Research Article

Exact Solution to the Extended Zwanzig Model for Quasi-Sigmoidal Chemically Induced Denaturation Profiles: Specific Heat and Configurational Entropy

G. E. Aguilar-Pineda¹ and L. Olivares-Quiroz²

¹ Colegio de Ciencia y Tecnología, Universidad Autónoma de la Ciudad de México-Centro Historico, Fray Servando Teresa de Mier 99, 06080 México, DF, Mexico

² Colegio de Ciencia y Tecnología, Universidad Autónoma de la Ciudad de México-Cuautepec, Avendia la Corona 320 Loma La Palma, 07160 México, DF, Mexico

Correspondence should be addressed to L. Olivares-Quiroz; gabriel.aguilar@uacm.edu.mx

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Temperature and chemically induced denaturation comprise two of the most characteristic mechanisms to achieve the passage from the native state N to any of the unstructured states D_j in the denatured ensemble in proteins and peptides. In this work we present a full analytical solution for the configurational partition function $\mathcal{Z}_{qs}$ of a homopolymer chain poly-X in the extended Zwanzig model (EZM) for a quasisigmoidal denaturation profile. This solution is built up from an EZM exact solution in the case where the fraction α of native contacts follows exact linear dependence on denaturant's concentration ζ ; thus an analytical solution for $\mathcal{Z}_L$ in the case of an exact linear denaturation profile is also provided. A recently established connection between the number ν of potential nonnative conformations per residue and temperature-independent helical propensity ω complements the model in order to identify specific proteinogenic poly-X chains, where X represents any of the twenty naturally occurring aminoacid residues. From $\mathcal{Z}_{qs}$, equilibrium thermodynamic potentials like entropy $\mathcal{S}$ and average internal energy $\langle E \rangle$ and thermodynamic susceptibilities like specific heat C_v are calculated for poly-valine (poly-V) and poly-alanine (poly-A) chains. The influence of the rate at which native contacts denature as function of ζ on thermodynamic stability is also discussed.

1. Introduction

The early recognition that ordered three-dimensional macromolecular structures, later known as the native state, play a fundamental role on biological activity of proteins and peptides led to a large interest in the fundamental mechanisms that drive formation and stabilization of such structures [1–4]. According to the classical view of protein folding, the native state N is a unique three-dimensional structure in which the protein displays biological activity [1, 5]. More recent studies, however, have shown that the structure-function paradigm may have remarkable exceptions. A large class of proteins known as *intrinsically disordered proteins* (IDPs) is one of the most outstanding examples of this category. IDPs can show a high level of biological activity even if

the protein is in one of the D_j states associated with the disordered ensemble [6–9]. The transition from $N \rightarrow D_j$ has been, accordingly, a subject of intense research. Temperature and chemically induced denaturation are two of the most common factors to induce such transitions being, the latter, probably the second most studied denaturation mechanism [10]. In order to chemically induce the $N \rightarrow D_j$ transition, urea and guanidine hydrochloride (GdnHCl) are the most usual organic compounds [11–13].

Despite the large amount of theoretical and experimental work, there is still an open debate on the underlying physical mechanism driving urea and GdnHCl mediated denaturation [14–16]. It is widely known that solvation of proteins and peptides in urea or GdnHCl decreases the free energy $\Delta G_{N \rightarrow D}$

compared to free energy of solvation in water $\Delta G_{N \rightarrow D}^\circ$ and that such decrease is linearly related to denaturant's concentration ζ according to $\Delta G_{N \rightarrow D} = \Delta G_{N \rightarrow D}^\circ - m\zeta$, where m is the so-called m value [17–19]. From theoretical or computational evaluation of m values it is expected to assess if the decrease in the free energy of solvation $\Delta G_{N \rightarrow D}$ in the protein-denaturant system is due to electrostatic, hydrogen bonding, or van der Waals interactions [10, 11]. A second central debate refers to whether denaturant molecules act directly on protein side-chains and backbone or instead act on bulk water altering hydrogen bond network and thus lessening or screening water-protein interactions [20, 21]. MD simulations suggest that whereas GdnHCl seems to decrease solvation free energy by electrostatic forces, urea seems to act through van der Waals forces in the first solvation shell of protein [13, 22].

From a theoretical point of view, statistical mechanics coarse-grained (SMCG) models have gained increasing popularity in order to carry out a physical-based analysis of both protein folding and protein denaturation [23–26]. The central feature of SMCG methods is the use of a small set of coarse-grained variables to describe average properties of the system instead of a fully detailed atom description. Common examples of such sets of variables include, but are not restricted to, pairs of dihedral angles (ϕ_j, ψ_j) for each residue, number of nonnative conformations ν per residue, or intrinsic helical propensity ω among others [27–29]. Such set of parameters are then used to perform statistical averages over specific ensembles following equilibrium statistical mechanics prescriptions [25, 30]. A pioneer example of this category of methods is the Lifson-Roig treatment of the helix-coil transition [29] in peptides in which the configurational partition function Z is written in terms of a transfer matrix $\mathbf{T}$ which depends on the conditional probabilities (u, ν, ω) of having a particular residue either in a coil or helical state.

Two additional recent examples of SMCG models are the elastic network model (EN) [31] and the Zwanzig model (ZW) [28]. The EN model considers a polypeptide as a chain of beads representing C_α carbons interacting to closest neighbors via harmonic potentials of varying strengths [26, 31, 32]. Whereas in the original version a cut-off distance r_0 is considered beyond of which residues do not interact at all [31], other versions of the EN model assign a decreasing value to the elastic constant κ_j as the distance from a reference point is increased. Albeit EN model is suitable to analyze vibrational properties of equilibrium states, the ZW model on the other hand is more appropriate to discuss equilibrium thermodynamic properties of homopolymer chains [33]. In the ZW model, an ansatz is proposed *a priori* for the energy levels E_j accessible to the system thus avoiding a quantum mechanical calculation of the energy spectra of the polypeptide chain through Schrodinger's equation $\hat{H}\Psi_j = E_j\Psi_j$ [28, 34]. Such energy spectra $\{E_j\}$ are minimally frustrated with a single ground state E_0 and multiple metastable states E_k with $k \neq 0$ [35]. In addition, a coarse-grained variable ν is used to describe the number of potential nonnative conformations per residue [28].

