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The internal rotational barriers about NC^{α} and $C^{\alpha}C$ backbone bonds of polypeptides

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Abstract In many studies on the protein folding problem it is assumed that the internal rotational barriers about NC^{α} and $C^{\alpha}C$ backbone bonds in unfolded polypeptides are quite small, around 0.7 kcal/mol, of an order comparable to the energy of kT at normal temperature (where k is Boltzmann's constant and T is the temperature in K) and hence that rotations about these bonds occur almost freely. Here it is highlighted that such consideration is an unfortunate mistake. Approximate values for the rotational barriers of NC^{α} and $C^{\alpha}C$ bonds are suggested from computations of $U(\phi, \psi)$ potential energy surface (PES) maps of a number of oligopeptides by a semi-empirical method for conformational analysis. The proposed values are about 16 kcal/mol for NC^{α} bonds and 6 kcal/mol for $C^{\alpha}C$ bonds. The values of the same barriers estimated from some ab initio quantum-mechanical PES maps for several dipeptides available in literature are also highlighted.

Keywords Barriers in proteins · Protein folding · Dipeptide · Potential energy surface · Peptide conformation

Introduction

The magnitude of the internal rotational barriers about the backbone and side-chain single covalent bonds of polypeptides are crucial in determining conformations, folding, dynamics, and functioning of proteins. In many studies on the protein folding problem carried out over about the last four decades, it is believed that the internal rotational barriers around NC^{α} and $C^{\alpha}C$ backbone single bonds in unfolded polypeptides are quite small, around 0.7 kcal/mol, i.e., of an order comparable to the energy of kT at normal temperature of about 0.6 kcal/mol (Scheraga 1968; Ramachandran and Sasisekharan 1968; Creighton 1990; Smith et al. 1996; O'Connell et al. 1999; Ellis and Pinheiro 2002; Finkelstein and Ptitsyn 2002; Fitzkee and Rose 2004; Ellis and Minton 2006). Based on this conviction, several fundamental conceptions have been advanced, in particular the flexibility of unfolded polypeptides and their capacity for relatively free rotations about NC^{α} and $C^{\alpha}C$ bonds, the representation of denatured proteins and unfolded polypeptides as random statistical coils, and the imitation of the protein folding process as a transition of a polypeptide chain from the random coil state to the native conformation (e.g., Creighton 1990; Smith et al. 1996; O'Connell et al. 1999; Ellis and Pinheiro 2002; Daggett and Fersht 2003; Fitzkee and Rose 2004; Ellis and Minton 2006; Baldwin 2007).

Usually, two brilliant reviews by Ramachandran and Sasisekharan (1968) and by Scheraga (1968) are quoted in studies for the origin of the existence of the small rotational barriers about NC^{α} and $C^{\alpha}C$ bonds. In these popular classical reviews, an empirical potential function for computational conformational analysis is given based on a number of theoretical studies performed in the 1960s. The presumed magnitudes of the rotational barriers about the covalent bonds of polypeptides are also suggested. The potential energy of a conformation of a polypeptide chain upon rotation about NC^{α} and $C^{\alpha}C$ backbone bonds (at fixed bond length and angle) as a sum of the contributions over all the molecule is expressed by the function

$$U = U_{\mathrm{nb}} + U_{\mathrm{es}} + U_{\mathrm{hb}} + U(\varphi) + U(\psi). \quad (1)$$

In (1), U denotes the total potential energy, and the terms U_{nb}, U_{es}, and U_{hb} denote the nonbonded van der

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Waals interaction, the electrostatic interaction, and the hydrogen-bond energy, respectively. $U(\varphi)$ and $U(\psi)$ are the intrinsic torsional potential functions for rotation about NC^{α} and $C^{\alpha}C$ bonds by φ and ψ dihedral angles. They are given by $U(\varphi) = (U_0(\varphi)/2)(1 + \cos 3\varphi)$ and $(U_0(\psi)/2)(1 - \cos 3\psi)$, where $U_0(\varphi)$ and $U_0(\psi)$ are the heights of the relevant intrinsic torsional barriers (Ramachandran and Sasisekharan 1968; Scheraga 1968). The values for $U_0(\varphi)$ and $U_0(\psi)$ are presumed to be small, in the range from 0 to 1.3 kcal/mol, being about 0.7 kcal/mol on average, i.e., comparable to kT at normal temperature (Ramachandran and Sasisekharan 1968; Scheraga 1968). It is emphasized that the barriers to internal rotation about NC^{α} and $C^{\alpha}C$ bonds can be accounted for by the sum of the intrinsic torsional barriers and nonbonded interactions. The total contribution of the nonbonded interactions, i.e., of the terms U_{nb}, U_{es}, and U_{hb}, is supposed to be small, too, comparable to $U(\varphi)$ and $U(\psi)$. Therefore, the suggestion is that the rotational barriers of both NC^{α} and $C^{\alpha}C$ bonds must not be as high as of the order of 3 kcal/mol (Ramachandran and Sasisekharan 1968; Scheraga 1968).

