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The importance of electrostatic charge and dielectric constant in conformational analysis of biologically active dipeptides

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Abstract Our aim was to use molecular modeling to determine the solution conformations of small peptides (2–6 residues), which are substrates for both peptide transporters and peptidases, and can be clinically important as the basis of various therapeutic agents including β-lactams and ACE inhibitors. We evaluated two conformational search strategies and, in particular, the influence of electrostatic charge and dielectric constant on the results. AlaAla (uncharged), AlaAla (charged), *N*-acetyl-Ala-Ala-*O*-methyl and *N*-acetyl-Ala-Ala-methylamide were modeled using grid search and random search as implemented within SYBYL 6.4 using distance-dependent dielectric constants of between 1 (in vacuo) and 80 (water). The two search procedures located similar energy minima for both forms of AlaAla at any given dielectric constant, indicating that random searches sample conformational space sufficiently well for dipeptides at least. Analyses of the minimum-energy conformers computed for each molecule showed that, whereas the dielectric constant had minimal effect for AlaAla (uncharged), *N*-acetyl-Ala-Ala-*O*-methyl and *N*-acetyl-Ala-Ala-methylamide, for AlaAla (charged), as the dielectric constant approached 1 (in vacuo), this conformer had a *cis* peptide bond and was the only conformer present. We conclude that it is essential to model peptides in their charged forms at dielectric constants of approximately 80 to be able to determine the solution conformations of peptides recognized by peptidases and peptide transporters.

Keywords Dielectric constant · Solution conformation · Dipeptide · Electrostatic charge · Random search

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Introduction

Peptides are an unrivalled source of nutrients in Nature and can be used as a sole source of carbon and nitrogen, and pre-formed amino acids [1, 2]. Consequently, transport systems for peptides are ubiquitous and found in all classes of organism ranging from bacteria and yeasts to higher plants and mammals. Furthermore, some peptides and peptide derivatives have potent biological activity as toxins, hormones, and neurotransmitters. Because of this, peptides are also of considerable importance in the pharmaceutical industry although, because of their inherent susceptibility to degradation by peptidase action, their effectiveness is frequently diminished by poor bioavailability. Much effort is, therefore, invested in designing peptidomimetics that retain biological activity with enhanced resistance to peptidase activity, thereby increasing their bioavailability.

Peptide transporters have the challenging role of transporting a highly varied set of substrates whilst retaining specificity, e.g. discriminating between peptides and free amino acids. Peptide transport has been most intensively studied in bacteria, which have three main peptide transporters with distinct, yet overlapping, specificities [2, 3, 4] and in the intestine and kidney [5, 6]. These systems transport all natural di- and tripeptides, indicating that they recognize features common to all peptides, i.e. the side chains are of lesser importance in determining specificity. Thus these transporters mainly recognize molecular features associated with the peptide backbone itself, e.g. amino and carboxyl termini, peptide bonds. The correct spatial orientation of these molecular features, or molecular recognition template (MRT) [7, 8], will be governed by the conformations of the peptides in solution. These conformations are, therefore, likely to be those commonly adopted by peptides within the di- and tripeptide pool, which comprise the natural substrates for these peptide transporters.

An approach to gain insight into the 3D conformations of peptides is to use molecular mechanics and conformational search strategies to simulate the behavior of

peptides in solution. Three conformational search strategies are in common use; systematic searches, grid searches, and random searches; these have been reviewed [9, 10]. The use of grid search and random search procedures specifically within the SYBYL package [11] have been evaluated for an extensive set of small (non-peptide) molecules [12, 13]. Systematic searches sample conformational space by altering user-defined torsion angles by fixed increments (e.g. 30°) to create sets of conformers representative of conformational space, although constraints are usually applied (e.g., distance ranges between hydrogen bond donor and acceptor groups) to reject unwanted conformations from consideration early on. Grid searches are a form of systematic search where every conformation generated is accepted (producing the theoretical maximum number) and can also be subjected to minimization procedures. Although grid searches can be set up to ensure that all available conformational space is rigorously sampled, this is computationally demanding, even for small peptides that have several torsion angles. Random searches might be less demanding in terms of computational time, although this might be at the expense of the proportion of conformational space actually searched, a quantity that may be difficult to assess. These separate limitations have profound implications when one is faced with the prospect of modeling many peptides to identify common conformations [7, 8].

