Journal of Computer-Aided Molecular Design, 2 (1988) 55–63
ESCOM

J-CAMD 028

Molecular modeling and dynamics of neuropeptide Y

Alexander D. MacKerell Jr.*

Department of Medical Biophysics, Karolinska Institutet, 104 01 Stockholm, Sweden

Received 12 November 1987
Accepted 15 December 1987

Key words: Molecular modeling; Molecular dynamics; Avian pancreatic polypeptide

SUMMARY

A combination of molecular modeling and molecular dynamics (MD) is used to determine a theoretical structure for neuropeptide Y (NPY). Starting with the X-ray structure for avian pancreatic polypeptide (APP), the substituted amino acids were mutated, the side chains oriented to local potential energy minima, and the entire structure minimized and subjected to an MD simulation. Comparison of the resulting NPY structure with APP X-ray and MD results showed secondary structural elements to be maintained and RMS fluctuations to be similar, although differences in both were observed. The approach presented offers a means to study the structure-function relationships of NPY and other similar polypeptides when combined with pharmacological measurements.

INTRODUCTION

Neuropeptide Y (NPY), a physiological polypeptide of 36 amino acids [1, 2], has been indicated to have a variety of functions, including cardiovascular [3], central neuroendocrine [4] and circadian rhythm [5] regulation, influence on sexual and feeding behaviour [6,7] and as a possible co-transmitter with noradrenaline [8]. Along with NPY there also exists a variety of other polypeptides, including the pancreatic polypeptides and peptide YY, all of which contain 36 amino acids with similar primary sequences [9]. Of these polypeptides only the X-ray structure of avian pancreatic polypeptide (APP) has been determined [10,11], showing the structure to contain a polyproline type II helix and an α-helix which are connected by a type II β-turn. In this communication a structure for NPY is reported, determined using molecular modeling and molecular dynamics (MD) simulations [12–14] and compared with the X-ray structure and MD simulations of APP [15,16], including one performed in the present work.

The combination of molecular modeling and molecular dynamics offers an effective means to obtain an unknown structure. Starting from a known structure, molecular modeling allows altera-

*Present address: Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, MA 02138, U.S.A.

tions of the primary sequence to be made followed by positioning of the altered residues and subsequent energy minimization allowing for an initial best fit structure to be achieved, an approach which has previously been used with NPY [17,18]. From this initial structure, MD simulations can be performed from which a time-averaged structure can be obtained. The advantage of using molecular dynamics is the presence of kinetic energy in the system [19], allowing barriers between local minima to be overcome, leading to a structure fluctuating around a stable potential energy minimum which is locally ergodic [20]. Such an approach has been used previously to determine free enzyme forms of the retinol binding protein [19] and ribonuclease T1 (A.D. MacKerell Jr., L. Nilsson, R. Rigler and W. Saenger, submitted to Biochemistry) when starting with the crystal coordinates for those enzymes in the presence of substrate or inhibitor. In the case of the 36 amino acid polypeptides such an approach should be effective due to the similarity of the primary sequences, the lack of any insertions or deletions in the primary sequence and spectroscopic evidence indicating the solution structures of APP, bovine, canine and ox pancreatic polypeptides to contain approximately the same amount of α-helix as the APP X-ray structure [18,21,22].

METHODS

Molecular modeling of porcine NPY, starting with the 1.4 Å coordinates for APP [10] which were obtained from the Brookhaven Protein Data Bank [23], was performed using the program HYDRA [24]. Mutation and initial positioning of the APP to NPY differing amino acids [25] (Table 1) was done as follows. First, the amino acid being mutated was changed from the APP to the NPY residue while maintaining the position of the backbone atoms (N, Cα, C, O). Subsequently, the side-chain torsion angles were manually rotated such that the angles corresponding to the lowest interaction energy between the side chain and the surrounding protein, according to the parameterization currently supported by HYDRA [24], were achieved. This procedure, starting at position 1, was repeated in succession for all the APP to NPY mutated residues. Following the initial mutation and placement of all the differing residues, polar hydrogen atoms were added using standard geometric parameters and the structure was energy minimized via 200 adopted-basis Newton Raphson (ABNR) steps using the program CHARMM [26]. The resulting NPY structure was considered the 'starting' structure.

