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The Effect of Side-Chain Branching on the Theoretically Predicted Conformational Space Available to Amino Acid Residues

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ABSTRACT: This study uses the most recently refined conformational energy parameters to define the conformational space available to both the backbone and side chains of the *N*-acetyl-*N'*-methylamide derivatives of alanine, β-methylalanine, valine, and β-methylvaline. The conformational space is described in terms of both conformational energy values and statistical percentage probabilities for 10° increments of ϕ and ψ and for different rotameric states of the side-chain torsion angles. Of the various regions of the (ϕ,ψ) map, the area surrounding the C_7^{eq} conformation is the most favored overall for alanine, β-methylalanine, and valine, whereas that containing the extended conformational energy minimum is the most probable for β-methylvaline. For both the valine and β-methylalanine residues the trans rotameric state for the C^α–C^β bond strongly predominates (88 and 62% probabilities, respectively).

A central tenet in the prediction of the conformations of larger peptides is the belief that the conformational space available to the peptide backbone immediately adjacent to any residue is largely governed by the nature and conformations of the latter's side chain. Thus, there has been increasing interest in the experimental conformational analysis of the side chains of biologically active peptides which has been facilitated by the application of high-resolution techniques such as NMR.[1,2] Conformational energy calculations have been used to predict the most probable conformation of a peptide where there is more than one conformation consistent with the experimental data.[3,4]

The number of possible side-chain conformations for most naturally occurring amino acids is sufficiently large to preclude a total search of the conformational space available to the backbone, exceptions being glycine, alanine, and proline. Attention has therefore been focused on establishing their minimum energy conformations.[5,6] Amino acids with short side chains, branched at the C^β atom, are small enough to allow an exhaustive description of the total space available to such amino-acid residues.

The side-chain conformations of these amino acids are uniquely defined by the value of the variable side-chain torsion angle χ^1. Only the three values which "stagger" the positions of the substituents at either end of the C^α–C^β bond need be considered. These values are $180 \pm 30°$, $60 \pm 30°$, and $-60 \pm 30°$, and the positions they define are designated trans, gauche$^+$, and gauche$^-$, respectively. Valine and threonine are the only amino acids that occur in proteins for which the above simplifications apply. However, β-methylalanine, an amino acid that is frequently used in synthetic analogues of biologically active peptides,[7,8] can also be treated in this way.

Substituting a third methyl group onto the C^β carbon atom of valine, to give β-methylvaline, gives a symmetric side chain for which only one value of χ^1 need be considered, namely that in which the three C^γ methyl groups are staggered with respect to the backbone bonds. β-Methylvaline is thus an interesting model for steric restrictions imposed by bulky side chains on the backbone conformations of the polypeptide chain.

The purpose of the present study is to explore the total conformational space available to β-methylalanine, valine, and β-methylvaline using a recently refined set of empirical potentials and parameters.[9]

Methods

Nomenclature and Abbreviations. The amino acid L-2-aminobutanoic acid has been given the trivial name β-methylalanine in this paper. It is also known as α-amino-*n*-butyric acid.[17] L-2-amino-(3,3)-dimethylbutanoic acid, which has been referred to as *tert*-leucine by other workers,[22] is called β-methylvaline in this study. Unless explicitly stated, all amino acid residues are considered in their L configuration. All other nomenclature and conventions used are those recommended by the IUPAC-IUB Commission.[10]

Energy Calculations and Minimizations. Conformational energies were calculated using ECEPP[11] (Empirical Conformational Energy Program for Peptides) with a Cyber 73 computer. This program was developed in the Department of Chemistry at Cornell University and uses the empirical potential energy functions and energy parameters described by Momany et al.[9] It was modified to be compatible with the Cyber system. The total conformational energy of the molecules is calculated as the sum of the electrostatic, nonbonded, and torsional energy components from interatomic interactions. Hydrogen atoms are considered explicitly and the hydrogen-bond energy is included in the nonbonded energy component.[9]

Energy minimizations were performed using ECEPP in conjunction with a function-minimizing subroutine.[12] Minimization was terminated when the conformational energy changed by less than 0.01 kcal/mol between successive calculations.

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Only side-chain torsion angles were minimized in this study and the values chosen as starting points were those at which the intrinsic torsional potential has a minimum. Thus, for side-chain rotations involving carbon atoms with nonsymmetric substitutions, starting values for χ were 180 and ±60° and for those involving symmetric substitutions, e.g., terminal methyl groups, only one starting value for χ was necessary and this was chosen to be +60°.

