

Intensity of the Infrared Amide I Band of Dipeptides in Heavy Water

Structural studies of polypeptides and proteins by the intensities of infrared amide bands have recently led to promising results.¹⁻⁴ In this connection we studied the intensities and other spectral parameters of the amide I band of eight dipeptides in heavy water at different pD. Optical properties of amide I of dipeptides can differ slightly from those of polypeptides. But in the latter case we cannot investigate many peculiar properties of vibration without the effect of conformation of the polypeptide backbone. The primary aim of this study is the estimation of the influence of various amino acid side chains on the spectral parameters of the amide I band.

EXPERIMENTAL

The spectra of dipeptides from L, α -amino acids were measured by the IR-27G spectrophotometer, Shimadzu, Japan. The complex spectra were resolved into individual components. The contour shape was described by the sum of the Gaussian and Lorentzian contours⁵ using parameter f_G equal to the portion of the Gaussian contour. The measurement error for the maximum frequency and the half-width was ± 1 cm⁻¹. The error for the molar absorption coefficient of maximum ϵ_0 was about 3-5%, and for the intensity $B = 2.3 \int \epsilon d\nu$, about 8-10%. Beer's law was satisfied at concentrations between 0.4 and 2%. This supports our assumption that association of molecules is absent. The concentration was determined by dry weight. The majority of the samples were from Reanal, Hungary. The purity of preparations was checked electrophoretically and by micro-Kjeldahl nitrogen analysis.

RESULTS

In a neutral aqueous medium the dipeptide-molecule has a dipolar structure. In an alkali medium only the carboxyl group is ionized, and in an acidic medium only the amino end group. The presence of the charged ends may lead to some changes in the spectral parameters of amide I vibrations generally due to the field effect. Besides, a number of rotating isomers can exist due to the restricted rotation around single bonds. In this sense, dipeptides containing glycine differ from those without this residue.

TABLE IA
Spectral Parameters of the Amide I Band of Dipeptides in Heavy Water.
Alkali Medium pD 11-13, NaOD

N	Compound	Frequency of maximum, ν , cm ⁻¹	Band half- width, $\Delta\nu_{1/2}$, cm ⁻¹	Band intensity		Shape of contour, f_G
				ϵ_0 , l mol ⁻¹ cm ⁻¹	$B \times 10^{-4}$, l mol ⁻¹ cm ⁻²	
1	Glycyl-glycine	1639	35	500	4.9	0.7
2	Alanyl-alanine	1632	35	420	4.1	0.7
3	Alanyl-glycine	1635	35	435	4.2	0.7
4	Glycyl-alanine	1633	35	440	4.3	0.7
5	Glycyl-D,L-alanine	1634	35	425	4.2	0.7
6	Glycyl-phenylalanine	1635	35	425	4.2	0.7
7	Lysyl-glycine	1640	35	505	5.0	0.7
8	Lysyl-valine	1627	35	430	4.2	0.7

TABLE IB
Spectral Parameters of the Amide I Band of Dipeptides in Heavy Water.
Neutral Medium pD 5-7

N	Compound	Frequency of maximum, ν , cm^{-1}	Band half- width, $\Delta\nu_{1/2}$, cm^{-1}	Band intensity		Shape of contour, f_G	
				ϵ_0 , l mol^{-1} cm^{-1}	$B \times 10^{-4}$, l mol^{-1} cm^{-2}		
<i>Amide I as one band</i>							
1	Alanyl-alanine	1665	30	440	3.5	0.9	
2	Alanyl-glycine	1670	28	455	3.4	0.8	
3	Glycyl-alanine	1668	30	465	3.8	0.8	
4	Glycyl-D,L-alanine	1669	31	460	3.5	1.0	
5	Glycyl-phenylalanine	1670	29	455	3.6	1.0	
6	Lysyl-glycine	1669	27	460	3.3	0.8	
7	Lysyl-valine	1664	30	455	3.7	0.8	
<i>Amide I as two bands</i>							
1	Glycyl-glycine	1674	28	485	3.6	5.0	0.8
		1650	28	200	1.4		

TABLE IC
Spectral Parameters of the Amide I Band of Dipeptides in Heavy Water.
Acidic medium pD 1-2, DCI

N	Compound	Frequency of maximum, ν , cm^{-1}	Band half- width, $\Delta\nu_{1/2}$, cm^{-1}	Band intensity		Shape of contour, f_G	
				ϵ_0 , l mol^{-1} cm^{-1}	$B \times 10^{-4}$, l mol^{-1} cm^{-2}		
<i>Amide I as one band</i>							
1	Alanyl-alanine	1668	30	440	3.9	0.6	
2	Alanyl-glycine	1671	30	490	4.3	0.6	
3	Glycyl-alanine	1672	30	500	4.4	0.6	
4	Glycyl-D,L-alanine	1674	30	500	4.4	0.6	
5	Lysyl-valine	1668	32	480	4.5	0.6	
<i>Amide I as two bands</i>							
1	Glycyl-glycine	1678	30	420	3.7	5.5	0.6
		1655	30	210	1.8		
2	Lysyl-glycine	1675	30	360	3.2	5.3	0.6
		1655	34	230	2.1		

Note: At these conditions glycyl-phenylalanine is nonsoluble.

