Folding degrees of azurins and pseudoazurins
Implications for structure and function
Ernesto Estrada a,∗, Eugenio Uriarte b

a Complex Systems Research Group, X-Ray Unit, Edificio CACTUS, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain
b Department of Organic Chemistry, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

Accepted 23 June 2005

Abstract

A quantitative measure of the degree of folding of azurins and pseudoazurins has been made. We have found that the reduction potential of azurins and pseudoazurins is a function of the contribution to the degree of folding of His117, a key amino acid in electron transfer which is directly bonded to copper in these proteins. The folding degree of His117 explains 95% of the variance in the experimental values of the reduction potential of azurins and pseudoazurins. The change in the folding degree of this amino acid influences several geometric parameters of the main backbones of these proteins. Among them, the angle formed between N(His117)···Cu···S(Cys112), which plays an important role in electron transport, but not the N(His117)···Cu distance, shows some non-linear correlation with the reduction potential of azurins and pseudoazurins. However, it is only able to explain less than 75% in the variance of the reduction potential of these proteins instead of the 95% explained by the folding degree of His117.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Folding degree; Blue copper proteins; Structure–function relation; Copper binding site; Protein geometry

1. Introduction

One of the principal goals of the proteome research is the study of protein structure and the understanding of the role it plays in the biochemistry, physiology and pathology of the cell (Domou and Broder, 2004). An understanding of these processes permits the successful search of disease markers as well as of possible targets for virtual drug screening. An important characteristic of the 3D structure of proteins is the degree of folding of the protein chain (Randić and Krilov, 1997, 1999; Estrada, 2004a,b). Because proteins fold to optimize the conformational preferences of amino acids subject to local and global constraints, it is expected that folding degree is related to the structure, evolution and function of proteins.

Families of proteins are comprised of members who share the same three-dimensional structure, perhaps as a consequence of evolution. One of these families is formed by proteins that have a structurally conserved 90-to 150-amino acid sequence module known as the “blue copper binding” domain (Nersissian and Shipp, 2002). The blue copper proteins (BCPs) are a subclass of the copper proteins that catalyze redox reactions in several types of organisms (Lappin, 1991), in which the copper changes from a diamagnetic, Cu(I), to a paramagnetic, Cu(II), oxidation state. Cu(II) is strongly coordinated to the sulfur atom of a cysteine producing an anomalously small parallel hyperfine coupling constant and a very strong absorption band at ca. 600 nm, giving the typical blue color to these proteins (Solomon et al., 1992). This coordination site, the so-called type-1, is completed with two imidazole nitrogens (histidines) in equatorial positions and a sulfur thioether (methionine) in an axial position (Katz et al., 2003). The type-1 site does not show the familiar tetrahedral or square planar/octahedral structures of small Cu(I) and Cu(II) coordination compounds (Broman et al., 1962; Shepard et al., 1990). In fact, it has been described as a strained structure intermediate between those of Cu(I) and Cu(II). Several authors have assigned to this distinctive
structure the significance of being ideal for the shuttling of electrons (Vallee and Williams, 1968; Williams, 1971). It has also been argued that this structure is responsible for the high redox potential of these proteins compared to the Cu(I)/Cu(II) couple in water (James and Williams, 1961; Gray et al., 2000).

However, recent calculations fail to support the suggestion that strain plays a significant role in the function of these proteins (Ryde et al., 2000).

The 3D structures of a large number of BCPs, such as azurins, pseudoazurins, stellacyanin, plastocyanins and ceruloplasmin, have been solved (Katz et al., 2003). Azurins are the most prominent members of the BCP family. They show the unique structural feature of a second axial group, the carbonyl oxygen of a glycine. Although the biological redox partner of azurins remains unknown, it is known that they are involved in anaerobic nitrite respiration and have been shown to donate electrons to nitrite reductase, a function thought to involve another BCP, pseudoazurin (De Rumezo et al., 2000). Azurins have been found to induce apoptotic death in macrophages. They form a complex with the tumor suppressor protein p53 stabilizing it and raising its intracellular level (Punj et al., 2003). On the other hand, pseudoazurins suppressor protein p53 stabilizing it and raising its intracellular level (Punj et al., 2003).

Here we study the role played by the folding of azurins and pseudoazurins on their structure and function. The degree of folding quantitatively measures how folded a protein backbone is. We have studied the global and local folding degree for a series of azurins and pseudoazurins and have found that their reduction potential is a function of the degree of local folding of one of the amino acids at the binding site. The explanations and possible implications of these findings are also discussed.