Geometric Parameters. The standard residue and end-group data supplied with ECEPP were used for alanine and valine and for the *N'*-methyl and *N*-acetyl end groups. These data are based on the work of Momany et al.[9] The peptide group was held in the planar trans position with ω = 180° and the variable dihedral angles of the end groups were also kept at 180° in the fully extended conformation. In the absence of direct crystallographic data on the structure of β-methylalanine and β-methylvaline the bond angles and bond lengths for these molecules were adapted from the standard residue data supplied with ECEPP.[9]

The geometry for the *tert*-butyl group of β-methylvaline was adapted from that of neopentane,[13] which is a regular tetrahedron of side 1.54 ± 0.1 Å and angle 109.5 ± 1°. This geometry was modified slightly, the C–C bonds being taken as 1.53 Å to conform with the value for other C–C bonds in amino acids.[9]

Appropriate partial electronic charges for β-methylalanine were selected from those for straight aliphatic side-chain amino acid residues published by Momany et al.[9] For β-methylvaline the partial electronic charges used were those of valine[9] except that the charges on the C^{γ} atoms were reduced from −0.072 esu in valine to −0.067 esu so that the molecule had a net charge of zero.

Energy Contour Diagrams. The energy distribution over the total conformational space available to the backbone of each residue was calculated in 10° increments of ϕ and ψ, i.e., at 1296 points in the (ϕ,ψ) plane. The side-chain torsion angles were first held fixed in the "staggered" positions of their torsional minima. From the energy map thus produced, conformations in regions of the surface with energies within 6 kcal/mole of the global minimum were recalculated allowing the side-chain angles torsional freedom around these positions to minimize the overall conformational energy. For β-methylvaline only a small region of the (ϕ,ψ) plane had energies which were even within 10 kcal/mol of the global energy minimum; in this case, therefore, all of this region was recalculated.

For the molecules with nonsymmetric β-carbon atoms, namely, valine and β-methylalanine, three energy contour diagrams were produced with χ^1 either in the trans, gauche$^+$, or gauche$^-$ positions. The most probable side-chain conformation for the residue at each point on the (ϕ,ψ) plane is that which gives the molecule the lowest total conformational energy. Thus, the total conformational space available to the backbone can be represented by the lowest conformational energy value of the three rotamers at each point on the (ϕ,ψ) plane. Composite maps for valine and β-methylalanine were constructed in this way.

Analysis of Rotamer Populations. The partition function for the entire conformational space available to the molecule is given by[14]

$$Z = \sum_{\phi}\sum_{\psi}\sum_{\chi^1}\cdots\sum_{\chi^n}\exp[-E(\phi,\psi,\chi^1,\ldots\chi^n)RT]$$

For those molecules with three side-chain conformations about χ^1, the first side-chain torsion angle, the statistical weight

$$w(\chi_k{}^1) = \sum_{\phi}\sum_{\psi}\exp[-E(\phi,\psi)/RT]$$

represents the distribution of backbone conformations for each rotamer k and the ratio $P(\chi_k{}^1) = w(\chi_k{}^1)/Z$ is the probability [14] for the occurrence of each rotamer over the entire range of backbone conformations available to the molecule. These probabilities were calculated for β-methylalanine and for valine, using a value of 293 K for T, the temperature.

Conformational Probability Diagrams. The statistical probability of the occurrence of each (ϕ,ψ) pair of values for an amino acid residue over the range 0–360° is given by

$$P(\phi,\psi) = w(\phi,\psi)/Z$$

In the case of multiple possibilities of side-chain conformations, the statistical weight for each point on the (ϕ,ψ) surface, $w(\phi,\psi)$, is given by[14]

$$w(\phi,\psi) = \sum_{i}\sum_{k}\exp[-E(\chi_k{}^i)/RT]$$

where $\chi_k{}^i$ refers to rotamer k of the ith side-chain torsion angle.

To represent the relative probabilities of various backbone conformations for the amino acid residue, the values of $P(\phi,\psi)$ may be expressed as percentages and plotted on a (ϕ,ψ) diagram.[14] This type of diagram should be particularly useful for residues with side-chain rotamer populations and should be a more accurate expression of the contribution of each type of side-chain population to the total probability of occurrence of a (ϕ,ψ) pair than the composite energy maps described in the section entitled "Energy Contour Diagrams".

Results

Minimum Energy Conformations for the *N*-Acetyl-*N'*-methylamides of β-Methylalanine and β-Methylvaline. The conformational energy minima of the β-methylalanine and β-methylvaline residues are listed in Tables I and II, respectively. Since the object of this study was to explore the effect on the flexibility of the backbone of increasingly "bulky" side chains, the entire (ϕ,ψ) conformational space was searched at discrete 10° increments with variations only in side-chain torsion angles χ^1 and χ^2 to achieve energy minimization. Therefore, these "minima" are minima only to the nearest 10° in ϕ and ψ. Conformational energy minima for alanine and valine have already been reported elsewhere by other workers[5,6,15] but the values obtained in the present study are given for comparative purposes.

