Journal of Inorganic Biochemistry 179 (2018) 135–145

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Relaxation of structural constraints during Amicyanin unfolding

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A B S T R A C T

We study the thermal unfolding of amicyanin by quantifying the resiliency of the native state to structural perturbations. Three signatures characterizing stages of unfolding are identified. The first signature, lateral extension of the polypeptide chain, is calculated directly from the reported crystallographic data. Two other signatures, the radial displacement of each residue from Cu(II) and the angular spread in the chain as the protein unfolds, are calculated using crystallographic data in concert with a geometrical model we introduced previously (J.J. Kozak, H. B. Gray, R. A. Garza-López, J. Inorg. Biochem. 155(2016) 44–55). Particular attention is paid to the resiliency of the two beta sheets in amicyanin. The resiliency of residues in the near neighborhood of the Cu center to destabilization provides information on the persistence of the entatic state. Similarly, examining the resiliency of residues intercalated between structured regions (beta sheets, the alpha helix) provides a basis for identifying a "hydrophobic core." A principal focus of our study is to compare results obtained using our geometrical model with the experimental results (C. La Rosa, D. Milardi, D. M. Grasso, M. P. Verbeet, G. W. Canters, L. Sportelli, R. Guzzi, Eur. Biophy. J.30(8),(2002) 559–570) on the denaturation of amicyanin, and we show that our results support a classical model proposed by these authors.

1. Introduction

Blue copper proteins (BCPs), their structures, properties, and roles in photosynthesis, in respiration, in oxidative deamination of primary amines, and in reduction of nitrite reductase have long attracted the interest of a large community of chemists. Fundamental advances in our understanding of these proteins have been discussed in seminal papers and influential reviews [1–9]. A physical chemical property underlying the structures and functions of these proteins is their structural stability. The intent of this study is to focus on one blue copper protein, amicyanin, and assess its response when the native state is systematically disrupted.

We have developed a geometrical model to follow quantitatively the early stages in the unfolding of a native protein. The main objective of the present study is to make comparisons of results obtained using our geometrical model with experimental unfolding data for amicyanin from T. versutus (now P. versutus) reported by La Rosa et al. [10]. Our study is based on amicyanin coordinates obtained from P. denitrificans (PDB# 1AAC). We expect the difference (if any) between results obtained using PDB# 1AAC and the amicyanin studied by La Rosa et al. to be minor, as the alpha-carbon coordinates of both proteins are essentially the same.

Our geometrical model has been elaborated in earlier publications [11–17]. We emphasize that we have now extended our approach to account for the native structure of amicyanin (as well as those of other BCPs), as this structure features several beta sheets [See Fig. 1]. Fundamental to our overall approach is that *first-nearest-neighbor* repulsive *and* attractive interactions between each residue i and its two nearest-neighbors are kept intact by introducing a triplet modular unit centered on residue i. The geometry of each triplet is determined by, and our subsequent analysis rests on, crystallographic evidence reported for this protein: See Refs [18–21] for amicyanin residue and metal ion coordinates (105 residues). By enforcing the constraint that nearest-neighbor interactions remain unchanged, we conserve the locally optimized structure of the protein.

In a globally optimized native state, based on crystallographic data, geometrical constraints delimit sterically the number of possible configurations accessible to a polypeptide chain, a fundamental insight that goes back to Ramachandran [22] (and can be seen in phi/psi plots). In our approach, structural perturbations of the native state are studied by relaxing steric (geometrical) constraints (*only*) between and among *non-nearest* neighbors.

Among the myriad of configurations that can be adopted by a segment of a polypeptide chain as the native state is disrupted, we adopt

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https://doi.org/10.1016/j.jinorgbio.2017.11.016
Received 5 July 2017; Received in revised form 28 September 2017; Accepted 17 November 2017
Available online 22 November 2017

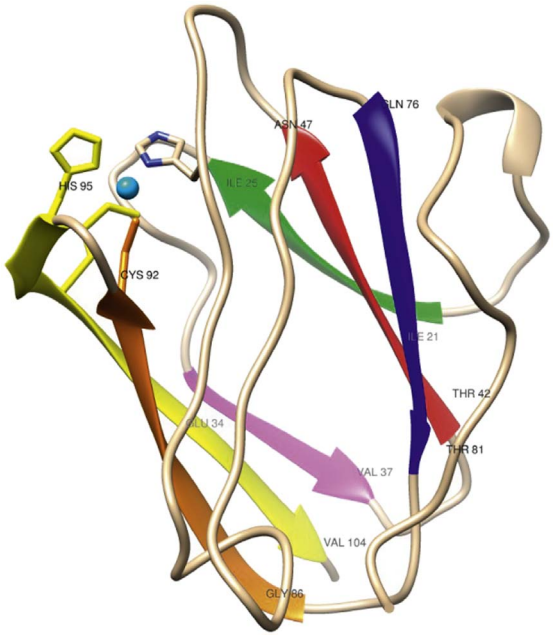

Fig. 1. Strands for amicyanin (PDB# 1AAC) considered.

one configuration as representative of the many that can result upon destabilization, viz., a maximally extended, linear sequence of triplets. By choosing this configuration for all proteins in a given family, or for proteins of different families with very different physiological functions (e.g., the cytochromes [11], or microbial proteins [14]), unfolding processes can be systematically compared (as the reference state is fixed).

