Homology Modeling of Human Leptin/Leptin Receptor Complex

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Leptin receptor mediates the weight regulatory signal carried by the adipocyte-secreted peptide hormone, leptin. It is important to understand the atomic interactions between leptin and the receptor for the therapeutic applications. However, the structure of leptin receptor has not yet been determined. Leptin shows structural similarity to G-CSF, while leptin receptor is similar in amino acid sequence to G-CSF receptor. Because of the similarity between leptin/leptin receptor complex and G-CSF/G-CSF receptor complex, we tried to build a model structure of leptin/leptin receptor complex with the crystal structure of the G-CSF/G-CSF receptor complex as the template. The obtained model for the complex was consistent with the results of the amino acid replacement and deletion experiments. The observation suggests that the model is useful to lead the experimental study on the interaction between leptin and the receptor. © 2000 Academic Press

Key Words: **homology modeling; leptin; leptin receptor; G-CSF; G-CSF receptor.**

Leptin is an adipose tissue-derived cytokine, which suppresses appetite by regulating activities of satiety centers in the brain and has effects on the control of body weight (see Ref. 1 for review). However, recent studies have suggested that leptin is involved in more diverse biological functions than expected previously. For example, it is reported that leptin is involved in the control of bone mass through the inhibition of bone formation (2). Bennet *et al.* (3) revealed that leptin shows proliferative effects of hematopoietic cells. The activity of leptin is mediated by the interaction with the membrane-bound leptin receptor. It is important to understand the mechanism of the interaction between leptin and the receptor, not only for the biological significance of the system, but also for the therapeutic applications. The tertiary structure of leptin has been already solved by Zhang *et al.* (4). On the other hand, neither the tertiary structure of leptin receptor nor the complex structure of leptin and the receptor has been determined yet. Instead, the interaction has been investigated only through amino acid replacements and deletion experiments.

Granulocyte-colony stimulating factor (G-CSF) specifically stimulates colony formation of neutrophilic granulocytes from bone marrow cells (5). G-CSF also exerts its activity through the corresponding receptor. Figure 1 shows a schematic diagram for the primary structure of G-CSF receptor. As shown in the figure, the extracellular region takes a mosaic structure, which consists of an immunoglobulin C_2-like (C2) domain, a cytokine receptor (CK) domain, and three fibronectin type3-like (F3) domains. Recently, the crystal structure of G-CSF complexed with the CK domain of G-CSF receptor has been solved (6). G-CSF receptor is a member of the class I cytokine receptor family, to which leptin receptor also belongs. We performed database searching with FASTA program (7), using the amino acid sequence of G-CSF receptor as a query. Then, we found that leptin receptor shows relatively high similarity in amino acid sequence to G-CSF receptor among the class I cytokine receptor family, although the sequence identity was about 24%. Figure 1 also shows a schematic diagram for the primary structure of leptin receptor. One of the major differences between the receptors is that leptin receptor possesses additional CK and F3 domains in the N-terminal region. However, the deletion and the reconstruction experiments of the extracellular domains suggest that the additional domains are not involved in the leptin binding, but that at least the second CK and F3 domains are sufficient for the leptin binding (8). On the other hand, G-CSF belongs to the long chain helical cytokine family by SCOP (9), as well as leptin. The two cytokines share a four-helix-bundle structure, although they do not show significant sequence similarity to each other. G-CSF binds to G-CSF receptor in a 2:2 ratio. Likewise, it is stoichiometrically demonstrated that leptin binds to leptin receptor in a 2:2 ratio

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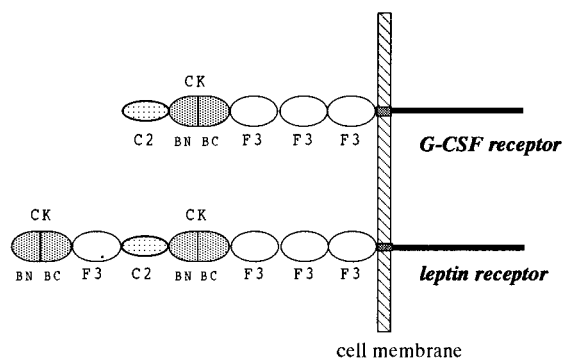
FIG. 1. Schematic diagrams for the primary structures of G-CSF receptor and leptin receptor.

(10). These observations suggest that the binding mode of leptin to the receptor is similar to that of G-CSF to the corresponding receptor. Therefore, we tried to build the model structure of leptin complexed with the CK domain of leptin receptor, using the tertiary structure of G-CSF/G-CSF receptor complex as the template.

