Complexes of Ca^{2+} and Mg^{2+} with *N*-acetyl-α,β-dehydrodipeptides: the state in an alcoholic solution and its relationship with asymmetric induction upon diastereoselective hydrogenation

I. N. Lisichkina, T. A. Larina, A. S. Peregudov, T. Yu. Vasil'eva, and V. M. Belikov*

A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 28 ul. Vavilova, 117813 Moscow, Russian Federation.
Fax: +7 (095) 135 5085. E-mail: inlis@ineos.ac.ru; vmbel@ineos.ac.ru

In an alcoholic solution, *N*-acetyl-α,β-dehydrodipeptides interact with Ca^{2+} and Mg^{2+} ions to form complex particles containing several dehydrodipeptide molecules per metal ion. The composition of these particles and steric interactions in them determine the acidity of the carboxyl groups and the degree of asymmetric induction upon diastereoselective hydrogenation.

Key words: calcium and magnesium complexes, dehydrodipeptides, diastereoselective hydrogenation, asymmetric induction.

Previously, we have studied the effect of complexation with Ca^{2+} and Mg^{2+} ions on asymmetric induction upon hydrogenation of *N*-acyl-α,β-dehydrodipeptides (DHDP) on a Pd/C catalyst to form diastereomers of *N*-acyldipeptides.[1,2] It was established that complexation in an alcoholic solution resulted in a substantial enhancement of the stereoselectivity of the reaction due to an increase in the conformational rigidity of the initial unsaturated molecule. However, the mechanism of this effect is still poorly understood. In this work, using *N*-Ac-ΔPhe-*S*-Pro (**1**), *N*-Ac-ΔPhe-*S*-Val (**2**), *N*-Ac-Δ(*p*-FPhe)-*S*-Glu (**3**), *N*-Ac-Δ(*p*-FPhe)-*S*-Pro (**4**), and *N*-Ac-Δ(*p*-FPhe)-*S*-Phe (**5**) (*p*-FPhe is the residue of *p*-fluorophenylalanine) as examples, we studied the state of calcium and magnesium complexes of DHDP in an alcoholic solution and revealed the relationship between this state and the diastereoselectivity.

Experimental

The ^1H NMR spectra were recorded on Bruker AMX-400 and Bruker WP-200SY spectrometers in CD_3OD. The ^{19}F NMR spectra were obtained on a Bruker WP-200SY spectrometer (operating at 188.31 MHz) in MeOH. Resonance conditions were stabilized with the use of a deuterium signal from D_2O placed between the walls of a 5-mm tube and a 3-mm insert containing a solution of a sample. The δ ^{19}F chemical shifts were measured relative to PhF as the external standard (in MeOH) by the substitution method. The accuracy of measurements was ±0.02 ppm. The UV spectra were recorded on a Specord UV-VIS instrument. The average molecular weights ($\bar{M}_n$) of compound **1** and its complexes in the **1**—$MgCl_2$—NaOH system (the molar ratio was 1 : 0.25 : 1) were determined on a Wescan 233 vapor-phase osmometer in EtOH at 30 °C and at the concentration [**1**] = 3—8 g L^{-1} (see Ref. 3). For **1**, found: $\bar{M}_n$ = 280. $C_{16}H_{18}N_2O_4$. Calculated: $\bar{M}_n$ = 302. For the complexes, the effective value was found to be $\bar{M}_n$ = 812. The true value ($\bar{M}_n$ = 1600—2000) was calculated taking into account corrections for contributions of Na^+ and Cl^- ions formed from NaOH and $MgCl_2$, respectively.

***N*-Acetyl-α,β-dehydrodipeptides** were prepared by the azlactone method. Condensation of the corresponding aldehyde with *N*-acetylglycine was carried out by the Erlenmeyer reaction. The azlactone that formed was introduced into the reaction with amino acid according to a procedure reported previously.[4]

***N*-Ac-Δ(*p*-FPhe)-*S*-Glu (3).** The yield was 65%, m.p. 169—170 °C (from H_2O). Found (%): C, 54.77; H, 4.92; N, 7.95. $C_{16}H_{17}FN_2O_6$. Calculated (%): C, 54.55; H, 4.86; N, 7.95.

