[CONTRIBUTION FROM THE LABORATORIES OF THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, GRADUATE SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF PITTSBURGH]

Dissociation Constants of Peptides. II. The Effect of Optical Configuration on the Infrared Spectra of Peptides Containing One Amino and One Carboxyl Group[1a,b]

BY ERIC ELLENBOGEN[2]

RECEIVED JUNE 3, 1955

Infrared spectra were obtained on the following peptides: glycylalanine (L,D), alanylglycine (L,D), glycylalanylglycine (L,D), trialanine (3L, LLD, LDL, DLL, 3D), and tetraalanine (4L, LLDL, LDLL, DLLL). They were carried out on mulls suspended in Nujol and fluorolube. The entire range from 5000 to 625 cm.$^{-1}$ was scanned. Certain frequencies were tentatively assigned to specific vibrations. It was observed that the optical configuration of the amino acid residues affected the type of spectra to a marked degree when configurational changes were introduced in those peptides containing two or more asymmetric carbon atoms. Substitution of a D-residue for a L-residue in the first three peptides did not change the infrared spectra. Similarly, the spectrum for trialanine (3L) was identical with that for trialanine (3D).

In the first paper of this series we have reported a survey[3] of the effect of optical configuration of amino acid residues in peptides on the ionization of their functional groups. We have since extended this study by obtaining infrared spectra on all of these peptides, as well as by determining the thermodynamic functions of representative peptides, in order to correlate physical-chemical properties with structures.[4]

Experimental

Peptides.—H·Gly-Ala·OH (L,D), H·Ala-Gly·OH (L,D), H·Ala-Ala-Ala·OH (3L, LLD, LDL, DLL, 3D) and H·Ala-Ala-Ala-Ala·OH (4L, LLDL, LDLL, DLLL) were part of the lots previously described.[3] H·Gly-Ala-Gly·OH (L,D) was obtained by courtesy of Dr. Jesse Greenstein.

Infrared Spectra.—The peptides were suspended in nujol and fluorolube, respectively, and spectra were determined in the Baird spectrophotometer at about 22°. Fluorolube is transparent from 5,000 to 1,400 cm.$^{-1}$, and nujol from 1,400 to 625 cm.$^{-1}$. This change of suspending medium is responsible for the breaks in the curves shown in Figs. 1 to 5. Figure 6 shows the spectra for fluorolube and for nujol.

Results

Tentative frequency assignments have been made for the compounds investigated[5] and are listed in Table I.

Discussion

Analogous to the observations made in connection with the effect of optical configuration on the ionization of the functional groups of peptides,[3] there are four compounds whose spectra remain unchanged upon complete isomerization. Three of them (glycylalanine, alanylglycine and glycylalanylglycine) contain only one asymmetric carbon atom, and in the fourth, trialanine (3L), all three centers of symmetry are changed to their isomers simultaneously (3D). Isomerization of one residue at a time in tri- and tetraalanine gives rise to marked differences. Some frequencies are shifted, new ones appear, while others disappear. This is probably due to strains and changes in spatial configuration, as has already been deduced from the titration curves.[3]

There seems to be general agreement that the bands near 3200 and 3060 cm.$^{-1}$ are indicative of a hydrogen bonded NH stretching frequency. From spectra of polyamino acids,[5] one might deduce that these bands arise from the NH stretching vibration of the *trans*-peptide and linkage itself hydrogen bonded, since such polymers contain so many peptide bonds relative to the single free α-amino group, that the latter's NH frequencies would not show up. This, of course, is incompatible with bands observed on relatively small peptides, where both the peptide NH as well as the free α-amino NH must contribute to those frequencies. The fact that three and four frequencies are observed in certain cases may be indicative that different types of hydrogen bonds are involved, the higher frequencies possibly belonging to the weaker hydrogen bonds.

