Structural Studies on C-Amidated Amino Acids and Peptides: Function of Amide Group in Molecular Association in Crystal Structures of Val–Gly–NH_2, Ser–Phe–NH_2, Gly–Tyr–NH_2 and Pro–Tyr–NH_2 Hydrochloride Salts

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As part of a series of elucidation of the structural features of peptides caused by C-terminal α-amidation, the crystal structures of H–Val–Gly–NH_2, H–Ser–Phe–NH_2, H–Gly–Tyr–NH_2, and H–Pro–Tyr–NH_2 hydrochloride salts were analyzed by the X-ray diffraction method. Although respective molecules take energetically allowable torsion angles concerning the backbone and side chains, their conformations are not necessarily the same as the corresponding unamidated ones. This results from the different molecular packing requirements, rather than from different conformational features inherent in the C-amidated and -unamidated peptides. As for the molecular packing feature, each peptide tended to form a repeated structure through those hydrogen bonds in which both amide NH and O=C groups participate. The chloride ions are located between the neighboring peptides and are hydrogen-bonded to the respective amide NHs, leading to the sheet structure. The hydrogen-bonding feature of the amide group and its function in molecular packing was discussed based on the results analyzed so far.

Key words H–Val–Gly–NH_2; H–Ser–Phe–NH_2; H–Gly–Tyr–NH_2; H–Pro–Tyr–NH_2; C-terminal amidation; crystal structure; molecular conformation

It is well known that C-terminal α-amidation is essential to the biological emergence of some mammalian and insect peptide hormones.[1,2] In spite of such biological importance of the C-terminal amide group, however, its structural/biological function is not yet well understood. As a possible approach to estimating the function of the amide group, it would be useful to examine the effect of the amide group on molecular conformation and intermolecular interaction. This information would give a clue concerning the function that the amide group plays in the binding of the C-amidated peptide with the receptor. Bearing these in mind, we have been studying the structural features of C-amidated amino acids and peptides by reference to their corresponding C-acids.[3—5] Consequently, it was suggested that the amide group significantly affects the molecular packing pattern, which is clearly different from the case of the C-terminal carboxyl group. In order to further accumulate data on the hydrogen-bonding features of the amide group and its effect on molecular association, this paper deals with the crystal structures of the hydrochloride salts of H–Val–Gly–NH_2, H–Ser–Phe–NH_2, H–Gly–Tyr–NH_2, and H–Pro–Tyr–NH_2. The results showed that the molecular packing is dependent on the hydrogen-bonding feature/ability of the amide group and its interaction mode with chloride ions. These would be useful in terms of the molecular association of C-amidated peptide and its interaction with biological ions. The atomic numberings of C-amidated dipeptides used in this work are given in Fig. 1.

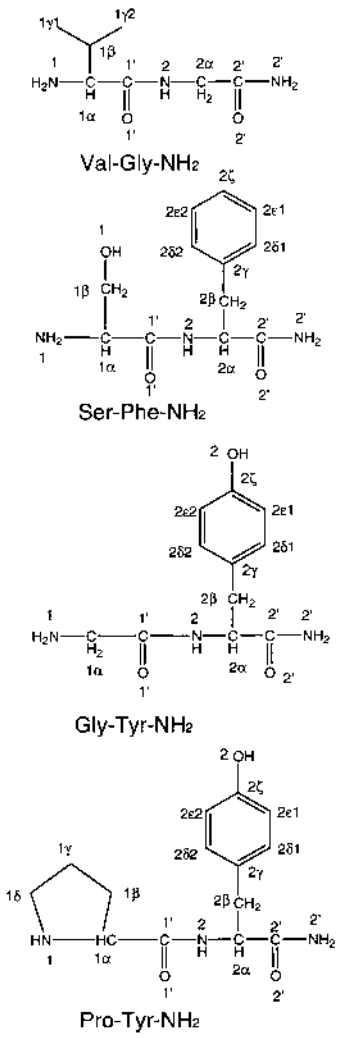

Fig. 1. Chemical Structures of Four C-Amidated Dipeptides and Their Atomic Numberings Used in This Study

Experimental

The C-amidated dipeptides used in this work were purchased from BACHEM AG (Switzerland). Single crystals were obtained from the slow evaporation of aqueous ethanol (H–Val–Gly–NH_2, H–Ser–Phe–NH_2, and H–Gly–Tyr–NH_2) or methanol (H–Pro–Tyr–NH_2) solution at room temperature in the form of transparent needles.

