Bioactive Conformations of Small Peptides: A Method for Selection of Candidates Based on Conformations of Active and Inactive Analogues and Its Application to Muramyl Dipeptide

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A method has been developed that selects a subset of candidates for the bioactive conformation based on the comparison of conformation clusters of the active and inactive ligands. Those conformations of the active ligand that cannot be reached by inactive ligands in spatially presenting preselected "target" atoms (conformations "unique" to the active ligand) are extracted. The method is applied to muramyl dipeptide (MDP); the selection of candidates was performed using two of its diastereomers that have no immunostimulative properties. A third diastereomer and a rigidified analogue, both inactive, and the protein-bound conformation of a related peptidoglycan containing the MurNAc-L-Ala-D-Glu sequence are used as test cases; the latter was found to be most similar to one member of the set of unique conformations of MDP.

INTRODUCTION

Search for the bioactive conformations of small, linear peptides (or other flexible molecules) in the absence of any template structure[1,2] (e.g., rigid analogue,[3] structure of the binding site[4−7]) is a difficult and rather hazardous undertaking. The NMR conformational analyses together with restrained molecular dynamics (MD) yield numerous families of low energy conformations which, at best, may contain the bioactive one. The number of candidates has to be reduced in order to facilitate the delineation of the three-dimensional (3D) pharmacophore required as a starting point for the design of mimetics. Rigidification of the lead peptide is the most often followed strategy, but the experimental work is lengthy and may lead to unpleasant surprises.[8]

Families of energetically possible but for the receptor unacceptable conformations of the lead peptide that are obtained from a conformational analysis may be excluded by considering the conformations of analogues that are inactive. The conformational space of active and inactive isomers will contain families of conformations that are very similar in the spatial presentation of potential binding groups irrespective of the configurational difference of the constituting amino acid residues; these families of conformations may be eliminated from the set of candidates for the bioactive conformation and thus the set reduced.

Following this reasoning we have developed a method that is an extension of Marshall's active analogue approach.[9,1] It assumes that the binding to the receptor is mainly accomplished by selected chemical groups of the ligand (the *target atoms*, e.g., hydrogen-bond donors and/or acceptors); the bioactive conformation presents the target atoms to the receptor in the spatially correct way, while the inactive ones fail to do so. The method searches for a subset of conformations of the active ligand that cannot be attained by any conformation of the inactive ligands in presenting the target atoms to the binding site of the receptor; this subset of conformations contains candidates for the bioactive conformation and is further examined for local structural features (e.g., turns, bends, and intramolecular H-bonds). Its members may be compared to the favored conformations in solution as determined by NMR if such a study has been performed.

The method was applied to the example of muramyl dipeptide (MDP = MurNAc-L-Ala-D-iGln, **I**, Figure 1a; MurNAc, *N*-acetyl/muramic acid) and its inactive diastereomers MurNAc-L-Ala-L-iGln (**II**) and MurNAc-D-Ala-L-iGln (**III**). A third diastereomer, MurNAc-D-Ala-D-iGln (**IV**), and the rigidified analogue from ref 8 (*N*-acetylmuramyl-L-alanyl-3-(carbomethoxymethyl)-D-proline methyl ester **V**; Figure 1b) are used to refine the data. MDP is an interesting immunostimulant, and some of its analogues are being clinically tested; one is already commercially available.[10] The activity of MDP critically depends on the configuration of both amino acids, Ala and iGln, respectively; L-Ala may only be replaced by Gly and a few nonpolar L-amino acids and D-iGln by D-Glu.[11] In view of its uncomfortable properties for application shared with most simple peptides the development of mimetics is indicated. However, the large conformational space of MDP and the complete lack of a rigid analogue or knowledge about its receptor are a serious obstacle in the search for a 3D pharmacophore. **I** has been studied in solution with NMR methods;[12] the preferred solution conformations contain a pseudo-β-turn that is characterized by the intramolecular H-bond acetamido-CO···HN-Ala,[12,13] while the conformation of iGln is less defined; similar features are found with some carbocyclic analogues of **I**.[14] Experimental data on **II** and **III** are not available. A candidate for the bioactive conformation of **I** has been proposed[12] but could not be underpinned by the synthesis of a more rigid analogue.[8]

METHODOLOGY

From a series of flexible analogues the biologically active lead molecule (designated A), a set of n_X biologically inactive analogues (designated X_i, $i = 1..n_X$), and for each molecule a set of n_T target atoms (i.e., the chemical groups of the

