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Factors influencing the thermal stability of buried protein mutants

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Abstract

Hydrophobic interaction is believed to be the most important factor for the stability of proteins upon buried mutations. In this work, we have analyzed the influence of different interactions to the stability of buried protein mutants by means of 49 various physical–chemical, energetic and conformational properties of amino acid residues. We found that the mutant stability is attributed with several factors including hydrophobicity. In lysozyme T4, the properties reflecting hydrophobicity, flexibility, turn and coil tendency, and long-range interactions show a strong correlation with stability. Entropy plays an important role and the contribution of hydrophobicity is minimal in barnase. The stability of human lysozyme is attributed with both hydrophobicity and secondary structure. The stability of buried mutants in staphylococcal nuclease is influenced by hydrophobicity and physical properties. Our results indicate that the stability of buried protein mutants are influenced not only with hydrophobicity but also other factors, such as, secondary structure, shape, flexibility, entropy and inter-residue contacts play an important role to the stability. We obtained the highest single property correlation of 0.83 between amino acid properties and thermal stability of buried protein mutants. The properties showing high correlation coefficient with thermal stability agree very well with experimental observations. Further, multiple regression technique combining three properties leads to the correlation in the range of 0.83–0.92 in the considered proteins.

Keywords: Thermal stability; Buried mutants; Amino acid properties

1. Introduction

Elucidating the factors influencing the stability of proteins upon mutations is one of the most challenging problems to understand the mechanism of protein folding and for designing stable mutants. Protein structures are stabilized by various free energies, including hydrophobic, electrostatic, van der Waals and hydrogen bonding interactions [1–4]. Site-directed mutagenesis studies provide a wealth of data about the stability of protein mutants and a deep insight to the contribution of these interactions [5–9].

The function of a protein mainly depends on its structure and stability and hence plenty of thermodynamic experiments have been carried out to reveal the stability of proteins upon amino acid substitutions, known as mutations [5–13]. Further, mutational studies are helpful to understand the thermal stability of thermophilic proteins, to delineate the important residues for protein function, activity, drug design etc. Due to the availability of enormous amount of thermodynamic data on protein stability and associated structural parameters [10–13] one can extract useful information about the mechanism of protein stability and develop empirical methods for predicting the stability change by the statistical analysis of these data.

The mutations are classified into buried, partially buried or exposed depending upon the location of the mutant residue in protein structure based on its solvent accessibility or accessible surface area (ASA). A mutant residue with ASA less than 2% has been considered as completely buried, 2–20% as intermediate between buried and partially buried, 20–50% as partially buried, 50–75% as partially exposed and more than 50% as completely exposed [14–17]. It has been reported that hydrophobic interactions play a dominant role in buried mutations whereas hydrophobic and other interactions are important for the stability of partially buried and exposed mutations [14–17].

The results obtained from stability analysis depend mainly on the data set and hence it is necessary to have a good data set with sufficient number of reliable data. The

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thermodynamic database for proteins and mutants, ProTherm (http://www.rtc.riken.go.jp/jouhou/protherm/protherm.html) is the unique source to provide such data, which contains the mutation and stability information for many proteins [12,13].

Recently, several investigations on protein stability have been carried out using the data available in ProTherm. Ooi [18] used this database to analyze the stability of globular proteins and proposed a method for predicting protein stability from its three dimensional structure. Guerois et al. [19] developed a potential for predicting the stability of protein mutants from the thermodynamic data of more than 1000 mutations. Kortemme and Baker [20] proposed a simple physical model for predicting free energy changes due to alanine mutations. Zhou and Zhou [21] developed a stability scale and atomic solvation parameters from the thermodynamic data of 1023 protein mutants. Further, computer aided methods have been developed for evaluating the thermal stability of proteins [22].

In our earlier works, we have analyzed the relationship between amino acid properties and protein stability upon mutations and reported the importance of difference amino acid properties for the stability of mutations at various ranges of solvent accessibility [14–17]. Gilis and Rooman [23,24] developed torsion and distance potentials and used them for predicting the stability of protein mutants. All these studies include the data from many proteins but the mechanism of individual protein stability differs from each other. Further, the inclusion of several proteins together in the analysis reduced the correlation between amino acid properties and Φ values [25] as well as thermal stability significantly. Hence, in this work, we have selected four well-studied proteins, lysozyme T4 (2LZM), barnase (1BNI), human lysozyme (1LZ1) and staphylococcal nuclease (1STN), in which thermodynamic data are known for several mutants and analyzed the important factors for the stability upon buried mutations in each protein. As the buried residues are important for the stability of protein structures, we considered the buried mutations in the present study. We found that the main contribution for the stability is different in these proteins. Further, apart from the major role played by hydrophobicity, other factors, such as, entropy, secondary structure and inter-residue interactions also make a significant contribution to the stability of buried mutations.

