Crystal and Molecular Structures of the Isomeric Dipeptides α-L-Aspartyl-L-alanine and β-L-Aspartyl-L-alanine

C. H. Görbitz

Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, N-0315 Oslo 3, Norway

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The crystal and molecular structures of the α- and β-L-Asp isomers of L-aspartyl-L-alanine have been determined at 120 K using 1226 and 1609 reflections ($I > 2.5\sigma I$), respectively. The space group for the α-isomer is $P2_1$, with cell parameters $a = 4.788(1)$, $b = 16.943(4)$, $c = 5.807(1)$ Å and $\beta = 107.55(2)°$; final R factor 0.042. The space group for the β-isomer is $P2_12_12_1$ with $a = 4.845(1)$, $b = 9.409(2)$ and $c = 19.170(3)$ Å; final R-factor 0.047. The two peptides crystallize as zwitterions with the side-chain acidic groups ionized. Each molecule adopts a *trans* configuration at the peptide bond with both carboxyl groups situated on the same side of the peptide plane. The geometries of the aspartyl moieties do, however, differ in the two structures. The peptide bond is significantly longer in the β-isomer than in the α-isomer, with C–N 1.344(3) and 1.328(4) Å, respectively. A very short intermolecular carboxyl⋯carboxyl hydrogen bond (O⋯O = 2.502(4) Å) is observed in the crystals of the α-isomer.

Acidic amino acid residues are biologically interesting in several aspects. Their importance in calcium binding proteins is well documented,[1] and the role of aspartic acid in peptide sweeteners has been dealt with in an earlier paper.[2] In the current project, interest focuses on these amino acids as constituents of oligopeptides believed to act as growth regulators during cell division in various tissues.[3]

Aspartic acid and glutamic acid are unique in that they have the possibility of forming β- and γ-peptide bonds via their side chain carboxy groups. Until recently, there was an almost complete absence of crystal structure determinations on peptides containing these residues, but several investigations have been presented in the last few years. All of these concern peptides with regular α-peptide bonds, and the only example of a β- or γ-peptide bond is in the long-known crystal structure of the biologically active tripeptide glutathione (γ-L-Glu-L-Cys-Gly).[4,5] The β-peptide link gives a chain with essentially the same conformational properties as a chain composed of β-amino acids. The additional free rotation around the C^α–C^β bond gives such a chain a flexibility which would prevent the spontaneous folding necessary for protein biological activity.[6] This is interesting from an evolutionary point of view, as nature has selected α-amino acids as building blocks.

The purpose of this work was to study the effect of the replacement of an α-peptide bond by a β-peptide bond on the molecular and crystal geometry. The two compounds chosen were α-L-aspartyl-L-alanine (αDA) and β-L-aspartyl-L-alanine (βDA) (one-letter amino acid symbols).

Experimental

The crystals of αDA were grown from ethanol, since the diketopiperazine had earlier been crystallized from an aqueous solution.[7] Large crystals of βDA were prepared by evaporation of an aqueous solution. They proved to be very flexible, and a number of crystals were tested on the diffractometer before a suitable specimen was found. The data collection procedures are sum-

Table 1. Data collection.

Instrument	Nicolet P3
Radiation	Graphite Crystal Monochromated MoK_α
Scanning mode	$\theta/2\theta$
Scan speed/° min^{-1}	3.0
Scan range/°	$2\theta_{\alpha1}$ −1.0 to $2\theta_{\alpha1}$ +1.0
Background count	For 35 % of scan time at scan limits
Temperature/K	120
2θ range/°	5.0–70.0

	α-L-Asp-L-Ala	β-L-Asp-L-Ala
Crystal dimensions/mm	0.55×0.35×0.20	0.35×0.25×0.15
No. of refl. measured	1359	2243
No. of unique refl. $I > 2.5\sigma I$	1226	1609

marized in Table 1. Cell parameters were determined by least-squares fit to the diffractometer settings for 25 general reflections. Standard deviations in the measured intensities were calcu-

lated as $\sigma I = [C_T + (0.02C_N)^2]^{1/2}$, where C_T is the total number of counts and C_N is the scan count minus the background count. The intensities were corrected for Lorentz and polarization ef-

Table 2. Fractional coordinates for α-L-Asp-L-Ala with standard deviations and equivalent isotropic temperature factors, B_{eq}, for non-hydrogen atoms.

