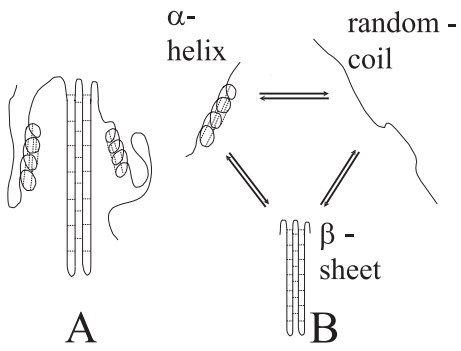

Figure 1: Representation of the random coil, β-sheet and α-helix secondary structures coexisting in a protein molecule (A), and of a closed sequence of chemical equilibria between these secondary structures taken separately (B).

These three states can coexist in the same protein molecule. However, when considering the macroscopic equilibria between them, we take into account the fraction of each of these states present in the whole chemical system at time t. In this paper we consider interactions between each other within a cycle of three sequential equilibria, as shown in Figure 2, where X, Y and Z represent the α-helix, β-sheet and random coils, respectively, x, y and z are the time-dependent concentrations of α-helix, β-sheet and random coils, while $\delta_1, \delta_2, \varepsilon_1, \varepsilon_2, \mu_1$ and μ_2 are reaction rate constants.

In each of the pseudo-first order reactions described in Figure 2 we assume, based on the conversion of stochastic dynamics of individual molecules into macroscopic properties of the system through the thermodynamic laws, that the quantity of the decay of reactant per time unit is equal to the quantity of reaction product itself multiplied by the corresponding constant, and conversely. Therefore, in our proposed mechanism the reaction rates are functions of the time dependent reaction product concentrations multiplied by their correspondent reaction rate constants.

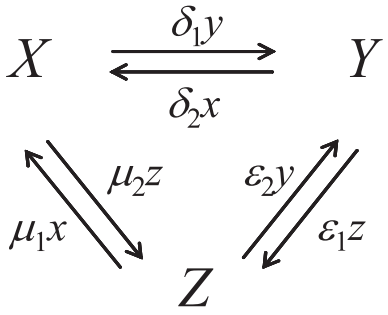

Figure 2: Closed sequence of chemical equilibria between α-helix (X), β-sheet (Y) and random coil (Z) (see text).

According to the law of mass conservation the dynamics of the reaction shown in Figure 2 is governed by the following system of nonlinear differential equations

$$\begin{aligned} \frac{dx}{dt} &= x[y(\delta_2-\delta_1)+z(\mu_1-\mu_2)] \\ \frac{dy}{dt} &= y[x(\delta_1-\delta_2)+z(\varepsilon_2-\varepsilon_1)] \\ \frac{dz}{dt} &= z[x(\mu_2-\mu_1)+y(\varepsilon_1-\varepsilon_2)], \end{aligned} \tag{1}$$

where $x, y, z : [0, T] \to \Re_+,\ T > 0$. Due to the conservation of mass the system (1) satisfies the conservation relation $x + y + z = 1$, therefore $0 \le x, y, z \le 1$ and the trajectories lie inside the triangle $\Delta = \{x + y + z = 1,\ x \ge 0, y \ge 0, z \ge 0\}$.
Moreover, by setting $\delta = \delta_1 - \delta_2,\ \mu = \mu_1 - \mu_2,\ \varepsilon = \varepsilon_1 - \varepsilon_2,\ z = 1 - x - y$, we can reduce the system (1) to

$$\begin{aligned} \frac{dx}{dt} &= x[\mu(1-x-y)-\delta y], \\ \frac{dy}{dt} &= y[(-\varepsilon)(1-x-y)+\delta x]. \end{aligned} \tag{2}$$

The trajectories of (2) lie inside the triangle $\Delta_1 = \{x + y = 1,\ x \ge 0, y \ge 0\}$, the projection of Δ onto the xy-plane. A simple computation shows that the system (2) is solvable and $H(x, y) = \delta[x^{\varepsilon/\delta}][y^{\mu/\delta}](1 - x - y)$ is another conserved quantity of (2), therefore $G(x, y, z) = x^{\varepsilon} y^{\mu} z^{\delta}$ will be a conserved quantity of (1). In 3d the trajectories may then be viewed as intersections of the triangle Δ with the level surfaces of the above function.

The fixed points of (2) are $P_1 = (0, 0), P_2 = (1, 0), P_3 = (0, 1)$ and

$$P_4 = \left(\frac{\varepsilon}{\delta+\varepsilon+\mu}, \frac{\mu}{\delta+\varepsilon+\mu}\right).$$

The Jacobi matrix of (2) at a fixed point (x_0, y_0) is

$$J = J_{(x_0,y_0)} = \begin{bmatrix} \mu(1-2x_0) - y_0(\delta+\mu) & -x_0(\delta+\mu) \\ y_0(\delta+\varepsilon) & -\varepsilon(1-2y_0) + x_0(\delta+\varepsilon) \end{bmatrix}.$$

The eigenvalues of the J at P_1, P_2, P_3 are all real. $J_{(0,0)}$ has the eigenvalues $\mu, -\varepsilon$, $J_{(1,0)}$ has the eigenvalues $\delta, -\mu$ and $J_{(0,1)}$ has the eigenvalues $\varepsilon, -\delta$. The only fixed point that can lead to complex eigenvalues, thus to oscillations, is P_4. The Jacobi matrix at P_4 has the trace zero and the determinant $D = \delta\varepsilon\mu/(\delta + \varepsilon + \mu)$. P_4 will be a saddle point if $D < 0$ and a center (purely imaginary eigenvalues) when $D > 0$. Since we are interested in oscillatory solutions of (2), we require that P_4 lie inside Δ_1, which happens if and only if δ, ε, μ have the same sign. In this case P_1, P_2, P_3 are unstable, P_4 is a center and the trajectories of (2) are closed curves around P_4, representing level curves of $H(x, y)$. In Figure 3 we represent the time series of two closed orbits for two different initial conditions and fixed parameters, as well as the corresponding trajectories in the phase plane.