In addition to the energy and dihedral angle values, the tables show the position of each minimum in the (ϕ,ψ) plane according to the conformational letter code devised by Zimmerman et al.[6]

Conformational Energy Contour Diagrams. The energy contour maps of the alanine and β-methylalanine residues are shown in Figure 1 and of valine and β-methylvaline in Figure 2. The maps for β-methylalanine and valine are composite maps. The maps for the individual trans, gauche$^+$, and gauche$^-$ rotamers for these two residues are given in the supplementary material.[16] The positions of the minimum energy conformations and the values of the energy contours (in kcal/mol), with respect to a global minimum normalized to zero, are marked.

Rotamer Populations for the *N*-Acetyl-*N'*-methylamides of β-Methylalanine and Valine. The probability of the occurrence of each side-chain rotamer over the entire conformational space available to the backbone of the two types of residues is shown in Table III, together with their statistical weights. The probabilities are expressed as percentages.

Conformational Probability Diagrams. The conformational probability diagrams for the alanine, β-methylalanine, valine, and β-methylvaline residues are shown in Figure 3.

Table I
Conformational Energy Minima for the *N*-Acetyl-*N'*-methylamide Derivatives of Alanine and β-Methylalanine

Confl. letter code	β-Methylalanine Torsion angles, deg ϕ^a	ψ^a	χ^1	χ^2	ΔE, kcal/mol ($E_0 = -2.476$)	Alanine Torsion angles, deg ϕ^a	ψ^a	χ^1	ΔE, kcal/mol ($E_0 = -3.116$)
C	−80	90	−173	57	0	−80	80	61	0
C	−90	80	−66	63	0.302				
E	−150	130	−175	55	0.503	−150	150	60	0.351
A	−70	−50	−175	56	0.594	−70	−50	60	1.143
E	−150	160	63	59	0.750				
G	−150	−60	−178	55	1.052	−160	−60	52	1.544
A	−80	−50	−67	62	1.118				
E	−130	150	−67	65	1.295				
D	−150	40	55	53	2.305	−150	70	59	0.648
A*	60	70	−165	60	2.454	50	60	63	2.442
A*	60	60	−61	65	2.582				
F	−60	150	73	67	2.604				
F*	60	−180	−60[a]	60[a]	5.724				
A	−70	−30	60[a]	60[a]	7.834				
C*	70	−80	−60[a]	60[a]	8.031	70	−70	60[a]	12.315
C*	70	−80	180[a]	60[a]	8.055				
A*	40	50	60[a]	60[a]	12.423				

[a] The values for ϕ and ψ and for the χ values marked have not been minimized in this study.

Table II
Conformational Energy Minima for the *N*-Acetyl-*N'*-methylamide Derivatives of Valine and β-Methylvaline

Confl. letter code	Valine Torsion angles, deg ϕ	ψ	χ^1	$\chi^{2,1}$	$\chi^{2,2}$	ΔE, kcal/mol ($E_0 = -1.790$)	β-Methylvaline Torsion angles, deg ϕ^a	ψ^a	χ^1	$\chi^{2,1}$	$\chi^{2,2}$	$\chi^{2,3}$	ΔE, kcal/mol ($E_0 = 3.364$)
C	−90	100	178	55	65	0	−130	140	61.8	58.9	67.1	53.5	0
E	−150	140	66	66	55	0.544							
A	−80	−50	174	54	63	0.716	−90	−20	74.7	65.9	74.5	58.2	3.125
E	−140	160	−67	68	54	1.441							
F	−60	140	73	70	57	1.954							
D	−130	30	−67	65	51	2.429							
A*	60	80	−175	59	69	3.058							
A	−60	−30	60[a]	60[a]	60[a]	6.775							
A	−70	−30	−60[a]	60[a]	60[a]	6.481							
C*	70	−70	180[a]	180[a]	180[a]	9.566							

[a] The values for ϕ and ψ and for the χ values marked have not been minimized in this study.

Since there are no conformations for any of those residues with a probability of occurrence greater than 0.1% for ϕ greater than 0°, only the areas of the diagrams for ϕ in the range −180 to 0° are shown. The total probability (%) for each conformational region (using the designations of Zimmerman et al.)[6] is shown to the nearest whole number in Table IV for those regions with probabilities of at least 1%.

The maxima on the probability diagram are marked there and are compared, in Table V, with the energy values of the conformational minima at those points. (The maxima represent the most probable backbone conformations in these regions, taking into account all side-chain conformations, whereas the energy minima are for specific χ values.)