All X-ray measurements were made on a Rigaku AFC-5 diffractometer with graphite-monochromated Cu $K\alpha$ radiation (λ=1.5418 Å) generated by a rotating anode generator operated at 12 kW. A summary of the crystallographic data and structural refinements are given in Table 1. Cell refine-

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Table 1. Summary of Crystal Data Collection and Structural Refinement

	Val–Gly–NH_2·HCl	Ser–Phe–NH_2·HCl	Gly–Tyr–NH_2·HCl	Pro–Tyr–NH_2·HCl
Crystallographic data				
Empirical formula	$C_7H_{15}N_3O_2$·HCl	$C_{12}H_{17}N_3O_3$·HCl	$C_{11}H_{15}N_3O_3$·HCl·H_2O	$C_{14}H_{19}N_3O_3$·HCl·H_2O
Molecular weight	209.68	287.74	291.73	331.80
Crystal system	Monoclinic	Orthorhombic	Orthorhombic	Orthorhombic
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions				
a, (Å)	7.765 (2)	8.226 (2)	8.868 (3)	9.187 (2)
b, (Å)	9.132 (1)	30.027 (2)	27.841 (2)	29.520 (2)
c, (Å)	8.408 (1)	5.605 (2)	5.874 (2)	5.877 (1)
β, (°)	115.40 (1)			
Volume, (Å^3)	538.5 (2)	1384.5 (6)	1450.3 (6)	1593.9 (4)
Z	2	4	4	4
F(000)	224	608	616	704
Crystal size, (mm^3)	0.2×0.1×0.8	0.2×0.1×0.9	0.2×0.05×0.8	0.6×0.1×0.9
Density (calculated), (g·cm^{-3})	1.293	1.381	1.336	1.383
Absorption coefficient, (mm^{-1})	2.973	2.531	2.476	2.321
Data collection				
Scan speed in ω, (° min^{-1})	10	12	10	12
Scan range in ω, (°)	1.73+0.3 tan θ	1.10+0.3 tan θ	1.42+0.3 tan θ	1.84+0.3 tan θ
Index ranges	$0\leqq h\leqq 9$	$0\leqq h\leqq 9$	$0\leqq h\leqq 10$	$0\leqq h\leqq 11$
	$-10\leqq k\leqq 0$	$0\leqq k\leqq 36$	$0\leqq k\leqq 33$	$0\leqq k\leqq 35$
	$-10\leqq l\leqq 9$	$0\leqq l\leqq 6$	$-6\leqq l\leqq 0$	$-6\leqq l\leqq 6$
Max/min. transmission	0.9975/0.5381	0.9988/0.7260	0.9989/0.7438	0.9993/0.4209
θ_{max}, (°) (Cu$K\alpha$)	67.57	67.59	67.60	67.62
No. of unique data measd.	1026	1467	1547	2796
No. of reflections with $I>2\sigma(I)$	992	1373	1227	2611
Structural refinement				
No. of variables refined	118	173	172	200
R (Rw)	0.039 (0.131)	0.028 (0.099)	0.037 (0.111)	0.058 (0.189)
R (Rw) (all data)	0.041 (0.135)	0.033 (0.104)	0.065 (0.135)	0.072 (0.219)
Goodness-of-fit on F^2	1.284	0.935	0.982	1.949
Extinction coefficient	0.054 (8)	0.0063 (10)	—	0.0034 (13)
Largest diff. peak and hole, (eÅ^{-3})	0.364 and −0.270	0.221 and −0.192	0.235 and −0.211	0.521 and −0.420
Max and mean shift/esd	0.039 and 0.010	0.021 and 0.003	0.033 and 0.005	0.052 and 0.011

ments, data collections and data reductions were done using MSC/AFC software.[6] Unit-cell dimensions were determined by a least-squares fit of 2θ angles of 25 reflections ranging from $45°\leqq 2\theta\leqq 55°$. Intensity data less than $2\theta=135°$ were collected at 293 K in a ω–2θ scan mode; the backgrounds were counted for 5 s at both extremes of each reflection peak. The weak intensities ($I<2\sigma(I)$) were rescanned (up to 7 scans) to ensure good counting statistics. The observed intensities were corrected for Lorentz and polarization effects. Empirical absorption corrections were also applied. Four standard reflections were monitored for every 100 reflection intervals throughout the data collection, showing a random variation of $<\pm 2\%$ without significant trends.

