Conformational Analysis of Leu-enkephalin by Molecular Dynamics Method

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Leu-enkephalin (Try-Gly-Gly-Phe-Leu) is an endogenous opioid pentapeptide with biological effects similar to morphine. Researches on its structure and conformational analysis have been reported in past few years[1-6], however, most of the researches on leu-enkephalin have concerned only with the crystal structure. Unlike another enkephalin — met-enkephalin, leu-enkephalin hasn't been fully studied by lots of conformational search methods. The global energy minimum conformation may not be the bioactive form that binds with the opioid receptors, so, to search a set of low energy conformations with energy close to global minimum are nesseary. In this paper, an extensive exploration of the conformation space of Leu-enkephalin have been carried out. Two kinds of MD (quenching and annealing) methods were used for this purpose. The lowest energy structure found with a distance — dependent dielectric constant was Gly — Phe β II '-type turn. Multiple fit have been performed with morphine for all the low energy conformational domains.

1　Methods

In order to identify the low energy conformation, molecular dynamic calculations were performed for this purpose with SYBYL/DYNAMICS option implemented on SGI workstation. Two kinds of molecular conformation sampling(quenching and annealing)were used. In general, it is believed that quenching covers more conformational space per CPU second. However, the conformations generated by annealing usually have lower conformational energies [7]. An extended X-ray structure of leu-enkephalin[8] was first fully optimized using TRIPOS force field then was subjected to MD quenching simulation at 1500 K. The dynamics were run for 10 ps in step of 1 fs with data collection every 0.05ps. The temperature coupling time is 10 fs. The high temperature was chosen in order to overcome high energy conformational barries. All the 200 conformations were fully optimized using the TRIPOS force field [9], when the energy gradient is lower than $0.005 \mathrm{kJ \cdot mol^{-1}} \cdot \mathrm{Å^{-1}}$, the minimization has been stoped. From the 200 minimized structure, all those within 20 $\mathrm{kJ \cdot mol^{-1}}$ above the lowest energy were collected for low temperature quenching and annealing. The low-temperature quenching simulations were run for 5 ps in step of 1 fs with data collection every 0.05 ps at 310 K. The temperature couping time was 10 fs. AMD was carried out for Leu-enkephalin using a distance-depedent dielectric constant $\varepsilon=r$. The system was run at high temperature for 250 fs followed by running at low temperature for 250 fs. Sampling was taken at the end of each low temperature period. Ten intervals were set. The low temperature was set to

50 K and several high temperature had been tried in order to find an appropreciate temperature. The conformations collected from the low-temperature simulations and AMD calculations were all optimized using TRIPOS force field, the criterium for termination of the minimizations is that the norm of the energy gradient is smaller than 0.005 kJ·mol^{-1}· Å^{-1}. All those within 20kJ·mol^{-1} above the lowest energy structure were chosen for multiple fit calculations with morphine.
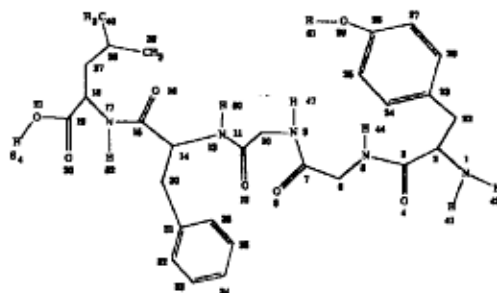

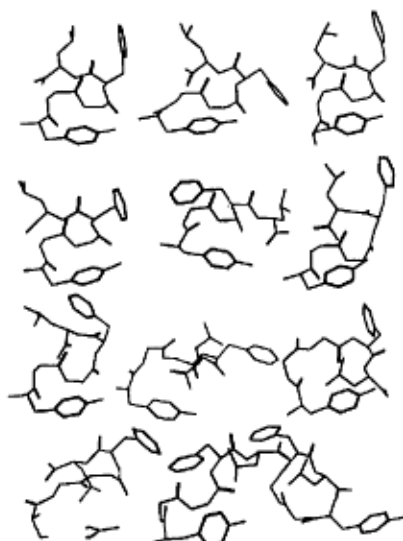Fig.1 The atomic number of Leu-enkephalin

Fig.2 Lowest energy conformers from each of different conformational domains obtained from the two kinds of MD calculations

From left to right and from up to down the domains are labled as A,B,C,D,E,F,G,H,I,J,K,L. Of the 12 domains, domains C,D,E,G,H,J, K obtained from the low temperature quenching simulations. Domains A,B,F,I,L came from the AMD calculations. All hydrogen atoms are omitted for clarity.