The knowledge outlined above has formed the exceptional reference background of the accepted belief that the rotational barriers about NC^{α} and $C^{\alpha}C$ backbone bonds of polypeptides are around 0.7 kcal/mol, i.e., comparable to kT at normal temperature. In this paper, it is highlighted that this conventional point of view is an unfortunate mistake.

First, it should be noted that the value of energy comparable to kT at normal temperature of around 0.7 kcal/mol does not represent the internal rotational barriers about NC^{α} and $C^{\alpha}C$ bonds per se. This value, as mentioned above, was recommended for the heights of the intrinsic torsional barriers, $U_0(\varphi)$ and $U_0(\psi)$, of the intrinsic torsional potential functions $U(\varphi)$ and $U(\psi)$ for rotations about NC^{α} and $C^{\alpha}C$ bonds. Besides, the functions $U(\varphi)$ and $U(\psi)$ are only the components of the empirical potential function (1) from which the barriers to internal rotation about NC^{α} and $C^{\alpha}C$ bonds can be estimated.

Second, many empirical potential functions and force fields with various values for the parameters have been developed on the basis of expression (1) (e.g., Ramachandran and Sasisekharan 1968; Scheraga 1968; Momany et al. 1975; Cantor and Schimmel 1980; Brooks et al. 1983; Weiner et al. 1986; McDonald and Still 1992; Vasquez et al. 1994). By using these tools, a large number of computations have been performed for $U(\phi, \psi)$ potential energy surface (PES) maps of numerous model dipeptides (Fig. 1), analogs of the dipeptide unit of polypeptides, which is believed to represent quite satisfactorily individual amino acid residues in polypeptides. Conformational analysis of many oligopeptides and polypeptides has also been carried out. However, different empirical methods give quite different estimations of the potential energy for

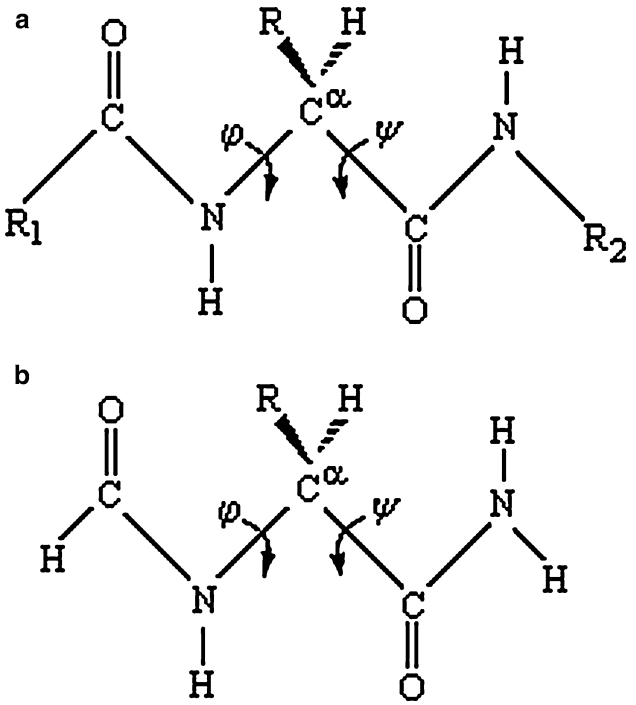


Fig. 1 Schematic of model dipeptides, analogs of the dipeptide unit of polypeptides, consisting of two neighborhood peptide bonds with an amino acid residue in the middle, with the ϕ and ψ dihedral angles indicated. R denotes an amino acid residue side-chain. **a** Common structure of model dipeptides R_1–C(O)NH–CH(R)–C(O)NH–R_2, where R_1 and R_2 are either a hydrogen or methyl group [R_1 and $R_2 = CH_3$, *N*-methyl-(amino acid residue)-*N*′-methylamide; $R_1 = H$ and $R_2 = CH_3$, *N*-formyl-(amino acid residue)-*N*′-methylamide, etc.]. **b** The simpler analog of model dipeptide (R_1 and $R_2 = H$) H–C(O)NH–CH(R)–C(O)NH–H, *N*-formyl-(amino acid residue)-amide used in the present study

the same conformation of the same peptide system. This inconsistency is usually ascribed by researchers to the well-known (Ramachandran and Sasisekharan 1968; Scheraga 1968; Vasquez et al. 1994) serious shortcomings of empirical potential functions of type (1). Therefore, no definite estimations of the rotational barriers of NC^{α} and $C^{\alpha}C$ bonds have in fact been reported. Nevertheless, it is noteworthy that the values of both barriers in almost all studies, including the popular classical reviews (Ramachandran and Sasisekharan 1968; Scheraga 1968), were estimated to be appreciably higher than 0.7 kcal/mol.

Third, it must also be emphasized that the values of the rotational barriers about NC^{α} and $C^{\alpha}C$ bonds can be estimated from ab initio quantum-mechanical calculations of the PES maps of model dipeptides. Several such studies have been performed since the 1970s (e.g., Hiller and Robson 1979; Peters and Peters 1981, 1982a, b, c; Wright and Borkman, 1982; Head-Gordon et al. 1991). However, these studies have remained virtually unnoticed as yet. The values for the rotational barriers about NC^{α} and $C^{\alpha}C$ bonds

obtained in these studies are much higher than 0.7 kcal/mol. Unfortunately, even the authors of the ab initio studies themselves have not concentrated intently on their results to announce decisively some definite values for the rotational barriers about NC^{α} and $C^{\alpha}C$ bonds.