2. Materials and methods

2.1. Data set

We have collected the thermal stability (ΔT_m) data for the four proteins, lysozyme T4, barnase, human lysozyme and staphylococcal nuclease from ProTherm [12,13], Thermodynamic database for proteins and mutants using the following conditions.

1. The solvent accessibility of mutant residues is between 0 and 2% in order to get the data for buried mutations within experimental error. The solvent accessibility was computed with the program ASC [26] as explained in our earlier article [14].
2. The pH was between 5 and 9 except for human lysozyme. This protein has several data at pH about 3.0 and hence we included the data obtained with pH 2–7.

2.2. Amino acid properties

We used a set of 49 diverse amino acid properties (physical–chemical, energetic and conformational), which fall into various clusters analyzed by Tommi and Kanehisa [27] in the present study. This set of properties has been used in our previous works [14–17], which includes several properties listed in Kidera et al. [28,29]. The amino acid properties are normalized between 0 and 1 using the expression, $P_{norm}(i) = [P(i) - P_{min}]/[P_{max} - P_{min}]$, where $P(i)$, $P_{norm}(i)$ are, respectively, the original and normalized values of amino acid i for a particular property, and P_{min} and P_{max} are, respectively, the minimum and maximum values. The list of 49 properties used in the present study and their brief descriptions are presented in Table 1.

2.3. Computational procedure

The mutation induced changes in property values $\Delta P(i)$ was computed using the equation:

$$\Delta P(i) = P_{mut}(i) - P_{wild}(i),$$

where $P_{mut}(i)$ and $P_{wild}(i)$ are, respectively, the property value of the ith mutant and wild type residues, and i varies from 1 to N, total number of mutants. The computed difference in property values $\Delta P(i)$ was related with experimental thermal stability $\Delta T_m(i)$ using single correlation coefficient.

2.4. Multiple regression analysis

We have combined the amino acid properties using multiple regression technique: multiple correlation coefficients and regression equations were determined using standard procedures [30]. When fitting the data by multiple regression technique, reducing the number of variables increases the reliability of results. Hence, we selected three properties by searching all possible combinations of the 49 properties (18,424 combinations) and computed the multiple correlation coefficients for all data sets. The highest correlation coefficient was selected and used in the analysis.

3. Results and discussions

We have computed correlation coefficients between each of the amino acid properties and thermal stability for all the four proteins and the r-values for all the 49 properties are presented in Table 2.