Discussion

Comparison of Conformational Energy Minima and the Total Conformational Space. Since the side chain of the β-methylalanine residue is larger than that of alanine its conformational space should lie within the low-energy regions of alanine. The conformational energy contour diagrams for the individual rotamers of β-methylalanine[16] each cover a part of the conformational space available to alanine. Together, as represented by the composite map (Figure 1), the conformational space available to β-methylalanine is only slightly more restricted than that for alanine. The minimum energy conformations for alanine do not have counterparts for all three rotamers of β-methylalanine. Only those minima appearing in regions A, A*, and E (see Table I) are also minima for each rotamer, but each backbone conformation which is a minimum for the alanyl residue has at least one side-chain conformation available to it for β-methylalanine. The overall minimum for each rotamer does not fall into the same area of the (ϕ,ψ) map. Thus, for the trans and gauche$^-$ rotamers the minima fall into region C, the region that is most favored for the alanine residue. For the gauche$^+$ rotamer, the most preferred conformation is in region E. This is in contrast with a previous study[17] on β-methylalanine where the molecule was not blocked by end groups as in this study but was considered as both a dipolar ion and a cation; the minimum for each rotamer of each ionic form occurred in slightly different positions in region A.

It is obvious from the relative contributions of the three-side-chain populations for β-methylalanine and valine (see Table III) that the substitution of another methyl group onto the β-carbon atom considerably restricts the rotation about the C^α–C^β bond. While the trans rotamer predominates for both types of residue, for valine it accounts for nearly 90% of the total side-chain rotamer population but only about 60% for the β-methylalanine residue in which there is a significant contribution (33%) from the gauche$^-$ rotamer.

The addition of a second β-methyl group to β-methylalanine to give valine reduces the total conformational space available to the molecule (see Figure 3) and also reduces the total number of minima from 17 for β-methylalanine to 9 for valine.

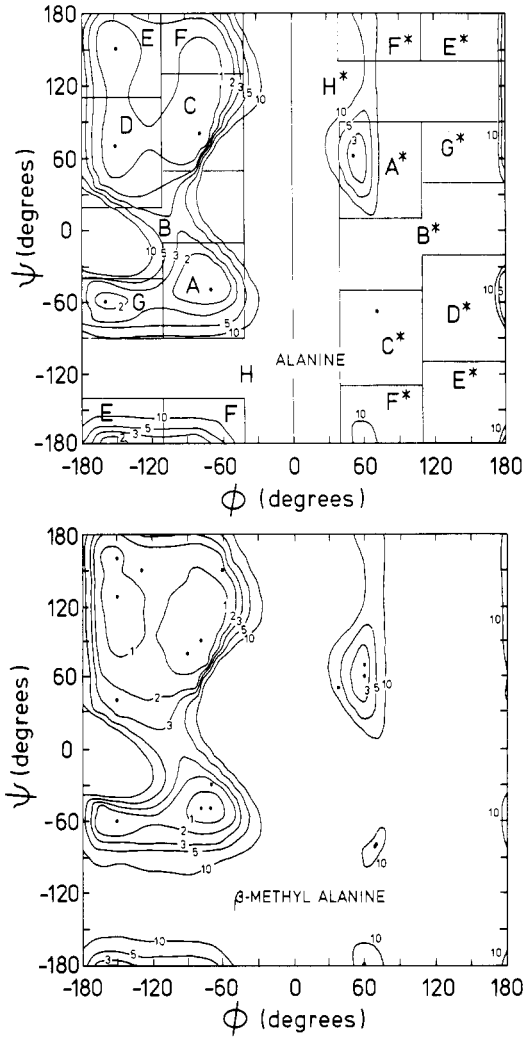

Figure 1. Conformational energy contour maps of the *N*-acetyl-*N'*-methylamide derivatives of alanine and β-methylalanine. The contour lines are labeled with energies in kcal/mol above the minimum energy points of $(\phi,\psi) = (-80,80)$ for alanine and $(\phi,\psi) = (-80,90)$ for β-methylalanine. The map for β-methylalanine is constructed from the lowest of the energy values of its three rotamers at each (ϕ,ψ) point. Locations of the minima listed in Table I are indicated by the filled circles. The regions defining the conformation letter code[6] are marked on the alanine map.

Table III
Side-Chain Rotamer Populations for the *N*-Acetyl-*N'*-methylamide Derivatives of β-Methylalanine and Valine

Rotamer	Statistical weight $(w) \times 10^{-3}$		Probability (P), %	
	β-Methylalanine	Valine	β-Methylalanine	Valine
t	2.102	0.496	62	88
g^+	0.179	0.049	5	9
g^-	1.123	0.015	33	3

Only one region A, that which contains the right-handed α helix, contains a minimum for all three staggered rotamers, and the extended region E contains minima for the two gauche rotamers. The regions C, F, D, and A* contain one minimum each and, unlike β-methylalanine, there are no low-energy minima in the regions F*, C*, and G.

