3) If, in fact, all PAH's containing a benzylic carbon atom are susceptible to nonphotochemical oxidation, as suggested herein, then the probable oxidative behavior of a large number of PAH's can be predicted (*6, 10*). For example, one would expect strongly carcinogenic compounds such as 9,10-dimethylbenz-[*a*]anthracene and 3-methylchloranthene (*3*) to be rapidly oxidized and thus detoxified. Possible evidence in support of this prediction is afforded by the fact that these compounds, if found at all in atmospheric particles, are present at barely detectable levels.

The widespread belief that particulate association of PAH's will promote their photochemical conversion is not substantiated by experiment. Nevertheless, substantial nonphotochemical conversion of PAH's adsorbed on coal fly ash can occur and may result in significant detoxification of several particulate PAH's.

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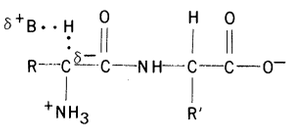
Racemization of Amino Acids in Dipeptides Shows COOH > NH_2 for Non-Sterically Hindered Residues

Abstract. *The relative rates of racemization for amino acid residues at the NH_2 and COOH ends of 37 different dipeptides were determined. In nine dipeptides containing alanine, leucine, phenylalanine, aspartic acid, and methionine, the amino acid residue racemized faster at the COOH-terminal position than at the NH_2-terminal position (COOH > NH_2). The sterically hindered amino acids isoleucine and valine showed NH_2 > COOH. Six proline dipeptides showed NH_2 > COOH. Intramolecular effects have been invoked to explain these surprising results.*

The significance of amino acid racemization and epimerization in biogeochemistry (geochronology and geothermometry) and paleobiology, as well as in peptide synthesis and the study of natural products, has been recognized in recent years. Several review articles have appeared (*1–5*). In polypeptides, racemization rates are reported to be altered by hydrolysis (*6*), which competes with racemization, complicating the study of racemization rates in peptides compared with those in free amino acids.

By employing optically active phases on a capillary gas chromatography column, we studied the racemization rates of eight amino acids, including isoleucine, in 37 different dipeptides. Unexpectedly, the dipeptides of alanine, leucine, phenylalanine, aspartic acid, and methionine, whether attached to a hindered or a nonhindered amino acid (valine and glycine, respectively), showed the COOH-terminal amino acid residues racemizing faster than the NH_2-terminal amino acid residues (Table 1). Emphasis is given these results because simple electrostatic theory predicts NH_2 > COOH. In nonproline dipeptides, only the sterically hindered amino acids isoleucine, valine, and serine showed NH_2 > COOH (Table 2). Proline, apparently, has a special effect. With proline dipeptides all amino acid residues showed NH_2 > COOH (Table 2), and the rates were faster at both ends with non-sterically hindered amino acids. All NH_2-terminal amino acid residues, including isoleucine and valine, racemized very rapidly in X-Pro dipeptides (33 to 53 percent D, Table 2). However, the sterically hindered amino acids isoleucine and valine racemized very slowly at the COOH-terminal position, particularly in Pro-X (~ 1 percent D, Table 2). Therefore, rate enhancement at NH_2 and rate retardation at COOH result in very high NH_2/COOH values for these sterically hindered amino acids. These data clearly show that position in the peptide, as well as amino acid structure, has a major effect on racemization and that steric hindrance affects racemization more at the COOH position than at the NH_2 position. Our results for isoleucine confirm those published recently by Kriausakul and Mitterer (*7*), who reported NH_2 > COOH in the epimerization of isoleucine in dipeptides of glycine, alanine, valine, tyrosine, and phenylalanine (Table 2). Because more of the amino acids racemize faster at the COOH position, the result NH_2 > COOH appears to be the exception rather than the rule. Serine falls in a class by itself. Its racemization was very fast at both positions, but its NH_2/COOH value was only slightly greater than 1.0 (1.2).

