ELSEVIER

International Journal of Pharmaceutics 199 (2000) 59–64

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Conformational analysis of 7-oxoacyl-L-alanyl-D-isoglutamines

T. Solmajer [a,b,*], O. Planinšek [b], S. Srčič [b]

[a] *Laboratory for Molecular Modeling and NMR, National Institute of Chemistry, and Lek, d.d., P.O. Box 3430, Hajdrihova 19, 1115 Ljubljana, Slovenia*

[b] *University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia*

Received 1 June 1999; received in revised form 21 January 2000; accepted 7 February 2000

Abstract

In a recent article (Planinšek, O., Srčič, S., 1999. Int. J. Pharm. 87, 199–207) some interesting physicochemical properties of a series *N*-(7-oxoacyl)-L-alanyl-D-isoglutamines with $n = 0–6$ methylene groups between the terminal methyl and 7-oxo group were measured. In view of the practical importance of these *N*-acetylmuramyldipeptide(MDP) immunomodulator analogues and their interesting biological properties a detailed conformational analysis was undertaken for the series with $n = 3–6$ methylene spacers between the 7-oxo and terminal methyl groups. The puzzle posed by the reversal of the measured water solubility and lipophylicity could be resolved by using the Monte Carlo approach to searching the conformational space of the molecules in this series. We have found that the increase in water solubility and drop in lipophylicity when the number of methylene groups is increased from 5 to 6 can be attributed to the change in predominant conformation in the conformational family as described by the Boltzmann distribution of conformations. Notwithstanding this, we point out the changes in biological response coupled to the nonlinearity of the physicochemical behaviour in the series.

Keywords: Desmuramyldipeptide; 7-Oxoacyl-L-alanyl-D-isoglutamines; Conformational analysis; Monte Carlo

1. Introduction

Conformational analysis can be loosely defined as the study of conformations of a molecule and their influence on its properties and behaviour (Leach, 1991). Many molecules of interest to organic, bioorganic and medicinal chemists can adopt more than one conformation. Stable conformations of a molecule correspond to local minima in the potential function. The relative population of the minima depend on their statistical weights which include contributions from both the potential energy and the entropy. An important consequence is that the global energy minimum on the potential energy surface does not necessarily correspond to the structure with the highest statistical weight. Thus, to perform a conformational search in a set of flexible molecules

* Corresponding author. Tel.: +386-61-1760277; fax: +386-61-1259244.

E-mail address: tom.solmajer@ki.si (T. Solmajer)

0378-5173/00/$ - see front matter
PII: S0378-5173(00)00368-9

requires some means of determining the energy of any given conformation and a method of determining minima on the surface described by the potential energy function.

It is obvious that conformational stereoisomers of acyclic molecules differ primarily in their torsional angles. By considering the least constrained internal degrees of freedom (i.e. torsions) a search problem formally having $3N-6$ dimensions may be treated as one which has at most N dimensions. Thus, the reduction in available space diminishes the scope of the search problem from one which expands as d^{3N-6} to one which expands as approximately d^N where N is the number of atoms and d is the sampling density along varied coordinates (Chang et al., 1989). Thus, the use of torsional internal coordinates facilitates selective sampling of the low-energy regions of conformational space where stable conformers are found.

In view of the importance of the series *N*-(7-oxoacyl)-L-alanyl-D-isoglutamines with $n=0$–6 methylene groups between the terminal methyl and the 7-oxo group, which possess immunomodulator properties analogous to *N*-acetylmuramyldipeptide(MDP), a detailed conformational analysis was undertaken with the goal o frationalizing the correlation between a peculiar increase in measured water solubility and drop in lipophylicity with changes in chain length from $n=5$ to $n=6$. Random-variation methods which were used for searching the conformational space generally lead not only to the lowest energy minimum (global minimum) but also to a set of all other significantly populated minima which together provide an objective basis for quantitative discussion of the model.

2. Methods

The most general conformational searching algorithms are those that aim to identify all minima on the potential surface. Since the number of minima increases dramatically with the number of rotatable bonds it is necessary to reduce the scope of the search. Random search methods are a viable alternative to perform this task. In the Metropolis Monte Carlo (Metropolis et al., 1953) the algorithm simulates the thermal behaviour of a system by tending to favour the lowest energy states of the system but also allowing random fluctuations into higher energy states. To evaluate the molecular potential energy in these minima we have used the molecular mechanics force field MM3 (Allinger et al., 1989) as implemented in the program package Spartan (Wavefunction Incorporated, 1995). Acyclic molecules of the series in which $n=3$–6 methylene units were included in the molecule *N*-(7-oxoacyl)-L-alanyl-D-isoglutamine between the 7-oxo and the methyl groups have between 10 and 13 single bonds which are rotated during a Monte Carlo cycle (all single bonds between the terminal methyl and the peptide bond were included). In order to limit the computational requirements we have computed the default population of 35 lowest energy minimized structures for each molecule of the series (Li and Scheraga, 1987). The energy of each conformational stereoisomer in the minima found by the Monte Carlo procedure was fully minimized in about 50 iterations. The computation thus provided a list of 35 low energy conformational stereoisomers which were ordered by energy, displayed and analyzed according to their Boltzmann factors determining the percentage of molecules residing in a given conformation.