Between 3000 and 2800 cm.$^{-1}$ many types of CH stretching vibrations may occur, such as CH_2 and CH_3, both symmetrical and non-symmetrical. No definite assignments can be made, although the 2925 frequency has in the past been assigned to non-symmetrical stretching, whereas the 2860 band is to be indicative of symmetrical stretching.[5a] The band 2100–2040 cm.$^{-1}$ is tentatively assigned to an NH frequency arising from the NH_3^+ configuration. This is based on an intensification of this band in some hydrochlorides of peptides (see paper III of this series,[4] and may be indicative of the zwitterion.

The bands at 1630, 1600 and 1580 cm.$^{-1}$ have been assigned tentatively to stretching vibrations arising from the resonating peptide linkage (based on CO stretch 1850 to 1650 and CN stretch 1700–1580). The band at 1670 cm.$^{-1}$ is tentatively assigned to a *hydrogen bonded* C—O stretching frequency.

The 1530 and 1270 cm.$^{-1}$ bands have been assigned to NH deformations or CN stretching or both, and the 1480–1410 bands to various CH deformations. The band at 1470 cm.$^{-1}$ arises most probably from the symmetrical vibrations of

(1) (a) Presented in part at the Third International Congress of Biochemistry, 1955; (b) supported by a grant from the U. S. Public Health Service, National Institutes of Health, RG 4014 (C).

(2) The author wishes to acknowledge his appreciation to Dr. F. A. Miller, Mellon Institute, Pittsburgh, Pennsylvania, for carrying out the infrared spectra determinations reported in this paper.

(3) E. Ellenbogen, THIS JOURNAL, **74**, 5198 (1952).

(4) Paper III of this series describes the infrared spectra of peptides containing more than two functional groups (THIS JOURNAL, **78**, 366 (1956)), and paper IV deals with the thermodynamic and infrared spectra of the isomeric alanylalanines (*ibid.*, **78**, 369 (1956)).

(5) (a) E. R. Blout and S. C. Linsley, *ibid.*, **74**, 1946 (1952); (b) S. E. Darmon and G. B. B. M. Sutherland, *Nature*, **164**, 44 (1949); (c) E. J. Ambrose and A. Elliott, *Proc. Roy. Soc. (London)*, **A205**, 46 (1951); (d) G. J. Szasz, N. Sheppard and D. H. Rank, *J. Chem. Phys.*, **16**, 704 (1948); (e) R. Neuman and R. M. Badger, *ibid.*, **19**, 1147 (1951); (f) N. B. Colthup, *J. Opt. Soc. Amer.*, **40**, 397 (1950); (g) M. Asai, M. Tsuboi, T. Shimanouchi and S. Mizushima, *J. Phys. Chem.*, **59**, 322 (1955).

TABLE I

TENTATIVE FREQUENCY ASSIGNMENTS, CM.$^{-1}$

Assignment	H·Gly-Ala·OH (L,D)	H·Ala-Gly·OH (L,D)	H·Gly-Ala-Gly·OH (L,D)	(3L)(3D)	H·Ala-Ala-Ala·OH (LLD)	(LDL)	(DLL)	(4L)	H·Ala-Ala-Ala-Ala·OH (LLDL)	(LDLL)	(DLLL)
NH stretch H-bonded	3180	3220	3225	3311	3390	3450	3225	3248	3244	3245	3244
	3060	3080	3210	3030	3245	3370	3125	3225	3030	3040	3225
			3040		3225	3140	3070	3030			3060
					3030	3060					
CH stretch	2930	2980	2910	2975	2975	2965	2970	2975	2950	2980	2980
	2835	2830	2860	2925	2915	2920	2920	2910	2918	2960	2908
						2864	2873			2915	
NH stretch from NH_3^+	2083	2100	2000	2045	2108	2040	2030	2090	2070	2118	2040
CO, CN stretch from	1623	1630	1636	1637	1636	1633	1646	1645	1647	1630	1650
peptide resonance	1570	1570	1608	1624	1614	1608	1608	1612	1628	1613	1626
			1578	1595	1574	1580	1565		1570		1609
C=O stretch H-bonded	1674	1676	1662	1667	1670	1654	1672	1676	1666	1651	1678
					1650						
CN stretch and/or NH	1510	1533	1511	1530	1534	1560	1510	1558	1557	1547	1540
deformation	1267	1270	1274	1274	1280	1270	1285	1540	1522	1520	1524
					1266	1240	1266	1508	1278	1278	1280
					1253		1250	1263			
Symm COO^- vibration		1470	1470	1470				1470	1470	1470	1472
CH deformation	1450			1490	1448	1496	1496	1485	1490	1485	1490
	1433	1458	1455		1434	1450	1453				
	1406	1414	1420	1445	1410	1434	1437	1446	1444	1446	1448
				1404		1417		1434	1434		1407
								1405			
CNC stretch	1000	1005	1020	1000	1000	1003	1002	1010	1010	990	1002
	774			774	775	766	983	773	772	769	995
							770				778
CH, CH_3 rocking	948	915	934	963	962	962	960	966	966	964	965
	920		914	940	938	938	936	935	936	936	933
				914	917	928	912	917	917	917	910
CH, NH rocking	725	727	736	733	725	730	720	729	710	702	710

