Underexposed polar residues and protein stabilization

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Received September 6, 2010; revised September 6, 2010; accepted September 16, 2010

Edited by Mauricio G. Mateu

Increasing protein stability is interesting for practical reasons and because it tests our understanding of protein energetics. We explore here the feasibility of stabilizing proteins by replacing underexposed polar residues by apolar ones of similar size and shape. We have compared the stability of wild-type apoflavodoxin with that of a few carefully selected mutants carrying Y → F, Q → L, T → V or K → M replacements. Although a clear inverse correlation between native solvent exposures of replaced polar residues and stability of mutants is observed, most mutations fail to stabilize the protein. The promising exceptions are the two Q → L mutations tested, which characteristically combine the greatest reduction in polar burial with the greatest increase in apolar burial relative to wild type. Analysis of published stability data corresponding to a variety of mutant proteins confirms that, unlike Y → F or T → V replacements, Q → L mutations tend to be stabilizing, and it suggests that N → L mutations might be stabilizing as well. On the other hand, we show that the stability changes associated to the apoflavodoxin mutations can be rationalized in terms of differential polar and apolar burials upon folding plus a generic destabilizing penalty term. Simple equations combining these contributions predict stability changes in a large data set of 113 mutants (Y → F, Q → L or T → V) similarly well as more complex algorithms available on the Internet.

Keywords: ASA, flavodoxin/polar residues/protein stabilization/underexposed residue

Introduction

Polar residues in folded proteins are usually, but not always, located on the protein surface. The potential role of buried polar residues in protein structure and stability has been investigated by many authors. Most polar side chains at core positions form at least one hydrogen bond, the disruption of which tends to destabilize the protein (Pace, 2001; Takano et al., 2001). In some cases, however, polar groups are buried without having an obvious hydrogende binding partner. In those cases, the extent to which those buried polar residues contribute to protein stability appears to depend on the surrounding structure (Serrano et al., 1992; Takano et al., 2003).

Trying to quantitate the contribution of buried polar residues to stability, several correlations with different protein properties (such as hydrophobicity, secondary structure, shape, flexibility or Van der Waals interactions) have been proposed (Gromiha et al., 1999, 2000). More elaborated force fields have also been developed to calculate the effect of replacing buried polar residues with non-polar ones (Gillis and Rooman, 2000; Capriotti et al., 2005; Schymkowitz et al., 2005; Parthiban et al., 2006). The issue has also been approached in a much simpler way by relating the contribution of polar residues to protein stability to their solvent exposures, as measured by their accessible surface areas (ASAs) (Loladze et al., 2002; Zhou and Zhou, 2004).

Typically, solvent accessibility calculations have been performed on the native structure. One good reason for this is the absence of detailed models for the unfolded state of proteins, which is almost always modelled as a random coil (Tanford, 1968, 1970) where all residues of a given type are equally exposed to solvent. Exposures of protein residues in random coil states have been proposed that are based in the exposures of the residues in tripeptides (Rose et al., 1985; Miller et al., 1987; Zielenkiewicz and Saenger, 1992) or in peptide fragments (Creamer et al., 1997). More recently, the unfolded state has been modelled as an ensemble of conformations with average properties in agreement with experimental measurements (Goldenberg, 2003; Bernado et al., 2005). One such model (Bernado et al., 2006) has been used to calculate sequence-specific solvent exposures in denatured ensembles (Estrada et al., 2009).