1.1. Degree of protein folding: global and local

The protein folding degree index $I_3$ transforms the qualitative criterion of folding to a quantitative scale, in which two proteins can be differentiated by measuring the degree of folding of their backbone chains (Estrada, 2002, 2004a).

The protein folding degree index is based on the torsion angles of the protein backbone chain, i.e., the so-called $\phi$, $\psi$ and $\omega$ torsion angles. These are all equal to 180° for a fully extended polypeptide chain, which corresponds to the least folded structure that a chain can hypothetically adopt. The angle $\omega_0$ defines the rotation about the C$_{\alpha}$-N$_{i+1}$ peptide bond, $\phi_i$ describes the rotation about N$_i$-C$_{\alpha}$ bond and $\psi_i$ describes the rotation about the C$\alpha_i$-C$_{\beta}$ bond. Fig. 1 shows a portion of a protein backbone indicating the these torsion angles. The folding degree index is defined from a graph whose nodes represent $\phi$, $\psi$ and $\omega$ torsion angles and two nodes are linked if, and only if, the corresponding angles are contiguous in the backbone chain of the protein as indicated in Fig. 1.

Let $A$ be the adjacency matrix of this chain representing the adjacency of torsion angles in the protein backbone and let $T$ be a diagonal matrix of the cosines of $\phi_i$, $\psi_i$ and $\omega_i$ angles (Estrada, 2002, 2004a). Define $B = A + T$ as a matrix representing the protein backbone, which has the spectrum $\sigma = (\lambda_1, \lambda_2, \ldots, \lambda_N)$, where $N$ is the number of atoms in the protein backbone and $t = N - 3$ is the number of $\phi$, $\psi$ and $\omega$ torsion angles. Then, the folding degree index $I_3$ is defined as (Estrada, 2000, 2002, 2004a):

$$ I_3 = \frac{1}{N - 3} \sum_{j=1}^{N-3} \mu_j \frac{1}{k!} $$

(1)

The index $I_3$ represents a global characterization of protein folding degree through the sum of contributions coming from individual torsion angles, pairs, triples, quadruples, etc. of contiguous torsion angles, in a way in which larger sequences of contiguous torsion angles receive lower weights than shorter ones. This interpretation follows directly from the fact that $I_3$ can be expressed as the infinite sum of spectral moments $\mu_k$ of $B$ divided by $k!$, where the spectral moment $\mu_k$ represents the sum of all closed walks of length $k$ in the chain (Estrada, 2000, 2002, 2004a):

$$ I_3 = \frac{1}{N - 3} \sum_{k=1}^{N-3} \mu_k \frac{1}{k!} $$

(2)

Each closed walk is associated with a sequence of torsion angles in the chain. For instance, closed walks of length zero represent the nodes of the chain, and corresponds to the single torsion angles, closed walks of length two stand for the edges of the chain, and symbolizes the pairs of adjacent torsion angles in the chain, etc.

It has been previously shown that Eq. (2) can be expressed simply as a sum of the local spectral moments of $B$ (Estrada, 2004b):

$$ I_3 = \frac{1}{N - 3} \sum_{k=1}^{N-3} \sum_{i=1}^{n} \mu_k(t) \frac{1}{k!} $$

(3)

where the local spectral moment $\mu_k(t)$ consists simply of the diagonal entries of the $k$th power of $B$ corresponding to the torsion angle $t(\phi, \psi$ or $\omega)$ in the chain. Let $t$ denote an amino acid of the protein with torsion angles $\phi_i$, $\psi_i$ and $\omega_i$.

The 3D structures of a large number of BCPs, such as azurins, pseudoazurins, stellacyanin, plastocyanins and ceruloplasmin, have been solved (Katz et al., 2003). Azurins are the most prominent members of the BCP family. They show the unique structural feature of a second axial group, the carbonyl oxygen of a glycine. Although the biological redox partner of azurins remains unknown, it is known that they are involved in anaerobic nitrite respiration and have been shown to donate electrons to nitrite reductase, a function thought to involve another BCP, pseudoazurin (De Rumezo et al., 2000). Azurins have been found to induce apoptotic death in macrophages. They form a complex with the tumor suppressor protein p53 stabilizing it and raising its intracellular level (Punj et al., 2003). On the other hand, pseudoazurins suppressor protein p53 stabilizing it and raising its intracellular level (Punj et al., 2003).
The contribution of this amino acid to the global protein degree of folding has been defined as the infinite sum of the local spectral moments corresponding to the $\phi_i, \omega_i$ torsion angles (note that $\omega_i$ corresponds to the peptide bond which is shared by amino acids $i$ and $i+1$) (Estrada, 2004b):

$$I_3(i) = \sum_{k=0}^{\infty} \frac{\mu_k(\phi_i) + \mu_k(\psi_i)}{4^k}$$

(4)