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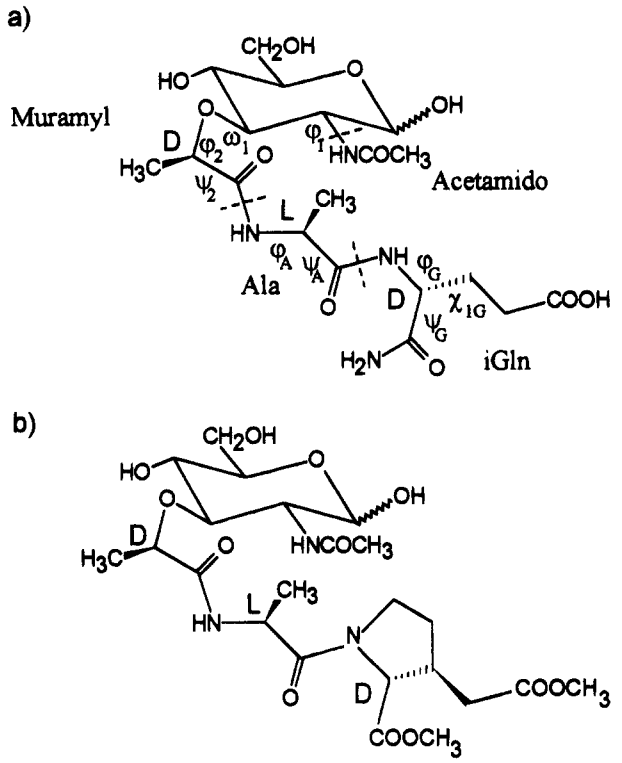

Figure 1. Chemical structure of (a) muramyl dipeptide (MDP, **I**) and (b) its rigidified analogue[8] **V**.

molecule that are presumably the main ones responsible for binding to the receptor) are chosen. The computer models of the molecules are built and subjected to an extensive conformational search. In our laboratory we use the program package INSIGHT/ DISCOVER;[15] the protocol for each molecule includes molecular dynamics (MD) at 500 K for 100 ps using a distance-dependent dielectric constant ($\epsilon = r$) starting from 20 different structures with random values of n_D selected dihedral angles; the coordinates are sampled every 0.5 ps. The resulting 4000 structures are subjected to energy minimization (EM) and clustered according to the values of the n_D dihedral angles. The clustering algorithm chooses a structure to be member of a cluster if

•the mean of the square root of the sum of squared differences (designated RMS differences) of the values of the dihedral angles of the observed structure and the running cluster average are less than *maxrms* (20°; value chosen arbitrarily) and

•none of the dihedral angles deviates by more than 2 • *maxrms* (40°) from the cluster average.

The numbers of conformation clusters found with the leading molecule and analogues are denoted NrC(***A***) and NrC($\boldsymbol{X}_i$) ($i = 1..n_X$), respectively. The coordinates of the member with the lowest energy are chosen to represent the cluster. The energy of the cluster is calculated as the Boltzmann averaged total DISCOVER energy of the cluster members.

In the next step each cluster representative of each analogue $\boldsymbol{X}_i$ ($i = 1..n_X$) is translated and rotated in a way to minimize the mean square root of the sum of squares of the differences between the coordinates of the target atoms of ***A*** and the corresponding target atoms of $\boldsymbol{X}_i$ (designated target atom least-RMS superposition) for each cluster representative of ***A***. The resulting values of the superpositions are stored in n_X matrices $\mathbf{M}_{X_i\text{-}A}$ with dimensions NrC($\boldsymbol{X}_i$)•NrC(***A***) ($i = 1..n_X$). The elements *(k,l)* in the matrices $\mathbf{M}_{X_i\text{-}A}$ contain the values of the target atom least-RMS superpositions of the representatives of cluster *l* of ***A*** and the representatives of cluster *k* of $\boldsymbol{X}_i$. In each column the smallest element is found and stored in a vector $V_{X_i\text{-}A}$ with dimension NrC(***A***); a large value of $V_{X_i\text{-}A}[l]$ means that no cluster of $\boldsymbol{X}_i$ is able to reach the arrangement of the target atoms of cluster *l* of ***A***. Finally the n_X vectors $V_{X_i\text{-}A}$ ($i = 1..n_X$) are compared at each *l* ($l = 1..$NrC(***A***)); again the value of the smallest of the n_X vector elements $V_{X_i\text{-}A}[l]$ is extracted and stored as *l*th element in the final vector V_{minRMS} with dimension NrC(***A***) (Figure 2).