Molecular dynamics simulations were performed using the program CHARMM [26] on a NORD-500 computer. Parameters for the simulation were those currently supported by

TABLE I
PRIMARY SEQUENCES OF AVIAN PANCREATIC POLYPEPTIDE AND NEUROPEPTIDE Y [25]

	5	10	15	20	25
APP:	Gly·Pro·Ser·Gln·Pro·Thr·Tyr·Pro·Gly·Asp·Asp·Ala·Pro·Val·Glu·Asp·Leu·Ile·Arg·Phe·Tyr·Asp·Asn·Leu·Gln·				
NPY:	Tyr·Pro·Ser·Lys·Pro·Asp·Asn·Pro·Gly·Glu·Asp·Ala·Pro·Ala·Glu·Asp·Leu·Ala·Arg·Tyr·Tyr·Ser·Ala·Leu·Arg·				

	30	35
APP:	Gln · Tyr · Leu · Asn · Val · Val · Thr · Arg · His · Arg · Tyr · NH2	
NPY:	His · Tyr · Ile · Asn · Leu · Ile · Thr · Arg · Gln · Arg · Tyr · NH2	

CHARMM. For the amidated C-terminal parameters for formamide were adapted for use. The SHAKE method was used to constrain covalent bonds involving hydrogen atoms [27], allowing an integration time step of 0.002 ps. The non-bonded interaction list was cut off at 8.0 Å and updated every 10 steps and the non-bonded interaction potentials were shifted such that both the energies and forces approached zero at a cut-off distance of 7.5 Å [26]. Prior to the simulations the APP 1.4 Å crystal coordinates with polar hydrogen atoms added and the NPY starting structure were energy-minimized for 200 steps using the ABNR method. Initiation of the MD trajectory was performed by instantaneously assigning the atoms with random velocities yielding an overall kinetic energy corresponding to 300 K. Integration of Newton's equations of motion was done using the Langevin algorithm where additional random-force and frictional terms are included to compensate for the lack of solvent in the simulation. For the frictional term, a friction coefficient of 50 ps^{-1} was used [28].

RESULTS AND DISCUSSION

The potential energy of the two simulations as a function of time is presented in Fig. 1A while the RMS difference between time-averaged structures from the two simulations and the APP 1.4 Å crystal structure vs. time is presented in Fig. 1B. As may be seen in the two simulations both the potential energies and RMS differences initially relaxed in a similar fashion followed by approximately stable values, indicating that energetically and dynamically stable structures had been reached. From these stable regions, the time range of 37–107 ps for both simulations was selected for analysis.