3. Results and discussion

In all cases computed, i.e. for $n=3$, 4, 5 and 6 the clustering of conformational states into conformational families was observed. The energies of conformational stereoisomers along with their populations given by Boltzmann factors and accounting for more than 5% of the population of molecules are given in Table 1. In each of the four cases considered the number of low lying energy states was different. Significantly, the sum of their occupancies is more than 59% in the case of $n=5$ and up to 89% for $n=6$.In Fig. 1a–d the conformational states of the molecules are presented for the stereoisomers listed in Table 1. It can be stated that conformers of the members of the series with $n=3$, 4 and 5 are clearly different

from the conformation predominant in the $n=6$ case. A significant change in structure of the conformational stereoisomers can be summarised as follows: instead of a compact, rigid, globular like shape present in the analogues with $n=3$ to $n=5$ (Fig. 1a–c) a distinct change of this pattern is seen in the case with $n=6$ (Fig. 1d): an extended hydrocarbon chain becomes available for intermolecular interactions with the solvent environment. In the latter case a distinct kink in the middle section of the hydrocarbon chain in question is observed (at the methylene group $n=3$ counting from the peptide bond). These observations can be correlated to the measured increase in water solubility and decrease in lipophylicity with the change of structure from $n=5$ to $n=6$.

The confomations of the molecules with $n=3$ to $n=5$ are compact with an approximately constant polar surface of the dipeptide part. In all cases we have observed a hydrogen bond connecting the carbonyl oxygen of the 7-oxacyl part of the alkyl chain and the amido nitrogen of the L-alanyl residue. Such a structural feature provides for the intrinsic similarity in the series (with the exceptionally dissimilar longest chain $n=6$).

The proportion of the solvent exposed hydrocarbon chain in the molecular surface increases with increasing alkyl chain length ($n=3$ to $n=5$), thus lowering water solubility. This is consistent with the experimentally observed water solubility data for the series (Planinšek and Srčič, 1999) which also show the decreasing water solubility in the series $n=3$ to $n=5$. As the hydrocarbon chain length gets even longer ($n=6$) there is no more folding around the polar dipeptide part and the freedom of movement can become greater. This exposure of the hydrocarbon chain to the aqueous environment provides a change of the interaction pattern of the molecule with the surrounding solvent for the case $n=6$. Such exposure would indicate the increase in entropy. The effect of an additional methylene group is smaller than for the small (more rigid) substituents and the solution phase properties are not dictated by the parent (polar dipeptide) structure any more.

The most plausible cause for the experimentally observed increased water solubility at hydrocarbon chain length $n=6$ seems to be the possibility of self-association (dimers, trimers, etc.). The exposed alkyl chain in the case $n=6$ appears to be suitable for such association, thus decreasing the entropy of the complex formed. The possibility of micelle formation cannot be excluded: on the contrary, such a process (Buckton et al., 1991) has been proposed to account for the anomalous solubility. The similarity of structures with $n=6$ (Fig. 1d) with detergent molecules corroborate such hypothesis.

We have been able to plot the striking influence of an additional methylene group to the statisti-

Table 1
Conformational analysis of *N*-(7-oxoacyl)-L-alanyl-D-isoglutamines with $n=3$–6 methylene groups between the terminal methyl and the 7-oxo group[a]

Conformer	$n=3$		$n=4$		$n=5$		$n=6$	
	Energy (kcal)	Population (%)	Energy (kcal)	Population (%)	Energy (kcal)	Population (%)	Energy (kcal)	Population (%)
1	−34.227	21.42	−27.872	77.13	−33.125	13.15	−33.060	26.40
2	−34.024	15.21	−26.287	5.31	−33.087	12.33	−32.940	21.56
3	−34.009	14.84			−33.024	11.07	−32.869	19.13
4	−33.957	13.59			−32.963	9.99	−32.749	15.63
5					−32.691	6.31	−32.218	6.37
6					−32.680	6.20		
Total population		65.1		82.4		59.1		89.1

[a] All stereoisomers with Boltzmann population >5% are listed.