Using graph spectral theory we prove that the local contribution of the $i$th amino acid to the global protein folding can be expressed as follows (see Appendix A):

$$I_3(i) = \sum_{j=1}^{N} e^{\psi_j([\nu_1; \nu_2])} + e^{[\psi_j([\nu_1; \nu_2])]}$$

(5)

where $\nu_1, \nu_2, \ldots, \nu_N$ is an orthonormal basis of $\mathbb{R}^N$ composed by eigenvectors of $\mathbf{B}$ associated to the eigenvalues $\lambda_1, \lambda_2, \ldots, \lambda_N$, $\nu_j(\phi_i)$ and $\nu_j(\psi_i)$ denotes the component of $\nu_j$ corresponding to the torsion angles $\phi$ and $\psi$ of the $i$th amino acid.

Eq. (5) was given by Estrada (2004b) in a different formula as well as the first chain is represented by the sequence ETTTE. The other chains have sequences: TETTE, TTETE, TTEET and TETET, respectively. This means that all these chains have 40% of E and 60% of T conformations. The only thing changing is the distribution of these regions along the chain. This situation is also typical in protein chains. For instance, the following three proteins have the same percentage of helix (38%) and strand (15%) but they have different values of the folding degree index (in parenthesis): 1OYB (3.0629); 1APS (3.1012) and ITCA (3.1226). Other examples are, for instance, 2HBG and 1CPD which have 78% of helix and 0% of strand and $I_3$ indices of 3.3718 and 3.3381, respectively as well as 1LEN and 2BBK with 3% of helix and 50% of strand and $I_3$ values of 2.6750 and 2.7165, respectively.

1.2. Proteins dataset

The dataset of azurins and pseudoazurins studied in this work is comprised of the following 15 proteins, for which PDB codes and crystallographic resolution (in parentheses) are also given (see Ryde et al., 2000 for coordination of Cu(I) and references for crystallographic data): A. xylosoxidans azurin (pH 5.0), A. denitrificans (M121Q) azurin (pH 5.5), A. denitrificans (M121H) azurin (pH 5.5), A. denitrificans (M121E) azurin (pH 5.5), 1OYB (3.0629); 1APS (3.1012) and ITCA (3.1226). Other examples are, for instance, 2HBG and 1CPD which have 78% of helix and 0% of strand and $I_3$ indices of 3.3718 and 3.3381, respectively as well as 1LEN and 2BBK with 3% of helix and 50% of strand and $I_3$ values of 2.6750 and 2.7165, respectively.

2. Results and discussion

The values of the degree of global folding index for the 15 proteins studied are given in Table I. The average folding degree for these proteins is 2.7675, a low value compared to that found in other protein families. For instance, the average value of $I_3$ for 152 proteins studied by Estrada (2004a,b) is 2.9767. The average value of $I_3$ for azurins and pseudoazurins agrees with the fact that blue copper proteins form mainly-\beta sandwich structures. Azurins show a slightly higher values of folding compared to pseudoazurins. This order of
Fig. 2. Representation of a chain of eight vertices with different folding conformations but having the same sum of dihedral angles. The matrices representing the adjacency of torsion angles in the protein backbone, their eigenvalues and the $I_2$ indices of each conformation are also given.
Table 1
Global folding degree indices ($I_3$) and other structural parameters of the azurins and pseudoazurins studied and their reduction potentials measured vs. a normal hydrogen electrode (NHE)