The original ZW model considers temperature as the only driving force to induce denaturation. However, it is possible to extend it to consider the concentration ζ of a chemical denaturant as a contributing factor to unfolding [34]. This extended Zwanzig model (EZM) uses the functional dependence of the fraction α' of native contacts with respect to ζ to derive an analytical formula for the configurational partition function $\mathcal{Z}$ of the polypeptide chain in terms of an integral over microstates whose energy E_j depends now on T , ν , K , and ζ . The fact that the experimental denaturation curve $\alpha'(\zeta)$ usually follows a sigmoidal pattern hinders the possibility of a closed analytical solution for such integral [34]. However, the EZM admits a full analytical solution for the case in which α depends linearly on ζ [36]. Although proteins and peptides do not follow in most situations this type of denaturation curve under chemical denaturants, it is possible to use this zeroth-order solution to build up a general solution for the sigmoidal case.

In this work we use the EZM framework to analyze thermodynamic equilibrium properties of ideal poly-X chains, where X represents any of the twenty naturally occurring aminoacid residues, under the influence of a chemical denaturation profile. In particular we focus our attention on thermodynamic susceptibilities like specific heat $C_v(T, K, \omega)$ and thermodynamic equilibrium potentials as configurational entropy $\mathcal{S}(T, K, \omega)$ and their connection to the mechanism of chemical denaturation imposed on the chain. In order to achieve this goal, we provide first an analytical solution for the configurational partition function $\mathcal{Z}_L$ in the EZM model for the case in which the fraction α' of native contacts is a *linear* function of concentration of chemical denaturant. From this result, we make an approximation for the full sigmoidal denaturation curve as a superposition of linear denaturation profiles. This quasisigmoidal representation provides us with a full analytical solution for the configurational partition function $\mathcal{Z}$ in EZM from which thermodynamic functions can be obtained straightforwardly. In addition, two types of denaturation mechanisms called A and B can be identified for this quasisigmoidal denaturation profile depending on the rate at which the fraction α of native structure is broken down by the chemical compound. Using a recently derived connection between helical propensity ω and the number ν of potential nonnative conformations per residue [37], an association with specific poly-X chains can be accomplished. Thermodynamics for poly-alanine (poly-A) and poly-valine (poly-V) in terms of the specifics of quasisigmoidal denaturation profile are discussed.

We proceed as follows. Section 2 contains a brief and self-contained summary of the physical foundations of the EZM. In Section 3 an analytical solution for the configurational partition function $\mathcal{Z}_L$ in the EZM for the linear $\alpha' = \alpha'(\zeta)$ is presented. Definition and properties of a quasisigmoidal denaturation profile are defined in Section 4 together with the implementation of the linear solution to obtain a full analytical solution for the quasisigmoidal $\alpha'(\zeta)$ case. For this case, $\mathcal{Z}_{qs}$ is obtained in terms of standard parameters of the EZM and also in terms of the slopes A_j of the individual linear profiles. As we shall see, such slopes weight

the different contributions of each linear denaturation profile to the full partition function. Finally, Section 5 presents the behavior of C_v and configurational entropy $\mathcal{S}$ for two poly-X homopolymer chains with different helical propensity. A further discussion is included on how thermodynamic equilibrium properties depend on denaturation curve.

2. Chemically Induced Protein and Peptide Denaturation: The Extended Zwanzig Model (EZM)

Statistical mechanics based coarse-grained models (SMCG models) provide a powerful tool to unveil intrinsic chain contributions to macroscopic thermodynamic observables of protein and peptide in solution [23–25, 38]. Depending on the details included, analytically solvable SMCG models may provide the fine tuning necessary to dissect the relative contributions of each major driving force for folding or unfolding. Even they are not so copious as expected, the ZW model [28, 34, 37] for ideal homopolymer chains and the EN model [26] together with on-lattice models with Ising-like interactions provide some outstanding examples of this category [39, 40] as mentioned before. In particular, ZW model proposes an *ansatz* for the energy spectrum $\{E_j\}$ of the chain which depends essentially on the number α of residues participating in a native contact. With this *ansatz*, ZW model overcomes the central obstacle encountered usually in applying the methodology of equilibrium statistical mechanics to calculate thermodynamic properties, which is the finding of the energy levels $\{E_0, E_1, \dots\}$ accessible to the system consistent with macroscopic restrictions and physical interactions encoded in the Hamiltonian operator $\hat{H}$ in quantum mechanics. Given this fact, the proposal of a phenomenological energy spectrum $\{E_j\}$ for a chain endowed with a single ground energy state and multiple metastable states satisfies the basic expectations for minimally frustrated energy landscape in proteins and peptides [41].

For a chain of K residues, the ZW model proposes that the energy spectrum E can be written as

$$E_\alpha = (K - \alpha)U - \epsilon\delta_{K,\alpha}, \quad (1)$$

where $\alpha = K\alpha'$ is the number of residues in a native contact, $U > 0$ is the energy penalty for a nonnative contact, $\epsilon > 0$ is the overall energy of the ground state, and $\delta_{K,\alpha}$ is the Kronecker delta function. It is worth underlining that here there is only one ground state which is associated with a native state and also that folding is a cooperative effect. Only when all residues have a native contact, namely, when $\alpha = K$, the second term of (1) contributes. Such functional form for E implies that there is a single ground state with energy $-\epsilon$ and a set of unstable states with energies $U, 2U, \dots, KU$. If we assume that all residue's conformations are statistically independent and there is a number $\nu + 1$ for each one, the partition function Z can be calculated readily to give

$$Z = \sum_{\alpha=0}^K g_\alpha \exp(-\beta E_\alpha) = \exp(\beta\epsilon) + [1 + \nu \exp(-\beta U)]^K - 1, \quad (2)$$

where g_α is the degeneracy associated with energy level E_α and $\beta = (RT)^{-1}$ is the Boltzmann factor as usual. From (2) equilibrium thermodynamic functions like free energy F , entropy S and specific heat C_v can be calculated [21].