Racemization is generally considered to proceed through the removal of the α-methine hydrogen by base. Applying Neuberger's (*8*) mechanism, the transition state leading to the carbanion is an incipient carbanion (**1**) with varying C–H



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bond polarization. Stabilization of this incipient carbanion transition state controls the rate of racemization, and factors that alter the entropy or enthalpy of transition, $\Delta S^{\ddagger}$ or $\Delta H^{\ddagger}$, or both, affect the rate. At *p*H 7.6, at which this study was carried out, the dipeptide is principally in its zwitterion form. The $-{}^{+}NH_3$ group, through its electrostatic effect, would stabilize an adjacent incipient carbanion, while the $-CO_2^-$ group, through its inductive and field effects, would destabilize an incipient methine carbanion formed at the COOH-terminal position. Simple electrostatic theory predicts that

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NH_2 > COOH would be favored in racemization, and it has been found that removal of the carboxylate anion by ester or amide formation enhances racemization in free amino acids (*9*). Apparently in dipeptides other, less apparent factors operate to cause low NH_2/COOH values and, in some cases, result in COOH > NH_2. In the work of Kriausakul and Mitterer and in our study, the NH_2/COOH values were modest—less than 10, and with most amino acids less than 5—except for Pro-Ile and Pro-Val, which appear to be very special cases. But more important, in our study nine dipeptides showed COOH > NH_2 (Table 1).

It now appears that inductive and resonance effects are not necessarily the most important factors in the racemization of both free and bound amino acids. Stabilization of the incipient carbanion controls racemization rates since the ground states of the dipeptides are all nearly the same. Stabilization results from numerous factors that alter both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, including inductive and resonance effects, solvation and neighboring group participation, and steric effects that alter these factors. As described below, a neighboring group may stabilize the incipient carbanion or act as a base to remove the methine hydrogen. In either case, a more favorable $\Delta S^{\ddagger}$ term is achieved and the rate is higher. When there is steric hindrance to solvation (inter- or intramolecular) or a favored conformation that enhances neighboring group participation, considerable alterations of racemization take place. We propose that these factors play a major role in explaining the interesting results reported.

Table 1. Amounts of racemization of amino acid residues in dipeptides: COOH > NH_2.

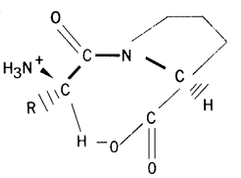
Dipeptide*	D-amino acid (%)†		COOH/NH_2‡
	NH_2 terminal	COOH terminal	
Ala-Gly, Gly-Ala	27.5 (Ala)	34.5 (Ala)	1.3
Ala-Val, Val-Ala	20.2 (Ala)	32.5 (Ala)	1.6
Leu-Gly, Gly-Leu	20.3 (Leu)	25.6 (Leu)	1.3
Leu-Val, Val-Leu	11.1 (Leu)	21.0 (Leu)	1.9
Phe-Gly, Gly-Phe	22.7 (Phe)	34.1 (Phe)	1.5
Phe-Val, Val-Phe	11.8 (Phe)	19.5 (Phe)	1.7
Asp-Gly, Gly-Asp	39.4 (Asp)	42.3 (Asp)	1.1
Asp-Val, Val-Asp	28.1 (Asp)	40.9 (Asp)	1.5
Met-Gly, Gly-Met	29.4 (Met)	34.8 (Met)	1.2

*The dipeptides listed here and in Table 2 were purchased from U.S. Biochemical Corp. and Vega-Fox Biochemicals. Abbreviations: Ala, alanine; Gly, glycine; Val, valine; Leu, leucine; Phe, phenylalanine; Asp, aspartic acid; Met, methionine; Ile, isoleucine; Pro, proline; Glu, glutamic acid; and Ser, serine. †Determined by gas chromatography (Hewlett-Packard HP-5830A) of the *N*-trifluoroacetyl-amino acid isopropyl ester on a stainless steel capillary column loaded with a mixed chiral phase. *N*-docosanoyl-L-valyl *t*-butyl amide, and *N*-octadecanoyl-L-valyl-L-valyl cyclohexyl ester. ‡Racemizations were carried out in Pyrex sealed tubes, in phosphate-buffered solution, 0.05*M*, ionic strength, 0.12, amino acid concentration, 0.02*M*, *p*H 7.6, 122.5°C, 8 hours. Percentages of D formation for free amino acids under the same conditions are Val, 0.3; Ile, 0.5; Leu, 0.5; Ala, 1.0; Phe, 1.9; Met, 2.1; Asp, 2.5; and Ser, 21.9. The values reported are the averages of triplicate analyses of triplicate racemization experiment.

Table 2. Amounts of racemization of amino acid residues in dipeptides: NH_2 > COOH.