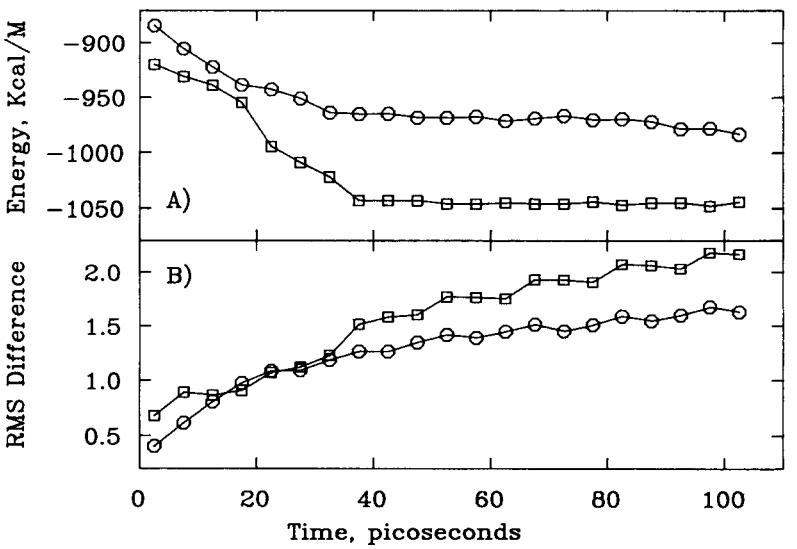

Fig. 1. (A) Potential energy of the APP (□-□) and NPY (○-○) simulations as a function of time. Energies were averaged over 5 ps. (B) RMS difference in Å of all the backbone atom (N, Cα, C, O) positions from the APP (□-□) and NPY(○-○) simulations vs. the APP crystal structure as a function of time. The coordinates were averaged over 5 ps using coordinate sets selected every 0.1 ps. RMS differences were calculated using $\{(\Delta x^2)+(\Delta y^2)+(\Delta z^2)\}^{1/2}$ where Δx, Δy and Δz are the differences between the coordinates for a certain atom following a least-squares fit of all the backbone atoms.

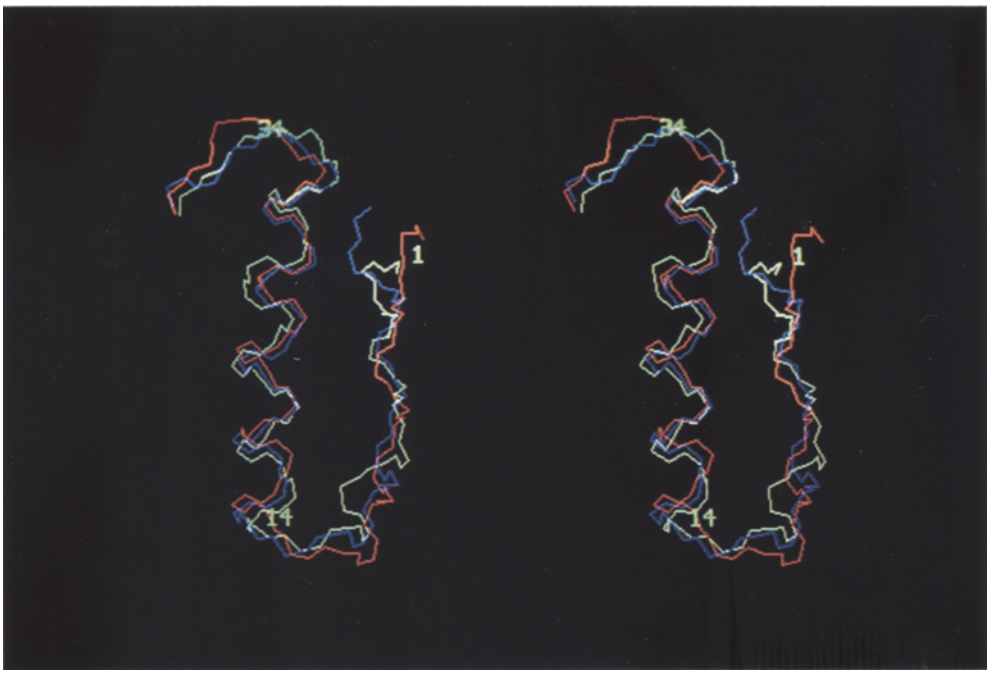

Fig. 2. Superimposed mainchain (N, Cα, C) structures from the 1.4 Å X-ray structure (blue), and the APP (red) and NPY (green) time-averaged simulation structures. The Cα atoms of residues 1,14 and 34 in NPY are labeled. The time-averaged structures were subjected to 100 steps of restrained ABNR energy minimization and all structures were oriented using a least-squares fit of the backbone atoms prior to viewing.

