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Molecular modeling of interaction between δ opioid receptor and 3-methylfentanylisothiocyanate[1]

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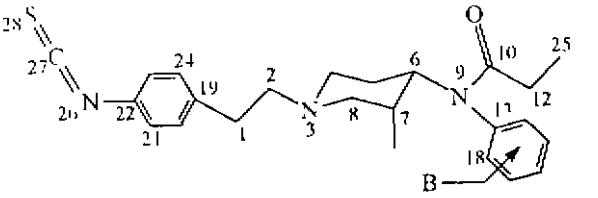
KEY WORDS delta opioid receptors; molecular models; binding sites; ligands; fentanyl

AIM: To construct a 3D structural model of δ opioid receptor (δOR) and study its interaction with 3-methylfentanylisothiocyanate (SuperFIT). **METHODS:** Using the bacteriorhodopsin as a template, the 3D structure of δOR was modeled; SuperFIT was docked into its inside. **RESULTS:** The interaction model between δOR and (3R,4S)-SuperFIT was achieved, in which the important binding sites possibly were Asp128, Ser106, Phe104, Tyr308, and Pro315. Asp128 formed the electrostatic and hydrogen-binding interactions with the protonated nitrogen on piperidine of the ligand. Ser106 formed the electrostatic interaction with the N atom of isothiocyano group of the ligand; whereas Phe104, Tyr308, and Pro315 formed the hydrophobic interactions with the S atom of isothiocyano group. In addition, there were some other interactions between δOR and the ligand. **CONCLUSION:** The residues Phe104, Tyr308, Pro315, and Ser106 of δOR are crucial to the δ selectivity of the ligand, which is beneficial for designing novel δ-selective ligand.

Fentanylisothiocyanate (FIT), 4 stereoisomers of 3-methylfentanylisothiocyanate (SuperFIT) and 8 stereoisomers of ohmefentanylisothiocyanate (OMFIT) were synthesized in our laboratory[1]. The preliminary pharmacological studies[1] revealed that their selectivity for δOR obviously increased, comparing to fentanyl, 4 stereoisomers of 3-methylfentanyl (3-MF) and 8 stereoisomers of ohmefentanyl.

The amino acid sequence of δOR had been determined from its cDNA sequence[2], which belongs to the G protein-coupled receptor (GPCR) family and contains 7 transmembrane (TM) segments, presumably adopting an α-helical conformation. The δOR consists of 372 amino acid residues and the N-terminal is in the extracellular space, the C-terminal the intracellular.

In this paper, to investigate the interaction of δOR with SuperFIT and explain the selectivity of SuperFIT for δOR, we have applied computer-aided model building techniques to generate 3D structural model of δOR.



(3R,4S)-SuperFIT

METHODS

Molecular modeling and display were performed with the molecular modeling package SYBYL 6.2[3] on SGI XZ 4000 workstation.

The 7 putative TM segments[2] were transformed into α-helical conformations with the normal φ and ψ values of −58° and −47°, but proline residue with φ −75°, which caused kinks in the helices. The arrangement of 7 helices of δOR was modeled using the structure of bacteriorhodopsin (bRh) as a template, in which the alignment between δOR and bRh was achieved on the basis of Sander's method[4]. The refined structure of bRh was read from the Protein Data Bank (PDB, entry 1BRD)[5].

The geometry of the receptor was optimized with the conjugate gradient minimizer until the RMS energy gradient <0.7 kcal·mol^{-1}·nm^{-1}, in which the other parameters were a distance-dependent dielectric constant of 5.0, nonbonded cut off of 0.8 nm and AMBER force field with Kollman all-atom charges. The all sidechains of 7 helices with fixed backbone were minimized to convergence; then, the whole receptor to convergence.

(3R,4S)-SuperFIT was docked into the possible binding sites in the 7 helix bundles of δOR. To obtain the better

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interaction model, the docking procedure was repeated 5 times by manually adjusting various starting orientations of the ligand. The ligand-receptor complex was optimized using Tripos force field with Kollman all-atom charges for the receptor and Gasteiger-Huckel charges for the ligand.

RESULTS AND DISCUSSION

Modeling of δOR As the feature of δOR is common to all GPCR and some residues are conserved in all GPCR, it is expected that these receptors have similar 3D architecture. However, no 3D structure has been determined for any of the GPCR.