Dipeptide	D-amino acid (%)		NH_2/COOH	
	NH_2 terminal	COOH terminal	This report	Kriausakul and Mitterer (*7*)
Ile-Pro, Pro-Ile	52.8 (Ile)	0.9 (Ile)	58.7	
Val-Pro, Pro-Val	34.6 (Val)	1.1 (Val)	31.5	
Phe-Pro, Pro-Phe	48.7 (Phe)	12.2 (Phe)	4.0	
Leu-Pro, Pro-Leu	44.1 (Leu)	16.8(Leu)	2.6	
Glu-Pro, Pro-Glu	33.0 (Glu)	15.8 (Glu	2.1	
Ala-Pro, Pro-Ala	43.2 (Ala)	26.5 (Ala)	1.6	
Val-Pro, Pro-Val	34.6 (Val)	1.1 (Val)	31.5	
Val-Asp, Asp-Val	29.5 (Val)	3.1 (Val)	9.5	
Val-Phe, Phe-Val	16.7 (Val)	2.2 (Val)	7.6	9.92 (Ile)
Val-Leu, Leu-Val	18.1 (Val)	3.5 (Val)	5.2	
Val-Ala, Ala-Val	26.0 (Val)	5.6 (Val)	4.6	1.91 (Ile)
Val-Gly, Gly-Val	13.0 (Val)	3.6 (Val)	3.6	
Val-Ile, Ile-Val	5.4 (Val)	3.5 (Val)	1.5	
Ile-Gly, Gly-Ile	20.6 (Ile)	5.6 (Ile)	3.7	1.35
Ile-Val, Val-Ile	11.8 (Ile)	4.0 (Ile)	3.0	1.20
Ser-Gly, Gly-Ser	46.5 (Ser)	39.1 (Ser)	1.2	

All amino acid residues gave higher rates of racemization in dipeptides of the type X-Pro than in nonproline dipeptides such as X-Gly (Table 2). When a sterically hindered amino acid was used (X-Val), the percentage of D was further reduced, implying steric effects. If we choose glycine as the standard, proline enhances racemization and valine reduces it. There appears to be a logical explanation for these results. It is perhaps not surprising that proline, isoleucine, valine, and serine act differently in racemization experiments than other amino acids. Blout *et al.* (*10*) showed some years ago that these and similar amino acids (sarcosine, hydroxyproline, and threonine) caused peptides to form the non-α-helical structure. These amino acids cause special steric or intramolecular effects. Because of the partial double-bond character of the C–N bond, the four atoms of the peptide link plus the two adjacent α-carbon atoms lie in the same plane, making possible *cis* and *trans* isomers. The *trans* peptide bonds are generally favored.



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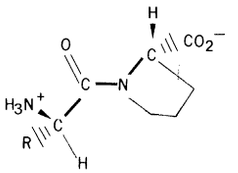

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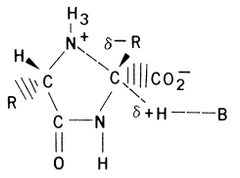
However, with amino acids such as proline, the X-Pro bond can be *cis* (**2**) or *trans* (**3**) (*11*). Using ^{13}C nuclear magnetic resonance, Dorman and Bovey (*12*) and Wuthrich *et al.* (*13, 14*) were able to distinguish between *cis* and *trans* conformers in polypeptides containing proline and other NH_2-substituted amino acid residues. Dorman and Bovey (*12*), in a study of Phe-Pro, reported that a negative charge on the COOH group of proline in H-X-ProO$^-$ favors the *cis* conformation. Wuthrich *et al.* (*14*) reported a similar result for Ala-Pro. Therefore, a logical explanation for the rapid racemization of all NH_2-terminal amino acid residues in X-Pro dipeptides is that the *cis* conformation (**2**) is more stable than the *trans* conformation (**3**) at *p*H 7.6. In the *cis* conformation the free carboxyl