Time-averaged structures for the main-chain atoms (N, Cα, C) from the two simulations and from the 1.4 Å X-ray coordinates [10] are presented in Fig. 2. The three main-chain structures were similar, containing the secondary structural elements observed in the APP X-ray structure. Examination of Table 2 supports this conclusion, showing both the α-helix and the polyproline helix for the structures to contain average φ,ω angles in agreement with the 0.98 Å X-ray [11] and

TABLE 2
AVERAGE Φ,Ψ ANGLES FROM MOLECULAR DYNAMICS TIME-AVERAGED STRUCTURES OF APP [16] AND NPY AND THE APP X-RAY STRUCTURE [11]

Structure	Φ	Ψ
α-Helix		
0.98 Å X-ray structure [11]	−64 (5)	−41 (4)
Crystal simulation [16]	−57	−48
Solvent simulation [16]	−55	−51
APP simulation	−61(2)	−41(2)
NPY simulation	−61(3)	−44(2)
Polyproline helix		
0.98 Å X-ray structure [11]	−72(8)	140(14)
APP simulation	−66(6)	132(15)
NPY simulation	−79(10)	124(12)

The α-helix includes residues 14–31 and the polyproline helix includes residues 2–8 for the Φ angles and 2–7 for the Ψ angles as previously published [11]. Values in parentheses are standard deviations.

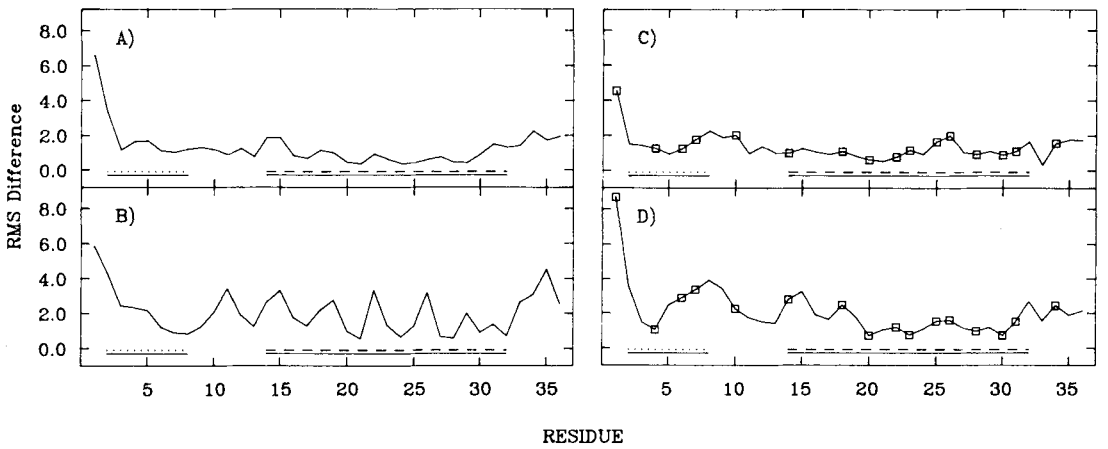

Fig. 3. RMS difference in Å between the APP time-averaged structure and the crystal structure for (A) the backbone (N, Cα, C, O) and (B) the side-chain atoms (Gly O and excluding hydrogens), and between the NPY time-averaged backbone structure and (C) the APP crystal structure and (D) the APP time-averaged simulation structure following least-squares fit of backbone atoms. In C and D, residues marked with a square are those which differ between APP and NPY (see Table 1). All structures were subjected to 100 steps of restrained ABNR energy minimization. Regions underscored with ..... are in the polyproline helix while those underscored with ---- are in the α-helix.

previous MD simulation structures [16]. Importantly, the non-bonded interactions between the polyproline helix (residues 2–8) and the α-helix (residues 14–32) were maintained [10,11], keeping the proteins' tertiary structures intact. Thus, the vacuum MD simulations provided stable structures for both APP and NPY which were similar to the X-ray structure of APP [10,11] and the previously determined solvent and crystal MD structures [16] as well as in agreement with circular dichroism measurements on the α-helix content of APP and other pancreatic polypeptides [18,21,22].

