# AMBER* Torsional Parameters for the Peptide Backbone

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Abstract: A new set of torsional parameters for peptides in the AMBER force field is described which reproduces *ab initio* calculations on the conformational preferences of simple peptides better than the original set. We designate the new parameter set as AMBER* and show how it compares with the performance of AMBER in reproducing the x-ray structure of the small protein crambin.

The AMBER molecular mechanics force field was first reported in 1984 and is widely used for modeling biopolymers.[1] In the intervening years, however, a great deal has been learned about the conformational preferences of simple peptides. This new data suggests that AMBER and other force fields may not accurately reproduce the conformational energies of simple peptides such as glycine dipeptide **la** and alanine dipeptide **2a.** In particular, the relative AMBER energies of the C5 and C7 conformations of 1 and 2 do not agree with recent *ab initio* calculations on these species.[2-4]

a) $R_1 = R_2 = CH_3$
b) $R_1 = R_2 = H$
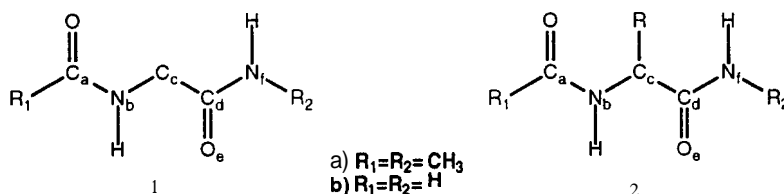
The original AMBER force field gives the C7 form of **la** as approximately 3.3 kcal mol^{-1} more stable than the C5 conformation, and a similar large energy difference is predicted for the C7$_{eq}$ form of **2a** (R = CH3) relative to its C5 conformation. In contrast, new *ab initio* calculations suggest that the energy differences are considerably smaller: SCF-MO[2] calculations using a DZP basis set predict that the C5 conformation of **1** is actually 0.3 kcal mol^{-1} more *stable* than the C7 form, while for the alanine dipeptide **2b,** the C7$_{eq}$ is more stable than the C5 form by 0.5 kcal mol^{-1}. Although the relative energies are basis set dependent, these and other high level calculations[3,4] consistently indicate that C5 and C7 conformers are significantly closer in energy than AMBER suggests. Consequently, we developed a new set of AMBER torsional parameters to fit the *ab initio* results. In this Letter, we describe the resulting force field which we term AMBER* and in the companion Letter we show how it compares with recent experiments by Gellman and coworkers.[5]
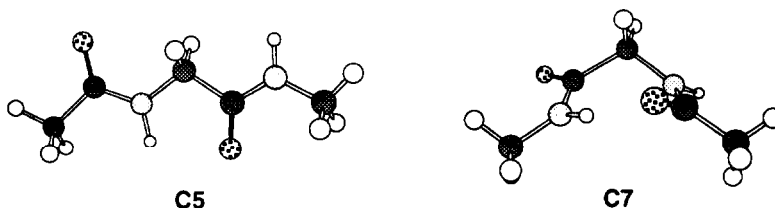
C5   C7

Figure 1.   Conformations of the glycine dipeptide (**la**).

We chose the calculations of Pople et al.[3] as the basis for our reparameterization because they fully defined the ψ and ϕ torsional potential energy surface for both 1 b and 2b. We began by selecting all stationary points reported by Pople for peptides 1 b (7 conformations) and 2b (16 conformations) as structures and relative energies to be reproduced. The molecular mechanics calculations started with the original AMBER united-atom parameter set[1a] and used Ferguson and Kollman's 6,12-Lennard Jones hydrogen bonding treatments and constant dielectric electrostatics with ε = 1. Keeping all other force field parameters unchanged, we adjusted the torsional (V1-V3) parameters associated with the ψ and ϕ bonds to minimize the differences between the relative energies of stationary points as reported for the HF/6-31+G* level of theory and calculated by molecular mechanics after energy minimization. Conformations reported to be maxima as well as some of the higher energy minima were minimized with ψ,ϕ torsional constraints which fixed the structures at the *ab initio* geometries. The C5 and C7 conformers were minimized without constraints. The torsional functions for the ψ and ϕ bonds turn out to be highly coupled, and a satisfactory agreement with all points on the potential energy surface could not be obtained by simply adjusting standard torsional parameters associated with the ψ and ϕ bonds. We therefore added a remote torsional interaction which depended on both ψ and ϕ and was defined by the C_a-N_b and C_d=O_e bonds in the diagrams above. Using an appropriate V1 parameter for this remote torsion (*), the HF/6-31+G* potential surfaces were well reproduced by the molecular mechanics and the final united-atom AMBER' parameter set is listed below in kcal/mol. We also reoptimized the all-atom AMBER[1b] and OPLS/AMBER[7] force fields in the same way and incorporated the new parameters into our BatchMin V3.5 force fields.