MATERIALS AND METHODS

Structure and sequence data used for model building. In this study, the model structure of human leptin/leptin receptor complex was constructed. The structure of G-CSF/G-CSF receptor complex (6) was used as the template for the model structure. The crystal structure of human leptin (4) was introduced into the model structure in a manner described below. The amino acid sequence of human leptin receptor (11) was subjected to the homology modeling. Figure 2 schematically shows the procedure of model building of the CK domain of leptin receptor complexed with leptin. According to the figure, the procedure is explained below.

Replacement of G-CSF with leptin by superimposition G-CSF in the G-CSF/G-CSF receptor complex was replaced with leptin by superimposition. For superimposition, residue-to-residue correspondence or alignment between a pair of structures under consideration is required. As described above, however, the sequence similarity between leptin and G-CSF was too weak to be correctly aligned according to the comparison of the amino acid sequences. Despite the weak sequence similarity, the tertiary structures are similar to each other (4, 6). Therefore, the tertiary structures of leptin and G-CSF were subjected to the structural alignment by the double dynamic programming algorithm. The algorithm was originally developed by Taylor and Orengo (12). Toh (13) recently modified the method by introducing two approximations, a distance cutoff and a ΔN cutoff. In the first version of the program, however, the structural environment of a residue was expressed by a set of residue-to-residue distance and a same value was assigned to the opening and extension gap penalties. The program was recently improved to adopt the vector expression of the structural environment and the affine gap penalty, like the original approach by Taylor and Orengo (12). The latest version of the program was used for the structural alignment. In the obtained structural alignment, the residue pairs constituting α helices were used as the reference of superimposition. Insight II Ver. 97.2 (Molecular Simulations Inc.) was used for the operation. Then, a hypothetical complex between leptin and the CK domain of G-CSF receptor was obtained.

Amino acid sequence alignment between leptin receptor and G-CSF receptor and homology modeling. The amino acid sequence of the CK domain of leptin receptor was aligned with that of G-CSF recep-

tor with an alignment tool, CLUSTAL W (14). The alignment thus obtained was slightly modified by visual inspection (Fig. 3b). According to the alignment, the CK domain in the hypothetical complex of leptin and G-CSF receptor was replaced with that of leptin receptor. At first, the residues of G-CSF receptor were substituted with the corresponding residues of leptin receptor when aligned residues were different from each other. Biopolymer module of Insight II Ver. 97.2 (Molecular Simulations Inc.) was used for the substitutions. At this stage, insertions and deletions were not introduced into the model structure yet. Then, the side-chains of the receptor were subjected to energy minimization, under the condition that the main-chains are restrained by a harmonic function. A protein simulation tool, PRESTO (16) Ver. 3, was used for the operation. Throughout the modeling procedure, energy minimization was performed by the conjugate gradient method with a force field, AMBER C96 (17), and the calculation was applied to the model in the vacuum. A cell multipole method (18) was used for the calculation of the electrostatic interaction. Next, the insertions and deletions were introduced into the model structure of leptin receptor with Insight II, according to the sequence alignment. The inserted segments were generated by the function of the Biopolymer module of Insight II Ver. 97.2. The regions corresponding to insertions and deletions, together with the two residues surrounding each region, were subjected to the energy minimization with PRESTO. Finally, the energy minimization was performed for all of the atoms of the model complex including leptin.

The solvent-accessible surface area was calculated with a program implemented the algorithm by Shrake and Rupley (19). The buried surface area of a protein was obtained as the difference in solvent-accessible surface area between the monomer protein and the corresponding constituent of the complex.

RESULTS AND DISCUSSION

We built a model structure of 2:2 leptin/receptor complex, which is shown in Fig. 4. Like the 2:2 G-CSF/receptor complex (6), the 2:2 leptin/receptor complex had the major and minor interfaces. Reflecting the pseudosymmetry of the 2:2 G-CSF/receptor complex (6), the 2:2 leptin/receptor complex also showed pseudosymmetry. Van der Waals contacts (within 4.2 Å) at the major interface of one 1:1 complex of leptin/receptor occurred between 20 residues of leptin and 21 residues of the receptor with a buried surface area of 961 Å^2, while those of the other 1:1 complex occurred between 18 residues of leptin and 19 residues of the receptor with that of 897 Å^2. The residues involved in the major interface were shown in Fig. 3. The number of residues that constituted the major interface of leptin/receptor was similar to that of G-CSF/receptor. The residue pairs involved in the interactions between leptin and the receptor were shown in Table 1. Especially, the common interactions of the major interfaces between two sets of 1:1 leptin/receptor complex were K5/D617, D9/R615, D85/R468, T12/N566, K15/N567, R20/G440, Q75/443T, 78N/502P, 82N/F504 (polar interaction), and 13L/506L, 86L/506L (hydrophobic interaction).