Crystal structures were solved by the direct method using the SHELXS97 program.[7] For structural refinement, reflections with $I>2\sigma(I)$ were used, and the atomic scattering factors and terms of anomalous dispersion corrections were taken from *International Tables for Crystallography* (*Vol. C*).[8] The refinement of non-H atoms was carried out by full-matrix least-squares calculations on F_o^2 intensities with anisotropic thermal parameters using the program SHELXL97.[9] H atoms which participated in hydrogen bonds were found from the difference Fourier maps, and others were geometrically located. These were treated as riding with fixed isotropic displacement parameters ($U_{iso}=1.2U_{eq}$ for the associated C or N atoms, or $U_{iso}=1.5U_{eq}$ for methyl C or O atoms); their atomic positions were not included as variables for the refinements. The function of $\Sigma w(F_O^2-F_C^2)^2$ was minimized by using the weighting scheme of $w=1/[\sigma^2(F_O^2)+(0.1000P)^2]$, where $P=(F_O^2+2F_C^2)/3$. Final R [$=\Sigma(|F_O|-|F_C|)/\Sigma|F_O|$]], Rw [$=(\Sigma w(|F_O|-|F_C|)^2/\Sigma w|F_O|^2)^{1/2}$] and S (goodness of fit) [$=(\Sigma w(|F_O|-|F_C|)^2/(M-N))^{1/2}$, where M=no. of reflections and N=no. of variables used for the refinement] are also given in Table 1. In the final stage of refinement, none of the positional parameters of non-H atoms shifted more than one-third from their estimated standard deviations. The final atomic coordinates, anisotropic temperature factors, bond lengths, bond angles, torsion angles of non-H atoms, and the atomic coordinates of H atoms have been deposited in the Cambridge Crystallographic Data Centre, Cambridge University Chemical Laboratory, Cambridge CB21EW, U.K. All numerical calculations were carried out at the Computer Center, Osaka University of Pharmaceutical Sciences.

The average estimated standard deviations for the bond lengths and angles of non-H atoms at the final stage were 0.006 Å and 0.2° for Val–Gly–NH_2, 0.004 Å and 0.2° for Ser–Phe–NH_2, 0.006 Å, 0.007 Å and 0.3° for Gly–Tyr–NH_2, and 0.2° for Pro–Tyr–NH_2.

Results and Discussion

Molecular Conformation By salt formation with hydrochloride, all N-terminal amino or imino groups take on cationic forms. The selected torsion angles defining the respective molecular conformations, together with the corresponding C-unamidated ones, are given in Table 2, where the crystal structures of Ser–Phe–OH (In *et al.*, unpublished data), Gly–Tyr–OH,[10] and Pro–Tyr–OH[11] are available, although that of Val–Gly–OH has not yet been analyzed. To clarify their conformational difference, the stereoscopic superimposition is shown in Fig. 2. As is obvious from this figure, these four peptides could be divided into two kinds of molecular conformations.

Val–Gly–NH_2 and Ser–Phe–NH_2: The same region of (ψ_1, ω_1, ϕ_2, ψ_2) torsion angles create type-II turn-like conformations, which are stabilized by two hydrogen bonds of the O(2′) atom of the neighboring peptide or of the Cl^- anion to the N(1)H and N(2′)H groups for Val–Gly–NH_2 or Ser–Phe–NH_2, respectively. (Table 3). The orientation of the Phe aromatic ring deviates from the most frequently observed one of the C-unamidated counterpart ($\chi^1=ca.$ 180°,

Table 2. Conformational Torsion Angles (°)[a] of Val–Gly–NH_2, Gly–Tyr–NH_2, Pro–Tyr–NH_2 and Ser–Phe–NH_2, Together with Corresponding Unamidated Dipeptides for Comparison

	Val–Gly–NH_2	Ser–Phe–NH_2	Gly–Tyr–NH_2	Pro–Tyr–NH_2
ψ_1	133.0 (3)	135.6 (2)	−168.7 (4)	173.3 (4)
ω_1	169.1 (4)	173.8 (2)	−178.7 (4)	177.1 (4)
χ^1	−58.3 (3)	−51.1 (2)		38.6 (4)
χ^2				−36.6 (4)
χ^3				21.0 (4)
χ^4				3.2 (4)
$\chi^{5c)}$				−25.9 (4)
ϕ_2	87.3 (4)	65.0 (2)	−62.1 (3)	−66.8 (3)
ψ_2[b)]	−0.1 (4)	20.9 (2)	143.2 (4)	132.2 (4)
χ^1		−44.5 (2)	−167.6 (4)	−167.7 (4)
χ^2		−67.8 (3)	−100.7 (5)	−104.1 (4)
		Ser–Phe–OH	Gly–Tyr–OH	Pro–Tyr–OH
ψ_1		153.1 (4)	169.8	162.2
ω_1		157.9 (4)	171.5	174.4
χ^1		−29.8 (4)		−26.0
χ^2				40.2
χ^3				−39.1
χ^4				23.0
χ^5				2.1
ϕ_2		−66.9 (4)	59.9	−67.9
ψ_2		−21.4 (4)	33.4	−35.0
χ^1		−163.3 (4)	170.2	−175.6
χ^2		76.6 (6)	−65.3	

a) The estimated standard deviations for the present X-ray analyses are given in parentheses. *b*) N2–C2α–C2–N2′. *c*) C1δ–N1–C1α–C1β.

χ^2=*ca.* 90°), due to the effect of crystal packing. The molecular conformation appears to be little affected by the difference between the amide and carboxyl groups, although the conformational difference between Ser–Phe–NH_2 and Ser–Phe–OH is significant at a ϕ_2 torsion angle. This means there is no significant contribution of an amide group to the molecular conformation.