In the next part of this paper, a quantitative estimation of the NC^{α} and $C^{\alpha}C$ rotational barriers in polypeptides is given based on the selected results of our computations of the $U(\phi, \psi, \chi_i)$ PES maps (where χ_i designates the side-chain dihedral angles of the nonglycine residues) of a number of model dipeptides and oligopeptides. The values of the same barriers estimated from the ab initio quantum-mechanical PES maps of some dipeptides available in the literature are also quoted in support of our results.

The peptide systems for study and the computational method

The peptide systems that were studied here are: model dipeptides of the glycine, alanine, serine, valine, tyrosine, and phenylalanine amino acid residues, the glycine and alanine homologous oligopeptides from three to decapeptide, and the middle fifth and the last ninth residues of the decapeptides of glycine and alanine. Model dipeptides are the *N*-methyl or formyl and *N*′-methyl or acyl derivatives of the dipeptide unit of polypeptides consisting of an amino acid residue between the two peptide bonds (Fig. 1), so that they represent individual amino acid residues in polypeptides. In the present study, dipeptides were modeled in the form of the simpler analog of model dipeptides as *N*-formyl-(an amino acid residue)-amide: H–[C(O)NH–CH(R)–C(O)NH]–H, where R designates an amino acid residue side-chain (Fig. 1b). In the same manner, we also designed oligopeptides as H–(CONH–CH(R)–CONH)$_n$–H, where n is the number of peptide units, so that $n = 1$ for dipeptides.

The semi-empirical bond–bond interactions (BBI) method (Basharov et al. 1984a; Basharov 1995) with a slight alteration of some parameters was employed for the calculations of the $U(\phi, \psi, \chi_i)$ PES maps of the peptides. The method is reliable enough, as it quite satisfactorily reproduces the available experimental or ab initio quantum-chemical data on the conformations, internal rotational barriers, and dipole moments of a wide range of organic molecules as well as the preferred structure, stable configurations, interaction energies, and dipole moments of dimers and complexes of such molecules (Basharov et al. 1984b, c, d, e; Basharov 1995). The method reproduces quite well also the $U(\phi, \psi)$ PES maps of dipeptides obtained by the ab initio quantum-mechanical methods available in the literature (Hiller and Robson 1979; Peters and Peters 1981, 1982a, b, c; Wright and Borkman 1982; Head-Gordon et al. 1991).

The $U(\phi, \psi, \chi_i)$ PES maps were calculated for the fixed standard values of bond lengths and bond angles of peptides given by Engh and Huber (1991). Conformational angles ϕ and ψ were varied at 10° intervals from −180° to 180°, and the side-chain rotational angles χ_i were varied at 30° intervals from 0° to 360° with consideration of rotational symmetry. Thus, the whole conformational space was sampled for the dipeptides. The $U(\phi, \psi)$ maps of the homologous oligopeptides were computed for the helical conformations of the oligopeptides. The $U(\phi, \psi)$ maps of the fifth and ninth residues in glycine and alanine decapeptides were calculated at the three fixed $C_5(\phi = -140°, \psi = 160°)$-, $C_7(-80°, 80°)$-, and $\alpha(-60°, -40°)$-helical conformations of the decapeptides. For each peptide system, the local and global minimums, the energies at the minimums, and the transition barriers between the minimums were determined from the PES maps. The conformational potential energy was estimated relatively to the lowest energy conformation. Computations were carried out on a Intel® Celeron® 1.7-GHz CPU PC using own computer programs. PES maps and preferable profiles of the potential energy were plotted using the demonstration version of SigmaPlot8.

Results

Figure 2 displays the $U(\phi, \psi)$ PES maps of the glycine and alanine dipeptides obtained in our computations. We denote conformations with energy (relative to the lowest energy) below 25 kcal/mol as accessible conformations. Four major distinct regions of such conformations will be specified on the PES maps, corresponding to each of the four quadrants. The largest region the lowest energy conformation $C_{7eq}(\phi \approx -80°, \psi \approx 80°)$ (eq-equatorial) and a local minimum of the potential energy near the conformation (−140°, 160°) in it is in the second quadrant ($-180° \le \phi \le 0°$, $0° \le \psi \le 180°$). The popular conformations of proteins such as parallel and antiparallel β-sheets and poly-Pro-II lie in this region, the region of lowest energy conformation. The other region with a weakly marked local minimum near the right-handed α-helical $\alpha_R(-60°, -40°)$ conformation is the region of α_R conformation in the second quadrant ($-180° \le \phi \le 0°$, $-180° \le \psi \le 0°$). Two other major regions of accessible conformations are the region of left-handed α-helical (α_L) conformation in the third quadrant ($0° \le \phi \le 180°$, $0° \le \psi \le 180°$) with a local minimum at the α_L (∼60°, 40°) conformation and the region of C_{7ax} (ax-axial) conformation with a local minimum at the C_{7ax} ($\phi \approx 80°$, $\psi \approx -80°$) conformation in the fourth quadrant ($0° \le \phi \le 180°$, $-180° \le \psi \le 0°$).