The 3D structure of bRh, as well as the arrangement of its helices and the position of side chains had been determined by electron cryo-microscopy[5]. Although there is little sequence homology between bRh and visual rhodopsin, a number of GPCR family, both proteins consist of 7 TM segments and bond a retinal molecule at a lysine residue in TM7. They are actived by light with *cis*-trans isomerization of retinal[6]. It seems reasonable to assume that visual rhodopsin and other GPCR, including δOR, have a helix arrangement similar to that of bRh. This assumption was the foundation of modeling 3D structure of δOR in this study.

According to Sander's method[4], when threading a putative TM segment of the receptor through a bRh helix and shifting its sequence ±8 position related to the original alignment (shift = 0), the plot of the sums of residue environmental preferences *vs* threading shift was obtained for each TM segment of δOR, all of which are plotted in Fig 1.

Theoretically, the alignment with the highest score should be chosen. But, the alignment is complicated. So, the several alignments with higher score were applied to the attemptation of modeling δOR. In this way, the final sequence alignments of TM segments between δOR and bRh were selected as Fig 2.

After energy minimization, the 3D structural model of δOR was achieved. In this model, the nonaromatic hydrophobic residues were mainly on the lipid-facing surface of the receptor; whereas the conserved and polar residues chiefly on the inside

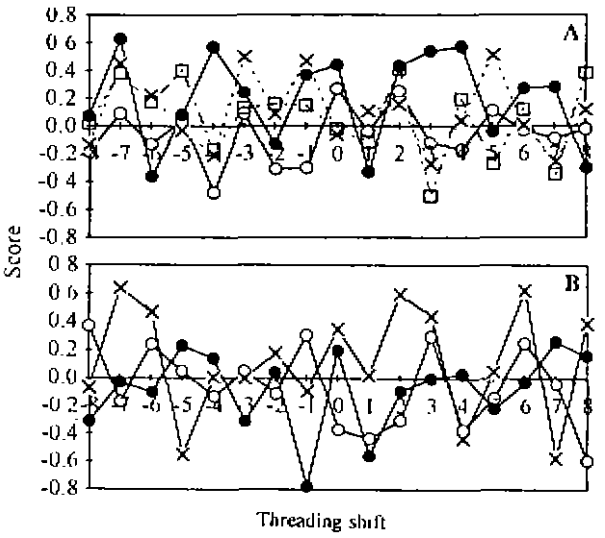


Fig 1. Scores when threading δOR sequence through bRh structure *vs* threading shift. A) TM1 (○), TM2 (●), TM3 (×), and TM4 (□) are the threading shift of 0, 4, −1, and 2, respectively. B) TM5 (○), TM6 (●), and TM7 (×) are the threading shift of 3, 0, and 3, respectively.

TM1	bRh	11	IWLALGTALMGLGTLYFLVKGM	32
	δOR	52	ITALYSAVCAVGLLGNVLVMFG	73
TM2	bRh	38	DAKKFYAITTLVPAIAFTMYLSML	61
	δOR	88	IFNLALADALATSTLPFQSAKYLM	111
TM3	bRh	81	ARYADWLFTTPLLLLDLALL	100
	δOR	123	AVLSIDYYNMFTSIFTLTMM	142
TM4	bRh	108	ILALVGADGIMIGTGLVGAL	127
	δOR	167	LINICIWVLASGVGVPIMVM	186
TM5	bRh	137	WWAISTAAMLYILYVLFFGFT	157
	δOR	215	ICVFLFAFVVPILIITVCYGL	235
TM6	bRh	167	VASTFKVLRNVTVVLWSAYPV	187
	δOR	261	VLVVVGAFVVCWAPIHIFVIV	283
TM7	bRh	204	ETLLFMVLDVSAKVGFGLI	222
	δOR	303	CIALGYANSSLNPVLYAFL	321

Fig 2. Alignment of TM regions of bRh and δOR. The underlined alphabets represent the binding sites, the double underlined are the key residues for δ-selectivity of (3R,4S)-SuperFIT.

surface of the 7 helix bundles, which is accordant with the helical arrangement of GPCR[7].