The effect on the conformational space available to the backbone when a third β-methyl group is added to valine, giving β-methylvaline, is profound. The energy contour diagram for this molecule (see Figure 2) shows that the only backbone conformations allowed for this molecule are, not surprisingly, those that are allowed for every one of the three valine rotamers. The global minimum for β-methylvaline (see Table II) occurs in the region E where the backbone is very extended and the only other minimum (in region A) has an energy 3 kcal/mol greater than this. Thus, although there are fewer backbone conformations available to valine than to β-methylalanine or alanine, the presence of a *tert*-butyl group on the C^α atom severly restricts the number of backbone conformations available to β-methylvaline so that it can adopt only a very extended conformation. This property of the residue would make it a particularly useful tool in synthetic analogues of biologically active peptides, especially in combination with the α-aminoiisobutyric acid residue which can only adopt the (ϕ,ψ) values close to those of the left-hand or right-hand α helix.[18] Either of these two residues could be substituted for other hydrophobic residues such as valine, leucine, and isoleucine to manipulate the conformation of a molecule and restrict local (ϕ,ψ) values to extended or α-helical ones. The biological activity of such analogues could provide evidence about the conformation of the native molecule. β-Methylvaline has been synthesized and incorporated into several dipeptides.[22] The steric effect of the *tert*-butyl group

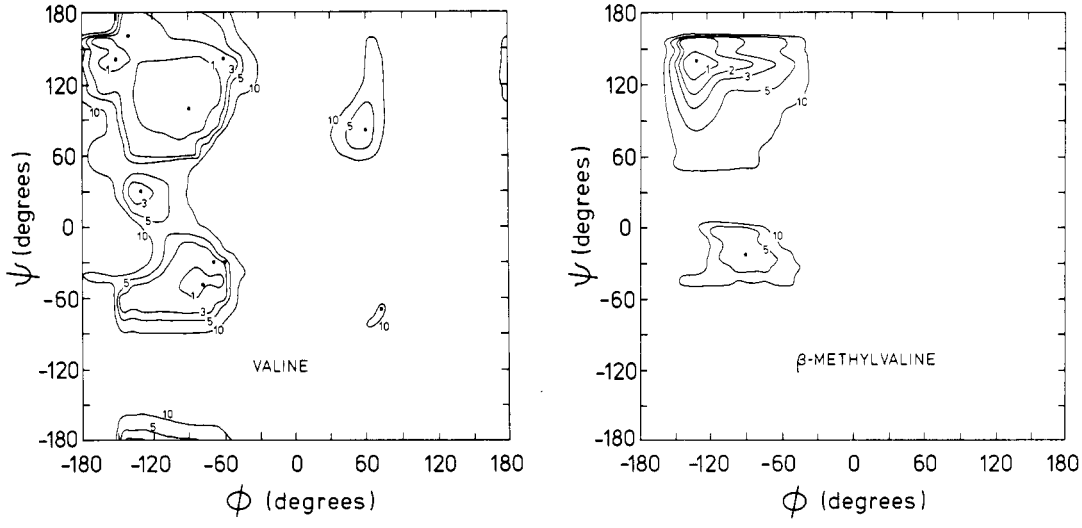

Figure 2. Conformational energy contour maps of the *N*-acetyl-*N'*-methylamide derivatives of valine and β-methylvaline. The contour lines are labeled with energies in kcal/mol above the minimum energy points of $(\phi,\psi) = (-90,100)$ for valine and $(\phi,\psi) = (-130,140)$ for β-methylvaline. The map for valine is constructed from the lowest of the energy values of its three rotamers at each (ϕ,ψ) point. Locations of the minima listed in Table II are indicated by the filled circles.

Table IV
Probabilities[a] for Each (ϕ,ψ) Conformational Region for the *N*-Acetyl-*N'*-methylamide Derivatives of Alanine, β-Methylalanine, Valine, and β-Methylvaline

Conformation letter codes	$> \phi \geq$		$> \psi \geq$		Ala[b]	β-Methyl-alanine	Val	β-Methyl-valine
A	−40	−110	−10	−90	4 (6)	10	10	0
C	−40	−110	130	50	37 (30)	45	52	0
D	−110	−180	110	20	20 (34)	10	7	0
E	−110	−180	180	110	24 (28)	19	21	91
			and					
			−140	−180				
F	−40	−110	180	130	13 (0)	13	10	9
			and					
			−140	−180				
G	−110	−180	−40	−90	1 (1)	2	1	0

[a] All values are given in percent to the nearest whole figure, for those regions on the (ϕ,ψ) surface that contain conformations that contribute to at least 1% of the total. [b] The values in parentheses are those calculated from Zimmerman et al.[6] using only the minima on the energy surface but taking into account the librational entropy. No minimum occurs in region F.