Two further problems arise when evaluating procedures to model peptides relevant to the situation found in-vivo for biological systems. Firstly, decisions have to be made on an appropriate solvation method for the simulations and, secondly, on the precise chemical nature of the molecules. The different approaches for mimicking solvation in molecular modeling simulations have been reviewed [14, 15]. Representation of the solvent by dielectric continua is the simplest and least demanding computationally, whereas explicit solvation, which might be a more accurate representation, drastically increases computational demands so that similar modeling procedures become unfeasible. Ösapay et al. [16] compared the effects of using a dielectric continuum with that of explicit solvation in the CHARMM-19 force field and concluded that the results they obtained for peptide solutes were in qualitative agreement, although some quantitative differences were apparent. When using the dielectric continuum approximation, a variety of dielectric constants, ranging from 1 (in vacuo) to 80 (water), has been used for the energy calculations. Commonly, dielectric constants of either 1 or 4 (simulating the situation in crystal structures) have been used in the energy calculations [17, 18, 19, 20, 21], although the use of dielectric constants of approximately 80 have also been reported [22, 23]. Alternatively, the solute and solvent can be assigned different values for their dielectric constant, e.g. 1 for the solute and 80 for the solvent [24]. This principle can be extended to consider the effect of the solute upon the solvent and vice versa with respect to the dielectric "constant" at their interface [25]. In this study the effects of salt concentration and peptide (Tuftsin) conformation upon the dielectric constants of water were examined by use of molecular dynamics simulations; both influenced the calculated dielectric constant. With regard to the nature of the starting structure, terminally blocked peptides, e.g. the "alanyl dipeptide" [18, 19, 24] or uncharged peptide species [26], are frequently used to simulate residues within a polypeptide chain. However, as free peptides exist in water as zwitterions with a protonated amino terminus and a dissociated carboxylate terminus, it is necessary to treat them in this form when modeling them as substrates of transporters and peptidases [25].

In this study we have compared grid search and random search methods for modeling peptide conformations, and investigate the influence of charge and dielectric constant on the results. Our aim was to identify optimum parameters for modeling di- and tripeptides to produce biologically relevant conformers that could be related to the substrate specificities of peptide transporters and peptidases [7, 8, 27]. These principles can then be applied to the rational design of peptidomimetics which can be transported by clinically important transporters.

Methods

Starting structures of dipeptides for conformational analysis. Analyses were performed using SYBYL 6.4 (Tripos, St Louis, MO, USA) [11] running on a Silicon Graphics Octane workstation (R10 K platform, 175 MHz processor). AlaAla (uncharged), AlaAla (charged), *N*-acetyl-Ala-Ala-*O*-methyl and *N*-acetyl-Ala-Ala-methylamide were constructed within SYBYL 6.4 as described elsewhere [8]. Atom types were assigned automatically, ensuring that for AlaAla (charged) the N-terminal nitrogen was assigned a protonated, tetrahedral N4 atom type and the C-terminal carboxylate group was dissociated, assigning atom types of C2 to the carbon and O.co2 to the two carboxylate oxygens. The N–C distance for all molecules was defined as the distance between the N-terminal nitrogen and C-terminal carbon of the common, central dialanine unit. Similarly, psi, omega, and phi torsion angles were defined using their usual definitions and also relate to this dialanine unit.

Conformational analysis using grid searches. For each of the four molecules, AlaAla (uncharged), AlaAla (charged), *N*-acetyl-Ala-Ala-*O*-methyl and *N*-acetyl-Ala-Ala-methylamide, two grid searches were performed with the omega (ω) torsion set at either *trans* (±180°) or *cis* (0°). The backbone torsion angles, ψ and ϕ, were varied from ±180° to +165° in 15° increments to generate 576 unique conformers for each search, covering the entire conformational space about the peptide bond of the central dialanine unit. Energy minimization was not performed on the conformers generated; this kept computational time to a minimum and had no effect on the energies of the generated AlaAla (uncharged and charged) conformers. The sets of *cis* and *trans* conformers for each of the four molecules were combined to form a single set of 1152 conformers. The energy of each conformer was calculated using the Tripos force field [28] with Pullman charges being applied and distance-based dielectric constants (ε) between 80 and 1. For conformer i, its Boltzmann distribution (Bi) was determined by comparison of its energy (Ei) with the minimum energy conformer (Eo) by using the equation $Bi=e^{-((Ei-Eo)/RT)}$, and the percentage contribution of conformer i determined by using the equation $((Bi/\Sigma B)\times 100)$.