***N*-Ac-Δ(*p*-FPhe)-*S*-Pro (4).** The yield was 84%, m.p. 190—191 °C (from H_2O). Found (%): C, 59.80; H, 5.39; N, 8.67. $C_{16}H_{17}FN_2O_4$. Calculated (%): C, 60.00; H, 5.39; N, 8.75.

***N*-Ac-Δ(*p*-FPhe)-*S*-Phe (5).** The yield was 86%, m.p. 217—219 °C (from 10% EtOH). Found (%): C, 65.09; H, 5.29; N, 7.58. $C_{20}H_{19}FN_2O_4$. Calculated (%): C, 64.87; H, 5.17; N, 7.57.

Hydrogenation. The metal salt (0.1 mmol) and MeONa (0.1 mmol) were added to a solution of DHDP (0.1 mmol) in EtOH. The mixture was stirred for 1—3 h and then hydrogenated under atmospheric pressure at ~20 °C on 10% Pd/C (the substrate : catalyst ratio was 10 : 1). The course of the reaction was monitored by UV spectroscopy. The completion of hydrogenation was determined from the disappearance of the absorption band with λ_{max} = 280 nm belonging to the system of conjugated double bonds. According to the data of UV and ^1H NMR spectroscopy, the hydrogenation product was obtained in nearly quantitative yield.

The diastereomeric excess of compound **2** was determined from the ^1H NMR spectra, which had two distinct sets of signals for the methyl protons of the valine residue belonging to two diastereomers. In other cases, in particular, in the case of compound **1**, when the assignment of signals to a particular diastereomer presented difficulties, the hydrogenation products were hydrolyzed with 2*M* HCl for 10 h. The resulting mixture

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 5, pp. 904—907, May, 1999.

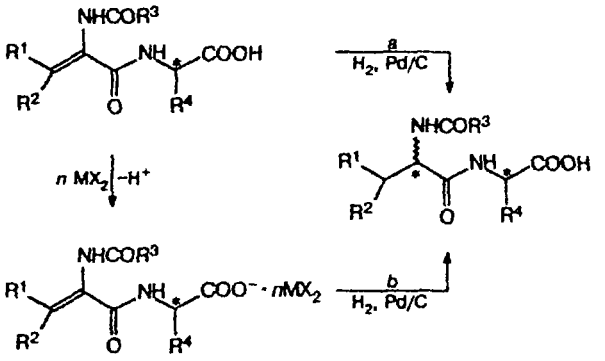
1066-5285/99/4805-0895 $22.00 © 1999 Kluwer Academic/Plenum Publishers

of free amino acids was isolated on a cation exchanger and analyzed by GLC on a chiral phase.[5]

Results and Discussion

The reaction under study is shown in Scheme 1.

Scheme 1



$R^1 = R^2 = Me$; $R^1 = Alk, Ar$, $R^2 = H$;
$R^3 = Me, Ph$; R^4 is the side chain of the amino acid;
$M = Ca, Mg$

As demonstrated previously using hydrogenation of complex **1** with $MgCl_2$ as an example,[2] the stereoselectivity of the reaction was enhanced, while the rate of hydrogenation decreased substantially as the $MgCl_2$: **1** ratio increased. Consequently, complexation favors the enhancement of asymmetric induction. Based on the data of potentiometric titration, it was found that the total acidity of the COOH groups increased as calcium and magnesium salts were added to an alcoholic solution of **1** (and other DHDP) and peaked when the salt was taken in a five- to eight-fold excess.[2] It was suggested that an equilibrium between free DHDP and its complexes exists in solution:

$$RCOOH + MX_2 \rightleftharpoons RCOOH \cdot MX_2, \qquad (1)$$
$$RCOOH \cdot MX_2 + MeONa \rightleftharpoons RCOO^- \cdot M^+X + NaX.$$