Table 1
List of amino acid properties used in the present study

No.	Property	Reference
1	K^0, compressibility	[40]
2	H_t, thermodynamic transfer hydrophobicity	[41]
3	H_p, surrounding hydrophobicity	[42]
4	P, polarity	[43]
5	pH_i, isoelectric point	[43]
6	pK', equilibrium constant with reference to the ionization property of COOH group	[43]
7	M_w, molecular weight	[44]
8	B_l, bulkiness	[43]
9	R_f, chromatographic index	[43]
10	μ, refractive index	[44]
11	H_{nc}, normalized consensus hydrophobicity	[45]
12	E_{sm}, short and medium range non-bonded energy	[46]
13	E_l, long range non-bonded energy	[46]
14	E_t, total non-bonded energy ($E_{sm} + E_l$)	[46]
15	P_α, α-helical tendency	[47]
16	P_β, β-structure tendency	[47]
17	P_t, turn and coil tendency	[47]
18	P_c, coil tendency	[47]
19	C_a, helical contact area	[48]
20	F, mean r.m.s. fluctuational displacement	[49]
21	B_r, buriedness	[50]
22	R_a, solvent accessible reduction ratio	[51]
23	N_s, average number of surrounding residues	[52]
24	α_n, power to be at the N-terminal of α-helix	[47]
25	α_c, power to be at the C-terminal of α-helix	[47]
26	α_m, power to be at the middle of α-helix	[47]
27	V^0, partial specific volume	[40]
28	N_m, average medium-range contacts	[53]
29	N_l, average long-range contacts	[53]
30	H_{gm}, combined surrounding hydrophobicity (globular and membrane)	[54]
31	ASA_D, solvent accessible surface area for denatured protein	[55]
32	ASA_N, solvent accessible surface area for native protein	[55]
33	ΔASA, solvent accessible surface area for protein unfolding	[55]
34	ΔG_h, Gibbs free energy change of hydration for protein unfolding	[55]
35	G_{hD}, Gibbs free energy change of hydration for denatured protein	[55]
36	G_{hN}, Gibbs free energy change of hydration for native protein	[55]
37	ΔH_h, unfolding enthalpy change of hydration	[55]
38	$-T\Delta S_h$, unfolding entropy change of hydration	[55]
39	ΔC_{ph}, unfolding hydration heat capacity change	[55]
40	ΔG_c, unfolding Gibbs free energy changes of chain	[55]
41	ΔH_c, unfolding enthalpy changes of chain	[55]
42	$-T\Delta S_c$, unfolding entropy change of chain	[55]
43	ΔG, unfolding Gibbs free energy change	[55]
44	ΔH unfolding enthalpy change	[55]
45	$-T\Delta S$, unfolding entropy change	[55]
46	v, volume (number of non-hydrogen side-chain atoms)	[39]
47	s, shape (position of branch point in a side-chain)	[39]
48	f, flexibility (number of side-chain dihedral angles)	[39]
49	$P_{\Phi-\psi}$, backbone dihedral probability.	[56]

Table 2
Correlation coefficient between amino acid properties and thermal stability of proteins

No.	Property	Correlation coefficient (r)			
		2LZM	1BNI	1LZ1	1STN
1	K^0	−0.35	0.12	−0.20	*−0.61*
2	H_t	0.44	−0.42	0.55	*0.67*
3	H_p	**0.76**	−0.30	0.51	*0.71*
4	P	−0.41	−0.19	−0.26	0.08
5	pH_i	−0.11	0.00	0.40	0.24
6	pK	−0.05	*0.61*	−0.52	0.28
7	M_w	0.29	−0.40	0.21	0.55
8	B_l	0.53	−0.33	0.25	*0.74*
9	R_f	0.59	−0.35	0.48	**0.82**
10	μ	0.45	−0.59	0.24	0.50
11	H_{nc}	*0.61*	−0.17	0.57	*0.76*
12	E_{sm}	0.08	0.31	−0.07	−0.40
13	E_l	*0.70*	−0.09	0.43	*0.64*
14	E_t	*0.67*	0.21	0.49	*0.71*
15	P_α	0.57	−0.57	0.23	0.29
16	P_β	*0.63*	−0.11	0.10	*0.61*
17	P_t	*−0.75*	0.49	−0.28	−0.55
18	P_c	*−0.78*	0.52	−0.24	−0.58
19	C_a	0.46	−0.40	0.33	0.60
20	F	*−0.79*	0.36	−0.37	*−0.74*
21	B_r	*0.71*	−0.13	**0.65**	*0.80*
22	R_a	*0.67*	−0.21	0.51	*0.68*
23	N_s	*0.65*	0.02	0.41	*0.78*
24	α_n	*0.63*	−0.36	0.25	*0.72*
25	α_c	−0.51	−0.42	−0.36	−0.33
26	α_m	0.47	−0.59	−0.08	0.20
27	V^0	0.48	−0.37	0.40	*0.69*
28	N_m	0.58	−0.36	0.41	0.29
29	N_l	*0.61*	−0.05	0.34	0.56
30	H_{gm}	*0.66*	−0.46	*0.61*	0.56
31	ASA_D	0.44	−0.46	0.39	*0.68*
32	ASA_N	−0.57	−0.30	−0.39	−0.26
33	ΔASA	*0.68*	−0.40	0.47	*0.72*
34	ΔG_h	0.50	0.11	0.50	0.19
35	G_{hD}	0.52	0.11	0.49	0.22
36	G_{hN}	0.50	0.12	0.46	0.27
37	ΔH_h	0.06	0.47	0.12	−0.34
38	$-T\Delta S_h$	0.59	−0.42	0.41	*0.70*
39	ΔC_{ph}	*0.63*	−0.32	0.52	*0.81*
40	ΔG_c	−0.35	−0.05	−0.47	−0.09
41	ΔH_c	0.12	−0.57	−0.14	0.28
42	$-T\Delta S_c$	−0.42	0.51	−0.15	−0.53
43	ΔG	0.12	0.44	−0.07	0.11
44	ΔH	0.17	−0.56	−0.10	0.14
45	$-T\Delta S$	−0.18	**0.83**	0.13	−0.15
46	v	0.24	−0.40	0.17	0.53
47	s	0.32	0.03	0.13	*0.78*
48	f	0.31	−0.40	0.51	*0.71*
49	$P_{\Phi-\psi}$	−0.42	*0.68*	−0.06	−0.58

