Side Chain Motions in Ternary Amino Acid-Palladium(II) Complexes as Measured from ^{13}C NMR Relaxation Times

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Received November 11, 1981

*Effects of electrostatic ligand-ligand interactions on the side chain motions in ternary amino acid-palladium(II) complexes have been studied by ^{13}C spin-lattice relaxation times, T_1. The NT_1 values (N = the number of hydrogen atoms bound to the carbon) of glutamate (Glu) in the systems containing palladium(II) and argininate (Arg), Pd(*L*- or* D*-Glu)(*L*-Arg), where side chain interactions exist, are considerably smaller than the values for Pd(*L*- or* D*-Glu)(*L*-Ala) (Ala = alaninate) without the interaction which are close to those of Pd(Glu)$_2$. Comparison of the NT_1 values reveals that the motions of the α-carbons are strongly dependent on the ligand-ligand interactions, whereas the β- and γ-carbons can move relatively freely even in their presence. The motions of the side chain of Arg are also affected by the interaction.*

Introduction

Specificity and efficiency of enzymatic reactions owe much to enzyme-substrate noncovalent interactions, which give rise to stereoselectivity and enable favorable placement of groups susceptible to subsequent catalytic actions of enzymes [1–3]. For metalloenzymes with a metal ion at the active center, the situation may be simulated by appropriate mixed ligand complexes with the two ligand side chains interacting with each other through noncovalent bonds. We have shown by synthetic and spectroscopic studies that ternary amino acid–copper(II) and –palladium(II) systems involving an acidic and a basic amino acid may serve as models for enzyme–metal–substrate complexes formed in the course of enzymatic reactions in the sense that the positively and negatively charged side chains of the coordinated amino acids can be close enough to each other to form ionic bonds which are similar to enzyme–substrate interactions [4–6]. Also, in the ternary systems involving histidine, an amino acid with a hydroxyl or an amido group, and copper(II) or palladium(II), a hydrogen bond is formed between the carboxylate oxygen of histidine and the polar group of the other amino acid coordinated to the same metal ion [7–9]. X-ray analysis of *L*-asparaginato-*L*-histidinatocopper(II) disclosed that the side chain of asparaginate is flexible, and this is consistent with such ligand–ligand interactions within complex molecules [10]. Ligand selectivity, probably attributable to the interactions, has been established by the stereoselective incorporation of amino acids into the ternary copper(II) and palladium(II) complexes isolated as crystals [6–8, 11, 12].

In order to shed light on the dynamic aspects of the ligand–ligand interactions, we investigated the motions of the ligand side chains under the influence of electrostatic interactions in the ternary palladium(II) complexes with an acidic amino acid A and a basic amino acid B. The present paper describes the side chain motions as measured from the ^{13}C spin-lattice relaxation times, T_1, and interpretations of the results.

Experimental

Materials

L-Alanine (*L*-Ala), *L*- and *D*-glutamic acid (*L*- and *D*-Glu), and *L*-arginine (*L*-Arg) were purchased from Nakarai Chemicals, Co. Palladium(II) chloride was obtained from Kishida Chemical Co. The complexes Pd(L-GluH)$_2$ and Pd(D-GluH)$_2$* were prepared from 1 mole of $PdCl_2$ dissolved in 2 M HCl (M = mol dm^{-3}) and 2 moles of *L*- and *D*-Glu, respectively, the pH being adjusted at ~3. The complex

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*Abbreviations for amino acids, Ala and Glu, in complexes refer to deprotonated forms; Arg denotes the zwitterionic form and GluH denotes the monoprotonated form of Glu.