Since equilibrium (1) is shifted to the right as the relative content of MX_2 increases, a large amount of free DHDP **1** should be present in a solution when the reagents are taken in an equimolar ratio. Consequently, in this case the reaction should proceed according to route *a* (see Scheme 1) at a high rate with low selectivity rather than follow route *b*. However, this conclusion is contradictory to the above-mentioned data on the effect of the relative content of the metal salt on the rate and diastereoselectivity of hydrogenation of DHDP. Even if MX_2 and DHDP were taken in a molar ratio of 0.25 : 1, the rate of hydrogenation was lower and the stereoselectivity was higher than those in the reaction of free DHDP. Therefore, the state of complexes **1** with MX_2 in solution has a pronounced effect on the mechanism of diastereoselective hydrogenation of DHDP complexes. The present study was devoted to elucidation of this question.

We assumed that all DHDP molecules occur as complexes in the presence of salts of alkaline-earth metals, which does not contradict the published data, according to which derivatives of amino acids interact with Ca^{2+} and Mg^{2+} ions to form complexes with coordination numbers of up to 8. Thus, X-ray diffraction study of the calcium complex of *N*-Ac-Met, which is a close analog of the DHDP under consideration, demonstrated that one Ca^{2+} ion has four coordination sites occupied by three *N*-Ac-Met molecules, two sites occupied by other Ca^{2+} ions, and two sites occupied by two water molecules.[6–8] High-coordinate Ca^{2+} complexes were also observed in solutions.[9]

We obtained direct evidence on the composition of complex particles in the system under study from measurements of their molecular weights. When measuring the average molecular weights in the **1**—$MgCl_2$—NaOH system (the molar ratio was 1 : 0.25 : 1) in 95% EtOH by vapor-phase osmometry, we detected the formation of large complex particles in the solution. The determined value $\bar{M}_n$ = 1600—2000 corresponds to complexes of the average composition ML_5. Therefore, in this case one Mg^{2+} atom bound *ca.* five DHDP molecules and, apparently, no free molecules **1** remained in the solution.

We also attempted to detect free DHDP molecules in the presence of the Mg^{2+} salt from the ^1H NMR spectra in the expectation that their signals would be observed along with signals for DHDP molecules that are incorporated into the complexes. The characteristic chemical shifts in the ^1H NMR spectra of the **2**—$MgSO_4$—NaOH system, which is a more convenient system for studies by this method, are given in Table 1. However, although complexation affects signals for the protons at the vinyl and asymmetric carbon atoms, this system did not give two sets of signals. Previously,[10] ^1H NMR spectra which we obtained in studies of the *N*-Ac-ΔPhe-Tyr · $CaCl_2$, *N*-Ac-ΔPhe-Tyr · $MgSO_4$, **1** · $CaCl_2$, **1** · $MgSO_4$, and *N*-Ac-ΔPhe-Leu · $MgSO_4$

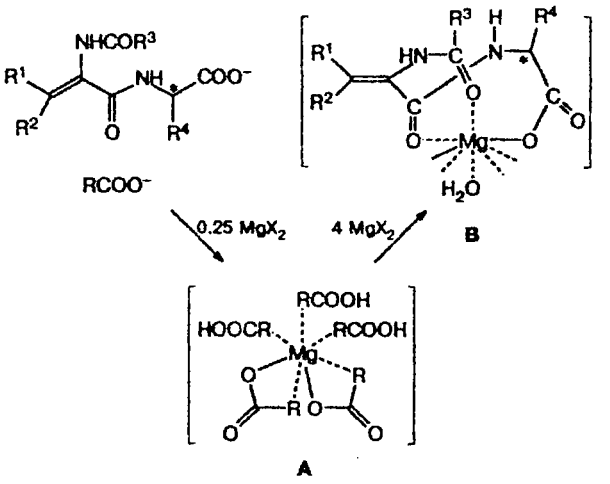
Table 1. ^1H NMR spectra (δ) of complexes **2** with $MgSO_4$ (in CD_3OD)