In the present modeling, loop regions connecting individual helices were omitted, because it was difficult to predict their conformation.

However, omission of the loop regions was not a big problem in the analysis of the receptor-ligand interaction as the ligand-binding sites was supposed to be located in the intra-TM region[8].

Modeling of (3R,4S)-SuperFIT-δOR complex In the above δOR model, there were 3 charged residues in TM region, *ie*, two negatively charged residues (Asp95 and Asp128) and one positively charged residue (His278). Of these amino acid residues, 2 Asp residues were conserved with all GPCR[9]. The Asp128 mutants decreased affinities of all agonists[10]. So, the residue Asp128 would be chosen as the binding site of the protonated nitrogen on piperidine of the ligand. Meanwhile, we considered that the positively charged residue His278, to some extent, probably interacted with the negatively charged carbonyl oxygen of the ligand.

In this way, the protonated (3R, 4S)-SuperFIT was docked into the cavity of 7 helix bundles. The bioactive conformation used in docking was selected as the common conformation of FIT, 4 stereoisomers of SuperFIT and 8 stereoisomers of OMFIT, which was attained by multi-conformational search and DISCO method. Undergoing manual adjustment, the docking procedure was carried out to convergence. The geometric optimization of the ligand-receptor complex was also performed to convergence. The 3D complex model of δOR with (3R,4S)-SuperFIT was achieved (Fig 3).

In the model, the possible binding sites could be the following amino acids (Fig 4): Asp128 (TM3), His278 (TM6), Tyr129 (TM3), Trp173 (TM4), Ser106 (TM2), Phe104 (TM2), Tyr308 (TM7), and Pro315 (TM7).

The negatively charged oxygen of Asp128 formed the potent electrostatic and hydrogen-binding interactions with the positively charged ammonium group on piperidine of SuperFIT. Comparatively, these interactions between His278 and the carbonyl oxygen in 4-phenylpropanamide of the ligand became exceedingly weaker, because the conformation of 4-phenylpropanamide of the ligand led to ligand's carbonyl oxygen far away from His278. There took place some other interactions between δOR and the ligand:

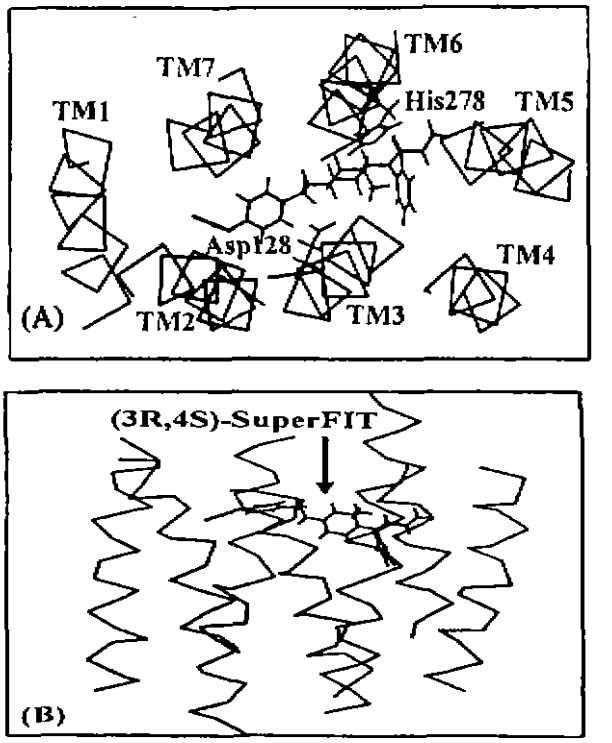


Fig 3. Stereoview of δOR with (3R,4S)-SuperFIT docked at binding sites. Only 2 residues (Asp128 and His278) and the trace of the main chain are displayed. (A) Top view; (B) Side view.

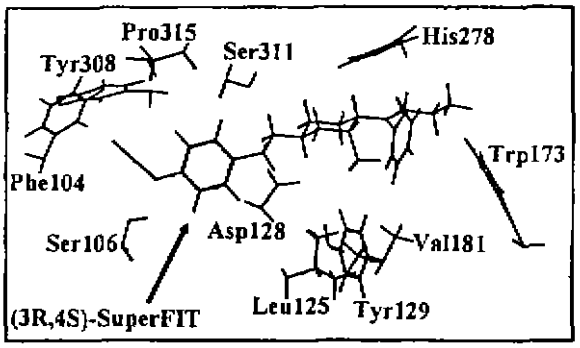
(1) Ser106 bound with the N atom of isothiocyano group of SuperFIT through the electrostatic interaction.