A large value (designated *maxLim* and chosen arbitrarily as 1.20 Å) of $V_{\text{minRMS}}[l]$ means that no cluster representative of any of the *n* inactive analogues $\boldsymbol{X}_i$ ($i = 1..n_X$) is able to reach the arrangement of the target atoms of cluster *l* of ***A***. All conformation clusters of ***A*** with $V_{\text{minRMS}}[l] \geq$ *maxLim* are called "unique (to ***A***)" and form a set of strong candidates for the bioactive conformation. On the other hand, a small value (< *minLim*, which is arbitrarily chosen as 0.30 Å) of $V_{\text{minRMS}}[l]$ means that at least one cluster representative of at least one of the n_X inactive analogues $\boldsymbol{X}_i$ ($i = 1..n_X$) is able to attain the arrangement of the target atoms of cluster *l* of ***A***; the conformation clusters of *A* with $V_{\text{minRMS}}[l] <$ *minLim* are called "common (to ***A*** and $\boldsymbol{X}_i$)" and are excluded from the set of candidates for the bioactive conformation.

Another group of conformations may be formed if an NMR conformational analysis of ***A*** in solution using NOE measurements has been performed. The spatial proximities of protons that are indicated by NOE measurements are most probably not found in one single conformation; therefore those lowest-energy conformation clusters of ***A*** that in ensemble fulfill all proximities of protons or proton groups (in the case of a measured NOE) and additionally all absences of proximities of protons (in the case of no measured NOE) are selected and denoted "solution clusters".

The collected groups of conformations may now be compared in local (distributions of dihedral angle values) and global (turns and intramolecular H-bonds) conformational features.

The information gathered can be refined by evaluating other analogues, e.g., ***Y*** (Figure 2). A conformational search of ***Y*** and a target atom least-RMS superposition calculation of all clusters of ***Y*** with all clusters of ***A*** have to be performed again. The vector $V_{Y\text{-}A}$ is extracted from the matrix $\mathbf{M}_{Y\text{-}A}$; a comparison of the V_{minRMS} values of the unique clusters and the corresponding values of $V_{Y\text{-}A}$ allows the elimination of member *l* of the set of "unique clusters" from the set of candidates for bioactive conformations if analogue ***Y*** is inactive and $V_{Y\text{-}A}[l]$ is small.

A first estimate whether a new, not yet tested (or even synthesized) analogue ***Y*** is biologically active can be made by calculating the mean deviation of the values of $V_{Y\text{-}A}$ of the unique clusters from V_{minRMS}; a small deviation indicates that bioactivity of the new analogue is unlikely.

The algorithm for clustering and further evaluation of clusters is implemented in our program CCLUES (Comparison of CLUsters: Evaluation of Superpositions) written entirely in ANSI C.

The algorithm is applied to **I** (=***A***) and its inactive diastereomers **II** and **III** (=$\boldsymbol{X}_1$ and $\boldsymbol{X}_2$, $n_X = 2$) using $n_D = 11$ variable dihedral angles and $n_T = 10$ target atoms (all carbonyl group C and O atoms). The potential activities of

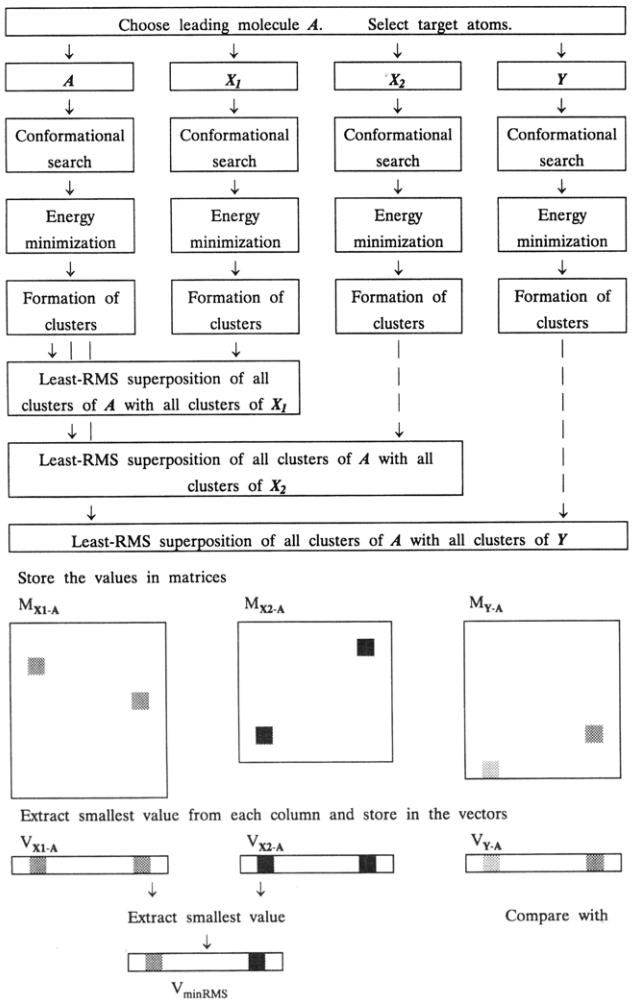

Figure 2. Scheme of the algorithm for selection of candidates for the bioactive conformation implemented in the program CCLUES.