Comparison of RMS differences between the APP time-averaged and the 1.4 Å X-ray structures is presented in Figs. 3A and 3B for the backbone and side-chain atoms, respectively. Overall, the backbone atom positions for the two structures were similar as indicated above (see Table 2), especially in the α-helix region, although a large difference occurred at the N-terminal. Greater differences also occurred with the side-chain atoms of residues which are located on the portion of the α-helix opposite the polyproline helix (Fig. 2) and at both termini, similar to that observed in the previous MD studies [16]. The N-terminal shift is probably attributable to both the N and O backbone atoms of residue 1 chelating the Zn^{+2} atom in the APP crystals [10,22]. In the case of the side-chain atoms opposite the polyproline helix and at the C-terminal the changes are probably related to the loss of protein–protein interactions [10] which occur in the crystal as well as to the increased mobility of those residues due to their lack of interaction with the polyproline helix (see below).

RMS differences between the backbone atom positions from the NPY time-averaged structure and the APP X-ray and time-averaged structures are presented in Figs. 3C and 3D, respectively, while the time-averaged structures are presented in Fig. 4. These results show the structures to be quite similar except at the N-terminal and in the regions around the C-terminal of the polyproline

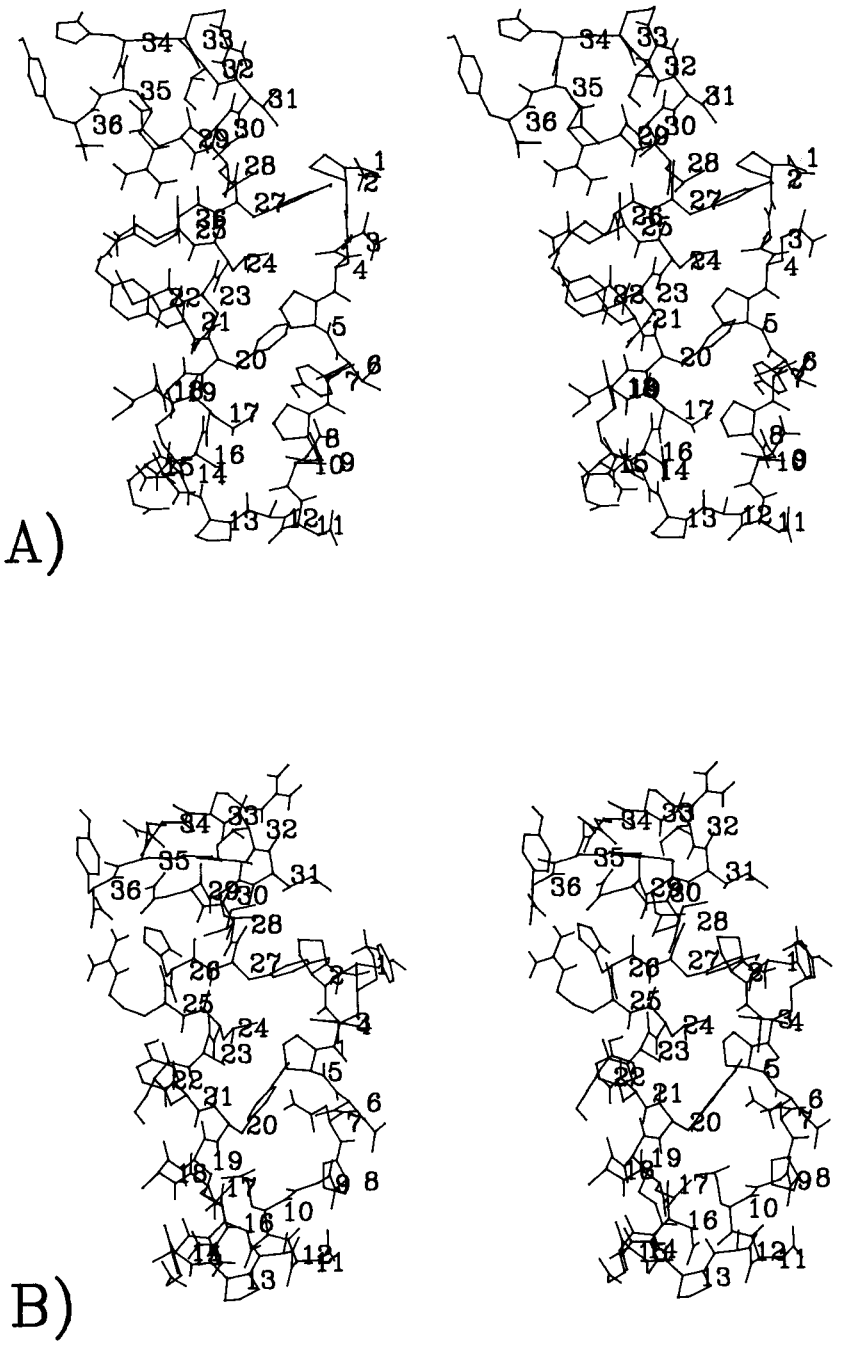

