Conformational Analysis of Benzyloxycarbonyl-Protected Peptides

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Peptide Conformations, Energy Calculations, Benzyloxycarbonyl Group

The benzyloxycarbonyl (Z-) residue, an important protecting group in peptide synthesis, was introduced into the input data set of the "Empirical Conformational Energy Program for Peptides" (ECEPP) developed by Scheraga and coworkers. With additional modifications ECEPP is now prepared for conformational analysis of peptides containing this amino end group. Preferred conformations of the Z-residue were studied by performing calculations on Z-Gly methylamide. The results obtained may be applied to the conformational analysis of Z-protected peptides.

Introduction

The benzyloxycarbonyl (Z-) protecting group plays a major role in peptide synthesis due to its universal applicability. For conformational studies on Z-protected peptides therefore it is of great interest to know how conformations are influenced by inter-residue interactions with this amino end group. Therefore conformational energy calculations on Z-Gly methylamide were carried out using our extended version [1] of the "Empirical Conformational Energy Program for Peptides" (ECEPP) originally developed by Scheraga and coworkers [2]. First geometric parameters and electronic charge distribution of the benzyloxycarbonyl group had to be determined, since these data are not included in the standard residue data set of ECEPP. As the Z-residue is a N-terminal end group containing more than two rotable bonds, some additional modifications of the program had to be applied.

Geometric Parameters and Electronic Charge Distribution of the Z-Residue

From published X-ray data obtained with compounds containing the benzyloxycarbonyl group [3–19], a set of average bond lengths and bond angles of the Z-residue was determined (see Fig. 1 a). Data failing to pass Student's t-test at the 99% level were not taken into account; the phenyl ring was assumed to be a regular hexagon. The standard deviations of the remaining set of bond lengths and bond angles are 0.08 Å and 1°, respectively.

Partial atomic charges for each atom of the Z-residue were obtained from molecular orbital calculations on Z-Gly methylamide, using the overlap normalized CNDO/2 method [20]. In order to maintain electrical neutrality, the results were rounded off insignificantly (see Fig. 1 b).

Minimum-Energy Conformations of the Z-Residue in Z-Gly Methylamide

Preferred conformations of the benzyloxycarbonyl group were determined by varying angles θ_1^1, θ_1^2, and θ_1^3 in Z-Gly methylamide (*cf.* Fig. 4). In a first step, energy contour maps in $\theta_1^2 - \theta_1^3$ space were computed for some values of θ_1^1 (all other angles were kept at 180°). The resulting map for $\theta_1^1 = 90°$ is shown in Fig. 2. From the low-energy regions of the maps starting conformations for energy minimization were selected. Minimization was carried out using program ECEPP/A [1]. Iteration was terminated as soon as, for two subsequent cycles, conformational energies differed by less than 0.001 kcal/mol, or if none of the variable dihedral angles θ_1^1, θ_1^2, and θ_1^3 deviated by more than 0.1° from its former value. In this way 8 minimum-energy conformations were found which are listed in Table I. The table shows that the folded conformations A, A*, B, and B* where the phenyl ring approaches the urethan bond ($d = 2.9$ Å) are more stable than the extended conformations C, C*, D, and D* with $d = 3.6$ Å.