Atom	x	y	z	$B_{eq}/Å^2$
OD1	0.9662(4)	0.6602(2)	0.6999(4)	1.5
OD2	0.7696(4)	0.6553(2)	1.0027(4)	1.4
O1	0.0875(4)	0.5000	0.2374(4)	1.7
O'	0.5610(5)	0.2791(2)	0.4173(4)	1.7
O''	0.7477(4)	0.2789(2)	0.1063(4)	1.4
N1	0.3820(5)	0.6472(2)	0.2751(5)	1.1
N2	0.5037(5)	0.4448(2)	0.2004(4)	1.1
CA1	0.5317(5)	0.5731(2)	0.3897(5)	0.9
CB1	0.5662(6)	0.5719(2)	0.6585(5)	1.1
CG1	0.7823(6)	0.6350(2)	0.7993(5)	1.1
C1	0.3543(5)	0.5023(2)	0.2653(5)	1.0
CA2	0.3659(6)	0.3706(2)	0.0959(5)	1.2
CB2	0.2892(8)	0.3697(3)	−0.1782(6)	1.8
C2	0.5694(6)	0.3038(2)	0.2233(5)	1.1
HO''	0.841(11)	0.235(3)	0.184(9)	
HN11	0.456(9)	0.657(3)	0.150(7)	
HN12	0.414(9)	0.688(3)	0.381(7)	
HN13	0.184(10)	0.643(3)	0.245(7)	
HN2	0.626(9)	0.456(3)	0.156(7)	
HCA1	0.703(8)	0.571(2)	0.346(6)	
HB11	0.627(8)	0.520(2)	0.714(6)	
HB12	0.387(8)	0.577(2)	0.699(6)	
HCA2	0.209(8)	0.364(2)	0.163(7)	
HB21	0.217(10)	0.326(3)	−0.235(7)	
HB22	0.456(9)	0.370(3)	−0.244(7)	
HB23	0.181(9)	0.415(3)	−0.242(7)	

Table 3. Fractional coordinates for β-L-Asp-L-Ala with standard deviations and equivalent isotropic temperature factors, B_{eq}, for non-hydrogen atoms.

Atom	x	y	z	$B_{eq}/Å^2$
O11	0.4539(3)	0.3888(2)	0.8090(1)	1.3
O12	0.7270(3)	0.5172(2)	0.7400(1)	1.1
OD1	1.1455(3)	0.3763(2)	0.6251(1)	1.3
O'	0.3976(4)	0.6777(2)	0.5514(1)	1.5
O''	0.7961(4)	0.7137(2)	0.6073(1)	1.4
N1	0.7714(4)	0.1493(2)	0.7950(1)	0.9
N2	0.7129(4)	0.3991(2)	0.5798(1)	1.1
CA1	0.8341(4)	0.2745(2)	0.7502(1)	0.8
CB1	0.7852(5)	0.2323(2)	0.6738(1)	1.0
CG1	0.8987(5)	0.3437(2)	0.6242(1)	1.0
C1	0.6568(5)	0.4033(2)	0.7693(1)	0.9
CA2	0.7750(6)	0.5151(2)	0.5307(1)	1.1
CB2	0.6628(7)	0.4794(3)	0.4590(1)	1.7
C2	0.6366(5)	0.6462(2)	0.5618(1)	1.0
HO''	0.706(7)	0.770(4)	0.633(2)	
HN11	0.773(6)	0.170(3)	0.840(1)	
HN12	0.891(6)	0.077(3)	0.787(2)	
HN13	0.601(6)	0.107(3)	0.784(2)	
HN2	0.534(6)	0.381(3)	0.589(1)	
HCA1	1.016(6)	0.302(3)	0.759(1)	
HB11	0.592(6)	0.219(3)	0.667(1)	
HB12	0.881(6)	0.148(3)	0.666(1)	
HCA2	0.973(6)	0.531(3)	0.531(1)	
HB21	0.701(6)	0.555(3)	0.427(1)	
HB22	0.466(7)	0.461(3)	0.462(1)	
HB23	0.748(6)	0.394(3)	0.442(1)	