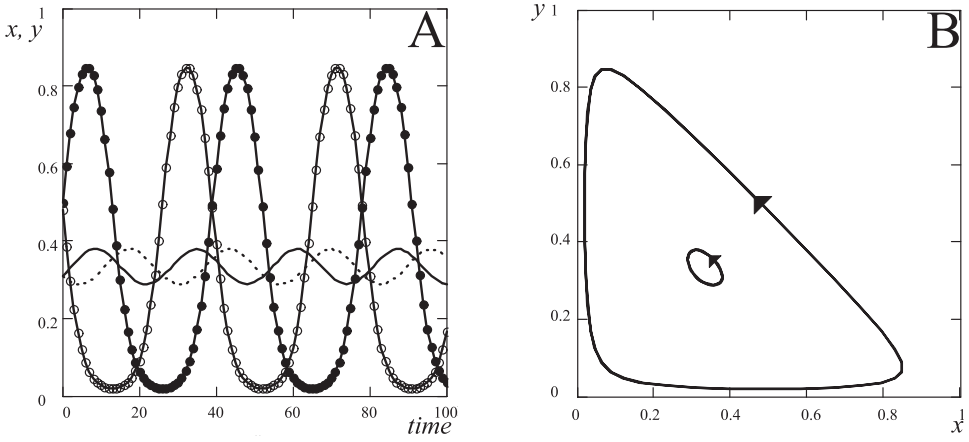

Figure 3: (A) Time series for two closed orbits of (2) corresponding to the initial conditions $x_0 = 0.31$ (solid line), $y_0 = 0.31$ (dotted line) and $x_0 = 0.48$ (solid line with white disks), $y_0 = 0.5$(solid line with black disks), for $\delta = \varepsilon = \mu = 1$. (B) Phase space representation of the two orbits.

There are three invariant subsets of (2): the lines $x = 0$, $y = 0$ and $x+y = 1$. Trajectories starting at the initial time in these subsets will remain there for all t. This corresponds to physical situations in which the system does not oscillate; it relaxes to an equilibrium where the system is characterized by the presence of only one chemical species. When the trajectories start inside the triangle and δ, ε, μ have the same sign, the system shows periodic and sustained oscillations around the center P_4. Once the coordinates of the center are specified, the closed orbits are defined by the initial concentrations of the chemical species. Closed orbits with $-\delta_0, -\varepsilon_0, -\mu_0$ are the same as the corresponding orbits for $\delta_0, \varepsilon_0, \mu_0$, only the direction of the vector field changes.

From Figure 2 we can see the consequence of flipping the sign of the parameters in terms of the general reaction. When δ, ε, μ are all positive the reaction occurs clock wise, while when the parameters have negative signs the reaction occurs counter clock wise. The reaction rate parameters are assumed to have Arrhenius-like form and they depend on temperature. This means that the proposed reaction mechanism allows flipping the reaction sense by

varying temperature.

3 Conclusions

In this paper we present a deterministic approach in the study of the oscillatory dynamics of a three-state protein folding system, formed by a closed sequence of equilibria between α-helix, β-sheet and random coil, and having pseudo-first-order kinetic rates. We assume that the reaction rate increases with the concentration of the reaction product. This formulation of the reaction rates proportional to the reactant's concentration is in agreement with the marked cooperative character of the phase transitions in protein folding; it is also applicable to other chemical systems showing cooperativity and formed by a closed sequence of equilibria.

The deterministic cooperative model we present here captures the possibility of sustained oscillations for the concentration of the species in equilibrium. Since the system (2) is a conservative oscillator we do not have limit cycle oscillations [9] (see also [10] for other types of oscillating reaction with conservative conditions). In our model the self-sustained oscillations are due to the existence of conserved quantities in the system and are conditioned by situations where all parameters have the same sign simultaneously. In such cases the system behaves as a closed cycle of three autocatalytic irreversible reactions [8]. When changing the sign of the parameters (but conserving the same sign for all parameters simultaneously), the global sense of these autocatalytic reactions in the cycle is flipped. This results bring new insight in understanding the state transitions and the role of the cooperativity in protein folding.

The system (2) is a modified version of the well known Lotka-Volterra predator prey systems used in ecological models [11]. In addition it has been used to model economical systems with increasing-diminishing return [12]. In both models it has been shown that the family of oscillations can be perturbed by including a carrier capacity for the prey and an auto-relaxation economical mechanism, respectively, to either approach a fixed point or a limit cycle oscillation (called *business cycle* in the economical model), revealing the universal aspect of the limit cycle. For our approach this will correspond to introduce higher order kinetic rates. Another avenue to model the damped oscillations recently observed in experiments [5, 6] is to take into account the time dependence of the rate constants δ, ε, μ, as described in [13] in the modelling of reaction kinetics with macromolecular crowding. This work is in progress and will be presented elsewhere [14].

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