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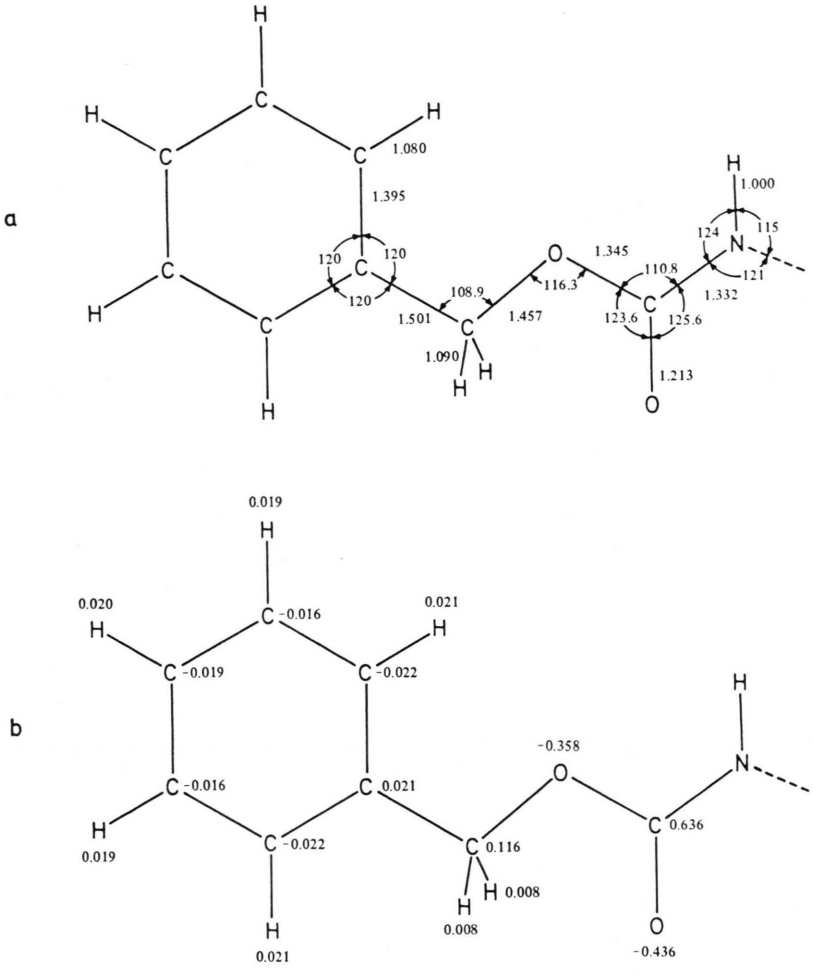

Fig. 1. Geometric parameters and electronic charge distribution of the Z-residue. a) Bond lengths (Å), and bond angles (deg). b) Partial atomic charges (electronic charge units).

Minimum-Energy Conformations of Z-Gly Methylamide

Up to this point angles φ_2 and ψ_2 (*cf.* Fig. 4) had been held constant. By including these angles into the minimization procedure, we now looked for conformational energy minima of the complete structure. By combining the 8 conformations of the Z-residue, summarized in Table I, with 7 minimum-energy states of Gly in N-Acetyl-Gly methylamide [21] we arrived at 56 different starting conformations for program ECEPP/A. Eventually, 34 low-energy conformations ($\Delta E \leqq 3.0$ kcal/mol) of Z-Gly methylamide were found, the more important of which are summarized in Table II. Conformational energies are given as $\Delta E = E - E_0$, where E_0 is the

Table I. Minimum-energy conformations of the benzyloxycarbonyl group in Z-Gly methylamide.

	ΔE[a]	d[b] [Å]	Dihedral angles [degrees][c]		
			θ_1^1	θ_1^2	θ_1^3
A	0.00	2.92	108	− 66	108
A*	0.00	2.92	72	66	− 108
B	0.64	2.97	114	− 71	− 105
B*	0.64	2.97	66	71	105
C	1.11	3.65	46	− 173	116
C*	1.11	3.65	134	173	− 116
D	1.18	3.65	133	− 177	115
D*	1.18	3.65	47	177	− 115

[a] $\Delta E = E - E_0$ with $E_0 = -11.23$ kcal/mol.
[b] Interatomic distance between the substituted C atom of the phenyl ring and the carbonyl carbon of the Z-residue.
[c] Only variable dihedral angles are listed, all others are 180°.