<table>
<thead>
<tr>
<th>Protein</th>
<th>PDB</th>
<th>$I_3$</th>
<th>SDA</th>
<th>$R_G$</th>
<th>%Helix</th>
<th>%Strand</th>
<th>$d_{N-Cu}$</th>
<th>$d_{N-Cu-S}$</th>
<th>$E_0$</th>
<th>$I_3$ (H117)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. xylosoxidans azurin (pH 8.0)</td>
<td>1RRK</td>
<td>2.7699</td>
<td>0.1600</td>
<td>13.53</td>
<td>21</td>
<td>35</td>
<td>1.906</td>
<td>124.89</td>
<td>8.0171</td>
<td>305</td>
</tr>
<tr>
<td>P. putida azurin (pH ∼ 7)</td>
<td>1NWP</td>
<td>2.7738</td>
<td>0.1615</td>
<td>13.52</td>
<td>19</td>
<td>36</td>
<td>1.944</td>
<td>119.53</td>
<td>7.8008</td>
<td>305</td>
</tr>
<tr>
<td>A. denitrificans (M121H) azurin (pH 3.5)</td>
<td>1A4C</td>
<td>2.7748</td>
<td>0.1611</td>
<td>13.51</td>
<td>18</td>
<td>36</td>
<td>1.948</td>
<td>112.30</td>
<td>8.5988</td>
<td>305</td>
</tr>
<tr>
<td>A. denitrificans (M121Q) azurin</td>
<td>1URI</td>
<td>2.7750</td>
<td>0.1615</td>
<td>13.52</td>
<td>16</td>
<td>34</td>
<td>2.111</td>
<td>123.62</td>
<td>8.2833</td>
<td>305</td>
</tr>
<tr>
<td>P. aeruginosa azurin (pH 5.5)</td>
<td>4AZU</td>
<td>2.7759</td>
<td>0.1628</td>
<td>13.52</td>
<td>16</td>
<td>34</td>
<td>2.111</td>
<td>123.62</td>
<td>8.2833</td>
<td>305</td>
</tr>
<tr>
<td>A. denitrificans azurin (pH 5.0)</td>
<td>2AZA</td>
<td>2.7820</td>
<td>0.1640</td>
<td>13.54</td>
<td>16</td>
<td>36</td>
<td>2.008</td>
<td>121.64</td>
<td>7.5784</td>
<td>285</td>
</tr>
<tr>
<td>P. aeruginosa (M121A) azurin (pH 5.1)</td>
<td>2TSA</td>
<td>2.7777</td>
<td>0.1619</td>
<td>13.44</td>
<td>15</td>
<td>34</td>
<td>2.390</td>
<td>127.72</td>
<td>8.7991</td>
<td>273</td>
</tr>
<tr>
<td>P. aeruginosa azurin (pH 9.0)</td>
<td>1HO</td>
<td>2.7781</td>
<td>0.1636</td>
<td>13.64</td>
<td>21</td>
<td>34</td>
<td>2.013</td>
<td>125.25</td>
<td>7.5008</td>
<td>285</td>
</tr>
<tr>
<td>A. xylosoxidans azurin II (pH 6.5)</td>
<td>1DDZ</td>
<td>2.7807</td>
<td>0.1624</td>
<td>13.51</td>
<td>19</td>
<td>34</td>
<td>1.988</td>
<td>121.05</td>
<td>7.9196</td>
<td>305</td>
</tr>
<tr>
<td>P. aeruginosa (M121E) azurin (pH 6.0)</td>
<td>1ETJ</td>
<td>2.7819</td>
<td>0.1639</td>
<td>13.52</td>
<td>19</td>
<td>36</td>
<td>2.020</td>
<td>125.15</td>
<td>7.8623</td>
<td>220</td>
</tr>
<tr>
<td>A. denitrificans azurin (pH 5.0)</td>
<td>1ZIA</td>
<td>2.7458</td>
<td>0.1551</td>
<td>12.80</td>
<td>20</td>
<td>35</td>
<td>1.988</td>
<td>113.49</td>
<td>7.4820</td>
<td>260</td>
</tr>
<tr>
<td>P. aeruginosa pseudoazurin (pH 6.8)</td>
<td>5AZU</td>
<td>2.7926</td>
<td>0.1676</td>
<td>13.50</td>
<td>17</td>
<td>34</td>
<td>2.056</td>
<td>122.74</td>
<td>7.9672</td>
<td>293</td>
</tr>
<tr>
<td>A. cycloclastes pseudoazurin</td>
<td>1PAZ</td>
<td>2.7448</td>
<td>0.1512</td>
<td>12.78</td>
<td>16</td>
<td>36</td>
<td>2.125</td>
<td>111.59</td>
<td>7.5416</td>
<td>260</td>
</tr>
<tr>
<td>A. faecalis pseudoazurin (pH 6.8)</td>
<td>8PAZ</td>
<td>2.7302</td>
<td>0.1511</td>
<td>12.86</td>
<td>17</td>
<td>37</td>
<td>2.013</td>
<td>113.50</td>
<td>7.5052</td>
<td>260</td>
</tr>
</tbody>
</table>

Protein database (PDB) codes are also given.