anion is suitably located for maximum assistance in the base removal of the α-proton through a seven-membered ring (**2**). It is well known that intramolecular base removal of a proton has a more favorable $\Delta S^{\ddagger}$. In this case, this would result in an increase in the racemization rate. Models show very clearly that only in the *cis* conformation is the carboxylate close enough to the methine hydrogen of the adjacent amino acid residue to assist in its removal. The *trans* conformation, the preferred conformation for amino acids other than proline and hydroxyproline, would require isomerization before those amino acids could assist in the intramolecular removal of the α-hydrogen. [The free energy ($\Delta G°$) value for this isomerization is 0.1 to 2 kcal (*14*).] This also explains why NH_2-terminal amino acids racemize more slowly in X-Gly and X-Val than in X-Pro. It is proposed that the lower rate for X-Val than X-Gly is due to steric hindrance. Steric factors (probably affecting solvation) are known to reduce racemization of free amino acids (*15*). When there are bulky amino acids at the reaction site, rates drop off precipitously. Valine in Val-Ile and Ile-Val racemized very slowly, forming only 5.4 and 3.4 percent D-valine, respectively (Table 2). The case of isoleucine in Ile-Val and Val-Ile was similar, with 11.8 and 4.0 percent D-isoleucine formed (Table 2). This steric effect may be better understood by considering intramolecular effects in racemization of dipeptides.

We propose that the unexpected result COOH $>NH_2$ for some dipeptides is due to a more favorable entropy effect resulting from the participation of another intramolecular neighboring group. At *p*H 7.6 the amino group in dipeptides is approximately 70 percent $-{}^{+}NH_3$ and 30 percent $-NH_2$. The $-{}^{+}NH_3$ group may "solvate" the incipient carbanion through a five-membered ring (**4**), and the free $-NH_2$ group may act as an intramolecular base to assist in removal of the methine hydrogen (**5**). Either or both of these would readily account for more competitive racemization at the COOH-terminal position. The geometry of the system for intramolecular assistance, as shown in **5**, is similar to that found in an S_N2-type mechanism (*16*) where steric hindrance is very effective in reducing rates. We propose that similar crowding occurs with X-Ile and X-Val at the COOH-terminal position, explaining the very slow rate of racemization of both isoleucine and valine at the COOH-terminal position (Table 2).

For proline to show similar intramolecular stabilization of the incipient



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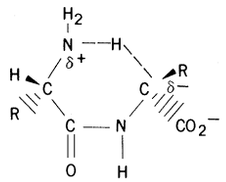

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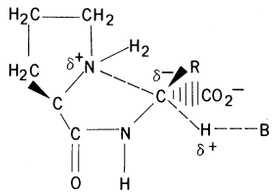

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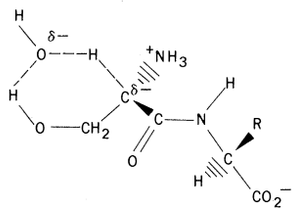

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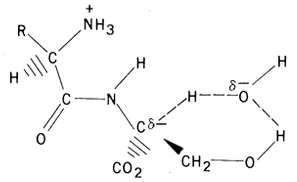

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carbanion would require the formation of two fused five-membered rings (**6**), which is rarely observed. Therefore, it is not surprising that the balance is shifted with proline dipeptides, resulting in faster racemization of the NH_2-terminal residues. With the exception of valine and isoleucine, however, the NH_2/COOH values are modest (less than 4), indicating very competitive rates of racemization even with proline dipeptides between the COOH- and NH_2-terminal positions.

Serine is a special case. It racemizes very rapidly and at about the same rate in both the NH_2- and COOH-terminal positions. The NH_2/COOH value is only 1.2 (Table 2). As the free amino acid it also racemizes rapidly, with 21.9 percent D formed at the same conditions. Inter- or intramolecular solvation of the incipient carbanion and the base are critical in racemization. We propose that the hydroxyl group in serine helps to solvate the base required to remove the α-hydrogen, bringing about rapid removal of the α-hydrogen. This would not be position-dependent, and hence assistance could occur at both the NH_2- and COOH-terminal locations (**7** and **8**).

The extent of hydrolysis of the dipeptide during the racemization was minimal, less than 1.5 percent. Dipeptides are considered to be more resistant to hydrolysis than are polypeptide bonds (*17*).

In summary, the relative rates of racemization of amino acid residues in dipeptides (NH_2 versus COOH terminal) are determined by a delicate balance of factors, including inductive and field effects, intramolecular base action, intramolecular solvation, and steric hindrance to solvation. The intramolecular affects appear to be more significant than inductive and field effects. Our results indicate that intramolecular neighboring group interactions play the major role in determining the relative rates of racemization of NH_2- and COOH-terminal amino acid residues in dipeptides. With X-Pro dipeptides, there is a high concentration of the *cis* configuration. When dipeptides are in the *cis* configuration, the carboxylate group can assist in the removal of the methine hydrogen of the adjacent amino acid residue, enhancing racemization of the NH_2-terminal residue (**2**).

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