In its original formulation, ZW model considers temperature as the central factor driving thermodynamic phase transitions. Although this is mostly true for a large variety of protein and peptide conformational transitions, there are other physicochemical factors that may trigger similar conformational phase transition. This is particularly the case of chemical denaturation which consists of the addition of chemical compounds, usually guanidine chloride (GdnCl) or urea, to an equilibrium water-protein solution to destabilize native structure. As discussed previously, details of the physical mechanisms by which GdnHCl and urea destabilize native structure are not fully understood; however the basics have been already devised [10, 14, 42]. For instance, for the case of urea, two central mechanisms have been proposed, a direct one in which a direct substitution of protein-protein and protein-water contacts is achieved by urea and an indirect one in which urea affects primarily the water hydrogen bond network of the first water shell around protein, thus making the exposure of nonpolar residues to protein's surface more favorable [15]. Recently it has been calculated that the passage of a urea molecule from the bulk to the first shell water that immediately surrounds protein is more favorable energetically $\Delta E = -12 \text{ kJ cal}^{-1}$ than the transfer of a water molecule from the bulk to the immediate vicinity $\Delta E = -0.25 \text{ kcal mol}^{-1}$. Thus the hypothesis of an indirect action of urea in protein denaturation is supported. The most probable picture for the action of urea is a two-step process. In a first stage formation of urea-water (U-W) contacts is achieved in the first water shell. Subsequently, when urea concentration has reached a value of approximately $\zeta = 8$ Molar, formation of protein-urea (P-U) is favored over protein-protein (P-P) contacts [14].

It is possible to propose a SMCG model to include the presence of a chemical denaturing agent as a major factor in protein denaturation. To achieve this an extension of the original ZW model was proposed in the recent past [34]. The corner stone of this extended Zwanzig model (EZM) considers that the action of a denaturing agent can be directly measured by its action on the fraction α' of native contacts along the chain, which has to be an explicit function of the concentration ζ of the chemical compound. Then, the EZM assumes that $\alpha' = \alpha'(\zeta)$ (or its equivalent $\alpha = \alpha(\zeta)$) which is a functional form that can be deduced experimentally. For instance, for a pure α helix-coil or β sheet-coil transition, the fraction α' of residues in a native conformation at a fixed temperature can be measured by circular dichroism (CD). For most chemical and temperature-induced conformational transitions, α' follows a sigmoidal relationship with either temperature or chemical concentration. Hence, for a sigmoidal denaturation profile, $\alpha'(\zeta)$ can be written as

$$\alpha'(\zeta) = p + q \tanh(\zeta - r), \quad (3)$$

where p , q , and r are parameters that have to be adjusted in order to fit a specific profile. Given the fact that the energy E

of a particular conformation is determined by the number α of native contacts and that $\alpha = \alpha(\zeta)$, the energy spectrum in the EZM is a function of the continuous variable ζ . Thus the EZM partition function $\mathcal{Z}$ can be written as [34]

$$\mathcal{Z} = \int_0^{\zeta^*} g_\zeta \exp[-\beta E(\zeta)] d\zeta, \quad (4)$$

where g_ζ is the degeneracy associated with the energy level $E(\zeta)$ and ζ^* is the concentration with a threshold value. Equation (4) represents the continuous version of (2) written in terms of the continuous variable ζ instead of the discrete one α . It describes a statistical average over energy conformations defined by variable ζ when energy spectrum E is a function also of ζ . Standard denaturation experiments show that ζ usually ranges from zero to a maximum concentration $\zeta^* = 8$ Molar in a $N \rightarrow D$ denaturation reaction.

Passage from a discrete model for Z as in the ZW model to a continuous model for $\mathcal{Z}$ in the EZM is not just a simple matter of substitution of sums by integrals [34]. Special care has to be taken since all variables that depend on the discrete variable α have to be correctly translated in terms of the continuous variable ζ . For instance, the degeneracy factor g_α in the ZW model can be written as

$$g_\alpha = \nu^{K-\alpha} \binom{K}{\alpha}, \quad (5)$$

with $\binom{a}{b}$ being the standard binomial coefficient. However, if α is a continuous function of ζ as it is stated in (3) the correct translation for the degeneracy factor g_ζ in (4) implies constructing a continuous approximation to the binomial coefficient. In the same spirit, the discrete Kronecker delta function $\delta_{K,\alpha}$ that represents the ground energy state in (1) and that mathematically is a well of energy can be best represented in the continuous case by δ -sequences $S_n(x)$. A particular useful δ -sequence S_n in one-dimension that describes a finite and continuous energy well is

$$S_n(x) = \frac{n}{\sqrt{\pi}} e^{-n^2 x^2}, \quad (6)$$

where n is any positive integer. By definition, δ -sequences satisfy that

$$\lim_{n \rightarrow \infty} \int S_n(x) f(x) dx = f(0). \quad (7)$$

Thus, for sufficiently large n , $S_n(x)$ is a strong peaked function [43].

The energy spectrum E as a function of denaturant's concentration is then obtained in two steps. Using remarks made above together with (3) one obtains

$$E(\zeta) = \frac{KU}{2} [1 + \tanh(\zeta - C_{1/2})] - E^* \exp\left(-\frac{n^2 K^2}{4} (1 + \tanh[\zeta - C_{1/2}])^2\right), \quad (8)$$

where parameters p, q , and r of (3) have to be determined to fit the mathematical properties of a particular sigmoidal

profile (see [34]); here $C_{1/2}$ is the concentration where denaturation has reached its midpoint, that is, where $\alpha(C_{1/2}) = K/2$ and E^* is defined as $E^* = n\epsilon/\sqrt{\pi}$ in order to fulfill integration requirements of δ -sequences with ϵ the ground state energy of the native state in the discrete version. As stated before, n is an arbitrary positive integer that can be adjusted accordingly. The partition function $\mathcal{Z}$ in EZM is thus obtained by substitution of (8) into (4). As it can be easily seen $\mathcal{Z}$'s integrand is a highly nonlinear function on ζ . Then, a full analytical solution is not attainable.