Conformational analysis using random searches. Random searches were performed allowing all torsion angles to vary, at dielectric

constants between 80 and 1, essentially as described by Treasurywala et al. [13] and Grail and Payne [8]. The Tripos force field was used for energy calculations with Pullman charges being applied, all other settings being left at default values. Each unique conformer (those with an RMS difference >0.2 Å) was minimized using 100 cycles of Powell conjugate-gradient minimization [29]. For AlaAla (uncharged and charged forms), 1000 search iterations were performed for each search; for the terminally-modified dipeptides, 2000 search iterations were used because of the increased dimensionality of conformational space. A relative energy cut-off value of 7 kcal mol^{-1} was used to eliminate high energy conformers and a gradient convergence of 0.050 for termination of the minimization algorithm. The Boltzmann distribution and percentage contribution for each conformer were determined as above.

Results

Considerations of conformational search strategies

When deciding how best to assess the effect of charge and dielectric constant upon the modeling of dipeptides in solution, the choice of residue is important. Ala was chosen as an ideal representative α-amino acid with minimal chi space, enabling more rapid computations because minimization of chi space geometry is not necessary, and has a neutral side chain that removes the need to consider effects of charged or aromatic side chains that could interact with terminal charges or backbone peptide bonds. Others have also chosen Ala for these and related reasons [30]. AlaAla, and other unsubstituted dipeptides without ionizable side chains, exist as doubly-charged zwitterions at physiological pH values, because of the pKa values of their amino and carboxyl groups (typically, 7.8 and 3.5, respectively). Consequently, AlaAla was modeled in its charged-form and, for comparison, also in its uncharged-form. Acylation of the N-terminal amino group has the dual effect of introducing an additional amide (peptide) bond whilst removing the positive charge. Similarly, the C-terminal carboxyl group can be esterified to remove the negative charge or amidated to remove the negative charge whilst also introducing an amide bond. Thus, AlaAla can be derivatized in these ways to produce *N*-acetyl-Ala-Ala-*O*-methyl (NAc-Ala-Ala-OMe) and *N*-acetyl-Ala-Ala-methylamide (NAc-Ala-Ala-MeAm), which are pseudo-tri- and tetrapeptides, respectively (c.f. "alanyl dipeptide" [18, 19]).

The inherent flexibility of dipeptides presents considerable computational demands to robust conformational analysis using molecular mechanics approaches. The grid searches used (at 15° intervals for the psi and phi torsion angles of the central dialanine unit) ensured that all conformational space was investigated but resulted in the production of 1152 conformers per molecule for analysis. Many of these conformers were highly improbable in solution (e.g. overlaps of van der Waal's radii) because of their high energies and none was subjected to energy minimization procedures because time constraints were considered important to selection of an optimum protocol. Random searches were also used to locate energy minima. In this approach, despite high energy conformers (such as some of those generated by the grid searches above) being discarded early on, the additional minimization of unique, lower energy conformers resulted in these searches being more time-consuming than the grid searches (up to ca. 2 h each for the two terminally-modified dipeptides).

For AlaAla (both uncharged and charged) (Table 1), grid searches and random searches located similar minimum energy conformers, the lower energy values found by the random search procedure being attributable to the minimization procedure used. The minimum energy conformers found by the two conformational search strategies had similar torsional values, indicating that the random search procedure was thorough enough to locate all potential energy minima within conformational space (data not shown). The same assumptions hold true for NAc-Ala-Ala-OMe and NAc-Ala-Ala-MeAm (Table 1) although, because these molecules have additional torsion angles compared with AlaAla, the difference between the energies of the grid searches and random searches are more pronounced. Had an amino acid residue with more extensive chi-space, e.g., Lys, been chosen the energies of the conformers generated by the two search procedures would be more different because the random search procedure would have optimized the side-chain geometry.

Effect of dielectric constant on conformations of dipeptides and modified dipeptides

Table 1 summarizes the relevant data for each of the four molecules under investigation for the grid search and random search conformational analyses.

For the grid searches, 1152 conformers corresponding to a 24×24×2 matrix of psi (ψ) (±180 to +165°, 15° increments), phi (ϕ) (±180 to +165°, 15° increments) and omega (ω) (either *cis* (0°) or *trans* (±180°)) were generated and the energy of each conformer was calculated using a dielectric constant between 1 and 80. This sample represents conformers from the shortest N–C distance possible (where ϕ=ψ=ω=0°) to the longest (where ϕ=ψ=ω=±180°). Because a common set of conformers was analyzed for each molecule, the mean N–C distances (±standard deviation) reported are identical and mean energies (±standard deviation) are at least very similar if not identical. With the random searches, the number of unique conformers found depends on the search parameters used (of which only the dielectric constant was varied) and, consequently, the values of all other reported parameters vary.