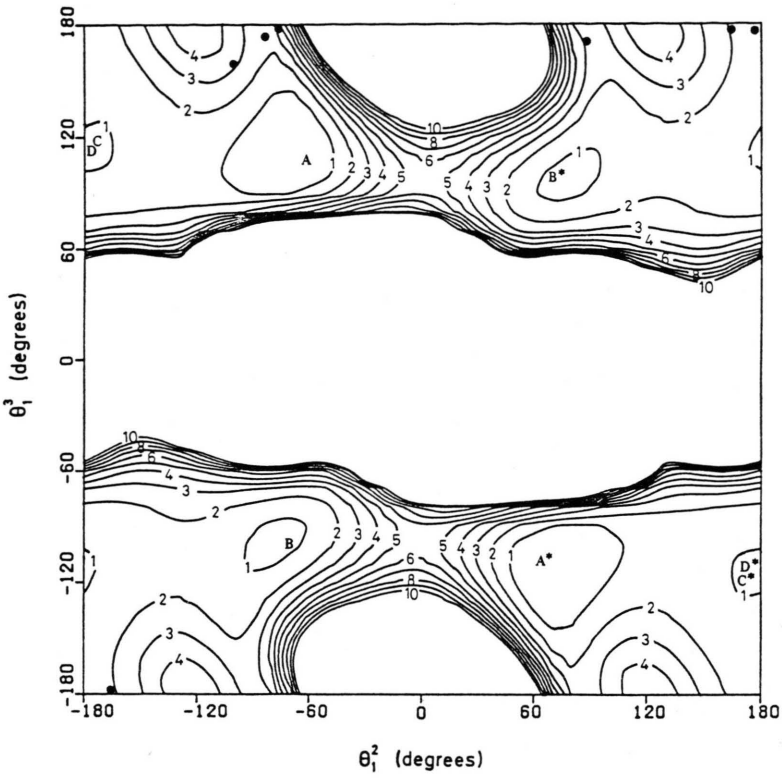

Fig. 2. Conformational isoenergetic contour map of Z-Gly methylamide in $\theta_1^2 - \theta_1^3$ space with $\theta_1^1 = 90°$, and 180° for the remaining angles. The minima listed in Table I are indicated by the corresponding capital letters. Conformations derived from X-ray data (see Table III) are indicated by filled circles. Numbers in lines are energy levels (kcal/mol) above the minima at $(\theta_1^2, \theta_1^3) = (-66°, 110°)$ and $(66°, -110°)$.

Table II. Minimum-energy conformations[a] of Z-Gly methylamide.

No.	ΔE[b]	W[c]	Dihedral angles [degrees][d]				
			θ_1^1	θ_1^2	θ_1^3	φ_2	ψ_2
1	0.00	0.2801	65	58	−121	− 85	73
2	0.00	0.2801	115	− 58	121	85	− 73
3	0.81	0.0708	60	65	−118	− 73	− 55
4	0.81	0.0708	120	− 65	118	73	55
5	0.94	0.0570	74	57	90	82	− 74
6	0.94	0.0570	106	− 57	− 90	− 82	74
7	1.45	0.0240	74	65	−115	83	− 76
8	1.45	0.0240	106	− 65	115	− 83	76
9	1.83	0.0127	118	− 72	−110	83	− 76
10	1.83	0.0127	62	72	110	− 83	76
11	2.06	0.0086	73	66	−106	−170	179
12	2.06	0.0086	108	− 66	106	170	−179
13	2.19	0.0069	75	66	−108	−174	− 64
14	2.19	0.0069	106	− 66	108	174	64
15	2.24	0.0064	133	173	−116	− 83	76
16	2.24	0.0064	47	−173	116	83	− 76
17	2.29	0.0059	48	177	−114	− 82	76
18	2.29	0.0059	133	−177	114	82	− 76

[a] Only conformations with $\Delta E \leqq 2.3$ kcal/mol are listed.
[b] $E_0 = -13.34$ kcal/mol.
[c] Boltzmann statistical weights w were calculated for $T = 298$ K.
[d] Only variable dihedral angles are listed; all others are 180°.

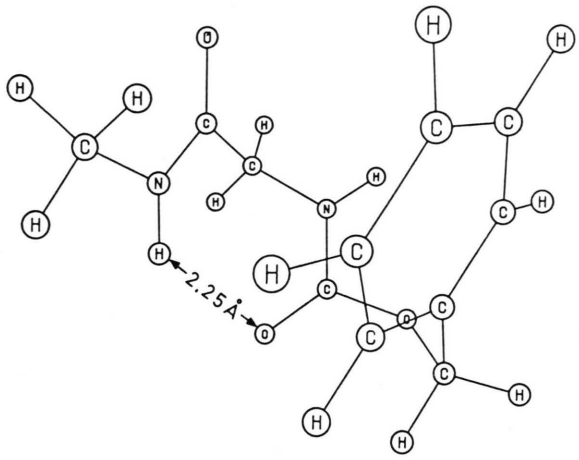

Fig. 3. Perspective view of global minimum conformation No. 2 (*cf.* Table II) of Z-Gly methylamide. One of the interactions stabilizing the structure is the H-bond indicated.

energy at the global minimum ($E_0 = -13.34$ kcal/mol). The structure of Z-Gly methylamide in that conformation (No. 2 in Table II) is depicted by Fig. 3.