3. Full Analytical Solution to EZM for Linear Denaturation Profiles

It is possible to develop an analytical approximation to $\mathcal{Z}$ taking into account that, for some values of $U, \epsilon, C_{1/2}$, and ν , the integrand of (4) has a well-defined peak at some value of $0 \leq \zeta \leq \zeta^*$ where ζ^* is the critical value at which secondary structure is fully unfolded. For these cases, integral in (4) can be approximated by a highly peaked single value and a zeroth-order solution for $\mathcal{Z}$ can be obtained. Nevertheless, this approach has a strong limitation. This zeroth-order solution $\mathcal{Z}_0$ retains a complex and rich dependence on $\nu, C_{1/2}, K$ and some other quantities of interest. However, temperature dependence essentially has been dropped out leaving only a first-order temperature on T . This yields that thermodynamic functions like free energy F or specific heat C_v shows no particularly interesting behavior on temperature. In fact since C_v is a second derivative of $\mathcal{Z}_0$ with respect to T , this zeroth-order approximation yields that $C_v = 0$ giving then no further detail on the existence of thermodynamic phase transitions [34].

EZM admits a full analytical solution for $\mathcal{Z}$ in the case of a linear denaturation profile. For such case, the number α of residues with native contacts follows a linear relationship with ζ . This is

$$\alpha(\zeta) = -A\zeta + B, \quad (9)$$

where A and B are adjustable constants to consider specific denaturation profiles. The general tendency described in (9) dictates that the rate at which urea or GdnHCl denatures protein is constant. Fully denatured systems are reached at a particular concentration ζ^* . Using (9) and (1) for the functional form of E as function of concentration, it is possible to obtain E as function of ζ as

$$E = (K + A\zeta - B)U - \epsilon\delta_{0,K-\alpha}. \quad (10)$$

The combinatorial part of the degeneracy $g_\alpha = \nu^{K-\alpha} \binom{K}{\alpha}$ can be written in terms of continuous variable ζ by means of a gaussian function centered at $K/2$ which is the value where the binomial term exhibits its maximum value. This is

$$\binom{K}{\alpha} \approx \frac{2^{K+1}}{\sqrt{2\pi K}} \exp\left[-\frac{2}{K}\left(\alpha - \frac{K}{2}\right)^2\right]. \quad (11)$$

Then, the partition function $\mathcal{Z}_L$ in the case of a *linear denaturation profile* can be written as

$$\begin{aligned} \mathcal{Z}_L &= \frac{2^{K+1}}{\sqrt{2\pi K}} \left\{ \exp\left(-\frac{K}{2} + \beta\epsilon\right) \right. \\ &\quad \left. + \lim_{j \rightarrow 0} \int_j^{\zeta^*} \nu^{A\zeta} \exp\left[-\frac{2}{K}\left(\frac{K}{2} - A\zeta\right)^2 - \beta UA\zeta\right] d\zeta \right\}, \end{aligned} \quad (12)$$

where the first term corresponds to $\zeta = 0$ and the integral is performed over an infinitesimal quantity of denaturant j and afterwards a limit when $j \rightarrow 0$ is performed. Second term in (12) can be written as

$$\begin{aligned} &\int_j^{\zeta^*} \nu^{A\zeta} \exp\left[-\frac{2}{K}\left(\frac{K}{2} - A\zeta\right)^2 - \beta UA\zeta\right] d\zeta \\ &= \exp\left[-\frac{K}{2} + \frac{(\beta U - 2 - \log \nu)^2 K}{8}\right] \\ &\quad + \int_j^{\zeta^*} \exp\left[-\frac{2A^2}{K}\left(\zeta + \frac{\beta U - 2 - \log \nu}{4A}K\right)^2\right] d\zeta. \end{aligned} \quad (13)$$

If we introduce the change of variable

$$x = \sqrt{\frac{2}{K}}A\left(\zeta + \frac{\beta U - 2 - \log \nu}{4A}K\right), \quad (14)$$

we can write the following equivalence:

$$\begin{aligned} &\exp\left[-\frac{K}{2} + \frac{(\beta U - 2 - \log \nu)^2 K}{8}\right] \frac{1}{A} \sqrt{\frac{K}{2}} \int_{X_0}^{X_1} e^{-x^2} dx \\ &= \frac{1}{A} \sqrt{\frac{K}{2}} \nu^{(K/4)(2-\beta U)} \\ &\quad \times \exp\left[\frac{K}{8}(\beta U(\beta U - 4) + (\ln \nu)^2)\right] \int_{X_0}^{X_1} e^{-x^2} dx. \end{aligned} \quad (15)$$

Hence, $\mathcal{Z}_L$ in the EZM for a linear denaturation profile reads as

$$\begin{aligned} \mathcal{Z}_L &= \frac{2^{K+1}}{\sqrt{2\pi K}} \exp\left(-\frac{K}{2} + \beta\epsilon\right) + \frac{2^{K-1}}{A} \nu^{(K/4)(2-\beta U)} \\ &\quad \times \exp\left[\frac{K}{8}(\beta U(\beta U - 4) + (\ln \nu)^2)\right] \frac{2}{\sqrt{\pi}} \int_{X_0}^{X_1} e^{-x^2} dx, \end{aligned} \quad (16)$$

with the integral limits given by

$$\begin{aligned} X_0 &= \frac{-2K + KU\beta - K \ln \nu}{\sqrt{8K}}, \\ X_1 &= \frac{4A\zeta^* - 2K + KU\beta - K \ln \nu}{\sqrt{8K}}. \end{aligned} \quad (17)$$

Since the integral $\int_a^b e^{-x^2} dx$ admits a closed solution given by

$$\int_a^b e^{-x^2} dx = \frac{\sqrt{\pi}}{2} (\text{Erf}[b] - \text{Erf}[a]), \quad (18)$$

where $\text{Erf}[x]$ is the error function at x , the function $\mathcal{Z}_L$ can be finally written as

$$\begin{aligned} \mathcal{Z}_L &= \frac{2^{K+1}}{\sqrt{2\pi K}} \exp\left(-\frac{K}{2} + \beta\epsilon\right) + \frac{2^{K-1}}{A} \nu^{(K/4)(2-\beta U)} \\ &\quad \times \exp\left[\frac{K}{8}(\beta U(\beta U - 4) + (\ln \nu)^2)\right] \\ &\quad \times (\text{Erf}[X_1] - \text{Erf}[X_0]), \end{aligned} \quad (19)$$

where $\beta = (RT)^{-1}$ as usual is Boltzmann's factor.