2 results and discussion

14 different conformations were founded after the high temperature quenching simulations. The lowest energy was −22.426kJ· mol^{-1}, which was dramatically lower than the energy of the crystal structure which was 35.058kJ·mol^{-1}. However, the low-temperature quenching simulations did not diminish the lowest energy efficently in our calculations, it dropped only 3.130kJ·mol^{-1}, from -22.426 to -25.556kJ·mol^{-1}. On the other hand, the lowest energy of AMD calculation was 13.890 kJ·mol^{-1} lower than that of the lowest energy conformation obtained from the high-temperature quenching simulations. In order to find a more proper high temperature for AMD, several high temperatures had been tested and 300 K was found as an ideal high temperature in our system. All the conformations obtained from the two procedures of low-temperture simulations and AMD were optimized using TRIPOS force field and there were only 320 having differences by at least 30 ° in one backbone, which is the criterion used for uniqueness. The 320 conformations could be grouped into 12 different structure domains according to the rms of the superimpose of two conformations. The lowest energy conformer of each domain was within 20 kJ·mol^{-1} above the lowest energy structures. Figure

2 shows the lowest energy conformer of each domain while their torsional angles are reported in Table 1, the hydrogen bonds are listed in Table 2.

Table 1 The main torsional angles and distances of the lowest 12 conformational domains

Domain					A	B	C	D	E	F	G	H	I	J	K	L
ψ_1	1	2	3	5	-103.5	-22.3	-91.6	-120.3	-126.7	-0.2	-98.8	-116.2	-131.1	145.8	-80.0	117.3
ω_1	4	3	5	6	0.9	3.5	2.8	-0.8	1.4	6.7	1.6	1.8	1.3	7.0	-3.7	-2.2
ϕ_2	3	5	6	7	-69.6	-147.3	-71.8	-68.7	-37.2	-76.6	-73.2	-46.5	-71.3	161.0	-88.6	-71.3
ψ_2	5	6	7	9	71.7	75.4	61.8	75.8	-45.6	61.8	62.0	-31.3	63.9	-164.7	-7.6	72.3
ω_2	8	7	9	10	-3.4	-2.4	-4.0	-0.3	-6.1	-1.3	-3.4	1.3	0.3	-5.0	6.1	4.3
ϕ_3	7	9	10	11	73.0	66.3	49.7	74.8	-70.7	65.8	49.9	-71.2	142.1	7.7	-73.5	67.5
ψ_3	9	10	11	13	-57.8	-71.1	-67.8	-58.9	68.9	-66.1	-86.0	54.8	169.3	72.8	55.6	-74.0
ω_3	12	11	13	14	-7.9	-0.1	-6.8	-9.2	3.1	-9.8	-4.4	2.6	-2.1	-180.0	0.4	-2.0
ϕ_4	11	13	14	15	-143.1	-134.4	-63.3	-152.8	-68.4	-134.3	-70.4	-73.2	-72.3	-131.9	-76.5	-138.4
ψ_4	13	14	15	17	36.0	46.0	-46.3	57.1	68.0	20.5	-37.3	68.1	67.6	49.1	67.2	39.9
ω_4	16	15	17	18	10.1	4.4	-6.6	3.6	3.5	-0.6	-2.3	3.4	1.1	-4.9	4.1	4.4
ϕ_5	15	17	18	19	-146.8	-164.8	-103.8	-155.7	-126.1	-124.2	-76.2	-118.0	-79.0	-141.2	-120.5	-141.8
ψ_5	17	18	19	20	-93.4	-36.1	-135.4	106.7	-14.4	-46.9	115.8	-123.6	-4.9	-129.1	-123.3	-21.1
χ_1^1	1	2	22	23	-175.1	-175.7	-174.6	-168.3	-179.5	81.4	-179.0	178.1	-163.7	67.9	-179.4	-55.6
χ_1^2	24	23	22	2	-106.7	79.7	76.1	75.3	-133.0	146.5	-108.5	-117.2	-103.9	-88.5	-101.2	-86.0
χ_4^1	13	14	30	31	57.2	-55.6	165.8	-173.2	-117.4	64.3	71.9	-61.0	-60.0	-60.1	-60.3	-54.1
χ_4^2	14	30	31	32	99.7	-86.7	-132.6	-107.7	-76.5	-83.2	85.0	-76.1	-75.3	-95.0	-75.7	-87.4
χ_5^1	17	18	37	38	-64.7	-173.7	-53.4	173.8	-54.8	-62.5	-166.9	-53.9	-51.2	45.0	-54.3	-60.1
χ_5^2	18	37	38	39	169.9	60.9	-179.9	58.6	172.3	-52.6	175.6	174.4	-176.5	63.7	173.4	168.6
d1	1–29				7.821	7.915	7.680	7.717	8.023	6.850	7.754	7.761	7.889	6.129	7.876	6.223
d2	29–78				7.162	5.408	7.568	5.182	7.929	7.220	8.608	7.003	5.913	7.057	6.518	13.372
d3	1–78				10.269	11.851	11.958	9.622	4.804	11.011	9.909	5.102	10.929	10.371	6.589	7.802
d4	1–79				5.070	5.172	5.097	5.110	5.267	4.331	5.121	5.145	5.173	3.795	5.154	3.824
d5	1–16				8.057	9.422	8.899	7.327	9.095	8.919	8.934	9.943	9.577	7.196	11.096	5.845
Energy					-36.32	-30.22	-25.56	-22.65	-20.73	-19.31	-17.98	-15.03	-14.86	-13.18	-11.66	-5.66