Protein stabilization is an important goal in protein engineering and, to some extent, it remains an art. The optimization of enzymes for industrial and medical purposes benefits from the development of new strategies of stabilization. In this respect, replacement of buried or partially buried polar side chains with hydrophobic residues could, in principle, constitute one such strategy and lead to substantial stabilization. Towards that end, the focus should be placed on side chains, the polar atoms of which become, upon folding, less exposed to solvent than they are in the unfolded ensemble, without establishing compensating hydrogen bonding interactions with neighbouring residues in the native state. We have assessed the feasibility of such an approach using the apoflavodoxin from Anabaena PCC 71119, a model protein with well characterized structure and stability (Genzor et al., 1996a,b; Maldonado et al., 1998; Irun et al., 2001; Campos et al., 2004a,b). Thus, we have substituted several buried polar residues of the protein by apolar residues of as similar size and shape as possible. Quite often, site-directed substitutions are destabilizing as a result of either debilitation of interactions present in the wild-type protein or due to the introduction of steric strains. The important question to address is, given the palette of the genetically encoded amino acids, how frequently the expected stabilization obtained by replacing severely underexposed polar residues...
by apolar ones will be large enough so as to out balance the also expected concomitant destabilizing effects?

Materials and methods

Mutagenesis and protein purification

The following mutations Y8F, T10V, Q63L, Y85F and Q99L, K81M and K164M were introduced in the cloned gene of *Anabaena* PCC 7119 flavodoxin by PCR mutagenesis using the protocol described in the Stratagene QuikChange kit. The mutations were confirmed by sequencing the gene. Expression (Fillat et al., 1991) was done in *Escherichia coli*. Purification and removal of the FMN cofactor were carried out as described (Genzor et al., 1996a).

Circular dichroism spectra

Circular dichroism (CD) spectra of wild type and mutants were recorded in a Jasco 710 spectropolarimeter at 25 (± 1) °C. Near-UV spectra were recorded from 250 to 310 nm in a 1 cm path-length cuvette using 20 μM protein solutions in 50 mM Mops (pH 7). Far-UV spectra were measured from 190 to 250 nm in a 1 mm path-length cuvette, using 30 μM protein solutions in 5 mM Mops (pH 7) with 15 mM NaCl. This buffer is of the same ionic strength, but less absorbance, as the 50 mM Mops buffer used for most of the experiments.

Urea denaturation curves

Protein solutions of 2 μM concentration in 50 mM Mops (plus 1 M NaCl, when indicated) and different urea concentrations from 0 to 5 M were prepared and equilibrated at 25 °C for 1 h. Unfolding curves were obtained by measuring fluorescence, using a ratio of emission 320/360 nm (excitation at 280 nm) to minimize protein concentration errors. All unfolding curves were performed in a thermostated Kontron SFM25 fluorimeter.

Differential scanning calorimetry

The heat capacity of the different flavodoxin mutants was measured as a function of temperature with a high precision differential scanning microcalorimeter VP-DSC (Microcal Inc., Northampton, MA, USA). Protein samples and reference solutions were properly degassed and carefully loaded into the cells to avoid bubble formation. Exhaustive cleaning of the cells was undertaken before each experiment. Thermal denaturation scans were performed with freshly prepared flavodoxin mutant solutions dialyzed in 50 mM Mops pH 7.0 with a protein concentration of 20 μM. Data were analysed using software developed in our laboratory.

Solvent accessible area determination

The solvent-ASA of amino acid residues in the native and unfolded states of apoflavodoxin and the other proteins for which stability data have been summarized (barnase, lysozyme, staphylococcal nuclease, ribonuclease Sa, ribonuclease Sa3, fibroenctin, ketosteroid isomerase, ribonuclease T1, U1a, Fk binding, ribonuclease HL, ubiquitin and Bs-Csp) have been calculated with ProtSA (http://webapps.bifi.es/protsa), a server which uses the Alphasurf part of the ProGeom software for ASA calculations.

The ASAs of the native state of the different proteins (ASA_p for polar atoms and ASA_ap for non-polar atoms) were calculated, using ProtSA (Estrada et al., 2009), from the crystal structures obtained from the Protein Data Bank (1FTG, 1A2P, 1EYD, 1LZ1, 1C54, 1MGW, 1TTG, 1OH0, 1RN1, 1OIA, 1FKJ, 2RN2, 1UBQ, 1C9O, 1RX1 and 1YCC). The mutations were modelled with Deepview (Guex and Peitsch, 1997). Average ASAs of the corresponding denatured ensembles (Bernado et al., 2006) were also calculated with ProtSA (Estrada et al., 2009).