With AlaAla (uncharged-form), the energy of the minimum energy conformer (E_0) rises slightly for both the grid search and random search with decreasing dielectric constant between 80 and 4, and then more sharply towards a dielectric constant of 1 (Table 1). The minimum energy conformer found in the grid search for dielectric constants between 80 and 4 is the same (ψ=+60°, ω=±180°, and ϕ=–150°, N–C distance=5.3 Å); at a di-

Table 1 Parameters for AlaAla (uncharged), AlaAla (charged), *N*-acetyl-Ala-Ala-*O*-methyl, and *N*-acetyl-Ala-Ala-methylamide from grid search and random search at selected dielectric constants

DC[a]	Grid search			Random search			
	E_0[b]	Energy[c]	N–C distance[d]	Number[e]	E_0[b]	Energy[c]	N–C distance[d]
a) AlaAla (uncharged)							
1	7.34	149±216	4.56±1.05	50	6.81	9.21±1.87	4.77±0.82
4	4.60	145±217	4.56±1.05	53	4.17	6.32±1.62	4.80±0.76
10	4.04	144±217	4.56±1.05	51	3.59	5.33±1.39	4.71±0.87
80	3.71	144±217	4.56±1.05	50	2.96	4.67±1.43	4.74±0.79
b) AlaAla (charged)							
1	–27.5	138±220	4.51±1.03	4	–48.5	–36.8±7.92	2.91±0.41
4	–0.82	152±226	4.51±1.03	20	–4.84	–0.56±1.81	4.41±1.04
10	1.50	155±228	4.51±1.03	26	1.25	3.18±1.57	4.58±0.88
80	2.86	156±228	4.51±1.03	13	2.40	3.66±1.22	4.83±0.77
c) *N*-acetyl-Ala-Ala-*O*-methyl							
1	8.68	157±212	4.57±1.05	143	1.95	5.37±1.48	5.11±0.63
4	9.54	157±212	4.57±1.05	164	3.16	5.77±1.54	5.06±0.69
10	9.71	157±212	4.57±1.05	163	2.91	5.76±1.54	5.06±0.58
80	9.81	157±212	4.57±1.05	189	2.75	5.71±1.43	5.03±0.59
d) *N*-acetyl-Ala-Ala-methylamide							
1	8.59	189±286	4.53±1.04	136	0.08	4.49±1.75	5.24±0.44
4	9.49	189±287	4.53±1.04	182	2.99	5.74±1.38	4.98±0.64
10	9.53	189±287	4.53±1.04	183	2.66	5.67±1.47	5.14±0.51
80	9.54	189±287	4.53±1.04	191	2.45	5.71±1.43	5.16±0.57

[a] Dielectric constant
[b] Energy of minimum energy conformer (kcal mol^{-1})
[c] Mean energy of sample of conformers±standard deviation (kcal mol^{-1})
[d] Mean distance between N- and C-terminus±standard deviation (Å)
[e] Number of unique conformers found in random search

electric of 1, the ψ and ϕ both decrease to +45° and –165°, respectively, with the ω remaining at ±180°. Several minimum-energy conformers were found with the random searches at the various dielectric constants used, although ω remained at ±180±2° (*trans* conformation). At dielectric constants between 80 and 20, there were two minimum energy conformers, which only varied in their ψ (either 167.7±0.4° or 57.4±1.5°) the ω and ϕ being 178.7±0.5° –64.1±1.4°, respectively, with an N–C distance of 5.2±0.0 Å. Although different minimum energy conformers were found, their difference in energy was very slight (data not shown). Three different minimum energy conformers were found at dielectric constants of 10 and below, although each had a *trans* peptide bond (~±180°).