In addition to the major interface, a leptin molecule had another interface, which is called the minor interface. A leptin molecule in 2:2 complex interacted with two receptors. The receptor interacting with the leptin molecule at the major interface was different from that

G-CSF   leptin   leptin   leptin

G-CSF   G-CSF   leptin   leptin
receptor receptor   receptor   receptor

Structural   Sequence   Energy minimization
alignment   alignment
                                (entire atoms)
   +   +
Super-   Homology
imposition   modeling

                 (1) amino acid replacement
                     & energy minimization
                 (2) introduction of insertions
                     and deletions & energy
                     minimization

FIG. 2. The procedure of model building of the CK domain of leptin receptor complexed with leptin.

at the minor interface. The number of residues involved in the interaction at the minor interface was smaller than that at the major interface. Van der Waals contacts (within 4.2 Å) at the minor interface of one 1:1 complex of leptin/receptor occurred between 7 residues of leptin and 7 residues of the receptor with a buried surface area of 249 Å^2, while those of the other 1:1 complex occurred between 5 residues of leptin and 6 residues of the receptor with that of 158 Å^2. The residues involved in the minor interface were shown in Fig. 3. The number of residues that constituted the minor interface of leptin/receptor was similar to that of G-CSF/receptor. However, the interaction mechanism at the minor interface of leptin/receptor complex may be different from that of G-CSF/receptor complex. The N-terminal region of G-CSF (residues 5–12, Fig. 3a) is a major constituent of the minor interface (6), while leptin lacked the corresponding region. The dimerization of G-CSF receptor is induced by G-CSF, and the minor interface is considered to be important for the dimerization (6). On the other hand, the dimer formation of leptin receptor occurs through receptor-receptor interaction, and leptin may not be required for the dimerization of receptors (8, 20). Therefore, the functional role of minor interface in the leptin/receptor complex may be different from that of G-CSF/receptor complex. Stabilization of the complex is one of the

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a
human G-CSF      5   GPASSLPQSFLLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWAPLSS
                       :          :              :
human leptin     3   -T-----QKVQDDTKTLIKTIVTRINDI---------LDF------I------PGLHPI-
                                           24        39
                     HHHHHHHHHHHHHHHHHHHHHHHH              GGHHHH
                     - ----- HHHHHHHHHHHHHHHHHHH ---------  ------ ------    -

human G-CSF     65   CPSQALQLAGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQLDVADFATTIWQQMEE
                        :: :   : :   :            :
human leptin    49   ----L-----TLSKMDQTLAVYQQILTSM-P-S----RNVIQISNDLENLRDLLHVLAFS
                     HHHHHHHHHHHHHHHHH          HHHHHHHHHHHHHHHHHHHHHHHHHH
                     ---- ----- HHHHHHHHHHHHGGG - - ----HHHHHHHHHHHHHHHHHHHHHHH

human G-CSF    125   LGMAPALQPTQGA--------M-PAFASAFQRRAGGVLVASHLQSFLEVSYRVLRHLAQP
                               :         :    : :: :        :
human leptin    94   KSCHL-PEASGLETLDSLGGVLEASGYS------TEVVALSRLQGSLQDMLWQLDLSPGC
                     -------- -           HHHHHHHHHHHHHHHHHHHHHHHHHHHHH
                     -       GGHHHH     ------HHHHHHHHHHHHHHHHHHHHGGGG

b
mouse G-CSF receptor    95   AGYPPASPSNLSCLMHLTTNSLVCQWEP-GPETHLPTSFILKSFRSRADCQYQGDTIPD-
                             :   : ::       :          :          :  :      :
human leptin receptor  424   ELYVIDVNINISCETDGYLTKMTCRWSTSTIQSLAESTLQLRYHRSSLYCSDIPSIHPIS
                             EEEEEE    EEEEEEE              EEEEEEE            EEEE-

mouse G-CSF receptor   153   ----CVAKKRQN-NCSIPRKNLLLYQYMAIWVQAENMLGSSESPKLCLDPMDVVKLEPPM
                                 :  :     : ::    : :: :  :             : :
human leptin receptor  484   EPKDCYLQSDGFYECIFQP-IPLLSGY-TMWIRINHSLGSLDSPPTCVLPDSVVKPLPPS
                             ----EE     EEEEE       EEEEEEEE   EEE   EEE          E