Correlation coefficients with more than 0.6 are shown in italics. The highest positive r-value for each protein is indicated in bold.

3.1. Lysozyme T4

We observed that the property H_p (surrounding hydrophobicity) has the highest positive correlation ($r = 0.76$) and other properties reflecting hydrophobicity (B_r, ΔASA,

R_a, H_{gm}, N_s and H_{nc}) show appreciable correlation ($r > 0.6$) with ΔT_m. This result indicates that hydrophobicity plays a major role to the stability of buried mutants, as observed in experiments [31]. On the other hand, flexibility, coil and turn tendencies have high negative correlation with ΔT_m. Recent experimental studies demonstrated that the increase of rigidity enhances the stability of T4 lysozyme buried mutants [32], which supports our observation. The negative correlation of coil and turn tendencies might be due to the fact that most of the mutants are in helical segments. Further, the properties showing long-range interactions (number of long-range contacts, N_l and long-range interaction energy, E_l) have good correlation with ΔT_m and these interactions play an important role to the folding and stability of globular proteins [25,33,34]. On the other hand, the correlation coefficient computed with a set of 49 random numbers yielded the r-value of 0.28 ± 0.16.

We have combined different sets of three amino acid properties and computed the multiple correlation coefficients. The properties with the highest r-value are selected and used these properties to derive the regression equation. The multiple correlation coefficient and the regression equation are given in Table 3. We observed an excellent agreement between experimental and predicted thermal stabilities ($r = 0.91$) by the combination of three properties, as seen in Fig. 1.

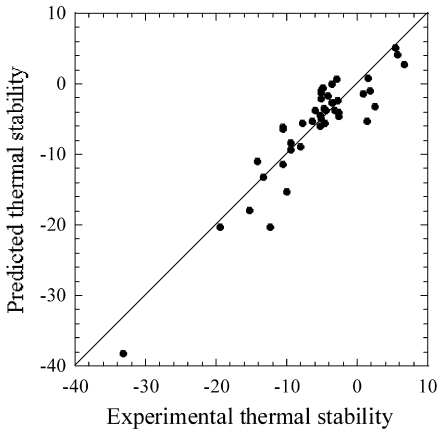

Fig. 1. Relationship between experimental and computed thermal stabilities in 2LZM by the combination of three properties. The multiple correlation coefficient is 0.91. The ΔT_m value of each mutant was computed using the regression equation, $\Delta T_m = -28.04F - 46.45ASA_D + 39.88f - 3.96$.

3.2. Barnase

In barnase, only three properties show a good correlation ($r > |0.6|$) with thermal stability and $-T\Delta S$ (entropy) has the highest correlation of 0.83 with ΔT_m (Table 2). Further, the backbone dihedral probability ($P_{\Phi-\psi}$) shows an appreciable correlation ($r = 0.68$) with stability. Axe et al. [35] reported that the local structure is an important determinant of stability and function of barnase, which strengthens our results. The effect of pK' to the stability of protein mutants has been analyzed by Oliveberg et al. [36] and the importance of pK' has been revealed from the high correlation ($r = 0.61$) between pK' and ΔT_m (Table 2). On the other hand, the properties reflecting hydrophobicity show a poor correlation with stability. The multiple correlation coefficients with the aid of three amino acid properties yielded the highest value of 0.88 (Table 3).