2. Computational details

To study the structural stability of the native state of a protein, we calculate three signatures (defined below) that characterize stages of unfolding of the native state. The first signature, the lateral displacement, is calculated directly from reported crystallographic data. Two signatures, the radial displacement of each residue relative to the metal center (in our case the copper of amicyanin) and the angular spread in the chain as the protein unfolds, are calculated using crystallographic data in concert with our geometrical model.

To illustrate the calculation of these signatures using our geometrical model, consider the second stage of unfolding for the $n = 7$ residue segment, Gly 86 to Cys 92, centered on Asp 89. This segment of amicyanin is H-bonded to the His 95 to Val 104 segment, forming one "ladder" of a beta sheet [23,24]. We specify the origin of the coordinate system to be the metal ion [Cu(II)]; and all unfolding processes are calculated relative to this fixed point.

The following distances can be calculated from the crystallographic data using the Theorem of Pythagoras:

D(Cu to R86) = 23.83 Å

D(Cu to R92) = 4.571 Å

D(R86 to R92) = 20.10 Å

Using these values and the Law of Cosines (Proposition 12 in Euclid's *Geometry*), the angles α, β, and γ are:

$\beta = 31.93^{\circ}$

$\alpha = 141.16^{\circ}$

$\gamma = 6.910^{\circ}$

Using the Law of Cosines, we determine the angles between residues 86 and 88, residues 88 and 90, and residues 90 to 92:

$\beta(86 \text{ to } 88) = 4.623^{\circ}$

$\beta(88 \text{ to } 90) = 7.910^{\circ}$

$\beta(90 \text{ to } 92) = 20.30^{\circ}$

The sum of these three angles, which characterizes the angular extension of a fully extended segment centered on Asp 89, is 32.83°, a value that can be compared with the angular extension between residues 86 and 92 in the native state calculated above, viz., 31.93°. The ratio of these two values, 1.03, gives a measure of the angular extension of the fully extended, seven-residue segment centered on Asp 89 relative to the angular extension of the same segment in the native state.

Using crystallographic data, we next calculate the distance between the alpha carbons of the two "terminal" residues in the seven-residue segment, Gly 86 to Cys 92, in the native state:

D(R86 to R92) = 20.10 Å

Again using crystallographic data, we determine the distance T_{89} for the fully extended configuration displayed in Fig. 2,

T_{89} = R86 to R88 + R88 to R90 + R90 to R92 = 20.23 Å

The ratio of these two distances,

$T_{89}/R(86 \text{ to } 92) = 1.01$

gives a measure of the lateral extension of the seven-residue segment, Gly 86 to Cys 92, in the fully extended state relative to the native state.

Finally, using the distance T_{89} in conjunction with the Law of Sines, we calculate the change in the radial distance of the alpha carbon of Asp 89 in the native state versus the seven-residue, fully extended state. The distance R (Cu to Asp 89) in the native state is 14.70 Å and in the fully extended state is 22.25 Å. The ratio of these two values defines the radial-extension metric f_{89} for Asp 89:

$$f_{89} = R(\text{Cu to Asp } 89)_{\text{extended state}}/R(\text{Cu to Asp } 89)_{\text{native state}} = 1.51$$

As evident from the above calculations, whereas the relative change in the angular spread and in the lateral extension between the native state and the fully extended segment is small, the change in extension metric is more pronounced. It is important to note, however, that the values of these signatures depend on the environment in which a particular residue is situated, a beta sheet (the above case), an alpha helix or, for example, a hydrophobic region: See, for example, Figs. (3–5), where the three signatures are displayed for all amicyanin residues in the second stage of unfolding.

Values of the above metrics have been calculated for each amicyanin residue and for each of the first six stages of unfolding. For example, see Table 1, where we display the signatures for the first six stages of unfolding for residues within ≈ 10 Å of Cu(II), several of which are associated with the protein entatic state. These three signatures change synchronously as amincyanin unfolds. To illustrate the simultaneous configurational changes in amicyain as a whole as the native structure is thermally destabilized, see Fig. (6a) and (6b), which display the third and sixth stages of unfolding calculated using our geometrical model.

3. Resiliency of beta sheets

To explore the resiliency of amicyanin beta sheets to structural perturbations we again focus on alpha-carbon to alpha-carbon distances, as determined from crystallographic data. Here, however, we consider residues in adjacent segments H-bonded to each other. To illustrate, the data show that the following residues are "coupled," forming one "ladder" of a beta sheet (H-bond distances in parentheses):

Thr 42 to Thr 81 (2.744 Å)
Val 43 to Leu 80 (2.815 Å)