	Glycine Residues			Other Residues		
Torsional Array	V1/2	V2/2	V3/2	V1/2	V2/2	V3/2
C_a - N_b - C_c - C_d	0.70	1.20	0.00	1.85	0.00	0.10
N_b - C_c - C_d - N_f	0.45	0.90	-0.50	1.85	0.50	-0.20
C_a - N_b ··· C_d = O_e *	2.50	0.00	0.00	-0.30	0.00	0.00

The final relative energies for the stationary points calculated with original united-atom AMBER and the modified AMBER* fields are compared with the results of Pople's *ab initio* study in Table 1 for the glycine peptide and its analog 1 b, and in Table 2 for the alanine peptide and its analog 2b. The root mean square error for all stationary points is given at the bottom of the table and compares HF/6-31+G* energies of b peptides with AMBER or AMBER* energies of a peptides.

**Table 1.** Calculated relative energies for the seven stationary points of 1a and 1 b.

Glycine Conformation	Relative Energy (kcal mol^{-1})		
	HF/6-31+G* 1b	Original AMBER 1a	AMBER' 1a
C7	0.58	0.00	0.66
C5	0.00	4.73	0.00
C7->C5	1.86	3.68	3.52
C7->C7	9.74	11.48	8.05
C5 cusp	8.95	8.10	8.27
C7->C7 (alternative)	10.27	7.79	9.12
C_s max.	22.98	22.32	23.93
		RMS Error 2.29	RMS Error 1.08

Table 2. Calculated relative energies for the sixteen stationary points of 2a and 2b.

Alanine Conformation	Relative Energy (kcal mol^{-1})		
	HF/6-31+G* **2b**	Original AMBER **2a**	AMBER' **2a**
C7$_{eq}$	0.00	0.00	0.00
c5	0.19	2.64	0.18
C7$_{ax}$	2.56	0.86	3.31
b$_2$	2.24	0.0	3.39
a$_L$	4.73	0.86	3.37
a'	5.52	5.62	5.23
C7$_{eq}$ -> b2	2.26	3.50	3.19
C7$_{eq}$ -> c5	1.11	3.14	0.54
C7$_{ax}$ -> a$_L$	4.76	5.19	6.47
C7$_{ax}$ -> a'	7.15	a.54	8.03
C7$_{eq}$ -> C7$_{ax}$	10.07	8.13	11.17
c5 -> C7$_{ax}$	7.39	11.46	8.01
b$_2$ ->a$_L$	7.30	6.18	6.11
C5 -> a'	6.67	8.10	6.27
C7$_{eq}$ -> a$_L$	9.74	8.31	9.70
a$_L$ -> C5	10.59	10.99	10.57
		RMS Error 1.97	RMS Error 0.88