4. Exact Solution of the EZM Model for Quasisigmoidal Denaturation Profiles

Albeit standard denaturation mechanisms usually follow a sigmoidal-like profile, the existence of an exact solution for the linear denaturation profile in EZM provides us with a powerful tool to proceed a step further towards a formulation of an analytically solvable SMCG model. While the sigmoidal denaturation profile is not analytically solvable within the EZM, it is possible to make a quite good approximation by means of a superposition of linear profiles. The idea is rather simple and it is illustrated in Figure 1. Sigmoidal denaturation profile is decomposed into the sum of three linear denaturation profiles with different slopes in such a way that sigmoidal denaturation is described as the superposition of three linear processes I, II, and III through which protein unfolds. Each process is characterized by a constant rate at which native contacts break. Initial and final processes (I and III, resp.) approximate quite well the sigmoidal profile since the derivative of hyperbolic tangent function is almost constant. However, intermediate process (II) has also a constant rate of native contact's breaking while hyperbolic tangent substantially differs from this. Nevertheless, linear and sigmoidal processes have a negative slope indicating a common decreasing mechanism. Except at the points P_j at which the derivative does not exist, the superposition of the processes I, II, and III approximates the rate at which native contacts are broken by denaturant.

Let us consider three linear denaturation processes labeled I, II, and III according to the following equations:

$$\alpha = \begin{cases} -A_1\zeta + B_1, & \text{for } 0 \leq \zeta < \zeta_1, & \text{Process I} \\ -A_2\zeta + B_2, & \text{for } \zeta_1 \leq \zeta \leq \zeta_2, & \text{Process II} \\ -A_3\zeta + B_3, & \text{for } \zeta_2 < \zeta \leq \zeta^* & \text{Process III} \end{cases} \quad (20)$$

with $\{A_1, A_2, A_3\}$ being their corresponding slopes and $\{B_1, B_2, B_3\}$ being constants that ensure mathematical continuity to the superposition. At zero denaturant concentration it is expected that $\alpha = K$, whereas at critical concentration ζ^* it is assumed that $\alpha = 0$. At intermediate concentrations

FIGURE 1: Sigmoidal and quasisigmoidal denaturation profiles for a chemically induced protein denaturation. Quasisigmoidal denaturation profile is composed by the superposition of three linear processes called I, II, and III with different slopes (gray solid line). Average rate at which native contacts break has a good correspondence between initial and final stages (processes I and III, resp.). The central discrepancy arises in the abrupt change artificially induced by the linear nature of process II. However, as we shall see, this is not a fundamental issue for the results obtained. Values used for quasisigmoidal pattern are given by $P_1(\zeta_1 = 3.5, \alpha'_1 = 39/40)$ and $P_2(\zeta_2 = 5.2, \alpha'_2 = 1/40)$, where process I intersects II and process II intersects process III, respectively. A chain of $K = 40$ residues is considered which is completely denatured at 8 Molar.

ζ_1 and ζ_2 , K_1 and K_2 residues are expected. This sets up the following equations:

$$\begin{aligned} \alpha(0) &= B_1 = K, \\ \alpha(\zeta_1) &= -A_1\zeta_1 + B_1 = -A_2\zeta_1 + B_2 = K_1, \\ \alpha(\zeta_2) &= -A_2\zeta_2 + B_2 = -A_3\zeta_2 + B_3 = K_2, \\ \alpha(\zeta^*) &= -A_3\zeta^* + B_3 = 0. \end{aligned} \quad (21)$$

This defines a 6×6 system which has a unique solution. From this, we obtain

$$\begin{aligned} A_1 &= \frac{K - K_1}{\zeta_1}, & B_1 &= K \\ A_2 &= \frac{K_1 - K_2}{\zeta_2 - \zeta_1}, & B_2 &= \frac{K_1\zeta_2 - K_2\zeta_1}{\zeta_2 - \zeta_1} \\ A_3 &= \frac{K_2}{\zeta^* - \zeta_2}, & B_3 &= \frac{K_2\zeta^*}{\zeta^* - \zeta_2}. \end{aligned} \quad (22)$$

The energy spectrum E can be written consequently as

$$E(\zeta) = \begin{cases} A_1\zeta U - \epsilon\delta_{0,K-\alpha}, & \text{for } 0 \leq \zeta < \zeta_1 \\ (K + A_2\zeta - B_2)U, & \text{for } \zeta_1 \leq \zeta < \zeta_2 \\ (K + A_3\zeta - B_3)U, & \text{for } \zeta_2 \leq \zeta < \zeta^*. \end{cases} \quad (23)$$