the ionized carboxyl group.[5] The bands at 1000 and 770 cm.$^{-1}$ are believed to arise from skeletal vibrations due to the CNC stretching and the band at 725 cm.$^{-1}$ is probably due to CH or NH rocking. The bands between 960 and 910 cm.$^{-1}$ are tentatively assigned to CH and C–R (R = side chain) rocking vibrations, based on a change in relative intensities of these bands. In alanylglycine, where the asymmetric carbon atom is in an amino terminal position, only the 915 band appears. In glycylalanine a second band, at 948 cm.$^{-1}$, less intense than that at 920 cm.$^{-1}$ is seen. This could be due to some effect exerted by the peptide linkage on the rocking vibration of the side chain. In glycylalanylglycine these two bands have similar intensities, and in the trialanines and tetraalanines the relative intensities shift from one to the other and are accompanied by the appearance of a third absorption band. Trialanine (3L,3D) has an intense band at 963, a weaker one at

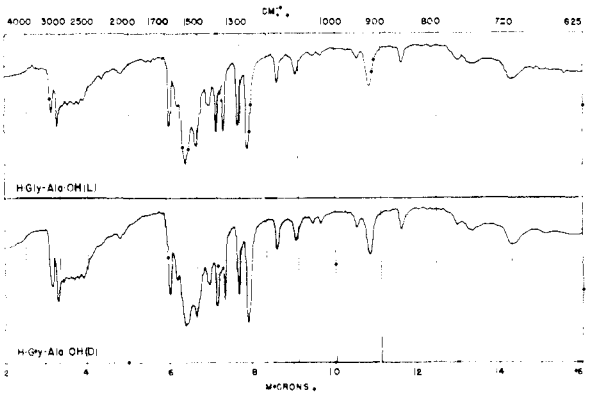

Fig. 1.

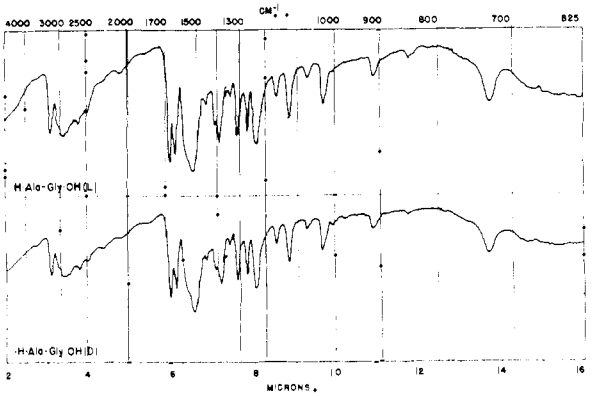

Fig. 2.

