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CONFORMATIONS OF ANGIOTENSIN II AND ENKEPHALIN RELATED TO N.M.R.

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SUMMARY

Statistical samples of conformations for Angiotensin II and Enkephalin, obtained from a Monte-Carlo method, are models proposed to simulate the behavior of these hormones under different physico-chemical conditions. Analysis of molecular conformations shows that, for the two peptides, extended conformations are more likely to be present in acidic solutions used for N.M.R. measurements and folded conformations due to interactions of charged terminal groups are favoured at neutral pH.

INTRODUCTION

Angiotensin II (ASN - ARG - VAL - TYR - VAL (or ILE) - HIS - PRO - PHE) is a linear octapeptide hormone with presor agent activity. Its conformational properties have been investigated with a wide variety of theoretical and physico-chemical methods (2-13). Enkephalin (TYR - GLY - GLY - PHE - MET (or LEU)) is an endogenous peptide with morphin-like activity. Conformational models of Enkephalin have been proposed on the basis of N.M.R. measurements (14-20) theoretical analysis (21-25) and similarities with morphin and related analgesics (26-28). Discrepancies between proposed conformations may result from differences in experimental conditions as well as from the possibly erroneous hypothesis of the existence of a unique active conformation.

Among techniques giving direct information about the geometry of peptides in solution, N.M.R. is particularly useful because of the correlation established between values of coupling constants and dihedral angles. Relationships of the Karplus type (29 - 32) are generally used for this purpose. But as a good molecular model is one giving the better agreement between calculated and experimental values, one has to test if a statistical sample of molecular structures can sometimes give a better model than a unique optimized conformation. Results from conformational analysis performed with a Monte-Carlo method (33) are presented as a contribution to this problem and molecular models for Angiotensin II and Enkephalin interpreting N.M.R. coupling constants are thus proposed.

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METHODS

Statistical values associated with a sample of peptide chains as well as generation of samples are determined with the method described and used previously (33-35). Potential functions for conformational energy calculations are those previously used (33-35) ; they are taken from Scheraga (36) and Poland and Scheraga (37) and modified in order to take into account hydrophobic effects (33). All side chains, except for GLY, PRO, TYR and PHE, are represented by a composite atom taken from Pletnev et al. (38). The four side chains noted above being represented with all their atoms. This way of doing has been proven to be efficient for statistical calculations (33-35). In the present molecular model charges of side-chains or chain-ends which are ionized depending on the pH are put equal to ± 0.5 e.u. in order to take into account the screening effect of water and counter ions (35). The dielectric constant is fixed at 3.5 for all calculations of coulombic terms.

Cung et al. (32) have established formulaes of the Karplus type for the proton-proton coupling constants J which are well adapted to amino-acids. Two different expressions are given ; one for amino acids with a side-chain and another for GLY for which the coupling constant is averaged over the two H on the C_α . These relations are used for the present calculations.

RESULTS

In the present work, the Monte-Carlo method is applied to four different molecular models of Angiotensin II. Hydrophobicity being simulated as explained and used previously, all models have the two first residues (ASN, ARG) hydrophilic and all others are hydrophobic. Backbone and side chain atoms of hydrophobic residues are considered as hydrophobic in models 1, 2, 3. Conversely, the peptide groups CONH are considered as hydrophilic in model 4. Additional charges make another difference between models. In model 1 the two first residues bear a complementary charge of + 0.5 e.u. as well as HIS_6 and the C-terminal group is negatively charged with -0.5 e.u. In model 2 complementary charges on HIS_6 and C-terminal group are withdrawn. With model 3, only the negative C-terminal charge is eliminated. This last model gives model 4 by defining all peptide groups as hydrophilic. Table I summarizes the features of the four models and indicates the order of magnitude of the pH simulated by this way. Flory's characteristic ratio are also given to indicate the degree of folding of chains. One can see that model 1 (neutral pH) presents the smallest characteristic ratio ; it contains many folded conformations. In this case averaged values of J's are not in agreement with experiment (table II). Samples obtained from model 2, 3, 4 (acidic pH) present more and more extended conformations and the values of the mean coupling constants are closer to experimental data which were obtained at acidic pH (14-20).