Hence, partition function $\mathcal{Z}_{qs}$ for the *quasisigmoidal denaturation profile* is defined as

$$\begin{aligned} \mathcal{Z}_{qs} &= \frac{2^{K+1}}{\sqrt{2\pi K}} \left[\exp\left(-\frac{K}{2} + \beta\epsilon\right) \right. \\ &\quad + \int_0^{\zeta_1} \nu^{A_1\zeta} \exp\left(-\frac{2}{K}\left(\frac{K}{2} - A_1\zeta\right)^2 - \beta U A_1\zeta\right) d\zeta \\ &\quad + \int_{\zeta_1}^{\zeta_2} \nu^{A_2\zeta + K - B_2} \exp\left(-\frac{2}{K}\left(B_2 - \frac{K}{2} - A_2\zeta\right)^2 \right. \\ &\quad \quad \left. - \beta U (A_2\zeta + K - B_2)\right) d\zeta \\ &\quad + \int_{\zeta_2}^{\zeta^*} \nu^{A_3\zeta + K - B_3} \exp\left(-\frac{2}{K}\left(B_3 - \frac{K}{2} - A_3\zeta\right)^2 \right. \\ &\quad \quad \left. - \beta U (A_3\zeta + K - B_3)\right) d\zeta \left. \right]. \end{aligned} \quad (24)$$

Each of the integrals involved in (24) has the same functional aspect as the one defined and solved previously in Section 3. Hence, an analytical full solution of each of the integrals in (24) can be accomplished to give an analytical expression for $\mathcal{Z}_{qs}$. That is,

$$\begin{aligned} \mathcal{Z}_{qs} &= \frac{2^{K+1}}{\sqrt{2\pi K}} \exp\left(-\frac{K}{2} + \beta\epsilon\right) \\ &\quad + \frac{2^K}{2} \nu^{(K/4)(2 - \beta U)} \exp\left(\frac{K}{8} [\beta U (\beta U - 4) + (\ln \nu)^2]\right) \\ &\quad \times \left[\frac{1}{A_1} (\text{Erf}(Y_1) - \text{Erf}(Y_0)) \right. \\ &\quad + \frac{1}{A_2} (\text{Erf}(Y_2) - \text{Erf}(Y_1)) \\ &\quad \left. + \frac{1}{A_3} (\text{Erf}(Y^*) - \text{Erf}(Y_2)) \right], \end{aligned} \quad (25)$$

where the quantities Y_j are defined as follows:

$$\begin{aligned} Y_0 &= \frac{K(-2 + \beta U - \ln \nu)}{\sqrt{8K}}, \\ Y_1 &= \frac{K(2 + \beta U - \ln \nu) - 4K_1}{\sqrt{8K}}, \\ Y_2 &= \frac{K(2 + \beta U - \ln \nu) - 4K_2}{\sqrt{8K}}, \\ Y^* &= \frac{K(2 + \beta U - \ln \nu)}{\sqrt{8K}}, \end{aligned} \quad (26)$$

and quantities A_j are given in (22). Configurational partition function $\mathcal{Z}_{qs}$ defined in (25) provides us with the main

frame to analyze equilibrium thermodynamic properties of polypeptide chains under the action of quasisigmoidal denaturation profiles. In order to investigate thermodynamic equilibrium properties we shall use $\mathcal{Z}_{qs}$ to evaluate configurational entropy $\mathcal{S}$, average energy $\mathcal{E}$, and thermodynamic susceptibilities like specific heat $\mathcal{C}_v$ which contain substantial information on the thermodynamic response of the system.

5. Thermodynamic Equilibrium Response to Quasisigmoidal Denaturation Profiles

Given a particular quasisigmoidal denaturation profile two possible mechanisms can be identified. Figure 2 shows two different possibilities for chemical denaturation between concentrations $\zeta = 0$ and $\zeta = \zeta^* = 6$ M. Central difference is associated with the rate at which native contacts are broken by denaturant. As discussed previously, for a strict sigmoidal process the rate [44] at which native contacts are broken is essentially constant in the initial and final stages of denaturation, whereas the middle process has a highly nonconstant rate. A quasisigmoidal denaturation profile can approximate this property and in addition includes two variants. Figure 2(a) shows a denaturation mechanism composed by a slow and fast denaturation modes, whereas Figure 2(b) depicts a mechanism with fast and slow denaturation modes. We shall call these two mechanisms type A and type B denaturation profiles, respectively. For type A, native contacts are broken in a slow mode initially and afterwards a fast denaturation mode is induced. In a type B process, native contacts are broken swiftly in initial stages and then a slow mode is induced. In both cases, initial and final concentrations are the same. A slow denaturation mode means that a larger amount of denaturant is needed to break down a certain fraction of native contacts in comparison to a fast denaturation mode.

If we restrict the model to helix-coil transitions, it is possible to establish a connection between the number ν of potential nonnative conformations which is a phenomenological parameter in the original ZW model and the helical propensity ω of a residue to participate in a helix-coil transition [37]. This relation proposes that in absence of an enthalpic energy barrier between helical and coil states, $\nu = \omega^{-1}$. This relation is a reasonable first-order approximation since as a particular residue is more prone to participate in a helical state (native conformation), the probabilities that it participates in a coil state (nonnative conformation) clearly diminish. Such quantitative relationship enables us to particularize the solution obtained for $\mathcal{Z}_{qs}$ for specific homopolymer chains with the replacement $\nu = \omega^{-1}$. Tables with measurements of ω_j for each of the twenty or so aminoacid residues are widely known. Here, we shall use data obtained from Chakrabarty et al. [45]. Figure 3 shows specific heat C_v as function of temperature T for poly-alanine (poly-A) and poly-valine (poly-V) chains composed of $K = 40$ residues in a slow-fast denaturation (type A) and fast-slow denaturation (type B) mechanisms. According to [45] for alanine, $\omega = 1.61$ whereas valine has $\omega = 0.23$. This scale suggests that alanine is one of the strongest helix formers whereas valine is basically a helix breaker. Since $\nu \approx \omega^{-1}$ [37], poly-A chains would

have a smaller number of *average* nonnative conformations in comparison to poly-V chains. Since helical propensity for valine, ω_V , is smaller than helical propensity for alanine ω_A , then $\nu_V > \nu_A$ holds and larger peak in poly-V chain is expected.