For AlaAla (charged-form), the energy of the minimum energy conformer (E_0) is reduced slightly for both the grid search and random search with decreasing dielectric constants between 80 and 10, and then markedly towards dielectric constants of 4 and 1 (Table 1). The minimum energy conformer found between dielectric constants of 80 and 4 is constant for the grid search (ψ=+165°, ω=±180°, ϕ=–165°, N–C distance=6.1 Å), changing to a shorter conformer with a *cis* peptide bond at a dielectric constant of approximately 1 (ψ=+105°, ω=0°, and ϕ=–60°, N–C distance=3.0 Å). For the random searches, between dielectric constants of 80 and 20, the minimum energy conformer found is constant although it differs from that found in the grid search in its ϕ value (ψ=+165.2±0.3°, ω=+178.2±0.2°, ϕ=–65.6±0.6°, N–C distance 5.3±0.0 Å) (Fig. 1). The minimum energy conformer found at a dielectric of 10 is similar to that found in the grid search (ψ=+166.6°, ω=–179.3°, ϕ=–159.0°, N–C distance 6.1 Å). At dielectric constants of 4 and 1, the minimum energy conformer shortens significantly and adopts a *cis* peptide bond (ψ=+100.2°, ω=+0.4°, ϕ=–55.7°, N–C distance 3.2 Å and ψ=+80.2°, ω=–5.2°, ϕ=–41.9°, N–C distance 2.5 Å, respectively) (Fig. 1). This *cis* conformer is stabilized by the presence of an intramolecular salt-bridge which, evidently, compensates for the penalty incurred by having the energetically unfavorable *cis* peptide bond. Also, the number of unique conformers found for the random search conducted at a dielectric constant of 1 (those with ΔE<7 kcal mol^{-1}) has been reduced to only 4 (Table 1).

For both NAc-Ala-Ala-OMe and NAc-Ala-Ala-MeAm, although the energies of the minimum energy conformers decrease with decreasing dielectric constant, the reduction is far less than that of AlaAla in its charged form (Table 1), and the number of unique conformers found using random search does not drop as sharply. The minimum energy conformer found for NAc-Ala-Ala-OMe using grid searches is constant throughout the range of dielectric constants used and similar to that found for the AlaAla (charged form) grid searches at dielectric constants above 4 (ψ=+165°, ω=±180°, ϕ=–150°, N–C distance=6.1 Å). Using random searches, the minimum energy conformer for dielectric constants between 80 and 4 are essentially the same (ψ=+165.2±0.3°, ω=+178.2±0.2°, ϕ=–65.6±0.6°, N–C distance 5.3±0.0 Å) changing as the dielectric constant approaches 1 whilst maintaining a *trans* conformation (ψ=+68.2°, ω=–178.0°, ϕ=–159.5°, N–C distance=5.4 Å). A similar situation was found with NAc-Ala-Ala-MeAm using grid search. The minimum energy conformer between dielectric constants of 80 and 10 is con-

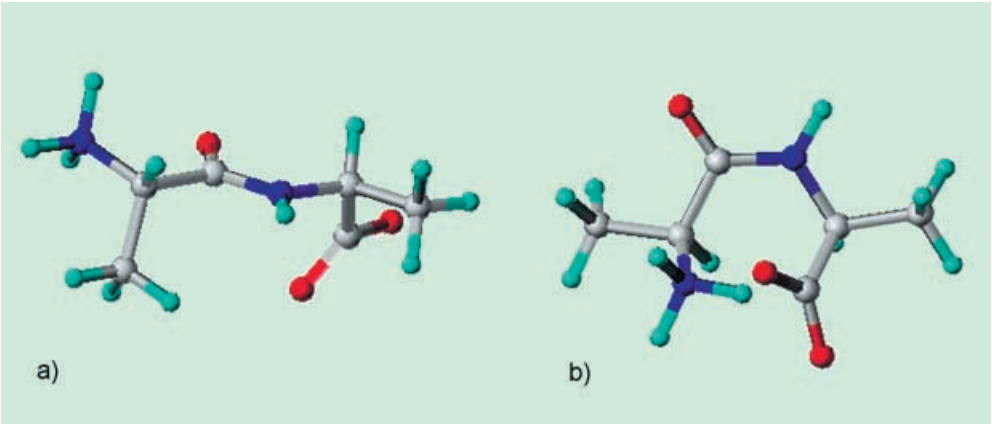

Fig. 1 Ball-and-stick models of the minimum energy conformers of AlaAla (charged) found with random searches at dielectric constants of (**a**) 80, and (**b**) 1. Conformer A has an energy of 2.40 kcal mol^{-1}, ψ, ω, and ϕ angles of +165°, +178°, and –65°, respectively, and an N–C distance of 5.3 Å; conformer B has an energy of –48.5 kcal mol^{-1}, ψ, ω, and ϕ angles of +80°, –5°, and –42°, respectively, and an N–C distance of 2.5 Å. The presence of a *cis* peptide bond (ω≈0°) in conformer B is allowed by the energetically favorable salt-bridge between the protonated NH_3^+ and dissociated COO^- groups