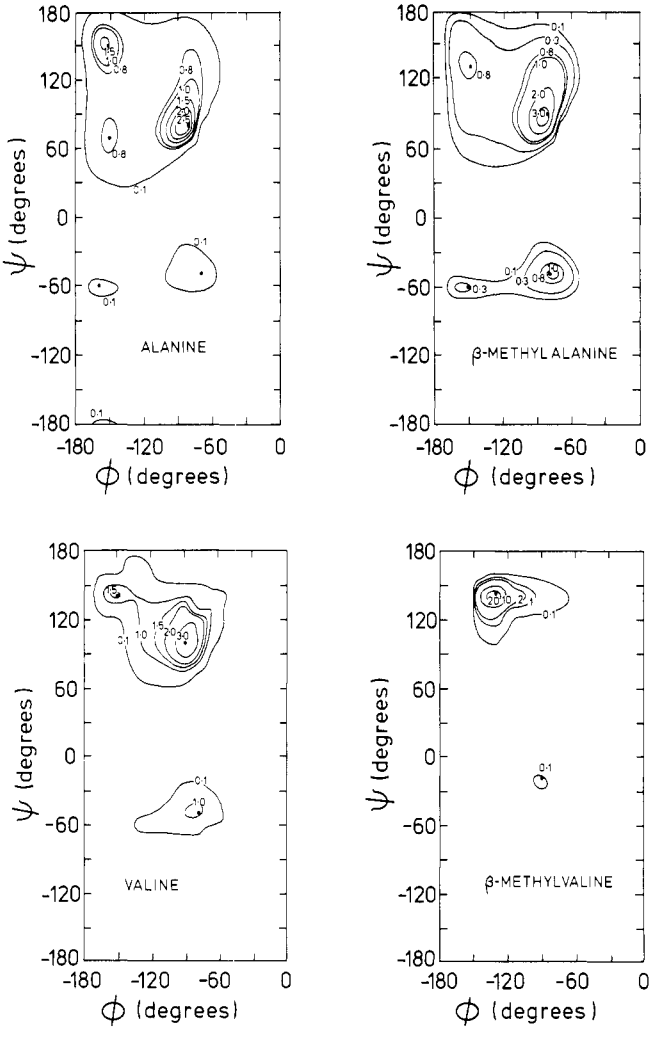

Figure 3. Conformational probability diagrams for the *N*-acetyl-*N'*-methylamide derivatives of alanine, β-methylalanine, valine, and β-methylvaline. The contour lines are labeled in percentages and the filled circles represent the backbone conformations with the maximum probability of occurrence for each residue (also see Table V).

of this amino acid rendered some aspects of the peptide synthesis difficult but analogues of TRF [L-Glu-L-His-L-β-methylvaline-NH_2] and of oxytocin (7–9) [L-Pro-L-β-methylvaline-Gly-NH_2] were successfully prepared.[22] The biological activities and conformations of these peptides have not yet been reported.

Table V
Backbone Conformations of the Valine and β-Methylalanine Residues. Statistical Probabilities and Minimum Conformational Energy Values

Valine				β-Methylalanine			
ϕ	ψ	ΔE	P, %	ϕ	ψ	ΔE	P, %
−90	100	0	3.9	−80	90	0	3.1
−150	140	0.54	1.5	−150	130	0.50	0.9
−80	−50	0.72	1.1	−70	−50	0.59	1.0
−130	30	2.43	0.1	−150	−60	1.05	0.3

The most favored (ϕ,ψ) region calculated for the residues studied, except for β-methylvaline, is region C (so designated because it contains those conformations that have an equatorial seven-membered ring with a hydrogen bond between the C=O and N–H groups adjacent to the central residues). This region accounts for 37 to 52% of the total conformational space of each type of residue (see Table IV). The next most favored region is region E which contains extended backbone conformations. The distributions among the regions for the residues alanine, β-methylalanine, and valine are somewhat similar but that of β-methylvaline is heavily biased toward the extended region of the map which accounts for 90% of the total conformational space. The normalized statistical weights of the alanine residue can be calculated from the data of Zimmerman et al.[6] using only the minima on the energy surface but taking into account the librational entropy at each minimum. These figures are given in parenthesis in Table IV for the regions in which each minimum falls. A study by Zimmerman et al.[19] on the statistical weights of dipeptides composed of glycine, alanine, or proline suggested that the results obtained by a total search of the conformational space (the method used in our study) gives similar results to those derived by using only the local minima and the librational entropy. Although the figures obtained for alanine are indeed in general agreement there is still some discrepancy. Using the approach of Zimmerman et al.,[19] the most favored regions are D > C > E whereas our figures show C > E > D. Also regions in which no minima occur such as region F are completely ignored when assessments are made solely on the basis of local minima and librational entropy. This region, by our calculations, makes a significant contribution (13%) to the total.

