Study on the Optical Rotatory Dispersion of Di-Peptides and its Application to the Recognition of Di-Peptides from Higher Peptides and Amino Acids

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Much works on optical rotatory dispersion (ORD) of peptides have been accumulated to elucidate helical conformation in proteins.¹⁾ Recently Beacham *et al.*²⁾ reported ORD of a series of diastereomers of tri- and tetra-peptides of alanine and serine and calculated the contribution of each chromophore to the molecular rotation.

In this paper use of ORD to differentiate di-peptides of L-amino acids from higher peptides and free amino acids of L-series is described. Hereafter peptides and amino acids of L-series are written as peptides and amino acids without a term of 'L-series.' The ORD of twenty-four di-peptides, including alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, glutamic acid, aspartic acid and lysine were measured. They were mainly prepared by mixed anhydride method³⁾ by a condensation of N-carbobenzoxyl amino acids⁴⁾ with benzyl esters of amino acids⁵⁾ and the

protecting groups were removed by catalytic hydrogenation. Secondary functional groups such as ω -amino group of N-terminal lysine were also protected with N-carbobenzoxyl group,⁴⁾ ω -carboxyl group of C-terminal and N-terminal glutamic acids^{5,6)} and that of C-terminal aspartic acid⁵⁾ with benzyl esters and the phenolic hydroxyl group of C-terminal tyrosine with benzyl ether.⁷⁾ The optical purity of each compound was checked by measurements of specific rotation in water or in diluted hydrochloric acid solutions.

The ORD were measured in the 210~400 m μ with a Japan Spectroscopic Model ORD/UV-5 recording polarimeter at room temperature by use of a quartz cell with an optical path of 1.0 cm. The concentrations of the peptides were 0.1% in 0.1 N potassium hydroxide solution or in 0.1 N hydrochloric acid solution. In order to avoid racemization especially in alkaline solution, all the solutions were prepared just before the measurements.

1) E. R. Blout in C. Djerassi, "Optical Rotatory Dispersion," Mc Graw-Hill, New York, 1960, p. 238; M. Goodman, F. Boardman and I. Listowsky, *J. Am. Chem. Soc.*, **85**, 2491 (1963) and preceding papers.

2) J. Beacham, V. T. Ivanov, P. M. Scopes and D. R. Sparrow, *J. Chem. Soc.*, **1966** (C) 1449; P. M. Scopes, D. R. Sparrow, J. Beacham and V. T. Ivanov, *Tetrahedron Letters*, **7**, 665 (1966).

3) J. R. Vaughan, R. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).

4) M. Bergmann and L. Zervas, *Chem. Ber.*, **65**, 1192 (1932).

5) J. P. Greenstein and M. Winitz, "Chem. of Amino acids" Vol. 2, Wiley & Sons Inc., 1961, p. 923; N. Izumiya, *et al.*, *Nippon Kagaku Kaishi*, **78**, 662 (1957); L. Zervas, M. Winitz and J. P. Greenstein, *J. Org. Chem.*, **22**, 1515 (1957).

6) J. P. Greenstein and M. Winitz, "Chem. of Amino acids" Vol. 2, Wiley & Sons Inc., 1961, p. 943.

7) E. Wunsch, G. Fries and A. Zwick, *Chem. Ber.*, **91**, 542 (1958); R. Schwyzer and W. Rittel, *Helv. Chim. Acta*, **41**, 159 (1961).

Figure 1 shows ORD curves of valyl valine as a typical example of di-peptides which gave a positive single Cotton effect curve with a peak at 225 m μ in acid and a negative single Cotton effect curve with a trough at almost the same wave length in alkali. For comparison, those of valine are shown in Fig. 1. ORD curves of amino acids in Table I were