* Domains A,B,H,I,L were obtained from the AMD calculations. Other domains were obtained from the quenching simulation calculations. The unit of energy is $kJ \cdot mol^{-1}$.

Of the 12 domains, A, B, C, F, L are G-F β II ' turn with varying hydrogen bonding patterns. All of the 5 domains have an interval hydrogen bond between Gly^2 and Leu^5. A, C, L have 4 internal hydrogen bonds. B has 3 internal hydrogen bonds. Domain F are stabilized by 5 internal hydrogen bonds. Other domains are less structural bends. No domain has a G-Gβ- II characteristic internal hydrogen bond (a hydrogen bond between Tyr^1 and Phe^4) just as some crystal structure has shown, it is because the molecule in solvent has a different character from that in crystal form. Multiple fit were carried out for all of the 12 conformers with morphine. The mutiple fit was involved 9 atoms: N_1, O_{21}, and seven atoms of phenol in Tyr^1, since it is widely believed that those atoms are important for recognization with opioid receptor [10,11]. Domain F was believed to be the most similar to morphine since its rms was only 0.0421.

In this paper, two kinds of molecular dynamics methods quenching and annealing, were used to sample the conformational spaces of Leu-enkephalin. Most of the domains obtained from the molecular dynamics calculations are G-Fβ II ' turn and domain F is the most similar to the structure of morphine. We can draw a conclusion that a high temperature quenching combined

with AMD is a better procedure to sample conformational spaces than the procedure of the low temperature quenching after the high temperature quenching.