The specific role of denaturation mode is showcased in Figure 3 as well. Figure 3(a) considers a poly-A chain subject both to a slow-fast denaturation mechanism (thick line) and a fast-slow denaturation mechanism (dotted line). It is observed that this factor plays a significative role in the thermodynamic stability of the chain. The transition temperature T_d varies appreciably for each case. The phase transition signaled by the peak in the specific heat C_v occurs earlier for the slow-fast denaturation mode (type A) than for the fast-slow denaturation mode (type B). This phenomenon is, however, inverted in the case of the poly-V chain. In this case, the transition temperature T_d associated with the type B denaturation is smaller than its counterpart in the type A mechanism. The intensity in the C_v maximum is also inverted. Type A mode is associated with a larger C_v peak for poly-A chain whereas for poly-V chain type A denaturation mode is associated with the smaller C_v peak. Within our model the difference between poly-A and poly-V chains lies exclusively in the helical propensity ω and its related variable ν . Hence, the number of potential nonnative conformations ν and the type of denaturation mechanism both play a fundamental role in thermodynamic stability of configurations, in particular in the transition temperature T_d . It is worth observing that $\nu_A < 1$ and $\nu_V > 1$; thus, $\nu = 1$ signals a threshold on thermodynamic stability. Homopolymer chains poly-X with a larger amount of conformational degrees of freedom are expected to have higher denaturation temperatures since more thermal energy is needed to overcome nonnative conformations. Larger values of ν tend to shift T_d to higher values. Such observation is consistent with results shown in Figure (3). The value $\nu \neq 0$ is not allowed since in that case each residue has only a single state and conformational transitions would be blocked.

For a further characterization of the equilibrium properties of the chains, we have calculated the configurational equilibrium entropy $\mathcal{S}$ as function of temperature for previous poly-A and poly-V chains. Figure 4 shows $\mathcal{S}(T)$ for poly-A (a) and poly-V (b) in type A and B denaturation mechanisms. Given the fact that $\mathcal{S}$ is a first-order derivative of the free energy $\mathcal{F}$ with respect to temperature, that is,

$$\mathcal{S} = -\left(\frac{\partial \mathcal{F}}{\partial T}\right)_{N,V}, \quad (27)$$

a discontinuous change of $\mathcal{S}$ at critical temperature T_d can be directed being associated with a first-order phase transition [30, 46, 47]. The formal definition of a first-order phase transition requires that entropy undergoes a *discontinuous* change at the transition temperature T_d . However, for a finite poly-X chain, it is expected that $\mathcal{S}$ changes continuously. As the number of residues K increases, a true first-order phase transition is expected since the peak observed in C_v becomes

FIGURE 2: Two possible denaturation mechanisms depending on the rate at which native contacts break can be devised for a given quasisigmoidal denaturation profile. In a slow-fast mode denaturation (a), the rate at which native contacts are broken is essentially constant at the beginning and then a higher increase of the rate is observed at the end. For the fast-slow mode denaturation (b), a small amount of denaturant induces a higher rate of denaturation in the beginning. Then, the process is essentially constant up to the critical concentration.

FIGURE 3: Specific heat C_v for a poly-alanine (poly-A) and poly-valine (poly-V) chains under a slow-fast denaturation mode (type A) and a fast-slow denaturation mode (type B). In both cases, the number of residues was set to $K = 40$, $U = 0.75 \times 10^3 \text{ J mol}^{-1}$, $\epsilon = 35 \times 10^3 \text{ J mol}^{-1}$. Type A mechanism is characterized by $A_1 = 0.32$, $A_2 = 16.19$, $A_3 = 6.25$, $K_1 = 39$, and $K_2 = 5$ whereas type B is defined through parameters $A_1 = 64$, $A_2 = 0.44$, $A_3 = 6$, $K_1 = 8$, and $K_2 = 6$. Helical propensity $\omega_A = 1.61$ was considered for alanine while $\omega_V = 0.23$ was considered for valine.

narrower and higher. Recently it has been shown that a first-order phase transition in the limit of large K is embedded in this type of coarse-grained models [37].

Figure 4 shows that although both poly-A and poly-V chains exhibit a first-order phase transition, such thermodynamic transition becomes steeper in the case of poly-V chains for the same values of parameters used. Smaller helical propensities imply then that thermodynamic transitions

occur in a smaller window of temperature. For poly-V chain, $\omega = 0.23$ and the temperature range in which the transition occurs is $\Delta T = 40 \text{ K}$ approximately. For poly-A chains, $\omega = 1.61$ and $\Delta T = 100 \text{ K}$, approximately. Entropy differences $\Delta \mathcal{S}$ between unfolded and folded structures $\Delta \mathcal{S} \approx 100 \text{ J K mol}^{-1}$ for poly-A and $\Delta \mathcal{S} \approx 500 \text{ J K mol}^{-1}$ suggest as well that poly-V denaturation is much more favorable in entropic terms than poly-A. This is consistent with the fact that within this

FIGURE 4: Configurational entropy $\mathcal{S}$ for poly-alanine (poly-A) and poly-valine (poly-V) chains under a slow-fast denaturation mode (type A) and a fast-slow denaturation mode (type B). In both cases, the number of residues was set to $K = 40$, $U = 0.75 \times 10^3 \text{ J mol}^{-1}$, and $\epsilon = 35 \times 10^3 \text{ J mol}^{-1}$. Profile A is characterized by $A_1 = 0.32$, $A_2 = 16.19$, $A_3 = 6.25$, $K_1 = 39$, and $K_2 = 5$ whereas profile B is defined through parameters $A_1 = 64$, $A_2 = 0.44$, $A_3 = 6$, $K_1 = 8$, and $K_2 = 6$. Helical propensity $\omega_A = 1.61$ was considered for alanine while $\omega_V = 0.23$ was considered for valine.

model poly-V residues have more nonnative conformations; thus more microstates are available to a poly-V chain in comparison with poly-A.