The conformational probability diagrams for the residues with side-chain populations, namely valine and β-methylalanine, give a much clearer representation of the conformational space available to the residue than do the composite energy diagrams. The statistically most favored backbone conformations are marked on the diagrams and these positions conform with the high contributions expected from the con-

formational energy minima. Nevertheless, the most probable backbone conformations do not rank in the same order as the values of the conformational energy minima (see Table V). This is because each minimum is for discrete values of ϕ, ψ, and χ^1 but the probabilities are calculated from the contributions of all three side-chain rotamers. Hence, although there is a value of χ^1 for which the conformational energy at (−150,130) is lower than that at (−70,−50) the probability of the other two rotamers occurring at (−150,130) is lower than at (−70,−50). This is reflected in a higher overall probability for the latter conformation. The values of the probabilities of the minimum energy conformations may seem low, the highest value for any one (ϕ,ψ) pair being only 4% of the total. These values, however, refer to the minima of regions which may contain many other conformations which are nearly as probable. If the total (ϕ,ψ) space is partitioned into regions containing similar conformations, by summing the contributions of each (ϕ,ψ) pair at each point in that region we obtain a clearer idea of the relative contributions of each *type* of conformation rather than conformations narrowly defined by specific (ϕ,ψ) values. This has been done in Table IV.

Comparison of the Theoretical Predictions with Published Experimental Data. The theoretically predicted minimum energy backbone conformations for β-methylalanine are consistent with the minima for the 20 naturally occurring amino acids predicted by Zimmerman et al.[6] These authors have already compared these conformations, that is, the C_7^{eq} and C_5 rings occurring in regions C and E, respectively, with experimental observations and our discussion will be restricted to side-chain conformations.

It is apparent from Tables I and II that backbone conformations exist for both valine and β-methylalanine for which each of the side-chain rotamers is permitted. There have been many published observations on the side-chain conformation of the valine residue in small molecules in which valine is the sole amino acid residue, as well as in other peptides and proteins, and each of the side-chain conformers of valine do occur to a significant degree both in the crystalline[14,20] and the solution state.[2,21] From our calculations on rotamer populations (see Table III) the trans rotamer should be strongly preferred for most regions of the (ϕ,ψ) map. However, under experimental conditions the conformations adopted by the backbone of valine or β-methylalanine will be influenced by factors that are not incorporated in our calculations, such as interactions with solvent and neighboring amino acid residues. Preferred populations of backbone (ϕ,ψ) values will require compatible side-chain rotameric states. Our calculations would indicate that for valine, extended backbone conformations would favor the gauche rotamers whereas α-helical or C_7^{eq} ring structures would demand the trans rotameric state for the side chain. However, where valine or β-methylalanine exist in a "structureless" situation where near-neighbor interactions are reduced, e.g., in proteins or peptides in a randomizing solvent, the trans rotamer should predominate.

Conclusions

The *N*-acetyl-*N'*-methylamides of a series of amino acids with increasingly bulky side chains, namely alanine, β-methylalanine, valine, and β-methylvaline, have been subjected to an exhaustive theoretical conformational analysis. The conformational energy values for 10° increments in ϕ and ψ values over the entire range available to these angles have been calculated. For those of the series with rotameric states the side-chain torsion angle χ^1 value was allowed to vary about the "staggered" values of ±60 and ±180° until a minimum energy for that conformation was obtained. It was found that the conformational space available to the β-methylalanine residue is only slightly less than that for alanine but that a second C^γ methyl group to give valine does, as is well known, restrict the conformational space of the residue. The addition of a third C^γ methyl group to valine, to give β-methylvaline, dramatically confines the (ϕ,ψ) values available to the backbone so that only very extended conformations in the region (−130,140) are allowed. The conformational energy minima of the alanine residue have counterparts for at least one of the rotameric states of β-methylalanine (which has a total of 17 minimal energy conformations), but the alanine minimum in the position (−160,−60) does not have a counterpart for the valine residue.

The total conformational space of these molecules is visualized both by energy contour diagrams and conformational probability diagrams. The statistical probabilities of various side-chain and backbone conformations are calculated. The region on the (ϕ,ψ) map that incorporates the C_7^{eq} ring is the statistically most probable set of conformations for the alanine, β-methylalanine, and valine residues (40, 45, and 52%, respectively), whereas the most probable conformational states of the β-methylvaline residue are extended conformations (91%). β-Methylalanine and valine show favored backbone conformations for each rotameric state. The C_7^{eq} ring is most likely for the trans rotamer for both residues and for the gauche$^-$ rotamer of β-methylvaline while the gauche$^-$ rotamer of valine and gauche$^+$ rotamers for both residues prefer extended conformations. Where (ϕ,ψ) values are randomly assigned and the backbone of each residue can be considered to be structureless the trans rotamer is strongly preferred with a probability of 62% for β-methylalanine and 88% for valine.

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Supplementary Material Available: Conformational energy contour diagrams for the *N*-acetyl-*N'*-methylamide derivatives of β-methylalanine and valine in the trans rotameric, gauche$^+$ rotameric, and gauche$^-$ rotameric states (Figures 4–6) (3 pages). Ordering information can be found on any current masthead page.