Fig. 4. Time-averaged structures from (A) the APP and (B) the NPY simulations. The structures were subjected to 100 steps of restrained ABNR energy minimization and oriented using a least-squares fit of the backbone atoms prior to viewing.

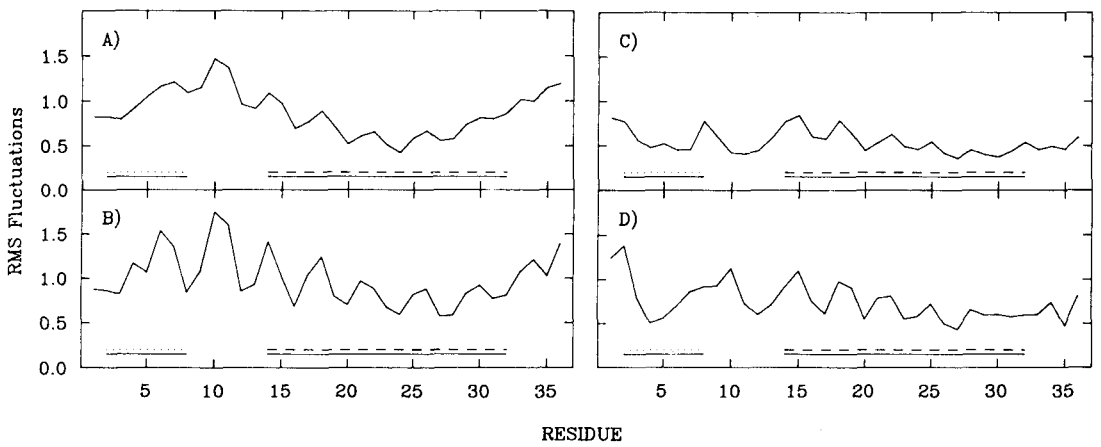

Fig. 5. RMS fluctuations in Å of (A) the backbone (N, Cα, C, O) and (B) the side-chain (Gly O and excluding hydrogens) atoms from the APP simulation, and (C) the backbone and (D) side-chain atoms from the NPY simulation. RMS fluctuations were calculated using $1/3\Sigma(<[(\triangle x_i)^2+(\triangle_i y)^2+(\triangle z_i)^2]>)^{1/2}$ where $\triangle x_i$, $\triangle y_i$ and $\triangle z_i$ are the differences between the coordinates at time i and a reference coordinate set, $< >$ is the time average and the summation is over the backbone or side-chain atoms. Regions underscored with ..... are in the polyproline helix while those underscored with ----are in the α-helix.

helix and the α-helix termini. Along with the interaction of the N-terminal with the Zn^{+2} atom in the APP crystals [10,22], the large difference in the N-terminal position was probably also influenced by the sequence change from Gly in APP to Tyr in NPY (Table 1). This change, when considering the close spatial orientation of position 1 and the C-terminal of the α-helix, along with the sequence differences at positions 25, 26, 28, 30 and 31, may also be responsible for the slight distortion of the NPY α-helix in that region (Fig. 2). Of note, however, is the overall structural maintenance of the α-helix (Fig. 2, Table 2) indicating the conserved nature of many of the mutations which are present.

