THE OPTICAL ROTATION OF OLIGOPEPTIDES CONTAINING L-PROLINE AND L-HYDROXYPYROLINE

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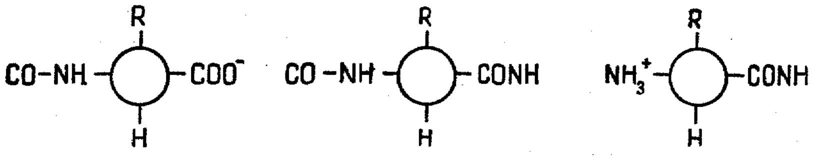
Erlanger and Brand [1–4] have shown that for relatively small peptides the specific rotation is an additive magnitude, and can be represented by the following simple expression

$$[\alpha]_D = \frac{100\sum R_i}{M}, \tag{1}$$

where M is the molecular weight of the peptides; and R_i is the rotation of the amino-acid residue equal, in the case of a peptide consiting of glycine and any other amino acid, to $M_1[\alpha]/100$ (where M_1 is the molecular weight of the peptide from the optical rotation of which the values of R_i are calculated). The magnitude of R_i for one and the same amino acid depends on its position in the peptide chain [1–4].

For each amino acid it is necessary to know three values of R_i, corresponding to the following positions: at the carboxyl end of the chain (I); within the polypeptide chain (II); at the amine end (III). For water, in which the peptides exist in the bipolar form, this corresponds to three different states of the substituents about the asymmetric carbon atom:



I II III

More remote interactions are not taken into account by the additivity rule, but they are probably small. Thus, Erlanger and Brand have found the following values of R_i for L-alanine in form III: + 18° C in L-alanylglycine, + 21° C in L-alanyl-L-alanine, and + 21° C in L-alanyl-L-lysine (I).

In all probability, the deviations from the additivity rule found are connected with conformational changes. In this case, the specific rotation of a peptide will have the form

$$[\alpha]_D = \frac{100\sum R_i}{M} + [\alpha]_{D\,\mathrm{conf}}, \tag{2}$$

where $[\alpha]_{D\ \mathrm{conf}}$ is the conformational contribution to the total rotation. A change in the optical rotation may occur as the result of hindered rotation around the C_α–CO and C_α–NH bonds. The presence of conformers of this type has been established by Kenner for oligopeptides [5] and by Shemyakin for depsipeptides [6].

The fixation of the conformations may take place as a result of intramolecular hydrogen bonds, while structures of the α-helix or polyproline (II) type may be formed (for peptides containing a large amount of imino acids and glycine). According to Goodman [7], the existence of conformers with an α-helical structure is actually the case for oligopeptides in nonpolar solvents.

In polar solvents, where intramolecular hydrogen bonds play a very small role, the first type of conformational effect must predominate, and in nonpolar solvents the second type.

Deviations from the rule of additivity may indicate different conformational changes in peptides and polypeptides. Unfortunately, at the present time R_i values have been calculated for only a few amino acids [1–4].

The present paper gives results confirming the correctness of the rule of additivity for peptides containing L-proline and L-hydroxyproline. We had synthesized the peptides previously [8-10]. The solvent selected was 96% acetic acid, a standard solvent for measuring the specific rotation of peptides, and 12 M lithium bromide. Table 1 gives the specific rotation of a series of peptides containing glycine and imino acids and the R_i values for L-proline and L-hydroxyproline in dependence on their position in the peptide chain. In addition, values of $[\alpha]_D$ and R_i measured in tris buffer at pH 7.2 are given.

The values of $[\alpha]_D$ measured and calculated by the additivity rule (using the R_i values from Table 1) for a number of peptides in 96% acetic acid are given in Table 2. As can be seen, the additivity rule is satisfactorily followed for all the compounds (the deviations do not exceed 5–7%), with the exception of (glycyl-L-prolylglycine)$_2$.

Table 1

Peptides*	Composition	M	$[\alpha]_D$ 96% CH_3COOH	$[\alpha]_D$ 12 M LiBr	$[\alpha]_D$ Tris buffer, pH 7.2	R_i 96% CH_3COOH	R_i 12 M LiBr	R_i Tris buffer, pH 7.2
			degrees					
Glycyl-L-proline	$C_7H_{12}N_2O_3$	172	−126	−98	−109	−217	−168	−188
L-prolylglycine	$C_7H_{12}N_2O_3$	172	−48	−51	−18.4	−82	−88	−32
Glycyl-L-prolylglycine	$C_9H_{15}N_3O_4\ 2H_2O$	265	−101	−51	−84	−267	−135	−223
Glycyl-L-hydroxyproline	$C_7H_{12}N_2O_4$	188	−119	−95	−99	−223	−178	−186
L-Hydroxyprolylglycine	$C_7H_{12}N_2O_4$	188	−49	−46	−25.6	−92	−86	−48
Glycyl-L-hydroxyprolyglycine	$C_9H_{15}N_3O_5$	245	−68	−33	−96	−166	−81	−235

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*In all experiments the concentration of the peptides was 0.2–0.3 g/100 ml, t = = 20° ± 1° C.