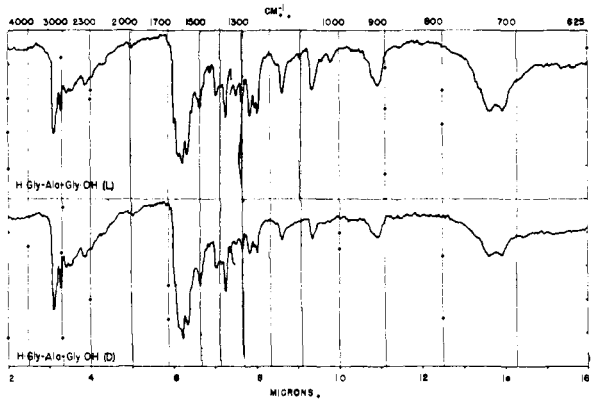

Fig. 3.

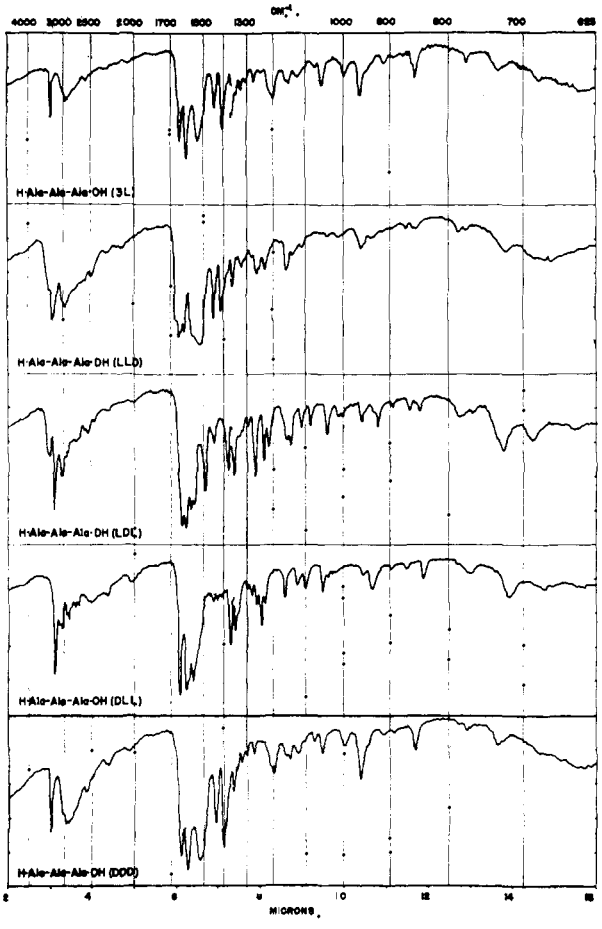

Fig. 4.

914, and a much weaker one at 940 cm.$^{-1}$. Replacing the COOH terminal L-residue by its D-isomer reduces the band at 962, increases the intensity at 938, and decreases the intensity of the band at 917. If the residue in the middle is replaced by its isomer (LDL), the previous band at 917 is shifted to 928 cm.$^{-1}$ and becomes the most intense one, the band at 938 is weakest, and the one at 962 is intermediate between the other two bands. Shifting the D-residue to the NH_2 terminal position diminished the intensity of the 960 cm.$^{-1}$ band, returns the previous 928 cm.$^{-1}$ band to its original frequency (and low intensity) at 912,