(2) Phe104, Tyr308, and Pro315, which could form one small hydrophobic pocket, might be occupied by the S termini of isothiocyano group of the ligand through hydrophobic interaction.

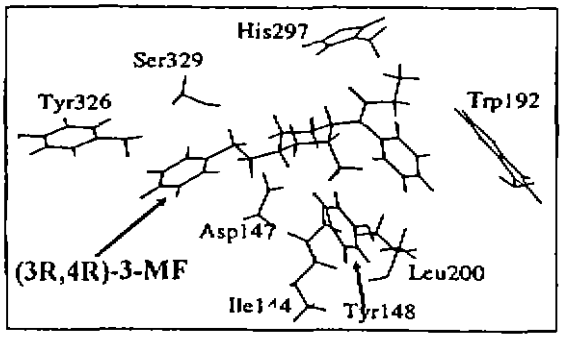
(3) Leu125, Tyr129, and Val181 would also form another small hydrophobic pocket, which might be accommodated by 3-methyl of SuperFIT and resulted in the hydrophobic interaction with the ligand.

(4) Tyr129 and Trp173 formed the very weak $\pi-\pi$ interactions with the phenyl ring in 4-phenylpropanamide of the ligand, because this phenyl ring escaped from the middle of Tyr129 and Trp173.

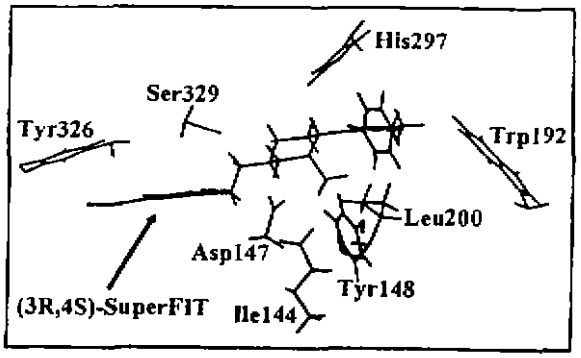
Ligand's key moieties and bioactive conformation From the (3R,4S)-SuperFIT-δOR complex model, it was obvious that the ligand's key



(A) δOR-(3R,4S)-SuperFIT complex model



(B) μOR-(3R,4S)-3-MF complex model



(C) μOR-(3R,4S)-SuperFIT complex model.

Fig 4. The ligand-binding sites.

moieties for interacting with δOR included isothiocyano group, nitrogen atom on piperidine, the carbonyl oxygen and the phenyl ring of 4-propanamide. These moieties are meaningful for us to study 3 dimensional quantitative structure-activity relationship of (3R,4S)-SuperFIT derivatives using comparative molecular field analysis method.

We extracted (3R, 4S)-SuperFIT from complex model, keeping ligand's conformation fixed, and compared this bioactive conformation with the initial conformation used in docking. We also made systematic conformational search for (3R, 4S)-SuperFIT extracted from the complex model, in which the nitrogen atom and 4-carbon atom on piperidine of the ligand kept fixed, and docked each conformation into δOR model. This method was named Sys-Docking, in which the binding energy ($E_{binding}$) of the δOR complex with each conformation was calculated according to the following equation.

$$E_{binding} = E_{complex} - E_{ligand} - E_{receptor}$$

In this way, the complex model with the lowest $E_{binding}$ was obtained, in which the ligand's conformation could be the extremely reasonable bioactive conformation. The bioactive conformations of (3R,4S)-SuperFIT achieved by the above different methods are contrasted in Tab 1, which showed that the values of the torsion angles of rotatable bonds as well as the distances between each key moiety for interacting with δOR are very similar. These facts proved that, not only did the

Tab 1. Bioactive conformations and geometric parameters of (3R,4S)-SuperFIT[a].