3.3. Human lysozyme

We observed that the properties reflecting hydrophobicity (H_{gm}, B_r) show a good correlation with thermal stability. However, the highest correlation is only 0.65, which is weaker than that obtained in other proteins ($r = 0.76$ in 2LZM, 0.83 in 1BNI and 0.82 in 1STN). Our further analysis revealed that the weak correlation might be due to the combination of mutants at various secondary structures. Hence, we separated the mutants according to their location in secondary structure and computed the correlation between amino acid properties and thermal stability. Interestingly, we observed very strong correlation in all secondary structures and the correlation coefficients are, 0.88, 0.84 and 0.78, respectively, for the mutants in helical, strand and coil segments. Takano et al. [37] experimentally observed that the relationship between hydrophobicity and stability could be well explained only if the secondary structure propensity has been taken into account. Hence the classification of mutants in secondary structures is important to understand the stability as evidenced from both computational and experimental observations.

Table 3
Multiple correlation of properties with the stability of protein mutants

Protein	N	$\|r_s\|$	r_{rand}	r_m	Regression equation
2LZM	43	0.79	0.16 ± 0.14	0.91	$-28.04F - 46.45ASA_D + 39.88f - 3.96$
1BNI	13	0.83	0.41 ± 0.24	0.88	$33.17\mu + 48.36N_s - 60.20\Delta ASA - 15.11$
1LZ1	35	0.65	0.28 ± 0.17	0.83	$25.45H_{nc} - 25.05C_a + 26.49f - 2.45$
1STN	23	0.82	0.29 ± 0.20	0.92	$56.30B_r + 50.08(-T\Delta S_c) + 44.40v - 0.49$

N : number of data; r_s : highest single property correlation coefficient; r_{rand} : average correlation coefficient obtained from 49 sets of random numbers; r_m : multiple correlation coefficient.

3.4. Staphylococcal nuclease

We observed that the property, R_f (chromatographic index) has the highest correlation with ΔT_m as reported earlier using the data from 19 different proteins [14]. However, the data with only 1STN shows remarkably stronger correlation ($r = 0.82$) than that with different proteins together ($r = 0.59$) and the relationship between R_f and ΔT_m in 1STN is shown in Fig. 2. Further, other properties reflecting hydrophobicity show a good correlation with stability. Our detailed analysis shows that in addition to hydrophobicity the physical properties (volume, size and shape) have appreciable correlation with ΔT_m. It has been reported that the shape (s) is one of the important factors for the stability the thermophilic proteins [38] and for predicting the stability of buried protein mutants [39]. The multiple regression technique improved the correlation up to 0.92.

3.5. Amino acid properties and random numbers

We observed strong correlations (0.65–0.83) between each of the individual amino acid properties and experimental thermal stability changes (Tables 2 and 3). In contrast, when we generated 49 sets of random numbers and computed the correlation coefficients, the r-values fell in the range of 0.28–0.41 (Table 3). This verifies that we could clearly discriminate amino acid properties from random numbers and emphasizes the validity of selecting amino acid properties.

3.6. Effect of sequence and structural information

We have analyzed the effect of sequence (contribution from neighboring residues on each side of the mutant site) and structural information (contribution made by the residues within 8 Å from the mutant site in space) to the stability of buried mutations. The computational details are described in our earlier article [16,17]. We observed no significant improvement due to the inclusion of sequence/structural information.

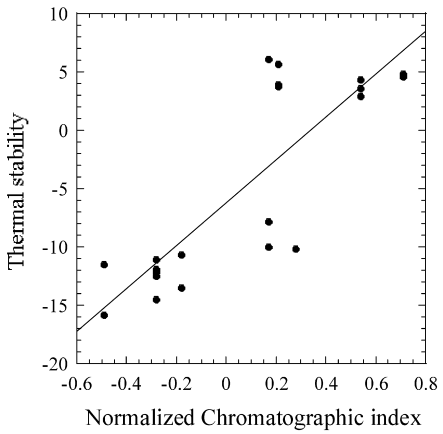

Fig. 2. Relationship between chromatographic index (R_f) and thermal stability (ΔT_m) in 1STN. The correlation coefficient is 0.82 and the regression equation is $\Delta T_m = 18.406 R_f - 6.229$.

4. Conclusions

We have systematically analyzed the relationship between amino acid properties and the stability of buried protein mutants with the aid of correlation coefficient approach. We found that the factors influencing the stability of proteins upon buried mutations are different in T4 and human lysozymes, barnase and staphylococcal nuclease. Although hydrophobicity plays a major role to protein stability other properties, such as, entropy, flexibility, size and shape, secondary structure and long-range contacts have significant contribution to the stability of buried protein mutants. We have delineated the important properties for the understanding the stability of each protein, which show an excellent agreement with experimental observations.

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