Table 1. Summary of the Conformational Search of **I**-**V**

	I	**II**	**III**	**IV**	**V**
no. of conformation clusters	1035	1121	1211	984	796
range of energies (kcal/mol)	31.6	28.2	33.9	28.8	31.0

IV and **V** (=Y_1 and Y_2) are tested using the approach described above. The protein-bound conformation of a peptidoglycan containing the MurNAc-L-Ala-D-Glu sequence is compared with the unique clusters of **I**.

RESULTS AND DISCUSSION

Cluster Formation. The summary of the results of the conformational search and subsequent clustering of **I**−**III** is shown in Table 1 (columns 1−4). No NOE distance restraining was applied during MD and EM. The clustering had to be performed using the nine dihedral angles shown in Figure 1a) and additionally χ_2 and χ_3 of the iGln side chain because a target atom pair (the $^{\delta}$CO group) is located at its end; in such a case the artifacts caused by the vacuum conditions during MD and energy minimizations are likely to influence the quality of the conformational search; a distance-dependent dielectric constant has been used in order to lessen these effects. The large numbers of sampled clusters (≈1000) with each diastereomer are an indication that the sampling of the conformational space is at least fair. The range of energies (the lowest energy found in each series is set to 0.0 kcal/mol; the use of Boltzmann averages for the energy of a cluster ensures that the lowest energy found with the members is emphasized) shows that energetically less favorable clusters of structures (with relative energies 20−30 kcal/mol) were also sampled; this is important in view of the fact that, additionally to the poor quality of force fields *in vacuo*, interactions with a receptor perturb the conformational energy surface of the ligand and even conformers with higher energy must be considered as potential candidates.[9,4,16]

In a recent article[17] the main weakness of clustering by dihedral angle values has been reviewed, but regarding the short length of **I**−**V** and small value of *maxrms* (20°; see Methods section) we believe it to be negligible.

Calculation of Superpositions and Formation of Conformation Groups. All carbonyl group C and O atoms were chosen as target atoms; the choice was based on known structure−activity relationships[11,18] and general considerations about the binding modes of peptides and related molecules. In the target atom least-RMS superposition calculations all pairs of representatives of all conformation clusters of **I**−**II** and **I**−**III**, respectively, were evaluated. The values ranged from 0.07 to 5.04 Å and were stored in the matrices $\mathbf{M}_{\mathbf{II-I}}$ and $\mathbf{M}_{\mathbf{III-I}}$, respectively. The smallest elements in each column were extracted to the vectors $V_{\mathbf{II-I}}$ and $V_{\mathbf{III-I}}$, respectively, and the smaller value of both vectors to V_{minRMS}. The latter was sorted and used to extract the conformation clusters of **I** "unique to **I**" (Table 2a, columns 1−6) and "common to **I**-**III**" (Table 2b, columns 1−6) as described in the Methods section.

The first 18 conformation clusters of **I** (sorted by increasing relative energy) are needed to fulfill all NOE data in DMSO-d_6 solution[12] and are included in the "solution" clusters; they range in energy from 0.0 to 3.65 kcal/mol.

Analysis of Conformation Groups. In the analysis the differences and common features in the local and global conformational characteristics of the three groups are emphasized.

The normalized dihedral angle distributions of the dihedral angles of **I** that were used for cluster formation are shown for each of the three groups in Figure 3a−k. Differences of the preferred values of some dihedral angles may be observed; most notably the values with the "common" clusters are usually most exclusive (especially with φ_1, ω_1, and φ_A), while the solution and the common clusters share many common features in the values sampled (except with ψ_A and χ_1). These findings are significant for the evaluation and the design of new molecules with equivalent covalent bonding pattern.