Table 2

Peptides	Composition	M	$[\alpha]$ Dfound	Contribution to the rotation: L-pro	Contribution to the rotation: L-hypro	Contribution to the rotation: L-ala*	$[\alpha]_{Dcalc}$	$\frac{[\alpha]_{calc} - [\alpha]_{found}}{[\alpha]_{calc}} \cdot 100$
				degrees				
(Glycyl-L-prolylglycyl)$_2$	$C_{18}H_{28}N_6O_7.H_2O$	458	−76	−58	—	—	−116	−34
(Glycyl-L-prolyl-L-alanyl)$_2$	$C_{20}H_{32}N_6O_7$	486	−140	−545	—	−15.1 −26.1	−150	−7.1
(Glycyl-L-prolyl-L-hydroxyprolyl)$_2$	$C_{24}H_{36}N_6O_9.2H_2O$	588	−155	−45.5	−28 −38	—	−157	−1.3
(Glycyl-L-prolyl-L-hydroxyproline)	$C_{12}H_{19}N_3O_5.H_2O$	303	−152	−88	−74	—	−162	−6.6
(Glycyl-L-prolyl-L-alanine)	$C_{10}H_{17}N_3O_4$ $1/2 \cdot H_2O$	252	−132	−106	—	−29	−135	−2.2
CBZ-glycyl-L-prolyl-L-alanylglycyl - L	$C_{27}H_{31}N_5O_9$	569	−71.4	−46.9	—	−22.3	−69.2	+3.2

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* L-Alanine in form (I) – 73.5° C [1], in form (II) – 127° C [13].

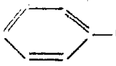
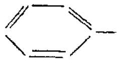
** CBZ represents the carbobenzoxy group —CH_2—O—CO—, NBE the p-nitrobenzyl ester group NO_2——CH_2—O.

Table 3 gives the values of $[\alpha]_D$ calculated and found for two hexapeptides in 12 M lithium bromide. It is interesting to note that when this solvent is used the additivity rule is well satisfied for (glycylprolylglycine)$_2$ as well.

Recently, it has become known that the majority of proteins increase their positive rotation in concentrated solutions of lithium bromide. Harrington and Shellman [11] have explained this by a fall in the activity of the water in such solutions, leading to a rise in the number of intramolecular hydrogen bonds in the protein molecules, i.e., to an increase in the percentage of helicalization.

Table 3

Peptides	Composition	M	$[\alpha]_D$ found	Contribution of the individual amino acids		$[\alpha]_D$ calc	Deviation from the additivity rule, %
				L-pro	L-hpro		
			Degrees				
(Glycyl-L-prolyl-glycine)$_2$	$C_{18}H_{28}N_6O_7 \cdot H_2O$	458	—72.9	36.7	—	73.4	—0.7
(Glycyl-L-prol-L-hpro)$_2$	$C_{24}H_{36}N_6O_9 \cdot 2H_2O$	588	—109	28.5	30.3 13.7	101	—7.9

However, it was shown subsequently that, on the one hand the activity of water decreases less markedly than was assumed by Harrington and Shellman [12] and on the other hand in 12 M lithium bromide the optical rotation of low-molecular-weight amino acids and peptides falls considerably. Thus, a considerable part of the effect of the increase in the positive rotation of proteins in 12 M lithium bromide is due to solvation changes of the state of the peptide bond. However, the theory of "supertwisting" requires at least additional experimental confirmation.

In our case, glycylprolylglycine and (Gly-Pro-Gly)$_2$ could hardly be expected to differ in the solvation of their bonds, but the $[\alpha]_D$ of the tripeptide changes markedly on passing from 96% acetic acid to 12 M lithium bromide, while that of the hexapeptide is practically unchanged. This interesting feature of the optical rotation of the hexapeptide on passing from one solvent to another and also the deviation of $[\alpha]_D$ from the calculated value in 96% acetic acid must be explained by the existence of a definite positive conformational contribution in the latter solvent.

Thus, the additivity rule, which is approximate by nature, is satisfied fairly accurately in a large number of cases. But marked deviations from the additivity rule are also possible. The latter cases probably deserve particularly great attention, since they may give information on the state of the polypeptide chain in a solvent.

Summary

1. Values of R_i for L-proline and L-hydroxyproline according to their position in the peptide chain in three different solvents have been calculated.

2. The rule of additivity of the optical rotation is satisfactorily followed in the majority of cases.

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