Gly–Tyr–NH_2 and Pro–Tyr–NH_2: These peptides form another type of conformation by nearly the same (ψ_1, ω_1, ϕ_2, ψ_2) torsion angles. The extended conformation of N(1)–C(1α)–C(1′)–N(2)–C(2α)–C(2β)–C(2γ) appears to be preferable in crystal packing, because a similar conformation is also observed for the C-unamidated counterpart. Although the amide group is located near the Tyr aromatic ring, no short contacts are observed between them. The Pro cyclic ring takes a C^{β}-*exo* conformation, which is in contrast with the C^{β}-*endo*-C^{γ}-*exo* conformation in Pro–Tyr–OH, although their whole conformations are very similar to each other.

Molecular Association *via* C-Terminal Amide Groups Hydrogen bonds and short contacts less than 3.4A are given in Table 3. In order to estimate the contribution of the C-terminal amide group to the molecular packing, the interaction networks in which the amide groups participate are shown in Fig. 3.

Val–Gly–NH_2: The molecules translated by diad screw symmetry are arranged along the *b*-axis (Fig. 3a). The oxo O(2′) atom as an acceptor is hydrogen-bonded to the amino N(1)H and amide N(2′)H groups of the neighboring molecule, thus forming a turned backbone structure. The chloride ions stabilize molecular packing through three hydrogen bonds (Table 3). The C(1′)···O(2′) short contact could be a carbonyl···carbonyl interaction, frequently observed in the crystal packings of C-amidated amino acids.[4)]

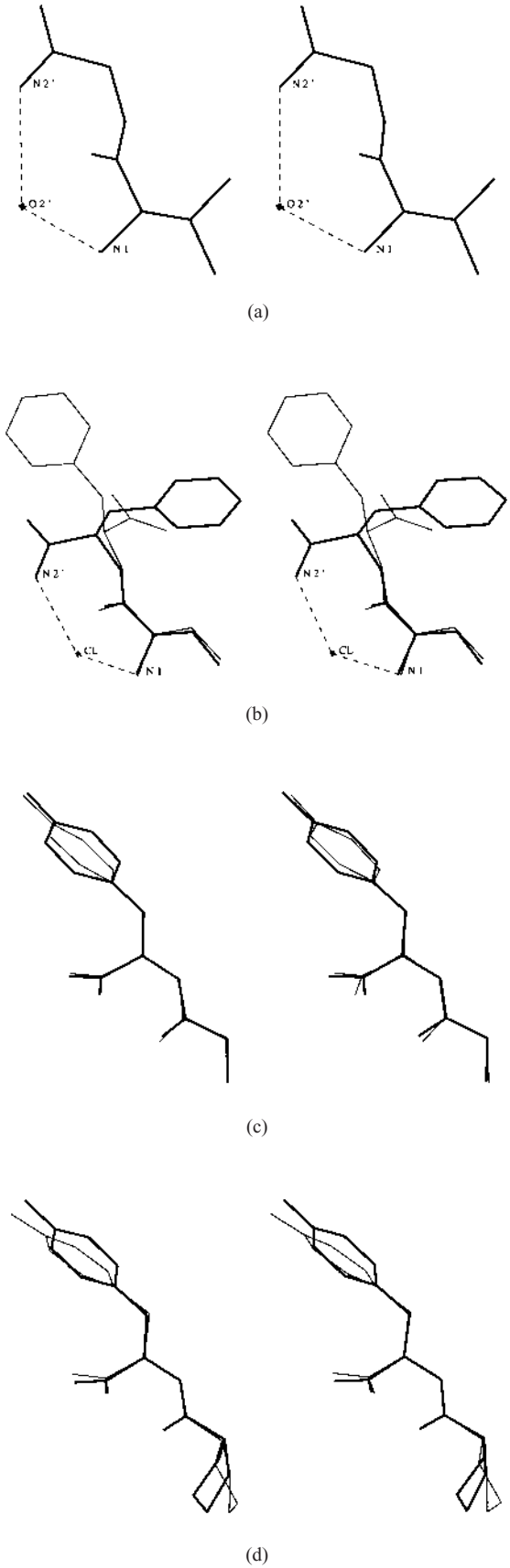

Fig. 2. Stereoscopic View of Molecular Conformations of Val–Gly–NH_2 (a), Ser–Phe–NH_2 (b), Gly–Tyr–NH_2 (c) and Pro–Tyr–NH_2 (d)

The corresponding C-terminal carboxyl peptides, except for Val–Gly–OH, are superimposed on the respective C-amides with thin lines. Two hydrogen bonds of a neighboring O(2′) atom or Cl^- anion to the amino and amide NHs of (a) or (b), respectively, are shown by dotted lines. Hydrogen atoms were omitted for clarity.