Results

Substitution of buried polar residues in *Anabaena* apoflavodoxin

The solvent exposure of all side chains in *Anabaena* PCC 7119 apoflavodoxin bearing polar atoms was calculated with ALPHASURF (Edelsbrunner and Koehl, 2003) as implemented in ProtSA (Estrada et al., 2009). Out of 45 polar residues in the protein (S, T, Y, N or Q), we selected five residues with <14 Å² side-chain polar solvent exposed area in the folded state, establishing just one (S, T or Y) or two (Q, N) hydrogen bonds, and showing less than 60% sequence conservation within flavodoxins. The low polar exposure threshold used was an arbitrary choice that allowed identifying the polar residues which were severely underexposed. Using high polar burial thresholds would also be possible if different residue-specific thresholds are defined. No single apoflavodoxin buried polar S, T, Y, N or Q residue was found not establishing at least one hydrogen bond, which will likely occur in many proteins.

In addition, we selected, out of 10 lysine residues in the protein, two lysines, the side-chain polar atoms of which exposed less than 25 Å² to the solvent in the folded state and were not hydrogen bonded. Seven residues were thus selected for mutation: Y8, T10, Q63, K81, Y85, Q99 and K164 (Table I). The seven residues were substituted by non-polar ones with as similar size and shape as possible (Y → F, Q → L, T → V and K → M). The near-UV and far-UV CD spectra of wild-type apoflavodoxin and mutants Y8F, T10V, Q63L, Y85F, Q99L, K81M and K164M are shown in Fig. 1. The spectra of the mutant proteins are very similar to those of the wild-type one, with K81M and Y85F displaying slight

<table>
<thead>
<tr>
<th>Residue</th>
<th>ASA (Å²)×</th>
<th>Side-chain hydrogen bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y8</td>
<td>13.26</td>
<td>OH E16 (OE2) 2.74</td>
</tr>
<tr>
<td>T10</td>
<td>12.16</td>
<td>OH SO4 (OP3) 2.66</td>
</tr>
<tr>
<td>Q63</td>
<td>5.11</td>
<td>O D65 (N) 3.35</td>
</tr>
<tr>
<td>Y85</td>
<td>0.78</td>
<td>OH H2O 2.89</td>
</tr>
<tr>
<td>Q99</td>
<td>0.24</td>
<td>N L143 (O) 3.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O G87 (O) 3.26</td>
</tr>
<tr>
<td>K81</td>
<td>7.29</td>
<td>None</td>
</tr>
<tr>
<td>K164</td>
<td>23.12</td>
<td>None</td>
</tr>
</tbody>
</table>

×Solvent exposure of the polar atoms of the residue calculated with ALPHASURF.
differences in intensity in the near-UV and far-UV spectra, respectively. The mutants, thus, seem to retain the structure of the wild-type protein.

Conformational stability of the mutants relative to wild type

The urea unfolding equilibrium of apoflavodoxin follows a simple two-state mechanism (Genzor et al., 1996a; Maldonado et al., 2002) and provides the simplest and more accurate way to determine small stability differences between point apoflavodoxin mutants. Table II shows the stability differences associated to the mutations analysed. Of all the mutants tested, one of the two Q to L mutations (Q99L) clearly leads to protein stabilization, the second one (Q63L) shows essentially the same stability as the wild-type protein, and the additional mutations give rise to less stable variants (Fig. 2). For the two substitutions involving charged residues (K81M and K164M), the unfolding curves were also recorded in the presence of 1 M NaCl to minimize potential destabilizing effects associated to the removal of a positively charged side chain from an acidic protein such as flavodoxin (Maldonado et al., 2002; Campos et al., 2004b). As expected, the destabilizing effect of these charge removal mutations was reduced in the presence of high salt concentrations, yet the mutations were still destabilizing (data not shown).