It is interesting to analyze the intramolecular H-bonds in the three groups even though their significance is reduced by the effects of the vacuum conditions. A geometric criterion is used to check for the existence of an H-bond (d(O···H−X) < 2.3 Å, θ(O,H,X) > 120°, X = N,O). Seven different H-bonds are found (Table 3). The most prominent is the intramolecular H-bond acetamido-CO···HN-Ala that characterizes a pseudo-β-turn (also reported by other authors[12,8,13] as the preferred conformational feature in solution); it is found in all three groups but dominates with the solution clusters. The H-bond muramyl-CO−HN-acetamido that cannot exist simultaneously with the former H-bond dominates in the common clusters and is not found at all with the unique clusters. The appearance of some other H-bonds

Table 2. The Clusters with the Largest (a, Unique Clusters) and Smallest (b, Common Clusters) Values of V_{minRMS} and the Corresponding Values of V_{II-I}, V_{III-I} and V_{IV-I} (See Text)

no.[a]	cluster no.[b]	energy (kcal/mol)	V_{II-I} (Å)	V_{III-I} (Å)	V_{minRMS} (Å)	V_{IV-I} (Å)	V_{V-I} (Å)
			a. Unique Clusters				
1035	80	6.14	1.51	1.67	1.51	1.17	1.49
1034	296	9.58	1.37	1.72	1.37	0.92	1.37
1033	7	2.62	1.37	1.55	1.37	0.85	1.30
1032	14	3.30	1.37	1.63	1.37	1.10	1.48
1031	218	8.67	1.37	1.65	1.37	0.99	0.82
1030	182	8.18	1.36	1.66	1.36	1.14	1.44
1029	278	9.43	1.35	1.54	1.35	1.31	0.69
1028	96	6.56	1.34	1.58	1.34	1.12	1.47
1027	243	8.99	1.34	1.34	1.34	1.04	0.89
1026	809	14.87	1.33	1.66	1.33	0.70	1.39
1025	756	14.27	1.32	1.70	1.32	1.23	0.62
1024	598	12.83	1.32	1.31	1.31	1.14	1.39
1023	109	6.88	1.31	1.63	1.31	1.15	1.35
1022	642	13.24	1.30	1.47	1.30	1.07	0.62
1021	401	10.88	1.30	1.51	1.30	1.10	1.02
1020	116	7.01	1.31	1.29	1.29	0.95	1.34
1019	90	6.39	1.29	1.49	1.29	0.86	1.24
1018	403	10.90	1.29	1.58	1.29	0.94	1.18
1017	816	14.96	1.29	1.32	1.29	0.72	0.64
1016	202	8.51	1.28	1.64	1.28	1.02	0.66
1015	436	11.19	1.28	1.36	1.28	0.93	1.24
1014	609	12.92	1.46	1.27	1.27	0.98	1.42
1013	506	11.92	1.27	1.42	1.27	0.95	1.02
1012	149	7.67	1.29	1.27	1.27	0.62	1.14
1011	856	15.59	1.27	1.61	1.27	0.95	0.51
1010	176	8.14	1.26	1.31	1.26	0.97	1.37
1009	340	10.09	1.27	1.26	1.26	0.63	0.81
1008	744	14.14	1.26	1.45	1.26	1.10	0.93
1007	688	13.62	1.25	1.27	1.25	0.77	1.13
1006	637	13.20	1.24	1.29	1.24	0.85	1.04
1005	226	8.83	1.23	1.44	1.23	1.07	0.71
1004	212	8.60	1.36	1.22	1.22	0.15	1.39
1003	257	9.14	1.22	1.32	1.22	0.95	1.28
1002	770	14.48	1.22	1.54	1.22	1.11	0.64
1001	629	13.12	1.21	1.25	1.21	0.58	1.55
1000	413	11.00	1.50	1.21	1.21	0.95	1.32
999	474	11.57	1.22	1.21	1.21	0.97	0.60
998	520	12.01	1.27	1.20	1.20	1.12	0.48
997	927	17.13	1.20	1.22	1.20	0.86	1.01
996	361	10.28	1.20	1.31	1.20	0.82	0.77
995	825	15.05	1.20	1.35	1.20	0.95	0.91
994	203	8.51	1.24	1.20	1.20	0.77	1.22
			b. Common Clusters				
11	294	9.57	0.30	0.85	0.30	0.80	0.71
10	134	7.46	0.29	0.87	0.29	0.72	0.63
9	166	7.95	0.29	1.02	0.29	0.73	0.62
8	477	11.58	1.28	0.29	0.29	0.18	1.26
7	24	3.98	0.28	1.11	0.28	0.85	0.57
6	304	9.64	0.28	1.00	0.28	0.88	0.64
5	458	11.42	1.26	0.27	0.27	0.14	1.27
4	40	4.83	0.27	1.18	0.27	0.86	0.54
3	62	5.50	1.15	0.27	0.27	0.16	1.24
2	92	6.49	1.19	0.25	0.25	0.37	1.26
1	54	5.14	0.20	0.92	0.20	0.69	0.45

[a] Serial number of cluster of **I** sorted by increasing V_{minRMS}. [b] Serial number of cluster of **I** sorted by increasing energy.

is mostly due to the vacuum conditions (e.g., the γ-turn muramyl-CO—HN-iGln).