6. Conclusions and Final Remarks

A full analytical solution for the EZM model in the quasisigmoidal approximation model is presented in order to calculate equilibrium thermodynamic properties of proteinogenic homopolymer poly-X chains under the action of a chemically induced denaturation mechanism. As discussed previously, the original EZM model considers the exact sigmoidal denaturation curve common to most chemical denaturation processes. The EZM partition function is then expressed in terms of a highly nonlinear integrand on the number of microstates as function of ζ . Given this fact, a full solution is not achievable. In this work, a quasisigmoidal representation for denaturation curve is used to obtain a full analytical solution to the EZM model. Results obtained here show that $\mathcal{Z}_{qs}$ can be obtained in terms of error functions $\text{Erf}(x)$ and the slopes A_j of the individual linear denaturation mechanisms that compose the quasisigmoidal approximation. With this approximation, the EZM model can be included in the class of analytically solvable SMCG models.

For a given quasisigmoidal denaturation mechanism two submechanisms can be identified depending on the rate at which native contacts are broken by denaturant. The first process which we called type A denaturation mode is defined by a slow rate of native contacts breaking followed by a fast denaturation rate. The second process which we called type B mode is characterized by a fast followed by a slow rate of native

contact breaking. Both type A and type B denaturation modes have the same initial and final stages. At $\zeta = 0$, $\alpha' = 1$ and at $\zeta = 8 \text{ M}$, $\alpha' = 0$ in both cases. A critical value $\zeta^* = 8 \text{ Molar}$ to achieve complete denaturation was set according to reports on the literature for urea denaturation experiments, although a different value can be used. Results obtained here do not depend qualitatively on the exact value of ζ^* . Using the fact that the number of potential nonnative conformations ν can be related thermodynamically to the helical propensity ω , thermodynamic properties like the specific heat $\mathcal{C}_v$ and configurational entropy $\mathcal{S}$ as function of temperature were calculated for poly-alanine (poly-A) and poly-valine (poly-V) homopolymer chains. For these homopolymer chains, the influence of the type of denaturation mode was also discussed.

Specific heat C_v calculations as function of temperature T and helical propensity ω show that the mode of denaturation (either type A or B) does play a significant role in thermodynamic stability, specifically in the value of the transition temperature T_d . For poly-A chains, type B denaturation mode shows a higher degree of thermodynamic stability, that is, a larger value of the transition temperature T_d . However, for poly-V chains, the opposite situation is observed. This implies that although both denaturation modes have the same initial and final stages, the rate at which native contacts are broken does play a role in the location of a thermodynamic transition. Such is consistent with the fact that $\mathcal{Z}_{qs}$ indeed depends on the slopes A_1 , A_2 , and A_3 of the linear processes I, II, and III that form the quasisigmoidal denaturation profile. C_v curves also show that the intrinsic nature of the X residue in the poly-X chain has a role in thermodynamic properties. Since $\omega_A > \omega_V$ and $\omega \approx \nu^{-1}$, poly-A chains exhibit a small

number of nonnative microstates whereas for poly-V chains this number is larger. This is in fact what is observed in Figure 3. The intensity of the C_v peak for poly-V is larger than its counterpart in poly-A. Main result obtained here is that the location of transition temperature T_d is both a function of the helical propensity ω and denaturation mode. For instance, poly-X chains with higher propensities to undergo a random coil helix transition shall denature at lower temperatures on type A denaturation mode.

Results obtained for configurational entropy $\mathcal{S}(T)$ for both poly-A and poly-V chains confirm the fact that poly-A tends to have a smaller number of microstates than poly-V since entropy differences $\Delta\mathcal{S}$ in poly-A and poly-V are different. For poly-A, $\Delta\mathcal{S} \approx 100 \text{ J K mol}^{-1}$ whereas for poly-V, $\Delta\mathcal{S} \approx 500 \text{ J K mol}^{-1}$. Since $\mathcal{S} = R \log \Omega$ with Ω the number of microstates available, Figure 4 confirms that $\Omega_A < \Omega_V$ as suggested by the specific heat plots. $\mathcal{S}(T)$ also contains information on the nature of the thermodynamic transition involved. Figure 4 suggests that ω is the main driving factor for a steeper transition (left panel). Poly-X chains with values $\omega < 1$ display a steeper thermodynamic transition. For such chains, transition temperatures T_d are slightly affected by the type of transition (A or B) in contraposition to poly-X chains with $\omega > 1$ like poly-A where sensitive dependence on denaturation mode is observed.

Comparison of the results obtained in this work with MD simulations of poly-alanine chains in vacuo shows that conformational transitions do occur even in absence of solvent interactions [48]. Conformational transitions predicted in this work signaled by a peak in the specific heat C_v (Figure 3) and configurational entropy (Figure 4) are in qualitative agreement with MD simulations regarding the fact that the steepness of the transition depends on the size of the chain [48]. Smaller chains have “softer” transitions in comparison with larger ones. This fact is indeed what is expected from the general theory of equilibrium statistical mechanics [49, 50] where true thermodynamic first-order phase transitions appear strictly as $K \rightarrow \infty$ and the volume of the system remains constant. Recently sodiated poly-alanine chains Ala_KNa^+ with $K = 8, 9, 10, 11$, which are poly-A chains with a Na^+ cation attached to the C terminus have been shown to exhibit helical stable conformations in vacuo as well suggesting that conformational transitions are robust phenomena in polypeptides with larger helical propensities even in the absence of solvent interactions [51]. Free energies reported for α helical conformations of these peptides range from -30 kJ mol^{-1} for Ala_8Na^+ up to $-44.2 \text{ kJ mol}^{-1}$ for $\text{Ala}_{10}\text{Na}^+$. This value can be compared with our results if we consider that the free energy change ΔF can be estimated as

$$\Delta F = \Delta E - T\Delta S, \quad (28)$$

where the internal energy change $\Delta E = -(KU + \epsilon) \approx -65 \text{ kJ mol}^{-1}$ for the values considered in Figure 3 and ΔS can be estimated from the results obtained in Figure 4. Since $\Delta E \approx 65 \text{ kJ mol}^{-1}$ and $T\Delta S = 20 \text{ kJ mol}^{-1}$ we can see that our results predict a value $\Delta F \approx -45 \text{ kJ mol}^{-1}$ which is in quite good agreement with these MD results for short Alanine peptides.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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