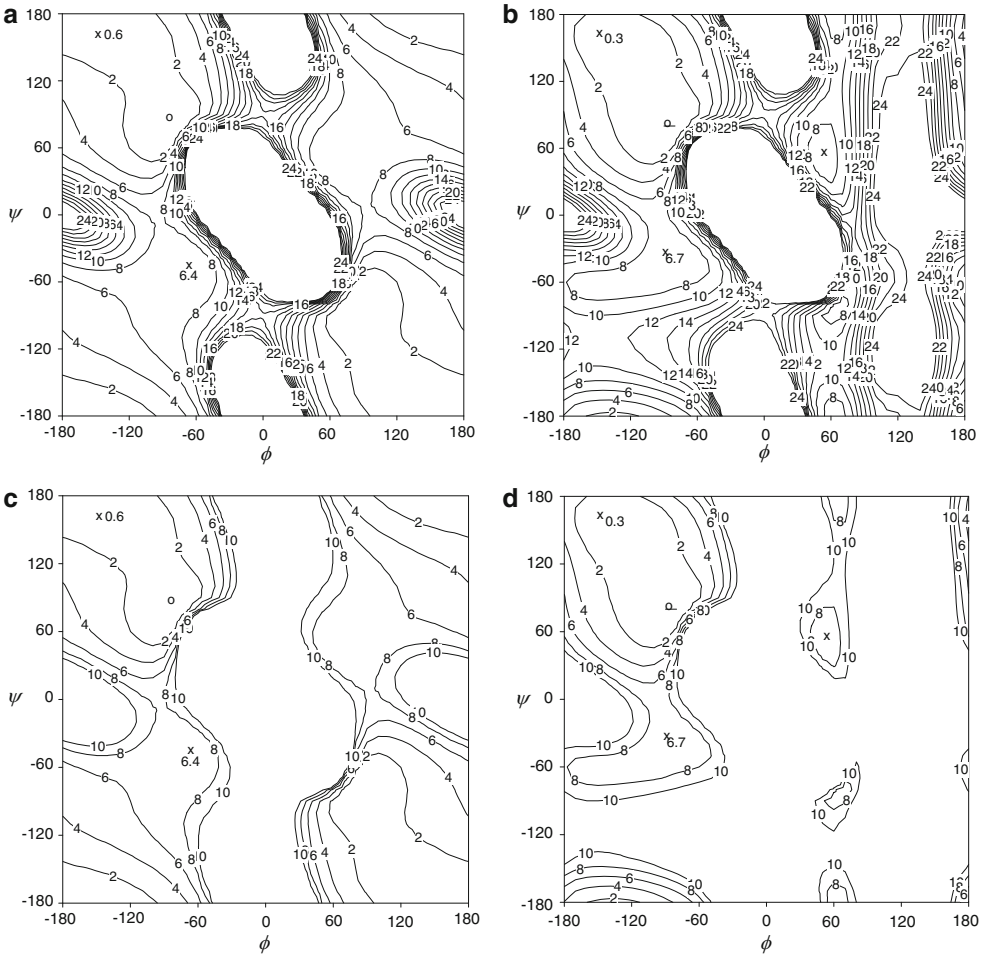
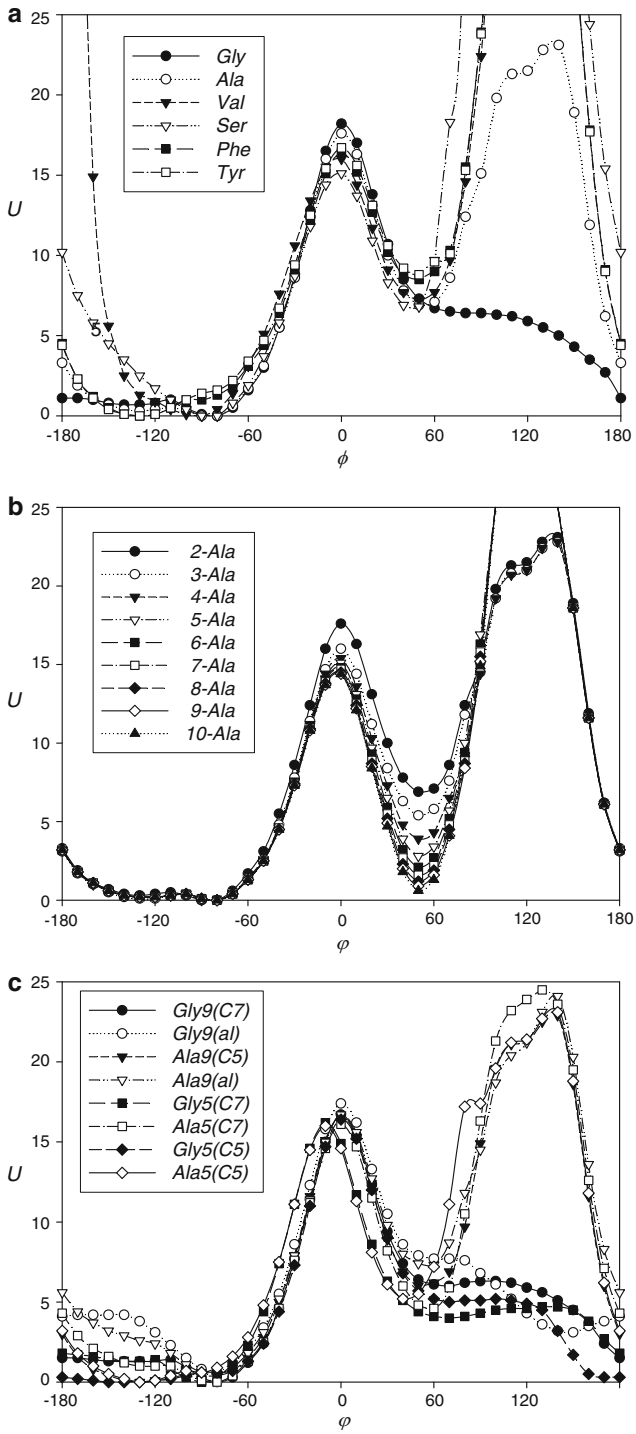