The set of *unique clusters* is most interesting since it probably contains the bioactive conformation. None of its low-energy members contains the pseudo-β turn characterized by the intramolecular H-bond acetamido-CO···HN-Ala. At this point all of them must be considered as potential candidates, but the conformation clusters that contain structures with low energies are more probable. Most remarkable therefore are the clusters number 7 and 14 (Figure 4a,b) that have (relative) energies of 2.6 and 3.3 kcal/mol (Table 2) and are also found with the solution clusters. This finding indicates that the bioactive conformation may be present in solution to a significant extent. Interestingly, no member of the set of the 'solution' clusters is common to the set of the common clusters. It must be emphasized that any conclusions regarding the set of solution clusters have to be taken with caution since it has not been obtained or checked with a simulation using explicit solvent molecules.

Table 3. Intramolecular H-Bonds Found with the Sets of Unique, Common, and Solution Clusters (in Percent)

H-bond	unique clusters (%)	common clusters (%)	solution clusters (%)
acetamido-CO—HN-Ala	10	27	39
acetamido-CO—HO1-muramyl	17		11
muramyl-CO—HN-acetamido		36	6
muramyl-CO—HN-iGln	33	45	11
Ala-CO—H_2EN-iGln	10	9	
iGln-δCO—HN-Ala			11
iGln-δCO—HN-iGln	17		
none	26	9	28

IV and V: Test Cases. In order to check the validity of the proposed approach and to further narrow the set of the unique clusters of **I** another diastereomer, **IV**, and a rigidified analogue with preserved peptide chirality, **V**, have been analyzed. **IV** has no immunostimulating properties and has been reported to act as an immunosuppressor under certain circumstances,[18] while **V** is reported to be inactive.[8] The conformational search, clustering of conformations, calculation of target atom least-RMS superpositions with the clusters of **I** and extraction of the vectors V_{IV-I} and V_{V-I} from $\mathbf{M}_{IV-I}$ and $\mathbf{M}_{V-I}$, respectively, have been carried out in exactly the same way as with **II** and **III**. As expected the number of conformation clusters of **V** (Table 1) is significantly smaller than the numbers of **I**—**IV** due to the rigidification involving ψ_A and χ_1. The values of the elements of V_{IV-I} and V_{V-I} corresponding to the unique and common clusters of **I** are listed in the last two columns of Table 2. A comparison with the values of V_{minRMS} at the unique clusters shows that the agreement is good (mean RMS deviation 0.37 Å in both cases) which would be expected for an inactive diastereomer. A low value of V_{IV-I} or V_{IV-I} at some unique cluster is an indication that this cluster should probably be excluded from the list of candidates for the bioactive conformation, but at this point no objective criteria are available to firmly set the limiting value of exclusion. At any rate the successful prediction of the activity of **V**, in accord with the experimental finding, is encouraging in that the method has properly classified the conformations of a molecule that possesses the same chirality as the active lead but obviously differs in the available conformational space.

Test Case of Peptidoglycan Binding Site. The X-ray coordinates of GlcNAc-MurNAc-L-Ala-D-Glu-DAP-D-Ala (**VI**; GlcNAc, *N*-acetyl/glucosamine; and DAP, diaminopimelic acid) bound to the T4 lysozyme mutant T26E[19] were used to additionally test the method. T4 lysozyme is a glycosidase specialized for cleaving bacterial cell walls; it specifically binds glycopeptides containing the MurNAc-L-Ala-D-Glu(X) sequence[20] (X = DAP or L-Lys). The set of corresponding target atoms of **VI** was determined and used for target atom least-RMS superposition calculations with all cluster representatives of **I**. The smallest value (0.85 Å)