0020-1693/1982/0000-0000/$02.75

TABLE I. ^{13}C Spin-Lattice Relaxation Times, NT_1, for Glu, Arg, and their Palladium(II) Complexes.[a]

System	pH	α-C	β-C	γ-C	δ-C
			NT_1 of Glu		
L-Glu	7 1	2.39	2.54	3.40	
Pd(*L*-Glu)$_2$	5.8	0.89	1.08	1.56	
Pd(*L*-Glu)(*L*-Ala)	5.9	0.86	1.02	1.56	
Pd(*L*-Glu)(*L*-Arg)	5.6	0.37	0.68	1.06	
Pd(*D*-Glu)(*L*-Ala)	6.3	0.98	1.12	1.50	
Pd(*D*-Glu)(*L*-Arg)	6.4	0.63	0.92	1.30	
			NT_1 of Arg		
L-Arg	5.9	1.67	1.74	1.86	2.00
Pd(*L*-Arg)$_2$	6.2	0.46	0.66	0.78	1.02
Pd(*L*-Arg)(*L*-Ala)	6.1	0.64	0.78	1.02	1.24
Pd(*L*-Arg)(*L*-Glu)	5.6	0.44	0 54	0.70	0.76
Pd(*L*-Arg)(*D*-Glu)	6.4	0.57	0.52	0.94	1.00

[a]NT_1 values are given in seconds. N is the number of protons bound to the carbon.

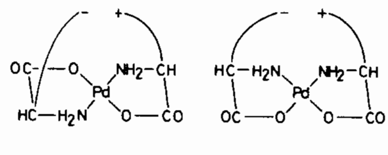
of *L*-Ala, Pd(*L*-Ala)$_2$, was prepared in the same manner at pH ~ 5. The complex Pd(*L*-Arg)$_2$(ClO_4)$_2$·2H_2O* was obtained by passing a solution of Pd(*L*-Arg)$_2Cl_2$·3H_2O [6] through the ion exchange resin IRA-410 in the ClO_4^- form. The isolated complexes were recrystallized from water in order to remove trace amounts of paramagnetic impurities.

Measurement of NMR Spectra

Samples for the binary systems were prepared from the isolated complexes by dissolving the crystals in deuterium oxide with or without a stoichiometric amount of NaOH. Samples for the ternary systems were prepared by mixing the solutions of the binary complexes, the total amino acid concentrations being 0.5 *M* in all the systems. The solutions were not degassed. A TOA HM-18A pH meter equipped with a TOKO CE 103 combination microelectrode was used without correction for readings in deuterium oxide.

The T_1 values were measured at 34 °C with a Hitachi R-900 Fourier transform (FT) NMR spectrometer at 22.6 MHz by the inversion recovery method [13] or the fast inversion recovery FT method [14] using the pulse series (180°-homospoil-τ-90°-PD)$_n$. The accuracy of the T_1 values was estimated to be within ±10% [15].

Anal. Calcd. for $C_{12}H_{32}N_8O_{14}Cl_2Pd$: C, 20.90; H, 4.68; N, 16.25. Found: C, 21.09; H, 4.57; N, 16.13.



Pd(L-A)(L-B) Pd(D-A)(L-B)

Fig. 1. Geometrical isomerism due to ligand–ligand interactions.

Results and Discussion

Estimation of T_1 Values

Although the samples were not degassed, the effects of dioxygen on the T_1 values may be negligible in the present study, because the observed T_1 values are much smaller than the relaxation times due to dioxygen. The T_1 value of a carbon atom with N hydrogen atoms attached is determined by dipole-dipole relaxation, and spin-lattice relaxation is assumed to be under the extreme narrowing conditions [16]:

$$\frac{1}{NT_1} = \hbar^2 \gamma_C^2 \gamma_H^2 r_{CH}^{-6} \tau_c \qquad (1)$$

where γ_C and γ_H are the gyromagnetic ratios of carbon and hydrogen, respectively, r_{CH} is the $^1H-^{13}C$ distance, and $\hbar = h/2\pi$ (h = the Planck constant). The effective correlation time, τ_c, which serves as a measure of the time necessary for rearrangement of a molecule, is expressed by eqn. 2 in terms of the molecular correlation time, τ_{mol}, describing the rotation of a molecule as a whole and the internal correlation time, τ_{int}, describing the internal segmental motion [17]:

$$\frac{1}{\tau_c} = \frac{1}{\tau_{mol}} + \frac{1}{\tau_{int}} \qquad (2)$$

The NT_1 values thus determined are shown in Table I.