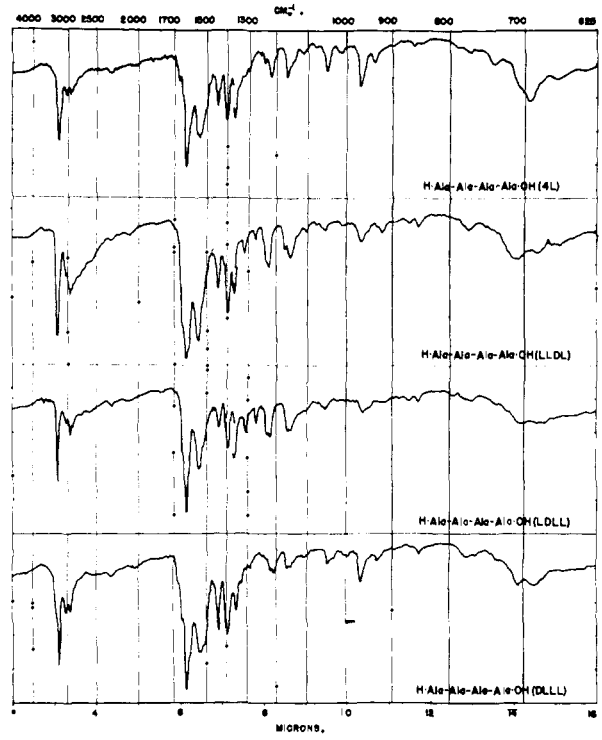

Fig. 5.

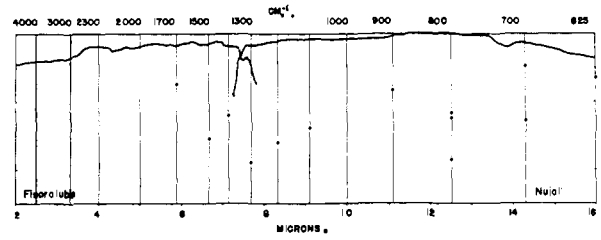

Fig. 6.

and leaves the band at 936 cm.$^{-1}$ the most intense. In tetraalanine (4L) the band at 966 is most intense compared with the ones at 935 and the one at 917 which is weakest. In the (LLDL) isomer, the intensity at 917 increases, whereas those at 936 and 966 decrease. In (LDLL) the intensity of the 964 band is further increased, the one at 936 remains apparently unchanged, and the one at 917 broadens considerably. The (DLLL) isomer has three frequencies at 965, 933 and 910 cm.$^{-1}$ similar in relative intensity to those seen in (4L). In a "pure" peptide, the side chain methyl groups are *trans* to each other in reference to the planar peptide linkage, as are the hydrogen atoms attached to the asymmetric carbons, and the rocking vibrations of these groups will produce a certain pattern in the infrared region. Replacing one internal L-residue of a "pure" peptide by its D-isomer gives rise to a "mixed" peptide, in which three methyl groups are *cis* to each other. The close proximity of these fairly bulky groups, as well as the closer approach to each other of the hydrogens on the asymmetric carbon atoms, may be responsible for repulsive forces which may modify the nature of these rocking vibrations so that changes in frequency as well as intensity are observed.

The latest paper on infrared spectra of peptides from the laboratories of Mizushima[5f] suggests that another band, at 1015 ± 15 cm.$^{-1}$, be considered as typical for the sequence...glycylglycyl ...in polypeptides or proteins. The argument is based on the observation that the 1015 band is present in peptides consisting only of glycine residues, as well as in polyglycine, and absent in poly-DL-alanine, poly-β-alanine, poly-glycyl-DL-phenylalanine, and the periodic polymer of (glycine-DL-alanine). From the spectra presented in this paper, one would have to draw the conclusion that these absences are fortuitous. It would seem that the explanation lies in the fact that Mizushima and his colleagues have prepared polymers composed of the racemic DL-amino acids. In tri- and tetraalanine bands at 1015 ± 15 cm.$^{-1}$ are observed in varying degrees of intensity. The band seems to be absent in trialanine (DLL) and tetraalanine (LDLL). This band is probably due to some type of skeletal vibration, since it shows up in varying degrees also in the spectra of polyfunctional peptides (paper III[4]), as well as in most of the racemic polyamino acids prepared by the Lossen rearrangement.[6] In conclusion, it should be pointed out that care must be exercised in applying information based on infrared spectra to the case of peptides in aqueous solution. It is quite possible that some frequencies are a function of the array of molecules in the solid state. Unfortunately, not enough material was available to undertake a study of the relation between infrared spectra and crystal lattice. It would also be interesting to apply spectra as a test for the existence of the helical configuration of peptides, since the tetraalanines should be able to form one hydrogen bond if they could fold into the 3.7 residue helix. There is no *a priori* reason to assume this should be the configuration for these relatively small molecules in the solid state, and none of the observed frequencies can so far be interpreted as representing such a folding.