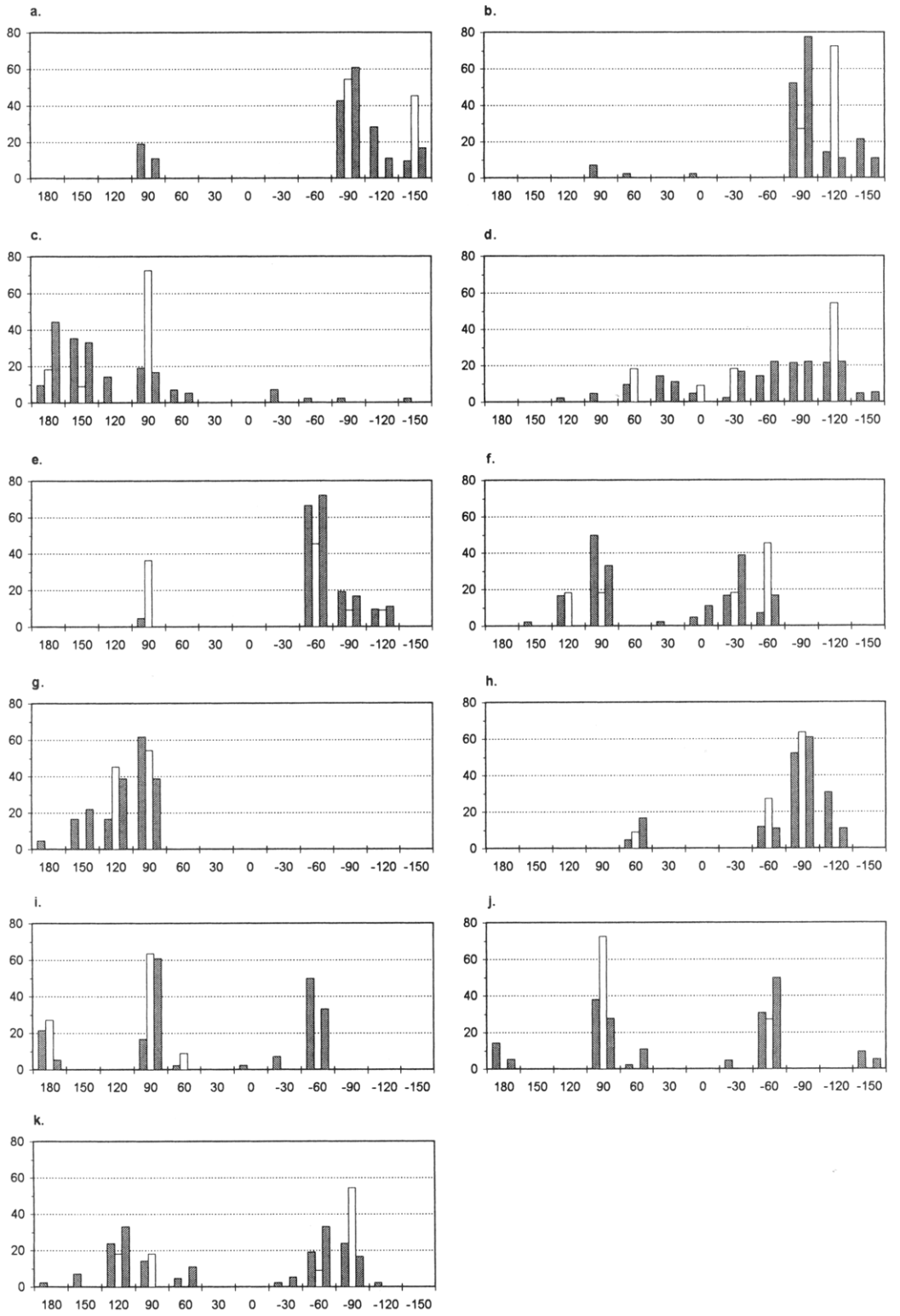

Figure 3. Distribution of dihedral angle (Figure 1) values in the unique (left), common (middle), and solution clusters (right column) of **I** (see text): (a) φ_1, (b) ω_1, (c) φ_2, (d) ψ_2, (e) φ_A, (f) ψ_A, (g) φ_G, (h) ψ_G, (i) χ_1, (j) χ_2, and (k) χ_3 (x-axis: interval of dihedral angle values in deg; y-axis: percent of structures).

was found with the representative of cluster number 825 of **I** (Figure 4c) that is found in the set of unique clusters (entry 995, column 1 of Table 2a). The method was therefore able to extract to the set of the unique clusters the MDP conformation that is most similar to the protein-bound conformation of a peptidoglycan containing the MDP-like MurNAc-L-Ala-D-Glu sequence.

Final Comments. The success of the method critically depends on two very demanding points: a thorough sampling of the conformational space of each molecule and the correct choice of target atoms. Except with the simplest cases the conformational search cannot be performed by systematically evaluating all possible combinations of dihedral angle values using a small step size which alone guarantees that no local minima are overlooked. The use of explicit solvent molecules that would eliminate some vacuum artefacts (e.g., γ-turn formation[21]) is also computationally too demanding with respect to the possible gains. The second point depends