Conforms	DISCO[a]	Extracted[b]	Sys-Dock[c]
$\varphi_{19-1-2-3}$°	−178.7	−155.1	−168.3
$\varphi_{1-2-3-8}$°	−88.8	−62.8	−72.1
$\varphi_{7-6-9-13}$°	125.2	123.4	123.7
$\varphi_{12-10-9-6}$°	179.4	176.0	175.4
$\varphi_{25-12-10-9}$°	−176.5	−175.0	−176.8
$\varphi_{6-9-13-18}$°	89.4	106.0	−72.2
$\varphi_{24-19-1-2}$°	−89.5	35.0	62.4
$\varphi_{27-26-22-21}$°	179.9	6.9	−5.5
d_{26-3}(nm)	0.7844	0.7787	0.7961
d_{26-11}(nm)	1.2831	1.2927	1.2974
d_{26-B}(nm)	1.3327	1.3262	1.3649
d_{28-3}(nm)	0.9878	1.0191	1.0252
d_{28-11}(nm)	1.4356	1.4976	1.4811
d_{28-B}(nm)	1.5648	1.5930	1.6159
d_{3-11}(nm)	0.5308	0.5356	0.5360
d_{3-B}(nm)	0.5894	0.5932	0.5989
d_{11-B}(nm)	0.4871	0.4895	0.4892

[a] $\varphi_{i-j-k-l}$(degree) is the torsion angle between i, j, k, and l atom; d_{m-n}(nm) is the distance between m and n atom, 2 key moieties of the ligand for interacting with δ opioid receptor; [a]The bioactive conformation used to docking, which was obtained by multi-conformational search and DISCO method; [b]The bioactive conformation extracted from the complex model; [c]The bioactive conformation achieved by Sys-Docking method.

initial conformation of the ligand used in docking be reasonable, but also δOR and its complex model with (3R, 4S)-SuperFIT had certain reliability. More importantly, this bioactive conformation may be chosen as the starting conformation of ligand for rational drug design.

Comparing δOR model to μOR model It is well known that there are at least 3 major classes of opioid receptors, designated μ, δ, κ, whose amino acid sequences had been determined from their cDNA sequences, respectively[2,11].

In our previous study, the 3D structural model of μOR was built up[12]. The alignment of the amino acid sequences of μOR and δOR revealed 61 % identity. On the basis of the alignment of bRh and μOR as well as that of μOR and δOR, the alignment of each TM region between bRh and δOR was obtained indirectly, which was the same as that listed in Fig 2. This result is reasonable and makes it possible to compare the ligand-binding sites of δOR to those of μOR. The similarities and differences between the δOR and the μOR model are shown in Tab 2.

To elucidate the selectivity of SuperFIT for δOR, we also docked (3R,4S)-SuperFIT into μOR model (Fig 4). Comparing the complex of (3R, 4S)-SuperFIT with δOR to its complex with μOR, we found that, in (3R,4S)-SuperFIT-μOR complex model, there were no the binding sites to interact with the isothiocyano group, *ie*, the residues corresponding to Ser106, Phe104, Tyr308, and Pro315 in the (3R, 4S)-SuperFIT-δOR complex model. This might be one of the reason of the δ-selectivity of SuperFIT.

We also contrasted the (3R, 4S)-3-MF-μOR complex model[12] (Fig 4) with the (3R, 4S)-SuperFIT-δOR complex model and found that there were some evident differences between them. Briefly, the main differences are as the following:

(1) In the (3R,4S)-SuperFIT-δOR complex, there were some interactions between the isothiocyano group of the ligand and Phe104, Tyr308 and Pro315 as well as Ser106; whereas these interactions were not existed in the (3R,4S)-3-MF-μOR complex, due to the lack of isothiocyano group in the ligand.

(2) The ligand's conformations were very

Tab 2. Similarities and differences between δOR and μOR.