TABLE I. MOLECULAR ROTATIONS OF PEPTIDES AND AMINO ACIDS AT EXTREMA IN ACID AND ALKALI

Name of compound	Molecular rotation at extrema near 225 m μ				Specific rotation			
	In 0.1 N HCl		In 0.1 N KOH		Conc.	Solvent	Temp. °C	[α] _D
	Wave length m μ	[M] $\times 10^{-2}$	Wave length m μ	[M] $\times 10^{-2}$				
Di-peptides								
H Ala-Ala OH	227 (max)	+ 18	227 (s)	-44	2%	0.5 N HCl	23	-37.5°
H Val-Val OH	224 (max)	+ 53	226 (min)	-32	2%	H ₂ O	17	+ 8.0°
H Glu-Val OH	225 (max)	+ 58	226 (min)	-54	2.65%	5 N HCl	15	+24.5°
H Lys-Val OH	225 (max)	+ 47	224 (min)	-42	2%	H ₂ O	14	+ 5.0°
H Ala-Leu OH	226 (max)	+ 47	227 (s)	-51	5%	H ₂ O	23	-16.8°
H Val-Leu OH	222 (max)	+ 50	223 (s)	-39	10%	0.1 N HCl	17	+ 6.0°
H Glu-Leu OH	225 (max)	+ 64	224 (min)	-38	1.8%	5 N HCl	14	+ 8.6°
H Lys-Ileu OH	226 (max)	+ 49	227 (s)	-22	2%	H ₂ O	16	+ 7.0°
H Val-Ileu OH	225 (max)	+ 53	225 (max)	+18	7.5%	0.1 N HCl	17	+ 8.1°
H Glu-Ileu OH	225 (max)	+ 55	226 (min)	-45	2%	5 N HCl	13	+33.0°
H Lys-Ileu OH	226 (max)	+ 53	226 (min)	-38	2%	H ₂ O	15	+ 7.5°
H Ala-Asp OH	224 (max)	+ 18	220 (min)	-35	2%	0.5 N HCl	23	+ 3.5°
H Val-Asp OH	221 (max)	+ 46	225 (min)	- 4.6	2%	H ₂ O	17	+25.0°
H Glu-Asp OH	222 (max)	+ 29	226 (s)	-16	1.05%	H ₂ O	15	+29.4°
H Lys-Asp OH	224 (max)	+ 26	227 (s)	-10	1.3%	H ₂ O	15	+20.8°
H Val-Glu OH	223 (max)	+ 27	225 (min)	- 1.5	1.5%	H ₂ O	17	+ 4.6°
H Glu-Glu OH	227 (max)	+ 61	225 (min)	-15	2%	5 N HCl	14	+18.0°
H Lys-Glu OH	226 (max)	+ 51	227 (s)	- 8	3.1%	H ₂ O	17	+19.0°
H Ala-Phe OH	227 (s)	+119	227 (s)	+34	2%	H ₂ O	23	+37.5°
H Val-Phe OH	223 (max)	+108	224 (min)	-17	2%	H ₂ O	17	+46.0°
H Glu-Phe OH	224 (max)	+135	225 (max)	+63	1%	5 N HCl	15	+24.0°
H Lys-Phe OH	227 (max)	+ 89	227 (s)	+48	2%	H ₂ O	16	+16.5°
H Val-Tyr OH	225 (max)	+ 81	225 (max)	+20	2%	2.5 N HCl	17	+33.0°
H Glu-Tyr OH	225 (max)	+ 26	220 (max)	+13	2%	5 N HCl	17	+30.0°
Tri-peptides*								
H Ala-Ala-Ala OH	227 (s)	- 4	227 (s)	-62	—		—	—
Tetra-peptides*								
H Ala-Ala-Ala-Ala OH	227 (s)	-45	227 (s)	-93	—		—	—
Amino acids								
Alanine	226 (max)	+ 7.3	224 (max)	+ 2.4	—		—	—
Leucine	226 (max)	+14	225 (max)	+ 8.3	—		—	—
Phenylalanine	225 (max)	+25	226 (max)	+16.1	—		—	—
Valine	224 (max)	+18	222 (max)	+ 6.9	—		—	—
Glutamic acid	224 (max)	+17	225 (s)	+ 7.5	—		—	—
Lysine	220 (max)	+17	225 (s)	+ 9.3	—		—	—

* Tri- and tetra-peptides of L-alanine were not measured but referred from the value of Beacham *et al.*²⁾ (max), (min) and (s) mean peak, minimum and the shortest wave length at which signal noise ratio reached about 10% respectively.

FIG. 1. Optical Rotatory Dispersion Curves of Valyl Valine and Valine of L-Series in Acid and in Alkali.

- 1 Valyl-valine in 0.1 N HCl
 - - - 1' " in 0.1 N KOH
 — 2 Valine in 0.1 N HCl
 - - - 2' " in 0.1 N KOH

measured in the same manner as those of di-peptides and they all gave positive single Cotton effect curves with a peak at about 225 $m\mu$ both in acid and in alkali as well as valine although the peaks in acid were higher than those in alkali.