fects, but not for absorption. Both structures were solved directly by MULTAN,[8] and isotropic refinements of the heavy atoms were followed by introduction of all but the carboxy group hydrogen atoms in theoretical positions. The latter were obtained from difference Fourier syntheses. All positional parameters and anisotropic temperature factors for the non-hydrogen atoms were refined by least-squares methods, giving $R = 0.042$ and $R_w = 0.044$ with goodness of fit $S = [\Sigma w \Delta^2/(m-n)]^{1/2} = 2.18$, and $R = 0.047$ and $R_w = 0.040$, with $S = 1.57$ for αDA and βDA, respectively. The final parameters are given in Tables 2 and 3. Atomic scattering factors for free heavy atoms and spherically bonded hydrogen atoms were taken from Ref. 9.

Lists of structure factors and anisotropic thermal parameters are available from the author on request.

Crystal data

α-L-Aspartyl-L-alanine, $C_7H_{12}N_2O_5$: monoclinic, $a = 4.788(1)$, $b = 16.943(4)$, $c = 5.807(1)$ Å, $\beta = 107.55(2)°$, $V = 449.2(2)$ Å^3, $M = 204.2$, $Z = 2$, $F_{000} = 216$, space group $P2_1$, $D_C = 1.509$ g cm^{-3}.

β-L-Aspartyl-L-alanine, $C_7H_{12}N_2O_5$: orthorhombic, $a = 4.845(1)$, $b = 9.409(2)$, $c = 19.170(3)$ Å, $V = 873.9(2)$ Å^3, $M = 204.2$, $Z = 4$, $F_{000} = 432$, space group $P2_12_12_1$, $D_C = 1.552$ g cm^{-3}.

Description and discussion

ORTEP[10] views of single molecules of both peptides are shown as Figs. 1 and 2 with atomic labelling schemes and bond lengths and bond angles indicated. Owing to the β-peptide link in βDA, there is no regular side-chain in the Asp-residue; however, the carboxy group will be referred to as a side-chain throughout this report.

In this context, it is very interesting to observe (Figs 1 and 2) that both molecules have the negative charge localized in the side-chains. The presence of unionized main-chain carboxy groups is, indeed, very unusual, the only other example of such an incidence in free peptides being seen in the tripeptide glutathione (GSH), mentioned above. The pK_a-value of the ω-carboxy group of glutamic or aspartic acid is about 4, this group being much less acidic than the α-carboxy group (p$K_a \approx 2.1$). Its pK_a is higher because its dissociation is not influenced by the presence of the neighboring positively charged amino group to the same extent as the α-carboxy group. These conditions may be somewhat different in peptides, but in all crystal structures with acidic residues so far investigated, the main-chain acidic group is ionized. In particular, this holds true for α-L-Asp-Gly,[11] which is the only other free peptide investigated having an N-terminal Asp residue.

In GSH an altered sequence is observed, with pK_{a1} (glutamyl) = 2.3 and pK_{a2} (main-chain) =
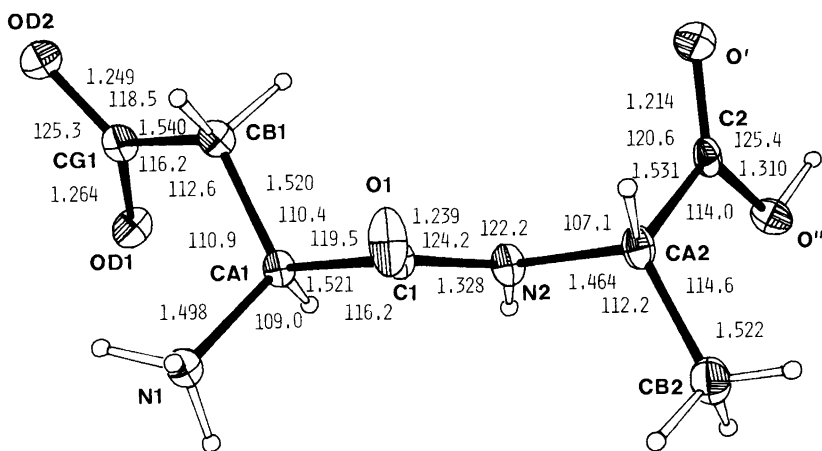