Table 3. Hydrogen Bonds and Short Contacts[a)]

Donor (D—H)	Acceptor (A)	Sym. code of A	D···A (Å)	H···A (Å)	D—H···A (°)
Possible Hydrogen Bonds					
Val–Gly–NH_2					
N(1)	O(2′)	1−*x*,*y*+1/2,1−*z*	2.781 (5)	2.01	154
N(1)	O(1′)	−*x*,*y*+1/2,−*z*	2.871 (4)	2.04	153
N(1)	CL	*x*−1,*y*,*z*−1	3.191 (3)	2.27	176
N(2)	CL	*x*−1,*y*,*z*	3.171 (3)	2.32	159
N(2′)	CL	*x*,*y*,*z*	3.241 (4)	2.32	154
N(2′)	O(2′)	1−*x*,*y*+1/2,1−*z*	3.078 (5)	2.26	151
Ser–Phe–NH_2					
N(1)	CL	*x*,*y*,*z*−1	3.159 (2)	2.40	144
N(1)	CL	1/2−*x*,2−*y*,*z*−3/2	3.197 (2)	2.35	154
N(1)	O(1)	3/2−*x*,2−*y*,*z*+1/2	2.874 (3)	2.04	156
O(1)	O(1′)	*x*,*y*,*z*−1	2.709 (3)	1.84	149
N(2)	O(2′)	*x*,*y*,*z*−1	2.746 (3)	1.86	164
N(2′)	CL	*x*,*y*,*z*−1	3.327 (2)	2.45	166
N(2′)	CL	*x*,*y*,*z*	3.640 (2)	2.83	142
Gly–Tyr–NH_2					
N(1)	CL	*x*+1/2,1/2−*y*,1−*z*	3.339 (4)	2.37	171
N(1)	O(1)W	*x*+1/2,1/2−*y*,−*z*	2.772 (5)	1.96	157
N(1)	O(1′)	*x*−1/2,1/2−*y*,−*z*	2.778 (5)	2.02	137
N(1)	O(2′)	*x*−1/2,1/2−*y*,1−*z*	2.984 (5)	2.42	119
O(2)	CL	3/2−*x*,1−*y*,*z*−1/2	3.164 (4)	2.17	165
N(2′)	CL	*x*+1,*y*,*z*	3.329 (4)	2.53	154
N(2′)	O(2)	5/2−*x*,1−*y*,*z*−1/2	2.927 (5)	2.11	157
O(1)W	CL	*x*,*y*,*z*	3.197 (3)	2.15	173
O(1)W	O(2′)	*x*−1/2,1/2−*y*,1−*z*	2.835 (5)	1.93	154
O(1)W	O(1′)	*x*−1/2,1/2−*y*,−*z*	3.127 (5)	2.56	118
Pro–Tyr–NH_2					
N(1)	CL	*x*,*y*,*z*−1	3.241 (4)	2.18	158
N(1)	CL	*x*+1/2,1/2−*y*,1−*z*	3.110 (4)	2.27	175
N(2)	O(2)	*x*+1,*y*,*z*	3.104 (4)	2.13	173
O(2)	O(1)W	*x*−1,*y*,*z*−1	2.614 (5)	1.82	162
N(2′)	CL	*x*−1/2,1/2−*y*,2−*z*	3.454 (4)	2.45	174
N(2′)	CL	*x*−1/2,1/2−*y*,1−*z*	3.345 (4)	2.41	176
O(1)W	O(2)	*x*+1,*y*,*z*+1	2.614 (5)	2.18	105
O(1)W	O(2)	1/2−*x*,−*y*,*z*+1/2	2.887 (5)	2.69	89
O(1)W	O(2′)	*x*,*y*,*z*	2.705 (5)	2.10	113

Short Contacts (less than 3.5 Å)