mouse G-CSF receptor   208   LQALDIGPDVVSHQPGCLWLSWKPWKPSEYMEQECELRYQPQLKGANWTLVPHLPSSKDQ
                                :  :  :: ::        :            :: :        :
human leptin receptor  542   SVKAEITI-----NIGLLKISWEKPVFPE-NNLQFQIRYGLSGKEVQWKMYEVYDAKSKS
                             EEE         EEEEE      EEEEEEEEE          EEEEEE

mouse G-CSF receptor   268   FELCGLHQAPVYTLQMRCIRSSLPGFWSPWS-PGLQLRPTM
                               :   :: : ::  :    : ::  ::  :
human leptin receptor  596   VSLPVPDLCAVYAVQVRCKRLDGLGYWSNWSNPAYTVVMDI
                             EEE      EEEEEEEEE            EEE
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FIG. 3. The alignments used to build the leptin/receptor model. The residue numbers of the leftmost residues in the line are shown at the left of the alignments. The capital letters, H, E and G, under the alignment indicate α helix, β strand and 3_{10} helix. "-" indicates gap for insertion/deletion. ":" between the aligned sequences indicates an alignment site occupied by an identical residue. (a) Structural alignment of human G-CSF and human leptin. The numbers at the bottom of the alignment indicate the residues of leptin neighboring a segment whose coordinates have not been determined yet. The coordinates of a segment of leptin have not been determined yet. The residues neighboring the segment are indicated by the residue numbers written under the alignment. On the basis of the structural alignment, every main chain atom constituting the four α-helices of leptin was superimposed onto that of each G-CSF in 2:2 G-CSF/receptor complex with a rms fit of 1.41 or 1.39 Å. (b) Sequence alignment of the CK domains of mouse G-CSF receptor and human leptin receptor. If the distance between a residue of a leptin and that of a leptin receptor is less than 4.3 Å, the residue pair is regarded as forming interface (red, major interface; light blue, minor interface; green, major and minor interface).

TABLE 1

Residue Interactions Observed in the Major and Minor
Interfaces of Leptin/Receptor Complex

		Leptin 1	Receptor 1	Leptin 2	Receptor 2
Major	Charge	K5	D617	K5	D617
		D8	R615	D9	R615
		D9	R615	D85	R468
		K15	E565	E81	R468
		D85	R468		
	H-bond	_D8_	N567	_T12_	N566
		T12	N566	T12	R615
		T12	N567	K15	N566
		K15	N567	K15	N567
		R20	T443	T16	N566
		R20	_G440_	R20	_Y441_
		Q75	T443	R20	_G440_
		N78	_P502_	Q75	T443
		N82	_F504_	N78	_P502_
				N82	_F504_
				N82	R468
				N82	_L505_
	Hydrophobic	13	L471	13	L506
		L13	L506	L86	L506
		L86	L505	L89	L471
		L86	L506	F92	L471
		V89	L505		
Minor	H-bond	_I3_	E587	_S95_	Q582
		_Q4_	E587	S95	_W583_
		_S93_	_M585_	S95	_M585_
		S95	_W583_		
		S143	Y586		
	Hydrophobic	13	Y586	13	V588
		13	M585	Q4	V588

Note. Hydrogen bond were selected so that the distance between
an oxygen atom of a residue and a nitrogen atom of another residue
is less than 3.5 Å. When the main chain atoms of a residue were
involed in hydrogen bond, the residue was underlined. When the
distance between the carboxyl group of a residue and the amino
group of another residue was less than 5.0 Å, the interaction between
the residue pair was regard as electrostatic interaction. On the other
hand, hydrophobic interaction was selected so that the distance
between the atoms of hydrophobic side-chains of leptin and the
receptor was less than 4.2 Å.

possible roles of minor interface in leptin/receptor
system.

As described above, the binding of leptin to the re-
ceptor has been investigated through amino acid re-
placements and deletion experiments. We found sev-
eral reports on mutation and deletion experiments of
leptin to investigate the binding activity of leptin to the
receptor. To evaluate the accuracy of the model, we
examined the results of the experiments so far with the
model structure.

For the X-ray crystallographic study, W100 of hu-
man leptin is substituted with Glu to improve the
solubility and propensity for crystallization (4). The
mutant protein, leptin-E100, has comparable biological
activity to the native leptin. As shown in Fig. 4, E100

was present on the surface of leptin, but the location of
the residue was apart from the interface region. That is
to say, E100 could not interact with the CK domain of
leptin receptor in the model complex. Thus, the loca-
tion of E100 in the model was consistent with the
result of the amino acid substitution.