Fig. 1. View of the dipeptide α-L-Asp-L-Ala. The e.s.d.'s in bond lengths are 0.003 Å for bonds involving O, for others 0.004 Å. The e.s.d.'s in bond angles are 0.3°.
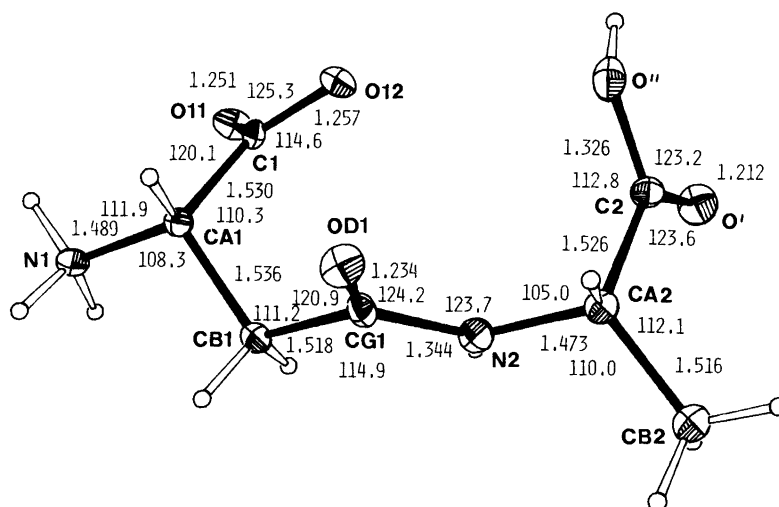
Fig. 2. View of the dipeptide β-L-Asp-L-Ala. The e.s.d.'s are 0.003 Å and 0.2° for bond lengths and bond angles, respectively.

3.3. This must certainly be a consequence of the presence of the γ-peptide linkage, which brings the glutamyl acidic group in close proximity to the protonated N-terminus. No determination of the pK_a-values for αDA and βDA has been carried out, but it seems reasonable that a similar change in the ionization sequence may occur in βDA. However, for αDA one must seek another explanation for the unique distribution of charge. This may be provided by the crystal packing, which will be discussed later.

A comparison of the bond lengths in the two isomers reveals only three significant differences. CB1–CG1 ($\Delta = 0.022$ Å) is situated in functionally different parts of the two molecules and will not be discussed further. Also, both values for the C2–O″ bond length (1.310 and 1.326 Å) are well within the rather wide limits observed for the single bond in carboxy groups. More interesting is the variation in the peptide bond length. In the isomeric L-Ala-LAsp[12] it is 1.335 Å, and in α-L-Asp-Gly it is 1.322 Å. An inspection of the available crystal structures with e.s.d.'s ≤0.007 Å, shows that 16 peptide bonds which involve an acidic residue[5,11–22] have a minimum length of 1.315 Å and an observed upper limit of 1.340 Å. The only exception is again GSH, where the γ-glutamyl bond has a length of 1.349 Å. The significant difference between the bond lengths in αDA (1.328 Å) and in βDA

(1.344 Å) is accordingly of more general interest. The available set of data for β- and γ-linked peptides is admittedly very restricted, but from the present data it appear that these compounds may have elongated peptide bonds. Attempts are now being made to crystallize the glutathione dimer, GSSG. If successful, the crystal structure of this compound will shed more light on this possible effect.

The difference between the two isomers manifests itself more clearly with respect to bond angles. Only the O–C–N angle in the peptide bond and the O–C–O angle in the side-chain are not significantly different in the two structures. The differences are particularly evident in the carboxy groups, which seem to have substantial flexibility in crystal structures. Noteworthy is the very small N2–CA2–C2 angle in βDA (105.0°).

Torsion angles for both molecules are shown in Fig. 3. The ψ_1 torsion angle in αDA is 132.2° and φ_2 is -134.5. A Ramachandran plot of (φ,ψ) falls in the fairly extended part of the β-region. The φ_2 torsion angle may be compared with those of two other peptides with C-terminal Ala residues: φ_2 is $-112.9°$ in L-Ala-L-Ala,[23] and φ_3 is -147.0 and $-159.9°$ for the two more elongated molecules in the asymmetric unit of L-Ala-L-Ala-L-Ala.[24] φ_2 in α-L-Asp-Gly (αDG)[11] is 152.8°, a value permitted only for Gly residues.