* Distance between N(His117)···Cu.

* Angle between N(His117)···Cu···S(Cys112).

* Contribution of His117 to $I_3$ index.

The folding degree follows the general trend of the percentages of secondary structures for these proteins (Hooft et al., 1996). For instance, azurins have about 19% helix and 34% strand on average, while pseudoazurins have 17% helix and 36.5% strand. The differences between $I_3$ index and the sum of dihedral angles as well as of the percentage of secondary structure of the proteins studied here is well illustrated in Fig. 3. Despite there is a general trend indicating a relationship between $I_3$ index and the sum of dihedral angles this relation does not exist for some particular series of proteins as those shown in Fig. 3. It is also remarkable that despite a linear correlation is observed between $I_3$ index and the percentage of secondary structure of several families of proteins this is not the case for specific families as the one studied here (see Fig. 3). These differences arise by the incomplete accounting of the folding degree by the sum of dihedral angles and the percentage of secondary structure, which consider the whole (backbone chain) as the sum of their parts (angles or amino acids) and do not consider the way in which such parts are distributed along the whole. This characteristic is typical of all complex system and it is simply stated as “the parts cannot contain the whole”.

In order to clarify the roles of individual residues in the function of azurins and pseudoazurins we have studied the influence of amino acid contributions to the global folding on the values of the reduction potential of these proteins. First, the contribution of all residues to the global folding degree of the BCPs studied were calculated. We have been able to obtain a very good model relating both properties for these proteins, showing that the reduction potential of these proteins is explained to a large extent by the folding degree of the His117 (His81 in pseudoazurins) residue according to the following linear regression model, which has a correlation coefficient of 0.974 (see Fig. 4):

$$E_0 = 75.2(±5.2)I_3(His117) - 297.8(±41.3)$$
Fig. 4. Plot of the reduction potential ($E^0$ mV vs. NHE) as a function of the contribution of histidine 117 (azurins) or histidine 81 (pseudoazurins). The straight line corresponds to the linear regression model between both variables.

This histidine residue serves as a conduit between the Cu ion and the protein surface and is solvent accessible, which makes it an important element for the function of these proteins (see Fig. 5). The His46 (His40 in pseudoazurins) residue is further away from the protein surface and is not accessible to solvent (Pozdnyakova et al., 2001).

According to this model, an increase of the folding degree of His117 (His81 in pseudoazurins) translates into an increment of the reduction potential of azurins and pseudoazurins. As we have previously remarked, this residue is important for the function of BCPs. In particular, it has been demonstrated (Jeuken et al., 2000) that the imidazole ring of His117 provides an excellent pathway for the electron to transfer to external partners, and its loss destroys the electronic coupling between the copper atom and the redox partner. It has been previously observed that replacing His117 by a glycine results in little change in the copper site geometry (Jeuken et al., 2000). However, the differences found in the reduction potential of wild type azurin and His117Gly azurin are probably due to slight changes in the structure of the protein close to His117 (Jeuken et al., 2000). This observation coincides with our findings that a change in the folding degree of His117 has a significant effect on the reduction potential of azurins and pseudoazurins. This difference in the folding degree of His117 of azurins and pseudoazurins can be reflected in different structural parameters of both the binding site and the protein backbone in the neighborhood of this residue. We have investigated two of these structural parameters: the distance between copper and the nitrogen atom of His117 (His81 in pseudoazurins) and the angle formed between the sulfur of Cys112 (Cys78 in pseudoazurins), the copper atom and the nitrogen of His117 (His81 in pseudoazurins). Our supposition is guided by the proposed scheme of electron flow in BCPs, for which (Cys112)···Cu···His117···H2O is an important path (see Fig. 5). In Fig. 6, we illustrate the enlargement of the N–Cu distance and widening of the S–Cu–N angle for 2TSA ($E^0 = 373$ mV) in comparison with the *A. denitrificans* azurin (PDB 1URI), which has a reduction potential of only 263 mV. We have found no relationship between the N–Cu distance and the...
Fig. 6. Superposition of the crystallographic structures of a portion of the binding site of two azurins with different reduction potentials: 2TSA has a reduction potential of 373 mV and 1URI of 263 mV. The first protein has a N(His117)···Cu distance of 239.03 pm and N(His117)···Cu···S(Cys112) angle of 127.72°, while the second has 202.83 pm and 116.98°, respectively. The dotted lines are provided as guides.