RMS fluctuations for the backbone and side-chain atoms in the APP and NPY simulations are presented in Figs. 5A,B and 5C,D, respectively. In both simulations the fluctuations were low in the α-helix, with higher mobility at both the N- and C-termini and in the transition region from the polyproline helix to the α-helix (residues 5–15). The results from the APP simulation qualitatively agree with the thermal factors for APP at 0.98 Å resolution [11], although the greatly increased C-terminal mobility in the X-ray study was not seen in the simulations. This difference appears to be due to hydrogen bonding between the C-terminal carbonyl and amide groups and the side-chain atoms of residues 26, 29 and 35 which did not occur in the APP crystals as well as the likelihood that the crystal thermal factor could be due to the C-terminal Tyr occupying a variety of conformational substrates which were not sampled in the MD simulation [11,29]. Again, the RMS fluctuations from the APP simulation were in good agreement with the previous MD work, especially with those for the APP monomer in solution [16]. In general, fluctuations were lower in the NPY vs. the APP simulation, with this difference most pronounced in the region including the polyproline helix and the β-turn. This increased mobility in the APP simulation appeared to be due to less favorable non-bonded interactions between the polyproline helix and the α-helix in the APP time-

averaged structure (−11 kcal/mol) as compared to the NPY time-averaged structure (−51 kcal/mol), which appears to be associated with the amino acid changes at sites 1, 4, 7 and 20. Furthermore, the flexibility in residues 5–15 of the APP simulation may be related to the RMS differences between the time-averaged NPY and APP structures in that region (Fig. 3D).

In conclusion, the presented results show that dynamically and energetically stable structures for APP and NPY were obtained which agree well with results for APP from crystal studies [10,11], from previous MD studies [15,16], and for APP and other pancreatic polypeptides in solution [18,21,22]. These structures, it should be emphasized, are limited by the potentials and assumptions used in the MD calculations including the lack of solvent and, therefore, should be considered working models allowing for the analysis of structure–function relationships within the limitations of the approach and assumptions made. The approach presented will be used as a starting point for further studies combining molecular dynamics simulations and pharmacological in vivo activity and in vitro binding measurements on various NPY fragments from which the structure–function relationships of NPY will be analyzed (A.D. MacKerell Jr., J.M. Lundberg, J.S. Lacroix and A. Hemsen, work in progress).

ACKNOWLEDGEMENTS

The author would like to acknowledge support from the NSF in the form of a NATO Postdoctoral Fellowship and grants from the Knut and Alice Wallenberg Foundation, the Swedish Natural Science Research Council and the Lisa and Johan Grönberg Foundation. Special thanks to Rudolf Rigler and Lennart Nilsson for helpful discussion and access to the facilities on which this work was performed. The author is also grateful to Thore Olausson and the Department of Medical Information Processing, Karolinska Institutet for assistance with and use of the NORD-500 computer.