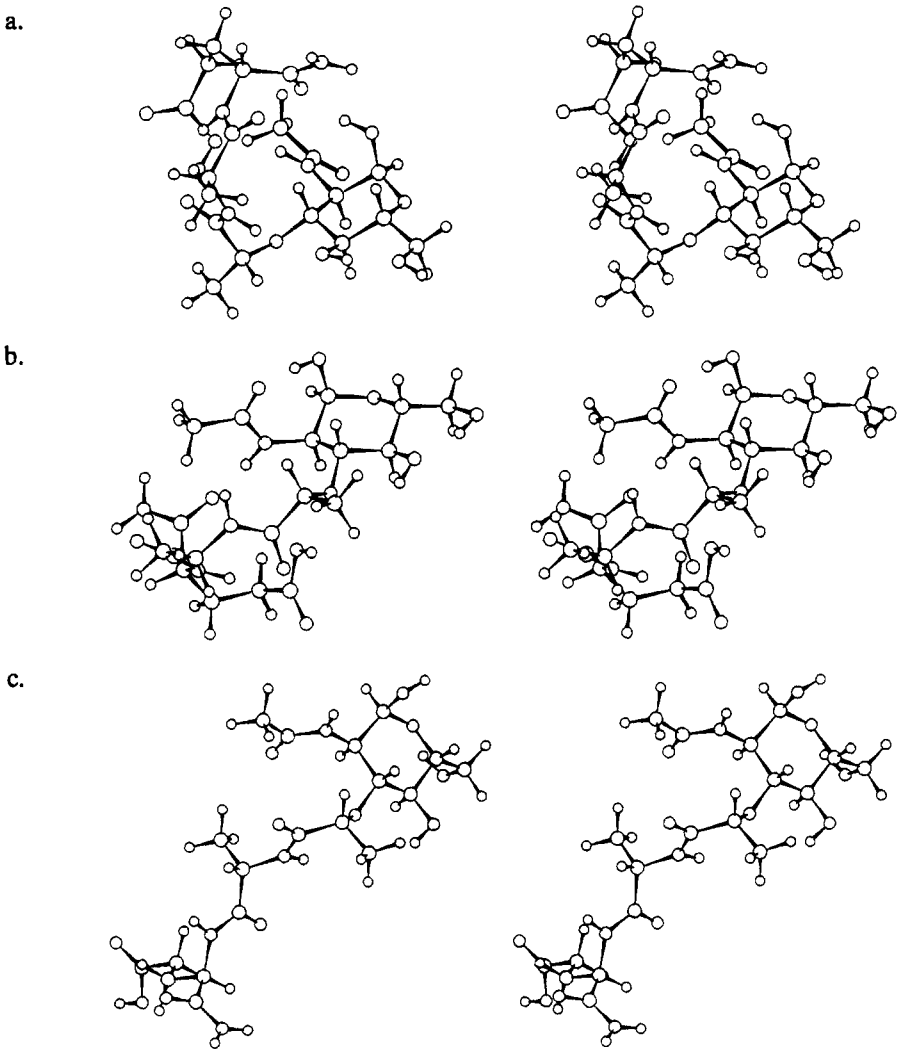

Figure 4. Stereoview of three candidates for the bioactive conformation of MDP representatives of conformation cluster 7 (a), 14 (b), and 825 (c) from Table 2 (column 2).

on the user's understanding of the structure−activity relationships of the series of analogues and his intuition. A recent article reports a spatial description of well-defined target atoms consistent with pharmacologic profiles of active ligands derived by a similar approach on smaller systems using systematic conformational search.[2]

A general problem regarding data on biological activity in the case of a series of analogues is that with structurally different molecules differences in biological activity *in vivo* may not only be related to the strength of binding to the receptor but also to differences concerning transport and passage through membranes in the living organism. In this study such a case is improbable for **I**−**IV** since they differ in chirality only not in overall hydrophobic character; different rates of digestion by enzymes still may play a role.

We are aware of some neglects and simplifications in our approach, e.g.

•The only criterion for successful binding to the receptor is the spatial position of selected target atoms (mainly hydrogen bond donors/acceptors); ligand-receptor binding that mainly depends on hydrophobic contacts is poorly described in this way.

•Possible close contacts of other parts of the molecule that may occur within the binding site are neglected.

CONCLUSION

The present work uses information gathered from the conformational search of inactive ligands to significantly reduce the set of candidates for the bioactive conformation of the active leading substance; this is achieved by excluding those conformations that present preselected target atoms in the same way as at least one of the inactive ligands. We believe that the method is generally applicable to a series of active and inactive flexible ligands with no known rigid template in all cases where a single receptor site is involved. At present the method is rather qualitative and user-dependent, but it is readily applied in this laboratory with new proposed analogues. It was able to put in the set of candidates for the bioactive conformation the conformation of **I** that is most similar to the protein-bound conformation of a related peptidoglycan containing the MurNAc-L-Ala-D-Glu sequence. An extension of the method that explicitly involves hypothetical receptor atoms and their interaction with the ligand atoms is being developed in this laboratory.

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