Table II. Stability changes brought about by mutation of underexposed polar residues in *Anabaena* PCC 7119 apoflavodoxin to apolar ones

<table>
<thead>
<tr>
<th>Mutant</th>
<th>ΔΔG° (Kcal/mol)</th>
</tr>
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<tr>
<td></td>
<td>Urea denaturation</td>
</tr>
<tr>
<td>Y8F</td>
<td>0.50 ± 0.05 (0.27 ± 0.11)</td>
</tr>
<tr>
<td>T10V</td>
<td>0.61 ± 0.04 (0.41 ± 0.06)</td>
</tr>
<tr>
<td>Q63L</td>
<td>-0.19 ± 0.05 (0.09 ± 0.05)</td>
</tr>
<tr>
<td>Y85F</td>
<td>0.35 ± 0.05 (1.31 ± 0.05)</td>
</tr>
<tr>
<td>Q99L</td>
<td>-0.42 ± 0.05 (-0.25 ± 0.08)</td>
</tr>
<tr>
<td>K81M</td>
<td>1.52 ± 0.06 (2.41 ± 0.07)</td>
</tr>
<tr>
<td>K164M</td>
<td>1.00 ± 0.05 (1.64 ± 0.5)</td>
</tr>
</tbody>
</table>

*Folding free energies of mutant minus wild type at 25°C. Negative values mean the mutant is more stable (positive values less stable) than wild type. The experiments were performed in 50 mM Mops, pH 7.

*Folding free energies have been calculated from urea denaturation curves as ΔΔG = mmut*ΔU(mut) - mWT*ΔU(WT), where m is the mean m value of the different protein mutants: 2.346 ± 0.073, and ΔU is the difference in urea concentration of half denaturation between wild type and mutant. The values within parentheses have been calculated without using an averaged m value for all of them: ΔΔG = mmut*U(mut) - mWT*U(WT).

*Folding free energies of mutant minus wild type at a reference temperature of 44°C using the Gibbs–Helmholtz equation from DSC thermal unfolding parameters.

Fig. 2. Urea-denaturation curves of wild-type and mutant proteins. The experimental data were fitted to a two-state equation (continuous lines). The unfolding was followed by fluorescence emission in 50 mM Mops pH 7.0 at 25°C.

Table II also shows the stability differences calculated from thermal unfolding followed by differential scanning calorimetry. Apoflavodoxin thermal unfolding is three-state (Irun et al., 2001; Campos et al., 2004a). Therefore, calculating overall stability differences between point mutants using thermal unfolding is not as easy as from urea denaturation data, particularly because of uncertainty in the exact values of the heat capacity changes of the two unfolding equilibria. In spite of this, the stability differences derived from the thermal unfolding experiments roughly agree with those derived from the two-state urea denaturation experiment, and they confirm that Q99L is stabilizing, Q63L is as stable as wild type and the rest of mutants are all destabilizing. Since the thermal unfolding data bear larger errors, only the more accurate chemical unfolding data will be further discussed.
Stability versus polar solvent expositions in native state and unfolded ensembles

To test whether stability effects due to substitution of buried polar residues by close to isosteric apolar ones can be related in a simple way to solvent exposures in the folded state, we have correlated the observed changes in folding free energies with the exposed polar areas of the mutated residues. The correlation (Fig. 3a) shows the expected trend indicating that replacing polar residues is more stabilizing (or at least less destabilizing) when they are more buried. However, the correlation is poor ($r = 0.54$).

This correlation does not take into account the actual changes in solvent exposures that occur in the wild type and in the mutant proteins upon folding. We have thus attempted a more physically meaningful correlation between the stability change and the differential polar area burial upon folding (wild type minus mutant) quantified as:

$$\Delta \Delta \text{ASA}_p = \Delta \text{ASA}_p^D - \Delta \text{ASA}_p^N$$

$$= (\text{ASA}_p^D\text{WT} - \text{ASA}_p^D\text{mut}) - (\text{ASA}_p^N\text{WT} - \text{ASA}_p^N\text{mut})$$

(1)