Fig. 2 PES (conformational energy) maps of the model dipeptides **a** glycine (*N*-formyl-glycyl-amide) and **b** alanine (*N*-formyl-alanyl-amide) constructed from computations at 10° intervals. Contours with potential energy below 25 kcal/mol are drawn at 2 kcal/mol intervals. *Crosses* show local minima, and *circles* designate global minima. The maps are similar to the corresponding maps of glycine and alanine dipeptides computed by the ab initio quantum-mechanical methods (Hiller and Robson 1979; Peters and Peters 1981; Wright and Borkman 1982; Head-Gordon et al. 1991). **c** and **d** display the same PES maps with the contours of the potential energy below 10 kcal/mol only drawn. These maps are similar in appearance to the classical maps of the allowed conformations of glycine and alanine dipeptides obtained by the empirical method at the lowest limit of nonbonded interatomic distances (Ramachandran and Sasisekharan 1968; Scheraga 1968)

The glycine dipeptide $U(\phi, \psi)$ energy landscape is centrosymmetrical (Fig. 2a); i.e., the energy of a conformation of the dipeptide from the third and fourth quadrants is the same as that with the corresponding centrosymmetrical conformation in the first and second quadrants. For the alanine dipeptide, the regions of the α_R and α_L conformations and the local minimum near the α_L conformation are much more pronounced, while the region of C_{7ax} conformation is inclined enough and the conformations from this region are quite unfavorable in energy (Fig. 2b). For both dipeptides, the regions of the lowest energy conformation in the first quadrant look similar in both appearance and energy.

Unquestionably, the values for the internal rotational barrier about NC^{α} and $C^{\alpha}C$ bonds may be estimated from the preferable paths of conformational changes of the dipeptides in the (ϕ, ψ) conformational space, that is, from the corresponding low potential energy paths of the dipeptides depending on the ϕ and ψ dihedral angles on the $U(\phi, \psi)$ PES map. These paths represent the minimal potential energy profiles of the dipeptides in the (ϕ, ψ) conformational space versus ϕ and ψ angles, namely the $U(\phi)$ and $U(\psi)$ profiles. Thus, to estimate the rotational barrier about NC^{α} and $C^{\alpha}C$ bonds in the glycine and alanine dipeptides, the $U(\phi)$ and $U(\psi)$ profiles should be plotted from the date on the $U(\phi, \psi)$ PES maps for clarity and convenience.

With the above features of the PES maps in mind, the following can be seen from the maps in Fig. 2 for both the glycine and alanine dipeptides: The $U(\phi)$ profile (the low energy path depending on ϕ) will follow the second and third quadrants, passing through the lowest energy conformation C_{7eq} in the first quadrant, the saddle point at the conformation (0°, 90°) or nearby, and the α_L conformation in the third quadrant. On the other hand, the $U(\psi)$ profile will follow the second and first quadrants, through the same C_{7eq} lowest energy conformation, the saddle point at the conformation (−90°, 0°) or nearby, and the α_R conformation in the second quadrant. The minimal value of the potential energy required for the transition of the dipeptides from the lowest energy conformation C_{7eq} to the region of α_L conformation will represent the internal rotational



◀ **Fig. 3** $U(\phi)$ potential energy profiles of a number of amino acid residues constructed from the $U(\phi, \psi)$ PES maps: **a** of the respective residues in model dipeptide, **b** of the alanine residue in the alanine oligopeptides from di- to decapeptide, **c** of the middle fifth and the last ninth residues in the glycine and alanine decapeptides in the $C_5(\phi = -140°, \psi = 160°)$-, $C_{7eq}(-80°, 80°)$-, and $\alpha(-60°, -40°)$-helical conformations of the decapeptides (selected representatives presented). For all the profiles, the zero of potential energy and the *right-side* local minimum, respectively, correspond to the lowest energy C_{7eq} and α_L conformations. For all the profiles, the zero of potential energy corresponds to the lowest energy C_{7eq} conformation and the *right-side* local minimum related to the α_L conformation