Table 2 List of the hydrogen bonds observed for the lowest energy conformational domains obtained from quenching simulations and AMD calculations

Domain	H-donor	H-acceptor	Distance	Domain	H-donor	H-acceptor	Distance
A	Leu5(20)	Gly2(44)	1.805	G	Gly2(8)	Phe4(50)	1.782
	Gly2(8)	Leu5(50)	1.776		Gly3(12)	Leu5(52)	1.815
	Tyrl(4)	Gly3(47)	1.843		Gly3(12)	Tyrl(61)	1.703
	Gly3(12)	Tyrl(61)	1.690				
				H	Tyrl(4)	Gly3(47)	1.789
B	Leu5(20)	Gly2(44)	1.733		Gly3(12)	Leu5(52)	1.801
	Gly2(8)	Phe4(50)	1.783		Leu5(20)	Tyrl(61)	1.680
	Gly3(12)	Tyrl(61)	1.676				
				I	Tyrl(4)	Gly3(47)	1.800
C	Leu5(20)	Gly2(44)	1.882		Gly2(8)	Phe4(50)	1.755
	Gly2(8)	Leu5(52)	1.843		Gly3(12)	Tyrl(61)	1.690
	Yyrl(4)	Gly3(47)	1.812				
	Gly3(12)	Tyrl(61)	1.674	J	Leu5(20)	Tyrl(41)	1.860
					Gly2(8)	Leu5(52)	1.705
D	Leu5(21)	Gly2(44)	1.851		Gly3(12)	Tyel(61)	1.656
	Gly2(8)	Phe4(50)	1.813				
	Tyrl(4)	Gly3(47)	1.875	K	Gly2(8)	Phe4(50)	1.799
	Gly3(12)	Tyrl(61)	1.686		Gly3(12)	Leu5(52)	1.870
					Gly3(12)	Tyrl(61)	1.678
E	Gly2(8)	Phe4(50)	1.843				
	Gly3(12)	Leu5(52)	1.740	L	Tyrl(4)	Gly3(47)	1.843
	Gly3(12)	Tyrl(61)	1.674		Gly2(8)	Phe4(50)	1.842
					Leu5(20)	Gly2(44)	1.835
F	Leu5(20)	Gly2(44)	1.804		Leu5(20)	Tyrl(42)	1.724
	Gly2(8)	Leu5(52)	2.246				
	Gly2(8)	Phe4(50)	1.745				
	Gly3(12)	Tyrl(61)	1.692				

Fig.3 Domain F conformation superimposed on the crystal structure of morphine
Multiple fit energy was 3.954kJ·mol^{-1}, rms was 0.0421. 9 pairs of atoms were used for multiple fit: N1 with the N atom in morphine; O21 with the O in 2-cyclohexene-1-ol of morphine; the seven phenol atoms of morphine with the phenol of Leu-enkephalin.

Reference

1 Kenny B,Lipkowitz,Donalld B. Reviews in Computational Chemistry, VCH, New York, 1990, 1

2 Smith G D, Griffin F. *Science*, **1978, 199:1214**

3 Blundell T L, Hearn L, Tickle R A, *et al. Science*, **1979**, 205:220

4 Camerman A, Mastropaolo D, Karle I L, *et al. Nature*, **1983**, 306:447

5 Andr é Aubry, Nikolaos Birlirakis, Maria Sakarellos- Daitsiotis. *J. Chem. Soc., CHEM. COMMUN.*, **1988**:963

6 Morales L B, Garduno-Juarez R, Romero D. *J. Biomol. Struct. Dyn.*, **1991**, 8:721

7 SYBYL Molecular Modeling Software, Tutorial Manual

8 Karle I L, Mastropaolo D, Camerman A, *et al. Acta Crystallogr., Sect. B*, **1983**, 39:625

9 Kolb V M. *Adv. Drug. Res.*, **1987**, 16:281

10 Morley J S. *Br. Med. Bull.*, **1983**, 39:5

11 Jacobson A E. *Handb. Psychopharmacol.*, **1978**, 12:39

亮氨酸脑啡肽构象的分子动力学方法研究

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摘要　采用淬火和模拟退火两种分子动力学的方法对亮氨酸脑啡肽进行了构象搜索，发现了多个 Gly-Pheβ- II' 转角的低能构象. 计算结果表明，高温淬火和模拟退火两者结合起来可以有效地寻找低能构象.

关键词：　亮氨酸脑啡肽，　构象搜索，　分子动力学