One would expect that, other things being equal, the larger the polar burial upon folding in wild type compared to mutant, the more stabilizing the mutation will be. This expectation is fulfilled (Fig. 3b) and the correlation between $\Delta \Delta \text{ASA}_p$ and $\Delta \Delta G$ is indeed better ($r = 0.72$). It seems thus clear than both the polar exposures in the native and denatured ensembles of both the wild-type and mutant proteins should be considered to try to correlate stability changes with modifications in polar exposure introduced by mutations. In this respect, the need of accurate models of protein denatured states becomes evident. The above correlation has been obtained using PROTSQA (Estrada et al., 2009) to calculate polar solvent exposures in the denatured ensemble. Using the generic solvent exposures proposed by Zielenkiewicz and Saenger (Zielenkiewicz and Saenger, 1992) and by Miller et al. (Miller et al., 1987) yielded correlations or $r = 0.72$ and $r = 0.34$, respectively.

Important contribution of concomitant changes in apolar exposures

Replacing underexposed polar residues by apolar ones will concomitantly increase the burial of apolar surface in the folded mutant. Since apolar burial is known to contribute to protein stabilization (Privalov and Gill, 1988), this ‘side-effect’ associated to this type of mutations should add to its potential stabilizing effect. As in the previous analysis of the correlation between differential polar burial and protein stabilization, the increases in apolar burial should be evaluated by taking into consideration both the native state and the denatured ensembles of both wild type and mutant. If we define the differential apolar burial upon folding (wild type minus mutant) as:

$$\Delta \Delta \text{ASA}_ap = \Delta \text{ASA}_ap^D - \Delta \text{ASA}_ap^N$$

$$= (\text{ASA}_ap^D\text{WT} - \text{ASA}_ap^D\text{mut}) - (\text{ASA}_ap^N\text{WT} - \text{ASA}_ap^N\text{mut})$$

(2)

the relationship between this magnitude and the stabilizing effect of the mutations analysed is clear (Fig. 3c). In fact, the correlation is even better ($r = 0.83$) than the one obtained for the differential polar burial. This correlation is obtained when solvent exposures are computed by modelling the unfolded state as an ensemble of conformations where each residue displays an average solvent exposure influenced by its sequence context (Estrada et al., 2009). Simpler models of the unfolded state (Zielenkiewicz and Saenger, 1992) and (Miller et al., 1987) lowered the correlation to $r = 0.36$ or 0.16, respectively (data not shown). It is worth noting that, among the mutants tested, the two Q to L mutations (one stabilizing and the other one as stable as wild type) are

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**Fig. 3.** Correlation between folding free energies of mutant minus wild type ($\Delta \Delta G$) and (a) the side-chain polar solvent exposition of the mutated residues in wild type ($r = 0.54$), (b) the differential increase in polar burial upon folding ($r = 0.72$) and (c) the differential increase in apolar burial upon folding ($r = 0.83$).
singularized by combining the higher differential polar burials with the lower (more negative) differential apolar burials. Therefore, these mutants bury upon folding less polar and more apolar area than the other mutants.

Discussion

Prediction of stability differences in underexposed polar replacement mutations with a simple equation

Since the stability of the apoflavodoxin variants is correlated with both polar and apolar differential burials, as defined in equations (1) and (2), we have considered relating the actual values of the stability differences to a simple combination of the two parameters and have obtained the following fit, represented in Fig. 4, which displays a reasonable correlation (r = 0.83)

\[
\Delta \Delta G = 0.0276 \times \Delta \Delta \text{ASA}_{\text{ap}} + 0.0072 \times \Delta \Delta \text{ASA}_{\text{p}} + 1.083
\] (3)

Interestingly, the value obtained for the coefficient that multiplies the differential apolar burial (0.028 per square Å) is reminiscent of analogous figures previously used in the literature to calculate the contribution of apolar area burial to protein stability (Chothia, 1974; Sanchez-Raiz, 1995; Vajda et al., 1995; Chan and Dill, 1997; Bueno et al., 2007). Indeed an average of recent quantitations of this effect was reported at 0.029 ± 0.003 kcal mol⁻¹/Å² (Campos et al., 2005).