Effects of Complex Formation

The ^{13}C NMR signals for free and coordinated amino acids are different from each other because the ligand exchange rates are slower compared with the ^{13}C NMR time scale. The NT_1 values for the carbons in the binary complexes Pd(*L*-Glu)$_2$ and Pd(*L*-Arg)$_2$ are smaller than those of *L*-Glu and *L*-Arg, respectively, indicating that τ_{mol} is longer and hence the molecular rotation is slower in the complexes than in the ligands alone owing to the increase of the molecular weight (Table I). The differences between the $1/\tau_c$ values of free and coordinated amino acids are roughly constant in going from α-carbon to γ- and δ-carbons.

TABLE II. Ratios, R of ^{13}C Spin-Lattice Relaxation Times of Amino Acids in Pd(A)(B) to Those in Pd(A or B)(*L*-Ala).

System Compared	Amino Acid	R			
		α-C	β-C	γ-C	δ-C
Pd(*L*-Glu)(*L*-Arg) / Pd(*L*-Glu)(*L*-Ala)	Glu	0.43	0.67	0.68	
Pd(*D*-Glu)(*L*-Arg) / Pd(*D*-Glu)(*L*-Ala)	Glu	0.64	0.82	0.87	
Pd(*L*-Arg)(*L*-Glu) / Pd(*L*-Arg)(*L*-Ala)	Arg	0.69	0.69	0 69	0.61
Pd(*L*-Arg)(*D*-Glu) / Pd(*L*-Arg)(*L*-Ala)	Arg	0.89	0.67	0.92	0.81

Effects of Ligand–Ligand Interactions

The steric requirements for the electrostatic ligand–ligand interactions, as viewed from space-filling models, suggest that the diastereomeric complexes Pd(*L*-A)(*L*-B) and Pd(*D*-A)(*L*-B) exist as geometrical isomers depicted in Fig. 1 [6]. The NT_1 values for Glu in the ternary systems Pd(*L*-Glu)(*L*-Ala) and Pd(*D*-Glu)(*L*-Ala) are close to those of Pd(*L*-Glu)$_2$, which shows that the motion of Glu is not affected appreciably by the ternary complex formation in the absence of ligand–ligand interactions. The NT_1 values for a complex are considered to be largely governed by local segmental motion rather than rotation of the molecule, since the difference in the molecular weights between the binary and ternary complexes does not seem to be reflected on T_1. On the other hand, Pd(*L*-Arg)$_2$ shows unexpectedly small NT_1 values probably because of the electrostatic repulsion between the long positively-charged side chains in the same molecule, and a reasonable comparison of NT_1 values may be made with Pd(*L*-Arg)(*L*-Ala) as a standard. In the systems involving Glu and Arg, the NT_1 values for the two amino acids are considerably smaller than those of Pd(*L*-Glu)$_2$, Pd(*L*- or *D*-Glu)(*L*-Ala), and Pd(*L*-Arg)(*L*-Ala), indicating that the electrostatic interactions between the carboxylato and guanidinium groups restrict the segmental motions of the ligands.

The ratios, R, of the NT_1 values for the ternary systems with side chain interactions to those for the systems containing *L*-Ala in place of *L*-Arg reveal that the value for the α-carbon of Glu is significantly smaller than the values for the β- and γ-carbons (Table II). This finding suggests that the motion of the α-carbon, which affects the distance between the two interacting side chain groups, is strongly dependent on the ligand–ligand interactions, whereas the β- and γ-carbons can move relatively freely even in their presence. As seen from the relative NT_1 values, the motion of the α-carbon of Arg appears to be less restricted, possibly because the motions of the Arg carbons are averaged owing to the longer side chain length. That the complex Pd(*L*-Glu)(*L*-Arg) exhibits smaller NT_1 values than those of the *meso* complex Pd(*D*-Glu)(*L*-Arg) may result from the separation between the two α-carbons, which is shorter in the latter than in the former (Fig. 1) and accordingly gives greater freedom of motion to the side chains of the latter.

Taken together, the results support the view that the ligand–ligand interactions in ternary systems affect the motions of amino acid side chains, which, however, are not fixed in a certain position but are flexible and in dynamic motion.

Acknowledgements

We are grateful to Prof. Yoshio Sasaki of Osaka University for suggestions. This work was supported by a Grant-in-Aid for Special Project Research (No. 56209007) by the Japanese Ministry of Education, Science, and Culture, to which our thanks are due.

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