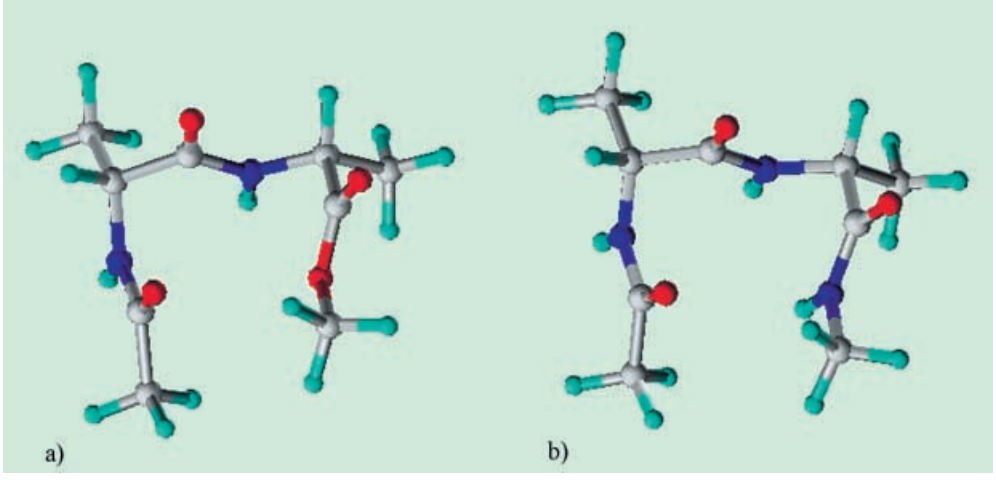

Fig. 2 Ball-and-stick models of the minimum energy conformers of (**a**) *N*-acetyl-Ala-Ala-*O*-methyl, and (**b**) *N*-acetyl-Ala-Ala-methylamide found with random searches at a dielectric constant of 80. The *N*-acetyl-Ala-Ala-*O*-methyl conformer has an energy of 2.75 kcal mol^{-1}, ψ, ω, and ϕ angles of –50°, +174°, and –67°, respectively, and an N–C distance of 4.4 Å; the *N*-acetyl-Ala-Ala-methylamide conformer has an energy of 2.45 kcal mol^{-1}, ψ, ω, and ϕ angles of –49°, +175°, and –63°, respectively, and an N–C distance of 4.5 Å

stant (ψ=+60°, ω=±180°, ϕ=–150°, N–C distance=5.3 Å), the ϕ changing around a dielectric constant of 4 (ϕ=–165°, N–C distance=5.2 Å) and the ψ changing as the dielectric constant approaches 1 (ψ=+165°, N–C distance=6.1 Å). Using random searches, the minimum energy conformer remains constant between dielectric constants of 80 and 4 (ψ=–49.8±0.6°, ω=+176.7±0.4°, ϕ=–64.2±0.9°, N–C distance 4.5±0.0 Å), changing at a dielectric constant of 1 but still having a *trans* peptide bond (ψ=+67.1°, ω=+179.7°, ϕ=–71.6°, N–C distance 5.4 Å). This conformer is different from that found using grid search conformational analysis. The reason different conformations for the energy minima have been found using grid searches and random searches for the NAc-Ala-Ala-OMe and NAc-Ala-Ala-MeAm molecules is that energy minimization was applied to conformers found in the random searches. However, conformers with similar torsional values to the minimum energy conformers found by grid searches were present as low energy conformations within the respective random searches (data not shown). The minimum energy conformers found for NAc-Ala-Ala-OMe and NAc-Ala-Ala-MeAm random searches at a dielectric constant of 80 are shown in Fig. 2.

The effects noted above are particularly pronounced when the percentage contributions that the minimum energy conformers represent of the sample of conformers found are compared (Fig. 3). For AlaAla (charged form), the minimum energy conformer accounts for approximately 7 and 20% of the percentage contribution for the grid search and random search, respectively, at dielectric constants between 10 and 80. However, at dielectric constants of 4 and 1 with the random searches, a single conformer accounts for 80 and 100% of the percentage contribution, respectively. Although the percentage contribution of the minimum energy conformers for AlaAla (uncharged form), NAc-Ala-Ala-OMe, and NAc-Ala-Ala-MeAm also vary with dielectric constant, they do not become the only significant conformer at dielectric constants of 4 and below. These con-