Table I shows the molecular rotations of amino acids and di-peptides at the extreme about 225 $m\mu$ in acid and in alkali. Measurements within 10% of signal noise ratio could be made until at about 220 $m\mu$ in acid, and the experimental errors were about 5~10% at 225 $m\mu$. However, in alkali the reproducibilities were slightly worse than in acid, and

the signal noise ratios in some compounds were more than 10% at 225 $m\mu$. In such cases, the molecular rotations at the shortest wave length at which signal noise ratio reached about 10% were measured instead of those at the extrema and the values were enough to determine the signs of the first extreme.

Di-peptides are amphoteric compounds containing chromophores of at least one amino group at N-terminal, one amide group between two asymmetric carbons and one carboxyl group at C-terminal.

In acid, amino groups are protonated and the carboxyl and amide groups remain non-ionized. On the other hand, alkali carboxyl groups are ionized to carboxylate and amino and amide groups remain nonionized. If the total ORD of oligopeptide is the sum of the contributions of the chromophores in the molecule, the first extremum of a dipeptide at 225 $m\mu$ is considered as a superposition of the first extrema of the Cotton effects of the chromophores which have the absorption maximum at the wave length region slightly shorter than 225 $m\mu$ and in this case they are carbonyl groups than amino or protonated amino groups which have absorption maxima at shorter wave length than those of carbonyl groups. When di-peptides do not contain other chromophores such as valyl leucine the change of the sign of extrema in acid and in alkali mean that the sum of the contributions of carbonyl chromophores in α -(protonated amino)-amide groups and C-terminal carboxyl group in acid is positive but the sum of the contributions of carbonyl chromophores in α -(amino)-amide group and C-terminal carboxylate in alkali is negative.

FIG. 2. The Forms of Carbonyl Chromophores of Dipeptides in Acid and in Alkaline Solution

When di-peptides contain other chromophores such as lysine, glutamic acid, asparatic acid, phenylalanine and tyrosine, the contributions of the additional chromophores should be counted. However, it is important that most of the di-peptides with other chromophores gave the same tendency of the change of sign in the similar measurements as shown in Table I. It should be also noted that although some di-peptides which have aromatic amino acid at C-terminal gave a weak positive extrema in alkaline solution as exceptions, they also gave considerable decrease of positive optical rotation in alkali. The discrepancies of valyl isoleucine needs further research.

The decrease of the positive molecular rotations of amino acids at the extrema around 225 $m\mu$ in alkali may suggest that the positive contribution of α -(protonated amino) carboxyl chromophore in acid is more positive than that of α -amino carboxylate in alkali.

On the contributions of various chromophores of tri- and tetra-peptides of L-alanine at 227 $m\mu$, Beacham²¹ showed that central amide which does not have terminal free amino at α -position gave a negative contribution independent of pH.

Although their quantitative data are limited in alanyl and seryl peptides, it can be expected that other tri-peptides or at least oligopeptides of higher than tri-peptides would give negative optical rotation both in acid and in alkali because of the increase of the number of negative contributing central amides, or the sum of the amide contributions would be negative enough to cancel the positive contribution due to terminal chromophores even in acid as well as in alkali.

It is also supported by the fact that all the natural proteins and synthetic polypeptides gave negative optical rotations at 225 $m\mu$ so long as they are in random state.

Although it is necessary to study the con-

tributions of chromophores in other amino acids, a possibility of application of ORD is proposed to differentiate di-peptides from higher oligopeptides and amino acids by measuring their optical rotations near at about 225 $m\mu$ in acid and in alkali, based on a hypothesis that the molecular rotations of oligopeptides at the wave length are the sum of the negative contributing central amides which is independent on pH and C-terminal carboxyl and N-terminal amide chromophores which are dependent on pH.

When a compound is identified by other methods as an amino acid or an oligopeptide of L-series and it gives positive optical rotations at 225 $m\mu$ both in acid and in alkali, it is most probable an amino acid. When it gives a positive optical rotation in acid and a negative one in alkali, it is highly probable to be a di-peptide or in few cases a tri-peptide. When it gives negative optical rotations both in acid and in alkali, it is considered to be larger than a tri-peptide.

In this paper no compound of D-series was studied. However, if oligopeptides are composed only of D-amino acids, their ORD curves should be symmetrical with the zero line against their enantiomorphs. Therefore the method may possibly be applied to D-series only by changing the signs of the optical rotations of L-series.

When a oligopeptide is composed of mixed D- and L-amino acids or of glycine, this method can not be applied.

The measurement of circular dichromism of the same condition will be used for the qualitative determination of the positive or negative sign of their optical rotations.

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