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The Effect of Refinements in Energetic Statistical Weighting on the Computed Chain Dimensions of Homopolypeptides

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ABSTRACT: In order to establish more rigorously the effect of side-chain length and branching on the theoretically derived unperturbed dimensions of homopolypeptides, the most recently revised covalent geometry has been used to calculate the characteristic ratios of polymers composed solely of glycine, alanine, β-methylalanine, valine, or β-methylvaline. All atoms in the side chains were included in the calculations and different methods of weighting for multiple side-chain conformations and of incorporating side-chain flexibility were compared. The effects of weighting with the conformational space of the backbone at 30 and 10° intervals of ϕ and ψ were compared. The characteristic ratios of alanine, β-methylalanine, and valine using the most rigorous of the methods explored were found to be very similar (8.06, 7.02, and 9.21). The side chain of β-methylvaline imposed profound steric restrictions on the backbone which resulted in a slowly converging characteristic ratio (94.8 at a chain length of 500 residues). Other than those on residues with sterically restricting side chains, these results are consistent with Flory's hypothesis that the side-chain atoms further removed from the backbone than the C^β atom need not be considered when calculating the unperturbed dimensions of most homopolypeptides.

Since Flory established the theoretical methods for calculating the dimensions of statistically coiling noncooperative polypeptide chains[1] most workers have accepted the approximations inherent in the methods. This study is intended to establish the conditions under which these approximations are valid and when more refined techniques are justified. To this end we have used the recently revised peptide geometry of Momany et al.[2] to compare the effect of side-chain length and branching on the unperturbed dimensions of homopolypeptides using the residues of glycine, alanine, β-methylalanine,[3] valine, and β-methylvaline[3] as examples. Side-chain atoms including hydrogen atoms were individually considered when constructing the backbone conformational energy surfaces used to weight the unperturbed dimensions (the conformational space available to the residues is reported elsewhere[4]). The effect of weighting with these surfaces at 10 and 30° intervals in ϕ and ψ was also compared. Methods of weighting with multiple side-chain conformations were explored and the effect of improving the treatment of flexibility in side-chain torsion angles by including minimized energy values in the weighting procedure is examined. The results obtained in this study are compared with those obtained in earlier work using less refined techniques.

Theoretical Methods

Generation of Random Polypeptide Chains. The unperturbed dimensions of homopolypeptides were each calculated as the mean-square end-to-end distance $\langle r^2\rangle_0$ and expressed as the characteristic ratio (C_∞) as the chain length (n) tends to infinity, by the method of Brant and Flory:[5]

$$C_\infty = (\langle r^2\rangle_0/nl^2)_\infty = [(\mathbf{E} + \langle\mathbf{T}\rangle)(\mathbf{E} - \langle\mathbf{T}\rangle)^{-1} - (2/n)(\langle\mathbf{T}\rangle)(\mathbf{E} - \langle\mathbf{T}\rangle^n)(\mathbf{E} - \langle\mathbf{T}\rangle)^{-2}]_{1,1} \quad (1)$$

where $\mathbf{E}$ is the identity matrix of order 3 and $\langle\mathbf{T}\rangle$ is given by:

$$\langle\mathbf{T}\rangle = Z^{-1}\sum_{\phi}\sum_{\psi}\mathbf{T}(\phi,\psi)\exp(-E(\phi,\psi)/RT) \quad (2)$$

where Z is the partition function for the conformational space available to the peptide residue:

$$Z = \sum_{\phi}\sum_{\psi}\exp(-E(\phi,\psi)/RT) \quad (3)$$

This expression for Z does not take into account side-chain rotational isomeric states.

$\mathbf{T}(\phi,\psi)$ is the matrix that transforms the coordinate system of the $(i + 1)$th residue to that of the ith residue.

The angles that define the peptide geometry of these transformations[5] were calculated from the geometric parameters of Momany et al.[2] and have the values $\xi = 14.9°$, $\theta = 70.7°$, and $\eta = 20.9°$. $E(\phi,\psi)$ is the conformational energy of the residue for each backbone conformation defined by the (ϕ,ψ) pair, and R is the gas constant. T, the temperature, was taken to be 293 K in this study.

Conformational Energy Calculations. The conformational energy space was calculated for the *N*-acetyl-*N'*-methylamides for each residue in this study using ECEPP[6] (Empirical Conformational Energy Program for Peptides) with a Cyber 73 computer. This program uses the empirical potential energy functions and energy parameters of Momany et al.[2] and calculates the electrostatic, nonbonded, and torsional energy components of the total conformational energy from interatomic interactions. Interactions involving side-chain atoms, including hydrogen atoms, are calculated explicitly.

The intraresidue hydrogen-bonded energy is included in these calculations (as part of the nonbonded energy compo-

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