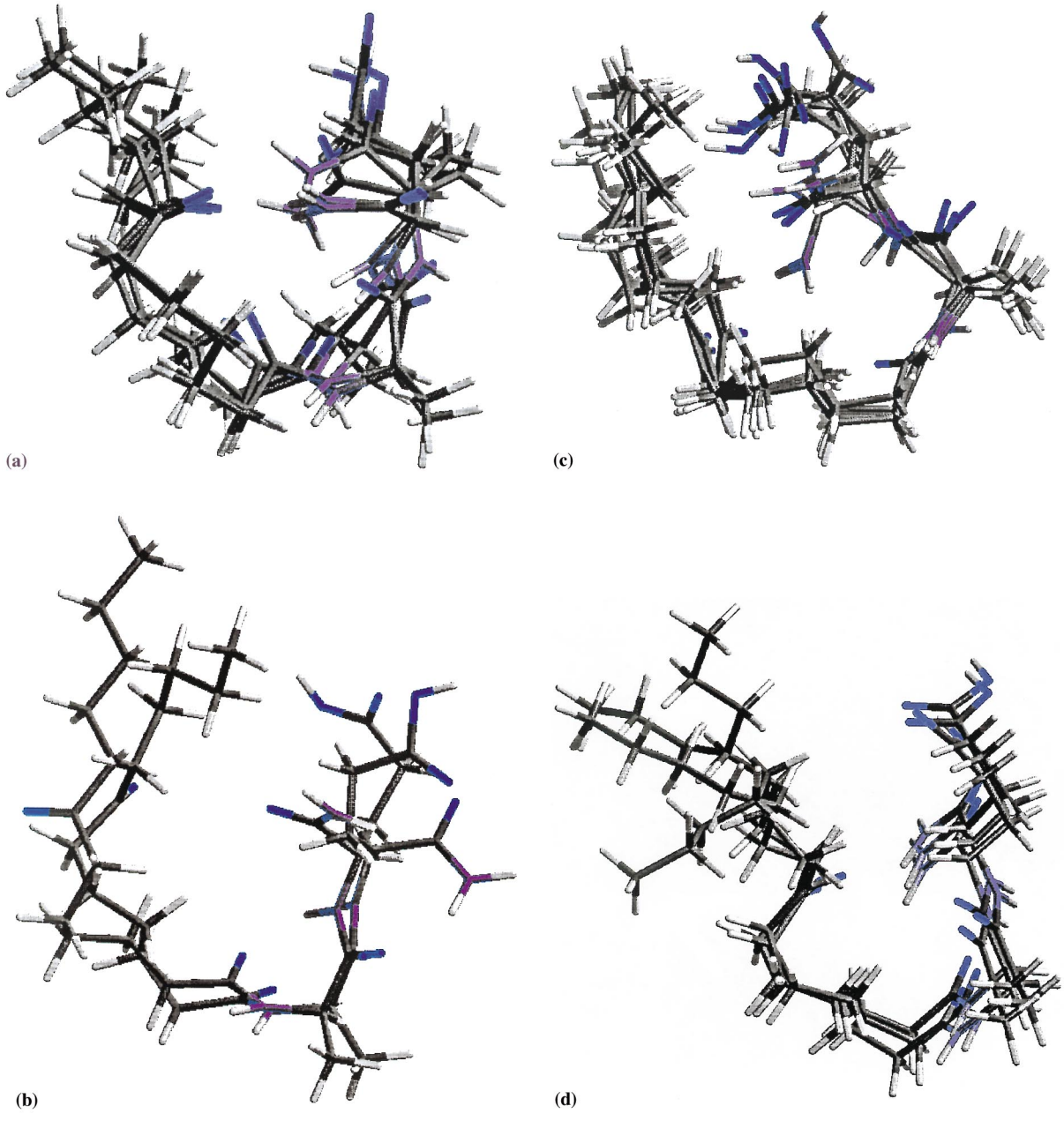

Fig. 1. (a) Conformational stereoisomers of *N*-(7-oxoacyl)-L-alanyl-D-isoglutamines with $n = 3$. Conformers are superimposed with the dipeptide part of the molecule on the right side of the figure. (b) As for (a) but $n = 4$. (c) As for (a) but $n = 5$. (d) As for (a) but $n = 6$.

cally significant conformational stereoisomers population. Such a relationship of aqueous solubility with the molecular structure points to the accommodation of solute molecules in the solvent cavities as the limiting factor as has been observed by Forster et al. (1991) in their study of alkyl *p*-amino and *p*-hydroxybenzoates. It is the subtle balance of hydrophobic and hydrophyllic forces

which controls the greater freedom of movement of the latter while the shorter chains have considerably less flexibility due to their more compact molecular shape.