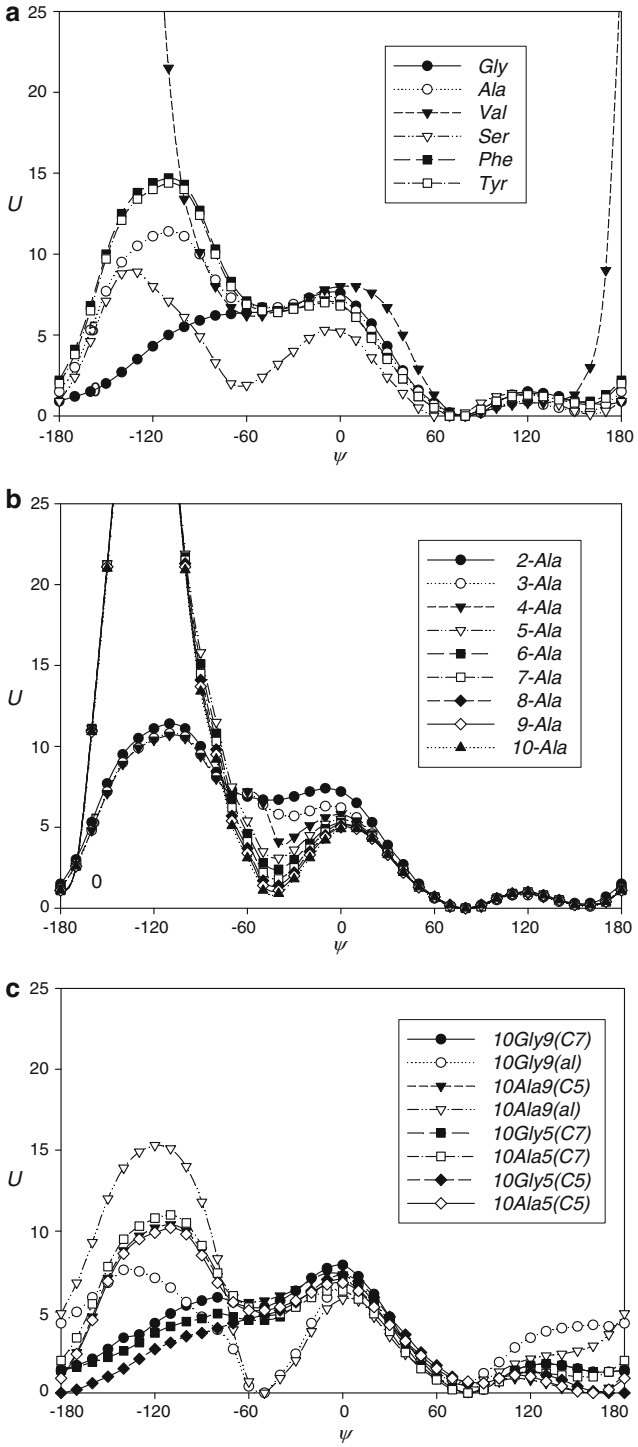
barrier about the NC^{α} bond, and that needed for the transition to the region of α_R conformation will represent the internal rotational barrier about $C^{\alpha}C$ bonds; i.e., the value of the (0°, 90°) saddle point energy will be the NC^{α} bond rotational barrier and the value of the (−90°, 0°) saddle point energy relative to the lowest energy will be the $C^{\alpha}C$ bond rotational barrier. Clearly, such estimates of the rotational barriers of NC^{α} and $C^{\alpha}C$ bonds suggest simultaneous rotations about both bonds.

For the dipeptides of the other amino acids considered here, the results of our computations testify against the following point. At the definite conformation of the side-chain of these residues, the overview of the PES maps, the locations of the minimums and saddle points on the maps, and the preferable paths of conformational changes described above for the alanine dipeptide are typical for these dipeptides. This is also evident from the computations of the $U(\phi, \psi)$ PES maps of dipeptides by either empirical or ab initio quantum-mechanical methods.

The curves in Figs. 3 and 4 represent the approximate $U(\phi)$ and $U(\psi)$ profiles (the preferable paths of the conformational changes) of a number of amino acid residues versus the ϕ and ψ angles, respectively. They were constructed from the data on the $U(\phi, \psi)$ PES map of the respective residue in the dipeptide and oligopeptides in the following way. In Fig. 3, in the $U(\phi)$ profile, for each residue the value of energy for the given angle ϕ_i is the minimum value of potential energy on the $U(\phi, \psi)$ map for that residue in the interval $0° \leq \psi \leq 180°$ at fixed ϕ_i. Similarly, in each $U(\psi)$ profile in Fig. 4, the value of energy for the given angle ψ_i is the minimum value of potential energy on the map in the interval $-180° \leq \phi \leq 0°$ at fixed ψ_i.