Fig. 7. Plot of the reduction potential of azurins and pseudoazurins as a function of the angle formed between His117 (azurins) or His81 (pseudoazurins), the copper atom and Cys112 (azurins) or Cys78 (pseudoazurins). The line is the quadratic model showing a correlation coefficient of 0.86.
a necessary prerequisite for the high reduction potential of these proteins. However, only the folding degree contribution of His117 appears to be quantitatively related to the reduction potential of the azurins and pseudoazurins as illustrated in Fig. 8.

3. Conclusions

We have shown that the degree of folding of azurins and pseudoazurins contains important 3D information that has probably been the result of evolutionary pressure over millions of years. The large range of reduction potentials in BCPs is also a result of evolutionary pressure, since it parallels different biological functions of the individual proteins. We have found that, at a minimum, the Ni(His117)–Cu–StCys112 angle which plays an important role in electron transport, could be involved in the differences in the reduction potential of azurins and pseudoazurins. The effect of the degree of folding of His117 on the efficiency of electron transfer from this residue to H2O is an interesting problem worthy of further study. We hope that these results shed some new light on the structure–function relationships of this important family of proteins.

Acknowledgement

E.E. thanks “Ramón y Cajal” program, Spain for partial financial support.

Appendix A

Let \( I_3(k) \) the contribution of the torsion angle \( k \) to the folding degree of the protein, where \( k \) is one of the following angles: \( \phi_i, \psi_i, \) or \( \omega_i \). Then, according to the original definition of folding degree index, we have that:

\[
I_3(k) = \sum_{j=0}^{\infty} \mu_3(jk)
\]

(A.1)

Let \( e_i \) be the orthogonal projection of the unit vector (the \( r \)th vector of the canonical base of \( R^n \)) on \( v_j \) is:

\[
p_{rj}(e_i) = \left( \frac{e_i \cdot v_j}{|v_j|^2} \right) v_j = e_i \cdot v_j v_j = v_j(k) \cdot v_j
\]

(A.2)

where \( v_j \) is the eigenvector associated to the \( j \)th eigenvalue of \( A \) and \( v_j(k) \) is its \( k \)th element.

Hence, the number of closed walks starting at torsion angle \( k \) can be expressed in terms of the spectral properties of the graph as follows:

\[
\mu(k) = (A^t)_i = \langle A^t e, e \rangle = \left\langle \sum_{j=1}^{N} \mu_r(e) \sum_{j=1}^{N} \mu_j(e) \right\rangle
\]

\[
= \sum_{j=1}^{N} \lambda_j^2 |v_j(k)|^2
\]

(A.3)

Substituting this expression in (A.1) we obtain:

\[
I_3(k) = \sum_{j=0}^{\infty} \left( \frac{\sum_{j=1}^{N} \lambda_j^2 |v_j(k)|^2}{\lambda_j^2} \right)
\]

(A.4)

which can be reorganized to obtain the absolutely convergent series:

\[
\sum_{j=1}^{N} \left( |v_j(k)|^2 \sum_{j=1}^{N} \lambda_j \right) = \sum_{j=1}^{N} |v_j(k)|^2 e^{\phi_j}
\]

(A.5)

and this clearly also converges to \( I_3(k) \) (Estrada and Rodriguez-Velázquez, 2005):

\[
I_3(k) = \sum_{j=1}^{N} |v_j(k)|^2 e^{\phi_j}
\]

(A.6)

Let \( i \) be the \( i \)th amino acid in the protein chain and let \( \psi_i \) and \( \phi_i \) be the two-torsion angle contributing to the folding degree of this amino acid. Then, if we substitute \( v_j(k) \) by \( v_j(\psi_i) \) and \( v_j(\phi_i) \) in (A.6) we obtain the expression for the contribution of \( i \)th amino acid to the global degree of folding of the protein:

\[
I_3(i) = \sum_{j=1}^{N} e^{\psi_i}|v_j(\psi_i)|^2 + |v_j(\phi_i)|^2
\]

(A.7)
References