On the other hand, the anomalous lipophilicity behaviour could be linked to the difference in the molecular conformations present during the crystallisation process. These forces are masked by intramolecular interactions of a predominantly hydrophobic nature for shorter substituent analogs (Planinšek and Srčič, 1999).

The physicochemical properties of compounds which have a common parent structure and differ only by the sequential addition of a methylene group to the alkyl side chain are expected to have a linear variation as a function of the length of the alkyl chain (Buckton et al., 1991). However, several examples in the literature are found where this pattern is broken at a chain length of five carbons. The NMR experimental data of Gillies et al. (1990) obtained by using relaxation techniques show that the carbon atoms C2–C5 of an alkyl chain are much more rigid than their counterparts C6–C8 in the hydrocarbon compounds studied. Greater flexibility of the latter as reflected in their internal correlation times and thus a different solvent structure has been used for the interpretation of the break in the observed solution phase properties such as a decrease in water solubility. Our results concur nicely with this finding: we have shown that a change in the overall conformational structure from the compact form observed for $n = 3$ to $n = 5$ significantly enhances the motional freedom of the hydrocarbon chain with $n = 6$ which in turn can be correlated to the solubility and lipophylicity anomalous behaviour.

The observed differential permeabilising action through biological membranes (Sollner, 1993; Sollner et al., 1996) appears to lead to high immunorestoration activity in vivo.

4. Conclusions

A conformational study using the Monte Carlo approach has given us a plausible clue towards the explanation of the anomalous increase in water solubility and decrease of lipophylicity in the series of *N*-(7-oxoacyl)-L-alanyl-D-isoglutamines with $n = 0–6$ methylene groups between the terminal methyl and the 7-oxo group. Several low energy conformational stereoisomers which occupy approximately 60% or more of the Boltzmann population conformational states fall into conformational families which allow the rationalization of the experimentally observed differences in the series.

Acknowledgements

We are grateful to the Ministry of Science and Technology of Slovenia and Lek. d.d. for financial support. Our thanks are due to a Referee for suggesting numerous improvements in the discussion of the data.

References

Allinger, N.L., Yuh, Y.H., Lii, J.-J., 1989. Molecular mechanics. The MM3 Force field for hydrocarbons I. J. Am. Chem. Soc. 111, 8551–9556.

Buckton, G., Beezer, A.E., Denyer, S.P., Russell, S.J., 1991. Observations on the biopharmaceutical importance of chain length in chemically related compounds. Int. J. Pharm. 73, 1–7.

Chang, G., Guida, W.C., Still, W.C., 1989. An internal coordinate Monte Carlo method for searching conformational space. J. Am. Chem. Soc. 111, 4379–4386.

Forster, S., Buckton, G., Beezer, A.E., 1991. The importance of chain length on the wettability and solubility of organic homologs. Int. J. Pharm. 72, 29–34.

Gillies, D.G., Matthews, S.J., Sutcliffe, L.H., Williams, A.J., 1990. The evaluation of two correlation times for methyl groups from carbon-13 spin-lattice relaxation times and NOE. J. Magn. Reson. 86, 371–375.

Leach, A.R., 1991. A survey of methods for searching the conformational space of small and medium -sized molecules. In: Lipkowitz, K., Boyd, D. (Eds.), Reviews in Computational Chemistry, vol. 2. VCH, New York, pp. 1–55.

Li, Z., Scheraga, H.A., 1987. Monte Carlo minimization approach to the multiple minima problem in protein folding. Proc. Natl. Acad. Sci. USA 84, 6611.

Metropolis, N., Rosenbluth, W., Rosenbluth, M.N., Teller, A.H., Teller, E., 1953. Equation of state calculations by fast computing machines. J. Chem. Phys. 21, 1087.

Planinšek, O., Srčič, S., 1999. Some physicochemical properties of 7-oxoacyl-L-alanyl-D-isoglutamines. Int. J. Pharm. 87, 199–207.

Sollner, M., 1993. Design and synthesis of biologically active 7-oxoacyl-L-alanyl-D-isoglutamines. PhD Thesis, University of Ljubljana, Slovenia.

Sollner, M., Pecar, S., Stalc, A., 1996. The influence of lipophylicity of 7-oxoacyl-L-alanyl-D-isoglutamines on their immunorestoration activity in vivo. Eur. J. Med. Chem. 31, 927–933.

Wavefunction Incorporated, 1995. Spartan version 4.0, Wavefunction Incorporated, 18401 Von Karman Avenue, 370 Irvine, CA 92715, USA.