In accordance with the discussion above, in each $U(\phi)$ profile in Fig. 3 the value of potential energy at the peak with the abscissa of $\phi \approx 0°$ represents the NC^{α} rotational barrier, and in each $U(\psi)$ profile in Fig. 4 the value of potential energy at the peak with the abscissa of $\psi \approx 0°$ represents the $C^{\alpha}C$ rotational barrier. These values of the rotational barriers around NC^{α} and $C^{\alpha}C$ bonds are given, respectively, in Tables 1 and 2. The tables for the dipeptides also list the values of the NC^{α} and $C^{\alpha}C$ bond rotational barriers estimated from a number of ab initio quantum-mechanical $U(\phi, \psi)$ maps available in literature.

According to the data in Table 1 and Fig. 3, the value for the NC^{α} bond rotational barrier in the dipeptides obtained in our computations is in the range 15.1–18.2 kcal/mol, being 16.7 kcal/mol on average, while that obtained by the ab initio methods varies from 16 to



◀ **Fig. 4** $U(\psi)$ potential energy profiles of a number of amino acid residues constructed from the $U(\phi, \psi)$ PES maps: **a** of the respective residues in model dipeptide, **b** of the alanine residue in the alanine oligopeptides (from di- to decapeptide), **c** of the middle fifth and the last ninth residues in the glycine and alanine decapeptides in the $C_5(\phi = -140°, \psi = 160°)$-, $C_{7eq}(-80°, 80°)$-, and $\alpha_R(-60°, -40°)$-helical conformations of the decapeptides (selected representatives presented). On all the profiles, the zero of potential energy corresponds to the lowest energy C_{7eq} conformation and the *left-side* local minimum related to the α_R conformation

21 kcal/mol. In the glycine oligopeptides, the value of the NC^{α} barrier is almost comparable to that of the same oligopeptides of alanine. The value of the barrier decreases slowly with increasing number of residues, from 17.8 kcal/mol in dipeptide to 14.4 kcal/mol in decapeptide. For the middle fifth and the last ninth residues of the glycine and alanine decapeptides, the value of the NC^{α} bond rotational barrier is in the range from 16 to 16.7 kcal/mol, being about 16 kcal/mol on average.

The value for the $C^{\alpha}C$ bond rotational barrier (Table 2; Fig. 4) in the dipeptides obtained in our computations is in the range 5.3–8 kcal/mol, being 6.6 kcal/mol on average. The value of the barrier obtained by the ab initio methods varies from 5 to 10 kcal/mol. In the oligopeptides of glycine and alanine, the value of the barrier decreases slowly with increasing number of residues, from 7.8 kcal/mol in glycine dipeptide to 4.9 kcal/mol in alanine decapeptide. For the middle fifth and the last ninth residues of the glycine and alanine decapeptides, the value of the $C^{\alpha}C$ bond rotational barrier is in the range 5.3–7.8 kcal/mol, being 6.5 kcal/mol on average.

Conclusions

Based on the presented data it is suggested that in polypeptides the rotational barrier around NC^{α} bonds is in the range of about 14–18 kcal/mol, being approximately 16 kcal/mol on average, and that around $C^{\alpha}C$ bonds is in the range 5–7 kcal/mol, being approximately 6 kcal/mol on average.

Obviously, the reliability of the values for the rotational barriers of NC^{α} and $C^{\alpha}C$ bonds suggested here can be justified by the reliability of the calculation method used. The reliability of the ab initio quantum-mechanical calculations cited here is indeed beyond question. The BBI method is also quite admissible, as its reliability has been tested on different organic molecules and dipeptides. The values suggested for the rotational barriers of NC^{α} and $C^{\alpha}C$ bonds do not pretend to be absolute, since there is no perfect theoretical computational method equivalent to a precise experimental tool for exact evaluation of internal rotational barriers. They truly highlight the range of possible values of the rotational barriers of NC^{α} and $C^{\alpha}C$ bonds, and they are considerably higher than the value of around 0.7 kcal/mol traditionally accepted for both barriers.

There is reliable indirect experimental evidence of the existence of a high rotational barrier at least about the NC^{α} bonds of polypeptides. This barrier, as seen from Figs. 2

Table 1 NC^{α} bond internal rotational barriers in oligopeptides (kcal/mol)

Dipeptide	Barrier	*n*-Peptide	Barrier[a]		Residue	Barrier[a]
			Ala	Gly		
Gly	17.8[a]	2	17.6	17.8	Gly9(C7)	16.7
	19.2[b]	3	16.0	16.3	Gly9(al)	17.4
	16[c]	4	15.4	15.7	Ala9(C5)	16.6
	21.7[d]	5	15.1	15.3	Ala9(al)	16.7
Ala	17.6[a]	6	14.8	15.1	Gly5(C7)	16.2
	16[c]	7	14.7	14.9	Ala5(C7)	16.1
	20.2, 17–18[d]	8	14.5	14.8	Gly5(C5)	16.4
Val	16.0[a]	9	14.4	14.7	Ala5(C5)	16.0
	>15, <20[e]	10	14.4	14.6		
Ile	>15, <20[e]					
Ser	15.1[a]					
	>15, <20[f]					
Phe	16.4[a]					
Tyr	16.7[a]					
Thr	>15, <20[g]					

Gly9(*C7*) the ninth residue of the glycine decapeptide in the C7 conformation of the decapeptide, *Gly9*(*al*) the ninth residue of the glycine decapeptide in the α-helix conformation of the decapeptide, *Ala9*(*C5*) the ninth residue of the alanine decapeptide in the C_5 conformation of the decapeptide, *Gly5*(*C7*) the fifth residue of the Gly decapeptide in the C_7 conformation of the decapeptide, etc.