Using AMBER' as described above, Ramachandran ψ, ϕ plots were generated for **la** and **2a** in order to ensure that the new energy surface has the same form as that reported for the HF/6-31G' calculations. Fully relaxed AMBER' Ramachandran maps for **la** and **2a** are shown below. These maps confirm that the major features of the surfaces described by Pople, et *al.* are reproduced with AMBER*. We also carried out Monte Carlo conformational searches8 with **la** and **2a** using AMBER* and found only those minima described in the above tables. Thus no new minima have been introduced into the potential energy surface of these peptides.
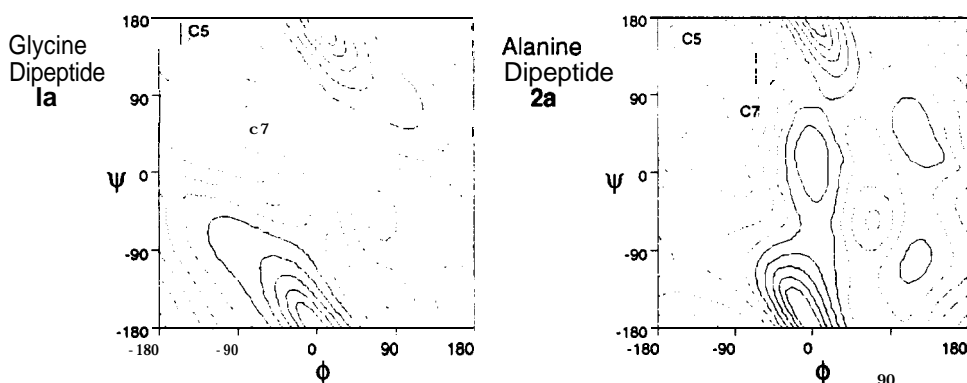
While the revised parameter set performs well for simple peptides, we wanted to see if the new parameters significantly altered the performance of the AMBER force field in reproducing the geometry of a simple protein. In such molecules, folding interactions can result in ψ and ϕ angles for the peptide backbone which may be different to those found in simple peptides. In order to test the AMBER' field, we chose minimization of the small protein crambin which has a well resolved X-ray crystal structure. The X-ray coordinates for a single molecule of crambin were taken from the Brookhaven Protein Databank entry 1CRN and were energy minimized with the original AMBER and

AMBER' force fields to a gradient <0.001kcal/mol A. The calculations were performed with a constant dielectric ($\varepsilon = 1$), a distance-dependent dielectric ($\varepsilon = 4R$), and with the GB/SA continuum solvent water models using a constant dielectric ($\varepsilon = 1$). Residue-based nonbonded cutoff distances of 7Å (van der Waals) and 12Å (electrostatics) were used. A measure of the effectiveness of the force fields in reproducing the experimental structure was obtained by least squares superimposition of the final minimized structures with the original X-ray coordinates. The results are summarized in Table 3. While neither force field reproduces the X-ray crystal conformation well in *vacuo* ($\varepsilon = 1$), both AMBER and AMBER' perform comparably with the previously suggested[10] $\varepsilon = 4R$ distance-dependent electrostatics. Though such a dielectric treatment gives reasonable structures, it is difficult to justify - especially for parts of the solute remote from solvent. We found best reproduction of the crambin crystal conformation by energy minimization using AMBER' with GB/SA water and the physically reasonable $\varepsilon = 1$ electrostatics.

Table 3. RMS Comparison of Crambin X-ray Structure with Energy Minimization Structures using the AMBER and AMBER' Force Fields.

	Original AMBER		AMBER*	
	All Atoms (Å)	Backbone Atoms (Å)	All Atoms (Å)	Backbone Atoms (Å)
Constant dielectric, $\varepsilon = 1.0$	1.54	1.40	1.73	1.68
Distance-dependent dielectric, $\varepsilon = 4.0R$	0.95	0.79	0.96	0.76
GB/SA water, constant dielectric, $\varepsilon = 1.0$	1.47	1.32	0.81	0.70

The AMBER' force field reported here is able to reproduce the results of HF/6-31+G* calculations for the simple peptides while maintaining the usefulness of the force field for larger molecules such as proteins. While even better peptide force fields can be created when higher level *ab initio* calculations on simple peptides become available, the force field described here provides a significant improvement over previous implementations.[11]

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11. We acknowledge the support of NIH Grant GM44525. All calculations reported here were carried out with BatchMin V3.5. The original AMBER energies were validated by authentic AMBER 3.0 calculations on peptides la and 2a.