From the data of Table II one can ascertain that the ensemble of conformations is dominated by conformation 1, and its mirror image 2. According to Boltzmann statistics these two global minimum conformations have a 56% probability of occurence. In the range of $\Delta E \leqq 2.2$ kcal/mol (92% of the low-energy conformations with $\Delta E \leqq 3.0$ kcal/mol) the Z-residue adopts exclusively the folded conformations A, A*, B, and B*.

Results and Discussions

From our results it is evident that the Z-residue is a rather flexible entity. In the $\theta_1^2 - \theta_1^3$ contour map (see Fig. 2) minima are surrounded by extensive low-energy regions. About 37% of all conformations are located within the range of $\Delta E \leqq 3.0$ kcal/mol.

The response of the conformational energy to small fluctuations of θ_1^1 to θ_1^4 about their values at the global minimum (No. 2 in Table II) is shown in Fig. 4.

Little change of E was observed when angles θ_1^1, θ_1^2, or θ_1^3 were perturbed. This finding again points to the fact that the benzyloxycarbonyl group has a high librational entropie. Due to the partial double bond character of the urethan bond, the corresponding angle θ_1^4 shows a stronger effect on conformational energy. In addition this dependency is considerably intensified by building up a C_7 hydrogen bond between the carbonyl oxygen of the urethan bond and the amide hydrogen (see Fig. 3).

The results of X-ray studies on Z-protected compounds [3, 6, 7, 13, 14, 16, 18] (Table III) are in good agreement with the calculations reported here. Both the folded and the extended conformations of the Z-residue were found in crystals as can be seen by comparing values of θ_1^2. The same is true for θ_1^1; however, considerable deviations exist with θ_1^3. Although the X-ray conformations are located in low-energy regions of the map (*cf.* Fig. 2), they are

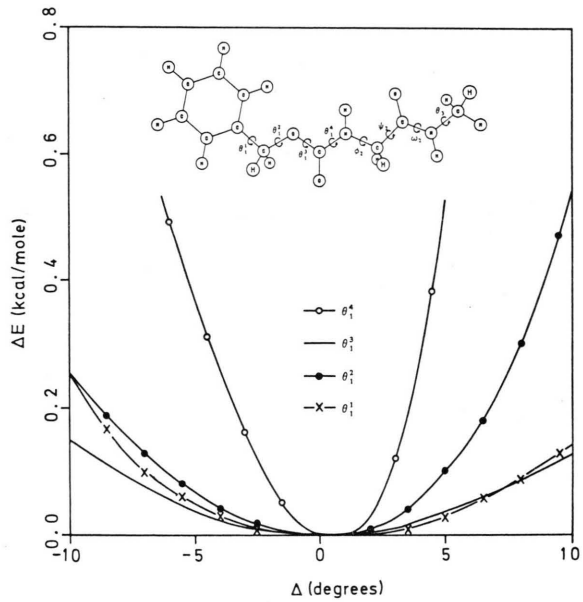

Fig. 4. Variation in conformational energy of Z-Gly methylamide as dihedral angles θ_1^1 to θ_1^4 are perturbed by small amounds from their values at the global minimum (No. 2 in Table II).

Table III. Dihedral angles of the Z-residue obtained from X-ray data.

Ref.	θ_1^1	θ_1^2	θ_1^3
3	100.3	162.5	178.6
6	174.6	178.0	178.5
7	16.1	−165.3	−178.7
13	109.3	− 85.0	174.9
14	61.8	−103.2	162.3
16	110.0	− 77.0	180.0
18	79.4	87.7	170.2

not at local minima of free Z-Gly methylamide. Unfortunately the corresponding X-ray data of Z-Gly methylamide are not available for comparison. Anyhow, the validity of such comparions is very limited, because intermolecular interactions, due to crystal packing, can alter the dihedral angles from those occuring in the isolated molecule.

During minimization the values of the variable angles were changed by only a few degrees. This is especially true for the dihedral angles φ_2 and ψ_2 of Gly, which are essentially the same in N-Acetyl- and Z-Gly methylamide.

From the above analysis, as well as from calculations on Z-Asp- and Z-Glu methylamide carried out recently, it is clear, that interresidue interactions in benzyloxycarbonyl-protected amino acids cause only small perturbations of the conformational space in a particular residue. If the Z-protected peptide contains Pro or if the peptide in question is larger, the urethan group may be strongly involved in intramolecular hydrogen bonds [19, 22, 23].

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