It seems thus possible to offer also an interpretation of the independent coefficient (1.08) which penalizes any mutation with a destabilization of around 1 kcal/mol. This term may simply represent an average of the variety of destabilizing effects associated to the different mutations introduced in the protein. Some of such destabilizing effects can be easily noticed. Tyrosine 8 forms a hydrogen bond with a charged residue, which disappears when it is replaced by Phe. Threonine 10 forms a hydrogen bond with anions that bind at the place where the phosphate group of the FMN cofactor is bound in holoflavodoxin (Rao et al., 1992; Genzor et al., 1996b). Therefore, mutating Thr10 to Val will debilitate that interaction, which will decrease the observed stability. Tyrosine 85 establishes a hydrogen bond with and internal water molecule which is lost in the Phe mutant, and it is known that perturbing the binding of structural water molecules may reduce protein stability (Drennan et al., 1999; Takano et al., 2003). Glutamine 63 and 99 form H-bonds that disappear in the corresponding Leu mutants. The two lysines (Lys 81 and Lys 164) will make electrostatic interactions with acidic groups all over the protein that will disappear when replaced by Met. Destabilizing effects such as those or others of different types are likely to accompany most point mutations introduced in proteins. In fact, the average destabilization observed in Anabaena apoflavodoxin mutants designed and analysed in our lab over the years (excluding those specifically aiming at protein stabilization) is of 1.39 ± 0.28 kcal/mol. We think it is thus sound to interpret the independent coefficient in equation (3) as an averaged destabilizing penalty associated to most mutations (for different reasons in each case). The existence of this term poses the question of whether replacing underexposed polar residues by apolar ones will suffice to offset such an effect in a significant fraction of mutations tested.

Comparison with predictions by a variety of more complex force fields

Equation (3) is very simple and has been derived using a small set of apoflavodoxin mutants, primarily selected to display large changes in polar burial relative to wild type. Not surprisingly, it predicts the stabilities of these seven training mutants better (r = 0.83) than a variety of Internet servers such as DFIRE (r = 0.49), FOLDX (r = 0.37), Imutant2.0 (r = 0.70), CUPSAT (thermal) (r = 0.76), CUPSAT (denaturant) (r = −0.09) or PoPMuSiC (r = 0.25) (Gilis and Rooman, 2000; Zhou and Zhou, 2004; Capriotti et al., 2005; Schymkowitz et al., 2005; Parthiban et al., 2006). Although an accurate prediction of stability differences of other type of mutations should not be expected in principle, we have calculated the stability changes brought about by a variety of apoflavodoxin mutations analysed in the lab over the years (excluding those designed with a specific stabilizing purpose). The correlation is shown in Fig. 4.
Fig. 4b. The correlation coefficient of this test set (28 apoflavodoxin mutants), which leaves outside the seven mutants used to derive equation (3), is $r = 0.56$. To compare the performance of equation (3) with that of servers available on the Internet (see above), we have calculated the stability changes predicted for the test set by those servers. The correlation coefficients for the corresponding fits are 0.20 (FOLDX), 0.16 (Imutant2.0) and 0.48 (PoPMuSiC). It seems that equation (3) performs no worse than more complex servers in predicting stability changes in previously published apoflavodoxin mutants that have not been used to train the equation.

However, the predictive performance of equation (3) for mutants introduced in other model proteins [barnase (Serrano et al., 1992), staphylococcal nuclease (Byrne et al., 1995), lysozyme (Yamagata et al., 1998; Takano et al., 1999), ribonuclease Sa (Pace et al., 2001; Takano et al., 2003), ribonuclease Sa3 (Pace et al., 2001), fibronectin (Cota et al., 2000), ketosteroid isomerase (Kim et al., 2000), ribonuclease T1 (Shirley et al., 1992), U1a (Kranz et al., 1996), Fk-binding (Main et al., 1998), ribonuclease HI (Haruki et al., 1994), ubiquitin (Loladze et al., 2002) and Bs-Csp (Perl et al., 2000)] significantly varies from protein to protein (data not shown). In an attempt to derive an equation of a more general applicability, we have fitted the stabilities and the incremental polar and apolar exposures of a larger data set of folding almost as well as more sophisticated force fields. To combine the largest side-chain polar and apolar area changes.