δOR	μOR
Asp128 (TM3)[a] formed the potent electrostatic and hydrogen-binding interactions with the protonated nitrogen on piperidine of (3R,4S)-SuperFIT.	Asp147 (TM3)[a] formed the potent electrostatic and hydrogen-binding interactions with the protonated nitrogen on piperidine of (3R,4S)-3-MF.
The interactions between His278 (TM6)[a] and the carbonyl oxygen in 4-phenylpropanamide of (3R, 4S)-SuperFIT are exceedingly weaker, even partly disappeared.	His297 (TM6)[a] formed the weak electrostatic and hydrogen-binding interactions with the carbonyl oxygen in 4-phenylpropanamide of (3R, 4S)-3-MF.
Ser106 (TM2) formed the electrostatic interaction with the N atom of isothiocyano group of (3R,4S)-SuperFIT.	
Phe104 (TM2), Tyr308 (TM7)[a], and Pro315 (TM7) formed one small hydrophobic pocket to interact with the S atom of isothiocyano group of (3R, 4S)-SuperFIT.	Tyr326 (TM7)[a] formed the π − π interactions with the β-phenyl ring of (3R4S)-3-MF.
Leu125 (TM3)[b], Tyr129 (TM3)[a], and Val181 (TM4)[b] formed one small hydrophobic pocket to interact with 3-methyl of (3R, 4S)-SuperFIT, which has steric selectivity.	Ile144 (TM3)[b], Tyr148 (TM3)[a], and Leu200 (TM4)[b] formed one small hydrophobic pocket to interact with 3-methyl of (3R, 4S)-3-MF, which has obvious steric selectivity.
Trp173 (TM4)[a] and Tyr129 (TM3) were nearly unsuitable to the π − π interactions with the phenyl ring in 4-phenylpropanamide of (3R,4S)-SuperFIT.	Trp192 (TM4)[a] and Tyr148 formed the π − π interactions with the phenyl ring in 4-phenylpropanamide of (3R,4S)-3-MF.

[a] The same amino acid; [b] Different amino acid residues in the alignment of TM regions between δOR and μOR.

dissimilar in the above 2 complex models, especially the conformations of 4-phenylpropanamide and β-aromatic ring. In (3R,4S)-3 − MF-μOR complex model, (I) the carbonyl oxygen of the ligand was closer to His297, which was suitable to the weak electrostatic and hydrogen-binding interactions between them; (II) the phenyl ring in 4-phenylpropanamide of the ligand was inserted between Tyr148 and Trp192, which resulted in the π − π interactions between them; (III) the β-phenyl

ring was nearly parallel to the aromatic ring of Tyr326, which was benefit to the π－π interactions between them. However, the similar interactions in (3R,4S)-SuperFIT-δOR complex model became extremely weaker, even partly disappeared, because the conformations of 4-phenylpropanamide and β-phenyl ring of the ligand were nearly unsuitable to the geometric arrangement of corresponding amino residues.

Summarily, the amino acid residues Phe104 (TM2), Tyr308 (TM7), Pro315 (TM7), and Ser106 (TM2) of δOR probably are the key ligand-binding sites to the δ-selectivity of (3R, 4S)-SuperFIT; whereas the residues His297 (TM6), Tyr148 (TM3), Trp192 (TM4), and Tyr326 (TM7) of μOR might be the significant ligand-binding sites to the μ-selectivity of (3R,4S)-3-MF.

In conclusion, the present δOR model and its complex model with (3R, 4S)-SuperFIT are preliminary, and further modification is necessary for quantitative analysis of the receptor-ligand interaction. It is, however, at least qualitatively useful for comprehending the interaction mechanism between δOR and its ligand and for designing novel δ-selective ligand.

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219-224

δ阿片受体与异硫氰基3-甲基芬太尼相互作用的分子模拟

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关键词 δ阿片受体；分子模型；结合位点；配基；芬太尼

目的：构建δ阿片受体(δOR)结构模型并研究它与异硫氰基3-甲基芬太尼(SuperFIT)的相互作用。**方法**：以细菌视紫红质为模板，模拟δOR的结构，并将SuperFIT对接于其内。**结果**：得到δOR-(3R,4S)-SuperFIT作用模型；其中，重要结合位点可能是Asp128，Ser106，Phe104，Tyr308及Pro315。Asp128与配基哌啶环上质子化氮原子形成强的静电和氢键相互作用；Ser106与配基异硫氰基N原子形成静电作用；Phe104，Tyr308及Pro315与异硫氰基S原子形成疏水作用。**结论**：Phe104，Tyr308，Pro315及Ser106对配基的δ选择性极重要，这将有利于设计新的δ选择性配基。