The χ^1 torsion angle is close to ÷gauche, which
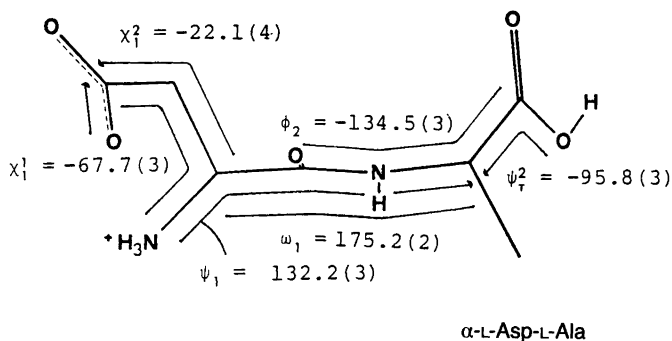
Fig. 3. Torsion angles (°) in both peptides.

α-L-Asp-L-Ala

β-L-Asp-L-Ala
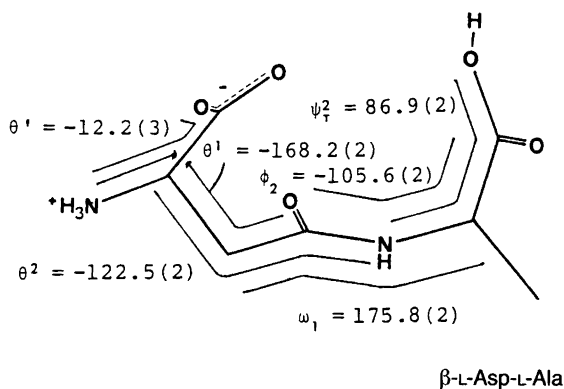
is the most commonly observed conformation for the Asp residue. The conformation of the aspartyl group is very similar to that observed in αDG. As in αDG, there is no sign of an intramolecular hydrogen bond between the protonated amino terminus and the carboxy group. In a third crystal structure with an N-terminal Asp residue, namely that of the peptide sweetener aspartame (α-L-Asp-L-Phe methyl ester),[25] a very weak hydrogen bond has been claimed. It seems that the need for strong intermolecular hydrogen bonding is decisive for the orientation of these groups. The formation of an intramolecularly bonded six-membered ring is of less importance, and such a ring may occasionally occur merely as a by-product of the intermolecular hydrogen bonding pattern. The situation is obviously different in the liquid phase.

Apart from the different location of the carboxyl group hydrogen atom, the C-terminal part of βDA is rather similar to that observed for αDA, with φ_2 −105.6°. The aspartyl moiety is however radically different from that in other peptides, owing to the presence of the β-peptide link. The θ^1 and θ^2 torsion angles may be compared with ψ torsion angles in the other peptides. The values of −168.2° and −122.5°, respectively, are prohibited for ψ for all other residues than Gly. This illustrates how the introduction of a special peptide bond in a chain facilitates the adoption of conformations unattainable for peptides with regular α-peptide bonds.

As in αDA, the two carboxy groups of βDA are both situated on the same side of the peptide plane, but whereas the aspartyl side chain in αDA is turned outwards, the corresponding group in βDA is turned inwards to point almost directly in the direction of the C-terminal carboxy group. Combined with the differences in the pep-
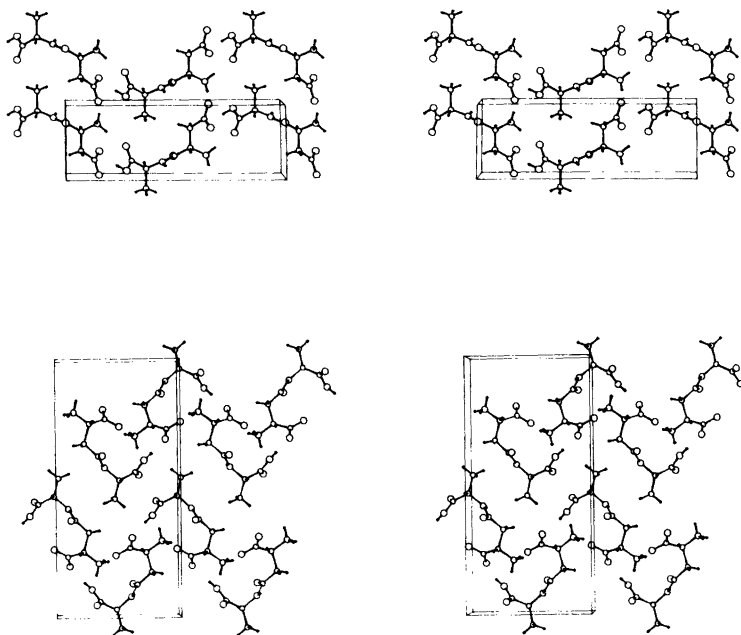
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Fig. 4. Stereoscopic drawings of the crystal packing of α-L-Asp-L-Ala (top) and β-L-Asp-L-Ala, both viewed along the a-axis.