[a] Current work; ab initio quantum-mechanical data on the dipeptides: [b] Hiller and Robson (1979), [c] Peters and Peters (1981), [d] Wright and Borkman (1982), [e] Peters and Peters (1982a), [f] Peters and Peters (1982b), [g] Peters and Peters (1982c)

Table 2 $C^{\alpha}C$ bond internal rotational barriers in oligopeptides (kcal/mol)

Dipeptide	Barrier	*n*-Peptide	Barrier[a]		Residue	Barrier[a]
			Ala	Gly		
Gly	7.6[a]	2	7.4	7.8	Gly9(C7)	7.9
	7.5[b]	3	6.3	6.6	Gly9(al)	6.7
	5.5[c]	4	5.8	6.6	Ala9(C5)	7.4
	5.5[d]	5	5.5	6.1	Ala9(al)	5.8
Ala	7.4[a]	6	5.3	5.9	Gly5(C7)	7.0
	8–9[b]	7	5.2	5.8	Ala5(C7)	6.3
	5.5[c]	8	5.1	5.7	Gly5(C5)	7.3
Val	8.0[a]	9	5.0	5.6	Ala5(C5)	6.8
	>6, <10[e]	10	4.9	5.6		
Ile	>6, <10[e]					
Ser	5.3[a]					
	6.3[f]					
Phe	7.1[a]					
Tyr	7.0[a]					
Thr	7.5[g]					

Gly9(*C7*) the ninth residue of the glycine decapeptide in the C7 conformation of decapeptide, *Gly9*(*al*) the ninth residue of the glycine decapeptide in the α-helix conformation of decapeptide, *Ala9*(*C5*) the ninth residue of the alanine decapeptide in the C_5 conformation of decapeptide, *Gly5*(*C7*) the fifth residue of the Gly decapeptide in the C_7 conformation of decapeptide, etc.

[a] Current work; ab initio quantum-mechanical data on the dipeptides: [b] Hiller and Robson (1979), [c] Peters and Peters (1981), [d] Wright and Borkman (1982), [e] Peters and Peters (1982a), [f] Peters and Peters (1982b), [g] Peters and Peters (1982c)

and 3, divides the (ϕ, ψ) conformational space of the amino acid residues into two regions: the region of negative conformations with negative values of ϕ and the region of positive conformations with positive values of ϕ. One can see from Figs. 2 and 3 that, for the glycine residue, the conformational transitions between the allowed negative and positive conformations will occur almost unhindered over the lines $\phi = \pm 180°$ and $\psi = \pm 180°$. From Figs. 2 and 3, it is also seen that, for nonglycine residues, the conformational transitions between the allowed negative and positive conformations are almost forbidden by quite high, 25 kcal/mol and higher, barriers (in the neighborhood of the line $\phi \approx 120°$). Such a transition will occur over the saddle point (0°, 90°), overcoming the NC^{α} bond rotational barrier. The value of this transition barrier is around 16 kcal/mol as estimated above, i.e., almost comparable to the *trans*-to-*cis* transition barrier of the peptide bond, which is well known to be about 18 kcal/mol. Such a high barrier, apparently, must make it difficult for nonglycine residues to transition from low-energy negative conformations to low-energy positive conformations.

Based on these arguments, one might assume that in proteins the glycine residues must appear in both negative and positive conformations with almost equal probabilities due to almost unhindered transitions between the centrosymmetrical low-energy negative and positive conformations, while nonglycine residues must predominantly occur in the negative conformations because of the $\sim$16 kcal/mol rotational barrier, taking into account that the polypeptide chain is synthesized on the ribosome most probably in the right-handed α-helical conformation (Lim and Spirin 1986; Bhushan et al. 2010, and references therein). The occurrence of nonglycine residues in positive conformations will be unlikely not only from a kinetics viewpoint because of the high rotational barrier. It will also be unlikely thermodynamically, since for the peptide systems of nonglycine residues considered here the minimum of the potential energy in the region of negative conformations is more favorable than that in the region of positive conformations (Figs. 1b, 2). These deductions can be tested using the available crystal structure data of proteins.

In the crystal structures of proteins, glycine residues occur in both negative and positive conformations while the occurrence of nonglycine residues in positive conformations is quite rare. To cite an example, according to an analysis of the Protein Data Bank, only 2% of the total population of 795,533 nonglycine and nonproline residues have positive dihedral angle ϕ (Fitzkee et al. 2005). These common observations provide a simple, reliable, indirect demonstration of the existence of a high rotational barrier about NC^{α} bonds in proteins. On the contrary, the high rotational barrier about NC^{α} bonds in polypeptides explains, for the first time, the aforementioned observations on the occurrence of glycine and nonglycine residues in the crystal structure of proteins in negative and positive conformations.

The data presented in this paper may be expected to be important in elucidating the stability, functioning, folding problem, and spatial structure organization of proteins.

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