REFERENCES

1 Tatemoto, K., Carlquist, M. and Mutt, V., Nature, 296 (1982) 659–660.
2 Tatemoto, K., Siimesmaa, S., Jörnvall, H., Allen, J.M., Polak, J.M., Bloom, S.R. and Mutt, V., FEBS Lett., 179 (1985) 181–184.
3 Kalra, S.P. and Crowley, W.R., Life Sci., 35 (1984) 1173–1176.
4 Fuxe, K., Luigi, F.A., Härfstrand, A., Zini, I., Tatemoto, K., Pich, E.M., Hökfelt, T., Mutt, V. and Terenius, L., Acta Physiol. Scand., 118 (1983) 189–192.
5 Zini, I., Pich, E.M., Fuxe, K., Lenzi, P.L., Agnati, L.F., Härfstrand, A., Mutt, V., Tatemoto, K. and Moscara, M., Acta Physiol. Scand., 122 (1984) 71–77.
6 Clark, J.T., Karla, P.S. and Karla, S.P., Endocrinology, 117 (1985) 2435–2442.
7 Stanely, B.G. and Leibowitz, S.F. Life Sci., 35 (1984), 2635–2642.
8 Lundberg, J.M. and Tatemoto, K., Acta Physiol. Scand., 116 (1982) 393–402.
9 Tatemoto, K., Proc. Natl. Acad. Sci. U.S.A., 79 (1982) 2514–2518.
10 Blundell, T.L., Pitts, J.E., Tickle, I.J., Wood, S.P. and Wu, C.-W., Proc. Natl. Acad. Sci. U.S.A., 78 (1981) 4175–4179.
11 Glover, I., Haneef, I., Pitts, J., Wood, S., Moss, D., Tickle, I. and Blundell, T., Biopolymers, 22 (1983) 293–304.
12 Karplus, M. and McCammon, J.A., CRC Crit. Rev. Biochem., 9 (1981) 293–349.
13 McCammon, J.A., Rep. Progr. Phys., 47 (1984) 1–46.
14 Warshel, A., Proc. Natl. Acad. Sci. U.S.A., 81 (1984) 444–448.
15 Haneef, I., Glover, I.D., Tickle, I.J., Moss, D.S., Pitts, J.E., Wood, S.P., Blundell, T.L., Hermans, J. and van Gunsteren, W.F., In Hermans, J. (Ed.) Molecular Dynamics and Protein Structure, Polycrystal Book Service, P.O. Box 26, Western Springs, 1985, pp. 145–147.

16 Kruger, P., Strassburger, W., Wollmer, A. and van Gunsteren, W.F., Eur. Biophys. J., 13 (1985) 77–88.
17 Allen, J., Novotny, J., Martin, J. and Heinrich, G., Proc. Natl. Acad. Sci. U.S.A., 84 (1987) 2532–2536.
18 Glover, I., Barlow, D.J., Pitss, J.E., Wood, S.P., Tickle, I.J., Blundell, T.L., Tatemoto, K., Kimmel, J.R., Wollmer, A., Strassburger, W. and Zhang, Y.-S., Eur. J. Biochem., 142 (1985) 379–385.
19 Åqvist, J., Sandblom, P., Jones, T.A., Newcomer, M.E., van Gunsteren, W.F. and Tapia, O., J. Mol. Biol., 192 (1986) 593–604.
20 Palmer, R.G., Adv. Phys., 31 (1982) 669–735.
21 Noelken, M.E., Chang, P.J. and Kimmel, J.R., Biochemistry, 19 (1980) 1838–1843.
22 Strassburger, W., Glatter, U., Wollmer, A., Fleischerhauer, J., Mercola, D.A., Blundell, T.L., Glover, I., Pitts, J.E., Tickle, I.J. and Wood, S.P., FEBS Lett., 139 (1982) 295–299.
23 Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer Jr., E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M., J. Mol. Biol., 112 (1977) 535–542.
24 Hubbard, R.E., In Ehrenberg, A., Rigler, R., Gräslund, A. and Nilsson, L. (Eds.) Structure, Dynamics and Function of Biomolecules (Springer Series in Biophysics, Vol. 1), Springer-Verlag, Berlin, 1987, pp. 122–126.
25 Tatemoto, K., Proc. Natl. Acad. Sci. U.S.A., 79 (1982) 5485–5489.
26 Brooks, B., Bruccoleri, R., Olafson, B., States, D.J., Swaminathan, S. and Karplus, M., J. Comp. Chem., 4 (1983) 187–217.
27 Ryckaert, J.P., Ciccotti, G. and Berendsen, H.J.C., J. Comp. Phys., 23 (1977) 327–343.
28 Brooks, C.L., Brunger, A. and Karplus, M., Biopolymers, 24 (1985) 843–865.
29 Kuriyan, J., Petsko, G.A., Levy, R.M. and Karplus, M., J. Mol. Biol., 190 (1986) 227–254.

ABBREVIATIONS

NPY: Neuropeptide Y.
APP: Avian pancreatic polypeptide.
ABNR: Adopted-basis Newton Raphson.
MD: Molecular dynamics.