We have found only two N–L mutations described (Linske-O’Connell et al., 1995; Arai and Iwakura, 2005). One of the mutants (N59L in E.coli dihydrofolate reductase, with the N atom exhibiting $\text{ASA}_\text{polar} = 8.62 \AA^2$ and establishing one hydrogen bond) is as stable as wild type (reported as 0.05 kcal/mol more stable than wild type). The other mutant (N52L in S.cerevisiae iso-cytochrome with a total $\text{ASA} = 0 \AA^2$ and one hydrogen bond) is 2.56 kcal/mol more stable than wild type.

Overall, it seems that replacing polar buried residues by apolar ones will not generally lead to more stable protein variants. However, it appears that replacement of buried Q or N by L residues gives a fair chance of stabilization.

Guidelines to mutate underexposed polar residues and achieve protein stabilization

The possibility of stabilizing the native conformation of proteins by replacing the rare apolar residues that appear overexposed in folded proteins was illustrated years ago and the term inverted hydrophobic effect was coined to describe it (Pakula and Sauer, 1990). We have explored here the feasibility of using the complementary approximation by replacing underexposed polar residues by apolar ones. To that end, we have selected seven underexposed polar residues in apoflavodoxin and replaced them by apolar residues as similar in shape as possible. Tyr has been replaced by Phe, Thr by Val, Lys by Met and Gln by Leu. The choice of replacing the residues by similarly large ones rather than by Ala may contribute to introducing strain but also to burying apolar area and to avoiding the creation of protein cavities. Although clear correlations between the decrease in polar area and the increase in apolar area buried in the mutants relative to wild type are observed, the fact is that, of these four types of mutations, only replacements of Q by L have increased the stability or left it unchanged. It appears [see equation (3) and discussion above] that most mutations are typically accompanied by some amount of destabilization and that only those where the changes in polar and apolar area burial are large will yield a net stabilization. Q to L mutations of buried Q residues are best among the four types tested (Y → F, Q → L, T → V and K → M) because they combine the largest side-chain polar and apolar area changes.

This is so because, on average, the polar solvent exposure of Y, T, K and Q residues is 50.1 $\AA^2$, 33.6 $\AA^2$, 58.1 $\AA^2$ and 79.2 $\AA^2$, respectively (Bernado et al., 2006), and because only the Q to L mutation introduces two new methyl groups in the chain. In fact, the average changes in folding free energies introduced by Y → F, T → V and Q → L mutations in the 78 mutants in Supplementary data, Table SI is $+0.79 \pm 0.18$ kcal/mol (49 Y → F mutations), $+0.61 \pm 0.19$ kcal/mol (28 T → V mutations), and $-0.24 \pm 0.11$ kcal/mol (6 Q → L mutations). It is also possible that Q side chains, having two hydrogen bonding groups, are more difficult to accommodate in buried environment than residues with only one hydrogen bonding group. This would contribute to a greater average stabilizing character of mutations that replace buried Q residues. Since N residues are, in these respects, analogous to Q residues, we have looked upon stability changes associated to replacement of N to L residues. We have found only two N–L mutations described (Linske-O’Connell et al., 1995; Arai and Iwakura, 2005).

Acknowledgements

We thank Jorge Estrada (University of Zaragoza, Spain) for help with ProtSA.

Funding

We acknowledge financial support from grants BFU2007-61376 (MICINN, Spain) and PI078/08 (DGA, Spain). S.A was supported by an FPI fellowship (MICINN-FSE, Spain).

References


Supplementary data

Supplementary data are available at PEDS online.

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