Imagawa *et al.* (22) make three human leptin ana-
logs in order to study the structure and function rela-
tionship of leptin. First analog lacks a disulfide bridge
between C96 and C146 by replacing the Cys residues
with Ser residues. Second analog lacks C-terminal re-
gion after S95. Third analog lacks N-terminal region
before C96. The *in vitro* binding activities of the native
leptin and the analogs to the soluble form of lep-
tin receptor are evaluated by autoradiography. The
breakage does not change the binding activity. The
C-terminal deletion decreases the binding activity, but
the analog shows about 40% binding activity. In con-
trast, the N-terminal deletion causes no binding activi-
ty. Then, Imanaga *et al.* (22) concluded that the
N-terminal region is essential for the receptor binding
activity. The N-terminal and the C-terminal regions of
a leptin are colored in orange and yellow in Fig. 4. As
shown in the figure, the N-terminal region of human
leptin faced the receptor to constitute the interface,
while the C-terminal region turned away from the re-
ceptor. Thus, our model structure was also consistent
with the experimental results.

Verploegen *et al.* (23) makes four human leptin mu-
tants, each of which includes single amino acid substi-
tution, in order to identify the critical residues for
receptor binding. The mutants are called R20Q, D40N,
S127D, and R128Q, according to the original residue,
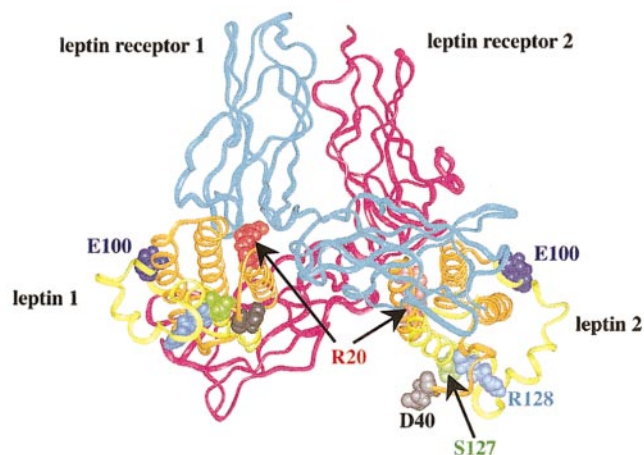the position in the sequence, and the substituted

FIG. 4. Two molecules of the receptors are colored in light blue
and purple, respectively. The N- and the C-terminal regions of lep-
tins are colored in orange and yellow (see text). R20, D40, E100,
S127, and R128 are expressed in the CPK model with different
colors.

residue. The receptor binding activity of R20Q decreases, while the remaining three mutants have binding activities comparable to the native human leptin. The locations of R20, D40, S127 and R128 of leptin are shown in Fig. 4. S127, R128 and D40 did not contact the CK domain of the receptor. In contrast, R20 was a constituent of major interface of leptin/receptor complex. The guanidyl group of R20 formed a hydrogen bond with the carbonyl group of the main-chain of G440, and the hydroxyl group of T443 or the carbonyl group of the main-chain of Y441 of the receptor. Bogan *et al.* (21) reported that there are hot spots of binding energy made up of a small subset of residues in a protein-protein interface and the hot spots are enriched in Trp, Tyr, Arg. There were Y441, R468 and R615 (leptin receptor) and R20 (leptin) in the major interface of leptin/receptor, while W583 and Y586 (leptin receptor) were found in the minor interface (Fig. 3). R20 (leptin) may be one of the constituent residues of the hot spots in the major interface.

Thus, all of the results of experimental studies by three independent groups were consistent with our model structure. Here, we discussed the receptor binding activity of leptin. However, the biological activities or signaling capabilities have also been investigated with mutant leptins. Verploegen *et al.* (23) observed that three mutants, D40N, S127D, and R128Q show reduced biological activity, although they bind normally to leptin receptor. Especially, R128Q cannot trigger intracellular signaling. As shown in Fig. 4, the sites of the single amino acid substitutions form a cluster at one edge of four helix bundle of a leptin molecule, which did not interact with either CK domains. Further experimental and theoretical studies are required to understand the mechanism of signaling by leptin/receptor system. As discussed above, however, our model structure is considered to represent an aspect of the binding of leptin to the receptor. Therefore, we believe that the model is able not only to lead experimental studies for the binding mechanism of leptin/receptor system, but also to provide a theoretical basis for rational drug design.

ACKNOWLEDGMENTS

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