tide linkages, the result is that the two molecules in the crystal structures are rather different in appearance.

The crystal packings of the two structures are shown in Fig. 4. Data for the intermolecular hydrogen bonds are given in Table 4. The aspartyl group of αDA is situated very close to the main-chain carboxy group of the neighbouring molecule, and the O″⋯OD1 hydrogen bond in αDA [2.502(4) Å] is among the shortest observed in crystal structures of peptides. One may then propose the existence of a double well potential with

Table 4. Hydrogen bond and hydrogen bond-like distances (Å) and angles (°).

| D	H	A	D–H	D⋯A	H⋯A	D–H⋯A
α-L-Asp-L-Ala						
O″	HO″	OD1	0.92	2.502	1.59	172
N1	HN11	OD2	0.91	2.781	1.94	152
N1	HN13	OD2	0.92	2.885	2.07	148
N1	HN12	O′	0.91	2.822	1.92	174
N2	HN2	O1	0.83	2.893	2.14	148
CA1	HCA1	O1	0.93	3.289	2.43	153
CB1	HB12	OD1	0.96	3.311	2.46	148
β-L-Asp-L-Ala						
O″	HO″	O11	0.84	2.599	1.76	175
N1	HN11	O′	0.89	3.068	2.24	156
N1	HN11	O″	0.89	2.891	2.35	118
N1	HN12	O12	0.91	2.811	2.00	147
N1	HN13	O12	0.94	2.798	1.86	178
N2	HN2	OD1	0.90	2.891	2.01	167
CA1	HCA1	O11	0.93	3.383	2.47	167
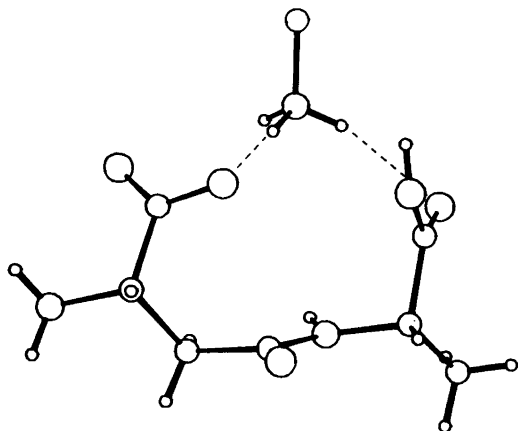
Fig. 5. Formation of a 12-membered hydrogen-bonded ring in the crystal structure of β-L-Asp-L-Ala. Only the protonated amino group of the neighboring molecule (at 1−x, 0.5+y, 1.5−z) is shown.

a low central barrier. Thus, the peptide may in fact be present in the liquid phase and crystallize with an ionized main-chain carboxy group as usual; subsequent solid-phase proton transfer would then give the situation observed in the crystals.

The hydrogen bond network is three-dimensional in αDA, but only two-dimensional in βDA. This may explain why the βDA crystals are so flexible. From Table 4 it can be seen that the HN11 hydrogen atom of βDA is involved in what may be characterized as a bifurcated hydrogen bond. Furhtermore, the formation of a 12-membered hydrogen-bonded ring structure which includes the carboxylic acid moieties stabilizes the special orientation of the acidic groups (Fig. 5).

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