(6) C. D. Hurd, L. Bauer and I. M. Klotz, THIS JOURNAL, **75**, 624 (1953).

PITTSBURGH, PENNSYLVANIA

[CONTRIBUTION FROM THE LABORATORIES OF THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, GRADUATE SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF PITTSBURGH]

Dissociation Constants of Peptides. III. The Effect of Optical Configuration on the Infrared Spectra of Polyfunctional Peptides[1a,b]

BY ERIC ELLENBOGEN[2]

RECEIVED JUNE 3, 1955

Infrared spectra were obtained on the following peptides: alanyllysylalanine (3L. LLD, LDL), alanyllysyldialanine (4L, LDLL), alanyllysyltrialanine (5L, LDLLL), α-alanylglutamic acid (LL, LD), α-glutamylalanine (LL,LD), α-glutamylglutamic acid (LL,LD), and γ-glutamylglutamic acid (LL,DD). Spectra were determined on mulls suspended in Nujol and fluorolube, and the range from 5000 to 625 cm.$^{-1}$ was scanned. Certain frequencies were tentatively assigned to specific vibrations. Changing the optical configuration of an amino acid residue caused marked changes in the spectra of those peptides when compared with the pure L-compounds. Some spectra were so "diffuse" that they resembled infrared spectra of protein.

In the preceding paper of this series[3] infrared spectra were reported on peptides of known configuration containing but a single amino group and a single carboxyl group. In this paper are reported infrared spectra of peptides containing two amino and two or three carboxyl groups. In each peptide the L-amino residue containing the additional functional group has been replaced by its D-isomer.

Experimental

Peptides.—H·Ala-Lys-Ala·OH (3L, LLD, LDL), H·Ala-Lys-Ala-Ala·OH (4L, LDLL), and H·Ala-Lys-Ala-Ala-Ala·OH (5L, LDLL) were part of the lots previously described.[4] The glutamyl peptides were obtained by the courtesy of Dr. H. A. Sachs.

Infrared Spectra.—For details of preparing the samples in nujol and fluorolube mulls, as well as for the spectra of the suspending media, see paper II of this series.[3] The spectra of the compounds are shown in Figs. 1 to 7.

Results

Tentative frequency assignments have been made for the compounds investigated,[5] and are listed in Table I.

Discussion

Frequency assignments were made on the basis of arguments similar to those already advanced.[3] The relative shifts of the intensities of the three bands tentatively assigned to CH and CH_2 rocking in alanyllysylalanine (3L, LLD, LDL) can again be due to mutual repulsions of the CH_3 and β-CH_2 groups of the side chains which are attached to the asymmetric carbon atom. The band near 2000 cm.$^{-1}$ is assigned to the NH vibration arising from the NH_3^+ group. It is present in all peptides containing lysine (monohydrochlorides) and is broadened in alanyllysyldialanine (4L) which was examined as the dihydrochloride. This particularly broad band might arise from the alpha and epsilon NH_3^+ groups whose frequencies could be so close together that they are not resolved by the particular instrument used.

Of great interest is the general nature of these spectra. On the whole, they begin to resemble spectra obtained on crystalline insulin.[6] In the tripeptides H·Ala-Lys·Ala·OH, distinct bands are

(1) (a) Presented in part at the Third International Congress of Biochemistry, 1955; (b) supported by a grant from the U. S. Public Health Service, National Institutes of Health.

(2) The author wishes to acknowledge his appreciation to Dr. F. A. Miller, Mellon Institute, Pittsburgh, Pennsylvania, for carrying out the infrared spectra determinations reported in this paper.

(3) E. Ellenbogen, THIS JOURNAL, **79**, 363 (1956).

(4) E. Ellenbogen, *ibid.*, **74**, 5198 (1952).

(5) Refs. 5a through 5f of (3).

(6) E. Ellenbogen, THIS JOURNAL, **77**, 6634 (1955).