Three different models are used for calculations simulating the behavior of Enkephalin. These models differ mainly by charge distributions on the C-terminal group and by hydrophobic or hydrophilic character given to backbone atoms (all side chains are hydrophobic). Properties and results concerning

TABLE I : Molecular models for Angiotensin II and characteristic ratio C_n

Model	Charges (pH)	Hydrophobicity * Side chain	Backbone	$C_n = \langle r^2 \rangle / nl^2$
1	ASN^+ ; ARG^+; HIS^+ ; PHE^- (pH ≈ 7)	VAL, THYR, PRO, PHE are hydro -	hydro -	1.9
2	ASN^+; ARG^+ (pH ≈ 5)	ASN, ARG, HIS are hydro +	hydro -	2.5
3	ASN^+; ARG^+; HIS^+ (pH ≈ 2)		hydro -	3.1
4	ASN^+; ARG^+; HIS^+ (pH ≈ 2)		hydro +	3.5

* hydro - : hydrophobic ; hydro + : hydrophilic.

TABLE II - Averages of $J(H_N - H_{C_\alpha})$ for the models of Angiotensin II

Sequence	Calculated $J(H - H_\alpha)$ for model 1	2	3	4	exp *	exp **
ASN	-	-	-	-	-	-
ARG	6.5	6.7	6.5	6.6	6.5	6.7
VAL	7.7	8.1	7.9	7.9	7.9	7.6
TYR	7.9	6.8	7.2	7.6	7.2	8.0
VAL	5.0	7.0	6.9	7.2	8.0	8.5
HIS	7.8	8.1	6.9	6.1	6.0	7.0
PRO	-	-	-	-	-	-
PHE	8.0	7.2	7.9	8.0	7.3	7.5

* J.D. GLICKSON et al., Biochemistry, 13, 11 (1974)

** H.E. BLEICH et al., Biochemistry, 12, 4950 (1973)

TABLE III - Molecular models for Enkephalin and associated characteristic ratio C_n.

Model	Charges (pH)	Hydrophobicity * Side chain	Backbone	$C_n = \langle r^2 \rangle / nl^2$
1	TYR+ ; LEU- (pH ≃ 7)	hydro -	hydro +	0.73
2	TYR+ (pH ≃ 2)	hydro -	hydro +	1.80
3	TYR+ (pH ≃ 2)	hydro -	hydro -	1.26

* hydro - : hydrophobic ; hydro + : hydrophilic

these models are given in table III. Many chains in model 1 (pH 7) are folded due to ionic interactions and mean values of J's do not agree with experiment which were performed at acidic pH. Results obtained with model 2 agree well with experimental measurements (table IV) and chains are here mainly extended. The neutrality of the C-terminal group promotes the destabilization of folded conformations as ionic interactions have disappeared. Model 3 is composed from extended and folded chain conformations and values of averaged J's are less in agreement with experiment than model 2.

TABLE IV - Averages of $J(H_N - H_{C\alpha})$ for the models of Enkephalin

Sequence	Calculated $J(H_N - H_{C\alpha})$ 1	2	3	exp *	exp **
TYR	-	-	-	-	-
GLY	5.2	5.4	5.3	5.4	6.0
GLY	5.4	5.2	5.2	6.1	6.0
PHE	9.2	7.7	6.4	7.1	7.5
LEU	6.1	7.8	7.3	7.6	8.0

* H.E. BLEICH et al., Proc. Nat. Acad. Sci. USA, 73, 2589 (1976)

** M. ANTEUNIS et al., Biochem., 16, 1462 (1977)

DISCUSSION

For Angiotensin II models adapted to mimic experimental conditions (models 3 and 4) mean values of J's are in much better agreement with experiments than any set of values associated to a particular conformation of the chain. In the samples, only one chain presents values of J's which all are at "distances" lower than 1,5 Hz from corresponding experimental data. Moreover, the conformations presenting the best agreement do not correspond to the lower values of the conformational energy and the lowest energy conformations present some values of J's more distant than 2.5 Hz from experimental data. Thus, one can conclude that there is no relation between a small set of "good conformations" and experimental values of J's and that statistical samples of molecular conformations constitute a better model than a unique conformation for explaining, in the present case, experimental measurements.

For Enkephalin, averaged J's for model 2 are in fairly good agreement with experiment. But, in the present case, it is possible to obtain from the sample of conformations, several different ones giving better agreement with experimental data. All these conformations are characterized by angles Ø of the two last residues equal to -160° or -80°. These results are close to those given by Isogaï et al. (22).

As computed J's are in agreement with experiments in so far as molecular models mimic conditions in which measurements were performed (different charge distributions for different pH's), one can suppose that models simulating pH 7 also give good representations of active molecules. For Angiotensin II as well as for Enkephalin conformations obtained in these conditions are mainly folded ones.

These samples of folded conformations are composed of many different conformations with very similar energies and a detailed analysis of these sets is presently under way.

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