Atom1	Atom2	Sym. code of Atom2	Distance	Atom1	Atom2	Sym. code of Atom2	Distance
Val–Gly–NH_2							
C(1γ1)	O(1′)	−*x*,*y*+1/2,−*z*	3.434 (6)	C(1α)	O(2′)	1−*x*,*y*+1/2,1−*z*	3.313 (5)
C(1′)	O(2′)	1−*x*,*y*+1/2,1−*z*	3.161 (5)	N(2)	O(2′)	1−*x*,*y*+1/2,1−*z*	3.476 (5)
Ser–Phe–NH_2							
N(2′)	C(1′)	*x*,*y*,*z*	3.156 (3)	N(2′)	O(1′)	*x*,*y*,*z*	3.190 (3)
C(2ε2)	O(2′)	*x*+1,*y*,*z*−1	3.476 (4)	C(1α)	CL	*x*,*y*,*z*−1	3.421 (2)
C(1α)	O(1′)	*x*,*y*,*z*−1	3.298 (3)	C(1β)	O(1′)	*x*,*y*,*z*−1	3.121 (3)
C(2α)	O(2′)	*x*,*y*,*z*−1	3.480 (3)	N(1)	CL	1/2−*x*,2−*y*,*z*−1/2	3.411 (2)
O(1)	C(1β)	3/2−*x*,2−*y*,*z*−1/2	3.312 (3)	O(1)	O(1)	3/2−*x*,2−*y*,*z*−1/2	3.345 (3)
Gly–Tyr–NH_2							
O(1)W	C(1α)	*x*,*y*,*z*	3.353 (5)	O(2′)	O(1′)	*x*,*y*,*z*+1	3.050 (5)
C(1α)	O(1′)	*x*−1/2,1/2−*y*,−*z*	3.243 (5)	C(1α)	O(2′)	*x*−1/2,1/2−*y*,1−*z*	3.326 (5)
C(1α)	O(1)W	*x*+1/2,1/2−*y*,1−*z*	3.128 (5)	O(1)W	C(1′)	*x*−1/2,1/2−*y*,1−*z*	3.066 (5)
N(2)	O(1)W	*x*+1/2,1/2−*y*,1−*z*	3.271 (5)				
Pro–Tyr–NH_2							
O(1′)	CL	*x*,*y*,*z*−1	3.452 (4)	C(2α)	O(2′)	*x*,*y*,*z*−1	3.485 (5)
C(2β)	O(2′)	*x*,*y*,*z*−1	3.472 (5)	C(2δ2)	O(2′)	*x*,*y*,*z*−1	3.460 (5)
O(1)W	C(2ε2)	*x*+1,*y*,*z*+1	3.480 (5)	O(1)W	C(2ζ)	*x*+1,*y*,*z*+1	3.458 (5)
C(1δ)	O(1′)	*x*+1/2,1/2−*y*,1−*z*	3.224 (7)				

a) The estimated standard deviations (esds) for atomic distances of non-H atoms are given in parentheses. The esds for those distances and angles including H atoms are not given because of the fixed refinement of H atoms.

Ser–Phe–NH_2: The molecules are piled up along the *c*-direction (Fig. 3b). The chloride ions are located among the molecules, and stabilize the piling through the hydrogen bond/electrostatic interactions of N(2′)H···Cl^-···N(2′)H. The chloride ion also forms a hydrogen bond with N(1)H, leading the molecule to a folded backbone structure similar