As a rule, the dipeptides had a normally expected spectrum consisting of one amide I band and one band of the carboxyl group in the ionized or non-ionized form depending on the solution pD (Fig. 1). For some dipeptides containing glycine the amide I band was occasionally seen as two components. Such a display can apparently be explained by the presence of rotating isomers, the charged end or side amino group of which is drawn near to the peptide carbonyl group. The results of spectra measurements are listed in Table I. Among the dipeptides studied some contain a glycine residue to the left or the right of

TABLE II
Spectral Parameters of the Amide I of Dipeptide Molecule in Heavy Water Solution
Depending on the Ionization of End Groups

pD	Type of molecule	Fre- quency of maxi- mum, ν , cm ⁻¹	Band half- width, $\Delta\nu_{1/2}$, cm ⁻¹	Band intensity		Shape of con- tour, f_G
				ϵ_0 , l mol ⁻¹ cm ⁻¹	$B \times 10^{-4}$, l mol ⁻¹ cm ⁻²	
11	ND ₂ -CHR ₁ -COND-CHR ₂ -COO ⁻	1635	35	450	4.4	0.7
6	N ⁺ D ₃ -CHR ₁ -COND-CHR ₂ -COO ⁻	1668	30	460	3.6	0.9
1	N ⁺ D ₃ -CHR ₁ -COND-CHR ₂ -COOD	1670	30	480	4.3	0.6

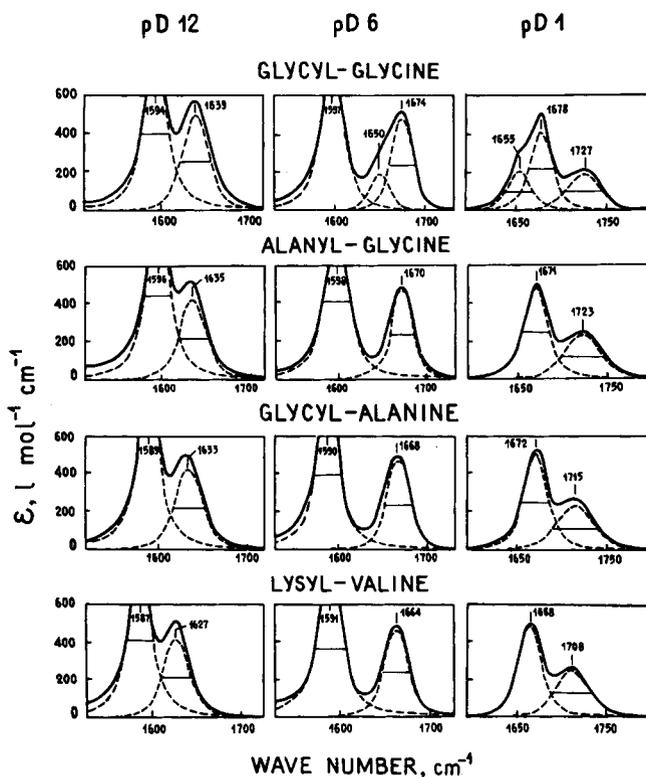


Fig. 1. Infrared spectra of several dipeptides in heavy water at different pD.

the peptide group, some contain amino acid residues of varying size, others have functional groups, and there are dipeptides containing D- and L-form residues. In all cases the intensity and other spectral parameters of the amide I band do not depend on the type of amino acid residues.

The ionization of the end groups leads to some regular changes of the band parameters. The mean values of these parameters are given in Table II. The ionization of carboxylic or amine groups leads to the decrease of the band intensity to about 20%. The frequency of the band maximum does not depend on the ionization of the carboxylic end,

but becomes higher at about 33 cm^{-1} due to the ionization of the amine group. We cannot accurately measure the amide I band in the spectra of dipeptides with an ionic side group of glutamic or aspartic amino acid residues. However, one can assume that the possible change of the band parameters in these cases must be no more than the change due to the ionization of the end groups of dipeptides.

We conclude that the intensities and other spectral parameters of the amide I band do not depend on the majority of amino acid side chains, and may be slightly changed for the side chain with the ionic group closely arranged near the peptide group.

Additional results of measurements are the spectral band parameters of the end carboxyl group. The average values for dipeptides are given in the same order as for amide I:

		ν_{\max}	$\Delta\nu_{1/2}$	ϵ_0	B	f_G
pD	11	1592	32	830	8.4×10^4	0.4
pD	6	1594	30	820	7.9×10^4	0.4
pD	1	1720	45	230	2.7×10^4	0.8

The authors thank Dr. N. P. Zapevalova for synthesizing lysyl-glycine and lysyl-valine and I. I. Bukatina for nitrogen microanalysis.

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Received January 18, 1973

Revised May 8, 1973