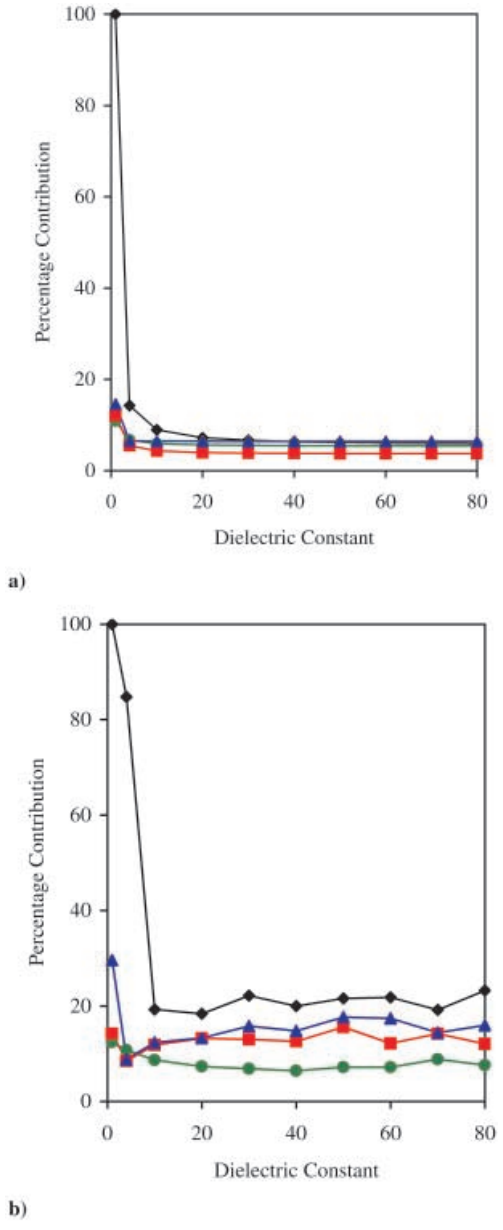

Fig. 3 Effect of dielectric constant on the percentage contribution of the minimum energy conformer for AlaAla (uncharged) (●), AlaAla (charged) (◆), *N*-acetyl-Ala-Ala-*O*-methyl (■), and *N*-acetyl-Ala-Ala-methylamide (▲) determined using (**a**) grid searches and (**b**) random searches. (**a**) At a dielectric constant of 1, the predominant conformer for AlaAla (charged) has a *cis* peptide bond. (**b**) At dielectric constants ≤4, the predominant conformers for AlaAla (charged) have *cis* peptide bonds

formers still have *trans* peptide bonds because the intramolecular stabilization afforded by a salt-bridge found for the AlaAla (charged form) *cis* peptide bond conformer is prevented by the lack of charged termini in these three molecules. Furthermore, the conformer profile varies little for these three molecules throughout the range of dielectric constants investigated, the same being true for AlaAla (charged) when a dielectric of ≥10 was applied.

Comparison with explicit solvation models

Knapp-Mohammady et al. [30] modeled AlaAla in its charged form using explicit solvation to simulate only the first solvation layer and found several low energy conformers with peptide bond torsions outside acceptable *trans* values. We also performed limited random searches for AlaAla (charged and uncharged forms) using explicit solvation to compare the results with those obtained by using distance-dependent dielectric continua. In these simulations, both forms of AlaAla were surrounded by approximately 1200 water molecules within a periodic box of dimensions ca. 28 Å×28 Å×28 Å, the bulk water outside this box being represented by a dielectric continuum (with dielectric constant of 80). These simulations took approximately 14 days to complete 100 search cycles with the random searches being set up as otherwise described for the implicit solvation model using distance-dependent dielectric constants. The conformers found for AlaAla (charged-form) had psi (ψ) values of +165±1°, –63±3°, and +75±4°, which were paired with phi (ϕ) values of –67±5°, +52±1°, and –159±2°. Conformers with *trans* peptide bonds (omega (ω) of ±180±2°) accounted for about 99% of the calculated percentage contribution. These conformational forms of AlaAla (charged) are very similar to those found using random searches with dielectric constants ≥10 (data not shown). The results for AlaAla (uncharged) were equally comparable using the two solvation methods (implicit and explicit solvation) (data not shown).

Computational demands of the random search procedures

Using grid search, generation of the conformers and calculation of their respective energies took approximately 40 min for each molecule. Since none of the conformations was energy minimized, however, it is unknown how long a complete conformational analysis using this procedure would have taken, although if each conformer took at least 10 s to minimize, this would have added at least an additional 180 min. Using the random search, for the four dipeptides, the CPU times were: (mean ± standard deviation) AlaAla (uncharged), 39.4±3.6 min; AlaAla (charged), 34.6±1.9 min; NAc-Ala-Ala-OMe, 106.3±7.9 min; NAc-Ala-Ala-MeAm 109.6±11.0 min. The computational times for these simple dipeptides are reasonable, making it feasible to model a large collection of dipeptides [8], with the expectation that the approach could be extended to tripeptides.