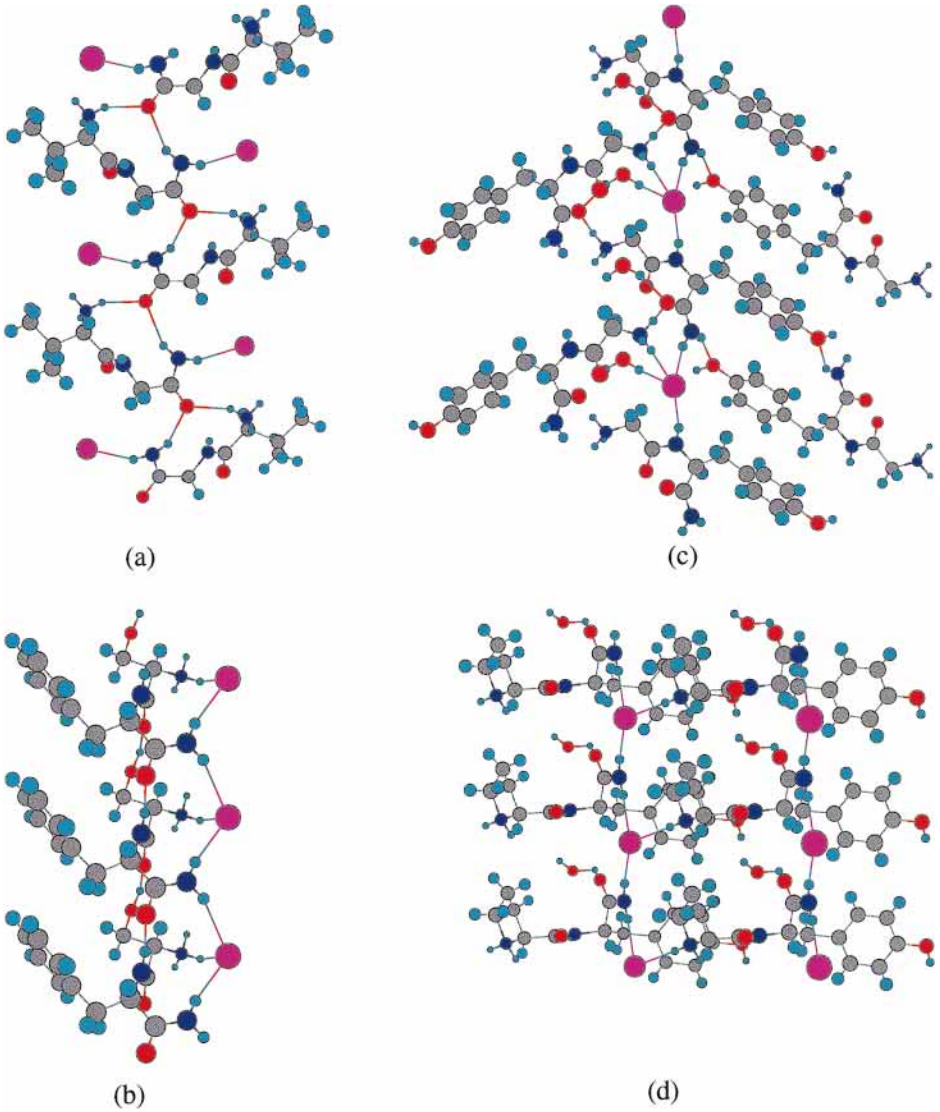

Fig. 3. Molecular Association Formed by Interactions of Amide Groups and Chloride Ions. Val–Gly–NH_2 (a), Ser–Phe–NH_2 (b). Gly–Tyr–NH_2 (c) and Pro–Tyr–NH_2 (d)

The respective atoms are classified by different colors: Cl^- (pink), O (red), N (purple), H (green). The thin lines between the polar atoms represent hydrogen bonds or short contacts.

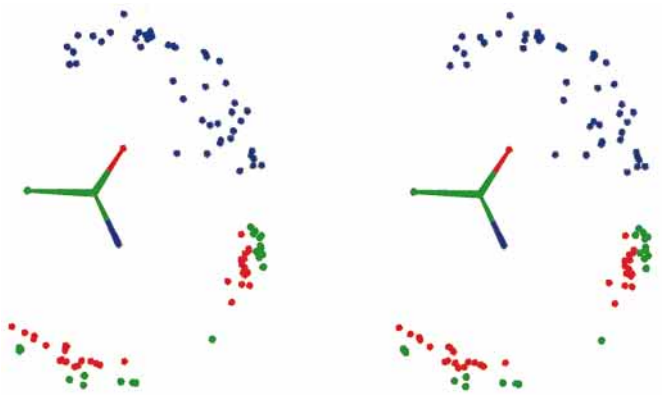

Fig. 4. Stereoscopic Views of Spatial Distribution of Acceptor O Atoms or Cl^- Ions and Donor OH or NH Groups Hydrogen-Bonded to a C-Terminal Amide Group

The donor groups, acceptor atoms and acceptor ions are shown by dots with purple, red and green colors, respectively. The purple- and red-colored bonds show the amide NH_2 and oxy O, respectively.

to that of Val–Gly–NH_2.

Gly–Tyr–NH_2: The amide group also plays the role of connecting the neighboring molecules, similar to other C-amidated peptide crystals. As is shown in Fig. 3c, however, the mode of linkage is specific in such a way that the N(1)H, N(2)H and amide N(2′)H groups of three neighboring molecules are hydrogen-bonded to the same chloride ion, leading to molecular arrangement along the *c*-direction. This linkage forms two different layers consisting of Gly and Tyr aromatic moieties. The hydrogen bonds of the N(1) amino group to a chloride ion and oxy O(2′) atom expand the molecular linkage the *b*-direction.

Pro–Tyr–NH_2: The molecular packing of Fig. 3d appears to be similar to Gly–Tyr–NH_2. The neighboring amide groups and chloride ions form a continuous hydrogen-bonded linkage of $\cdots HNH\cdots Cl^-\cdots HNH\cdots$, which runs almost perpendicular to the extended molecular conformation. The ring orientations of Pro and Tyr side chains are stabilized by two O(2)H$\cdots$O(W1) and O(2)$\cdots$HN(2) hydrogen bonds, and by a N(1)H$\cdots Cl^-$ hydrogen bond, respectively.

Binding Mode between Chloride Ion and Amide Group By salt formation with hydrochloride, the peptides take a monocationic structure protonated at the N-terminal amino or imino group. In this situation, the amide groups participate directly in the formation of continuous pleated molecular association through the hydrogen bonds with chloride ions, in addition to the neighboring amide and/or other polar atoms. It is important to note that these interactions combine the molecules into a 'head-to-head' parallel arrangement, leading to a unique binding mode between the Cl ion and amide group. This is in contrast to the tendency of the C-terminal carboxyl group to form a linear 'head-to-tail' molecular connection *via* the hydrogen bonds, which is one of the most popular packing patterns in C-unamidated peptides.[3] The characteristic interaction mode in Ser–Phe–NH_2 and Pro–Tyr–NH_2 crystals (Figs. 3b, d), the hydrogen-bonded linkage of Cl^- ions to two neighboring amide NH_2 groups translated by one unit-cell, is most frequently observed in the crystal structures of C-amidated amino acid hydrochloride salts.[4] The Cl^- ions in Gly–Tyr–NH_2 (Fig. 3c) also show an interaction mode similar to Fig. 3b, where the peptide N(1)H and amide NH are hydrogen-bonded to Cl^- ions. On the other hand, the molecular association of Val–Gly–NH_2 (Fig. 3a) is rather exceptional, and belongs to a pattern frequently observed in the crystal structure of a free form C-amidated peptide.[3] The pleated sheet structure is primarily formed by the O(2′)···N(2′)H hydrogen bonds, and Cl^- ions secondarily stabilize the structure by a hydrogen bond with amide NH_2.

Hydrogen Bonding Character of the Amide Group The crystal structures showed that a C-amidated peptide could adopt different crystal packing from the corresponding unamidated one, mainly due to the different hydrogen bonding character/ability of the amide and carboxyl groups. In order to clarify the hydrogen-bonding feature of the amide group, the statistical distribution of the acceptor atoms or donor groups, which are hydrogen-bonded to the amide group, is shown in Fig. 4. Judging from the spatial disposition of acceptor atoms into two separated regions, it could be said that the amide NH_2 forms two relatively strong hydrogen bonds with these atoms. On the other hand, the oxy O atom forms one to two hydrogen bonds with the donor NH or OH groups, depending on the interaction pattern with the neighboring molecules. Since these donor groups are widely distributed so as to surround the oxy O atom, this hydrogen-bonding force would not be strong enough to fix the NH position in a definite position. This suggests that the hydrogen bonds *via* the amide NH_2 are primarily used for molecular association with the neighboring acceptor atoms, and those *via* the O of the amide group serve secondarily to the association. In contrast, the reverse tendency has been reported for the anionic carboxyl oxygen atoms,[3,4] *i.e.*, potent and less potent abilities of the oxy O and anionic O^- as hydrogen-bonding acceptors, respectively. Therefore, the different association modes of C-amidated and -unamidated peptides could be related to the different hydrogen-accepting abilities of their oxy O atoms.

Function of Amide Group for Molecular Association To consider the function of an amide group in the association with neighboring molecules, the relative orientation of the neighboring peptides or chloride ions (which are linked to the amide group by hydrogen bonds or short contacts) with respect to the amide group of the host peptide is shown in Fig. 5. Several common features were observed. The number of neighboring peptides hydrogen-bonded to an amide NH_2 group or oxy O atom of the host peptide is usually restricted to one, because of the limited space for the interaction. Chloride ions and/or solvent waters occupy the remaining spaces. Interestingly, when the oxy O atom of an amide group forms a hydrogen bond with the donor group of a neighboring molecule, the *trans*-oriented amide NH (with respect to the C–O bond) always participates in a hydrogen bond with the acceptor atom of the other molecule, as shown in Val–Gly–NH_2 and Gly–Tyr–NH_2 (Figs. 5a, c). In these peptides, the *cis*-oriented amide NH usually forms a hydrogen bond with a chloride anion. A similar association is also formed in the crystal structure of hydrochloride salts of Tyr–Lys–NH_2.[3] On the other hand, Figs. 5b, d correspond to the absence of direct binding of peptides to an amide NH_2 group. In this case, chloride ions occupy the acceptor position and form a continuous ···HNH···Cl^-···HNH··· hydrogen-bonded linkage.

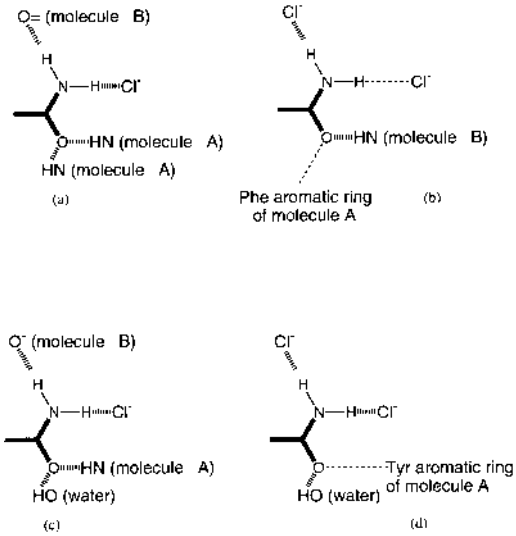

Fig. 5. Schematic Drawing of Relative Orientation of Neighboring Peptides, Chloride Ions, and/or Water Solvents Located Close to Amide NH_2 and O of Host Peptide Val–Gly–NH_2 (a), Ser–Phe–NH_2 (b), Gly–Tyr–NH_2 (c) and Pro–Tyr–NH_2 (d)

Hydrogen bonds and short contacts are shown by the broken lines of large width, and the relatively gentle contacts are shown by the usual broken lines. Molecules A and B represent the neighboring molecules translated by the different crystallographic symmetry operations of the host peptide.

The present work indicates that the association mode of peptides is largely changed by C-terminal amidation, and by the coexistence of biological anions such as the chloride ion. This interaction change may be necessary for the bioactivity of C-amidated peptides, because the hydrogen-bonding ability of the amide group and its preferred interaction mode with anions could play an important role in the specific interaction with the receptor.

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