Discussion

In this study we have demonstrated that the random search procedure implemented in SYBYL locates energy minima for dipeptides that are comparable with those identified using grid searches. With AlaAla, the respec-

tive energies of the minima were similar, the slight differences arising from optimization in the backbone torsion angles ψ and ϕ during the minimization procedure implemented in random search. The terminally-blocked dipeptides had extra torsion angles either side of the central dialanine peptide unit that were not varied during the grid search procedure (they were set at ±180°) and, therefore, the energy differences between the minima identified by grid searches and random searches were more pronounced and not directly comparable. The random search procedure has been shown to explore conformational space for dipeptides sufficiently well that the results can be relied upon as an acceptable approximation to the situation found in solution. Such a conclusion was also reached by Treasurywala et al. [13] for the SYBYL random search procedure on a collection of small molecules, and endorsed further by a related study on a collection of dipeptides [8]. Thus, the parameters (search iterations, minimization cycles, energy cut-off, distance-dependent dielectric constant of 80) selected for random search here have been optimized for small peptides with the SYBYL package, but are equally applicable to many other small, flexible peptide analogs of biological and therapeutic interest such as β-lactams and ACE inhibitors, and also peptide mimetics. The use of a distance-dependent dielectric constant has given results for the peptide conformations similar to those from explicit solvation simulation in a time-scale more suitable for modeling many peptides/peptidomimetics to establish their potential as putative substrates for peptidases and/or peptide transporters. Furthermore, the conformers generated have greater biological relevance (*trans* peptide bonds) than those previously suggested using a more sophisticated ab initio calculation [30].

For the terminally-blocked dipeptides and the uncharged AlaAla dipeptide, changing the effective dielectric constant between 80 and 20 had little effect upon the identity of the minimum energy conformer or its energy and percentage contribution. Changes were apparent at dielectric constants below 20, especially between 4 and 1, although the conformers retained a central, *trans* peptide bond. With AlaAla in its charged form, the situation was markedly different. At dielectric constants between 4 and 1, the only energetically relevant conformer of the population had a *cis* peptide bond. The reason for this is the electrostatic interaction between the oppositely charged amino- and carboxyl termini (Fig. 1), which would be strengthened at low dielectric constants by reduced ionic shielding. The Tripos force field used in SYBYL [28] incorporates a term to account for electrostatic energy which depends on the product of the two atomic charges (here the amino and carboxyl termini) divided by the product of their separation distance and the dielectric constant used. Thus, for AlaAla in its charged form in solution, this term will vary by a factor of approximately 80 over the range of dielectric constants used. Molecular dynamics simulations of two conformations (*cis* and *trans*) of the tetrapeptide Tuftsin in water containing 0 and 1 mol L^{-1} NaCl revealed that the calculated value of the dielectric constant of water varies between 80 and 45 [25]. In conclusion, using low dielectric constants (<10) to model charged molecules such as peptides is unsound and leads to results that are difficult to interpret in relation to molecular recognition of substrates by peptidases and transporters [17, 31, 32].

Our overall objective is to evaluate molecular modeling as a tool enabling prediction of the conformations adopted by simple (2–6 residues) peptides in aqueous solution as an aid to describing the basis for the molecular recognition of substrates by proteins such as peptide transporters [7, 8, 27], and to apply this to the rational design of therapeutic peptidomimetics that can exploit these transporters for drug delivery [33]. The focus of most other studies has been different in that the primary interest was in using small peptides to model structural features within protein chains where solvation might have negligible influence upon their energetic stabilization [see, e.g., 16, 18, 19, 22] An important conclusion from the modeling studies here is that use of dielectric constants of 1–4 with charged peptides can produce conformers that are not relevant biologically and can result in the finding of low-energy conformers with *cis* peptide bonds, which occur infrequently in Nature. Use of a dielectric constant of 80 avoids this problem and has been used successfully to model a collection of over 50 dipeptides to identify their predominant, shared conformations in solution [8] and to determine a molecular recognition template of dipeptides [7, 27]. Further experimental support for this modeling protocol is provided by results of high-performance capillary electrophoresis used to measure the *cis*/*trans* ratio of several dipeptides [34, 35]; the measured results for GlyPro and GlySar and those computed as described here agree to within ±2% (B.M. Grail and J.W. Payne, unpublished results).

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