Compound, system	>CMe_2 (m)	MeCO (s)	CH* (m)	=CH— (s)	Ph (m)
2	1.15, 1.19	2.33	4.62	7.36	7.62
2—NaOH (1 : 1)	1.14, 1.19	2.34	4.50	7.49	7.56
2—NaOH—$MgSO_4$ (1 : 1 : 1)	1.15, 1.19	2.33	4.48	7.43	7.56

Table 2. ^{19}F NMR spectra of fluorodehydrodipeptides 3—5 and their complexes with metals (in MeOH)

F-DHDP	Metal ion	[MCl_2] (equiv.)	[MeONa] (equiv.)	^{19}F NMR, δ
3	—	—	—	−2.06
3	Ca^{2+}	8	—	−2.58
3	Zn^{2+}	8	—	−2.53
4	—	—	—	−1.1, −0.91
4	Mg^{2+}	0.5	—	−1.15, −0.95
4	Mg^{2+}	1	—	−1.18, −0.97
4	Mg^{2+}	4	—	−1.31, −1.12
4	Ca^{2+}	4	—	−1.61, −1.34
4	—	—	1	−0.6, −0.3
4	Mg^{2+}	0.5	0.5	−0.92, −0.51
4	Mg^{2+}	4	1	−1.33, −0.99
5	—	—	—	−2.09
5	—	—	1	−1.90
5	Ca^{2+}	2	1	−2.26
5	Ca^{2+}	5	1	−2.27

Scheme 2



complexes also did not contain signals indicative of the presence of two types of particles in the solution.

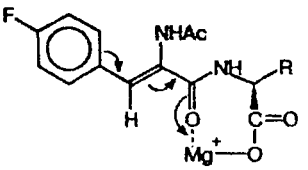
The ^{19}F NMR spectra of the complexes of *p*-fluorine-substituted dehydrodipeptides *N*-Ac-Δ(*p*-FPhe)-AA (F-DHDP) **3—5** with metal ions are also indicative of the absence of free ligands in alcoholic solutions of the complexes (Table 2). When different amounts of metal salts were added, only a downfield shift of the signal for the F atom was observed. Other signals were not manifested whatever ligand to metal salt ratio was used.

The ^{19}F NMR spectrum of free DHDP **4** has two signals corresponding to two conformers of **4** that occur due to hindered rotation about the —C(O)—N= peptide bond. In the presence of metal salts, these two signals were shifted downfield. However, no new signals appeared. Neither splitting nor noticeable broadening of the signals was observed upon cooling of samples to −50 °C.

All the above-mentioned data of NMR spectroscopy confirm the suggestion that free DHDP molecules are absent in solution although rapid (within the NMR time scale) ligand exchange must not be completely ruled out. The absence of kinetically independent DHDP molecules in solution (in the concentration range under study) is also confirmed by decrease in the rate of dehydrogenation in the presence of even 0.25 mol of the metal salt.

Complex particles formed as the relative content of MgX_2 is gradually increased are shown in Scheme 2. Apparently, the ligands are tightly held in these complexes, the metal ions in them are reversibly bound with the carboxyl and carbonyl groups of the ligands, and rapid migrations of metal atoms occur within particles **A**.

This change in the structure of the complex particles in going from structure **A** to structure **B** is confirmed by the parameters of the ^{19}F NMR spectra given in Table 2, from which it can be seen that shielding of the indicator F atom decreases as the relative content of Mg^{2+} ions in the complex particle increases. This is apparently attributable to strengthening of interactions between Mg^{2+} and all carbonyl groups of the ligands and, consequently, to a formal increase in the electronegativity of the fragment bound to the *p*-fluorophenyl group.



The formation of complex particles occurs relatively slowly, which can be followed with the use of UV spectroscopy. The UV spectra of DHDP in EtOH have two maxima with λ = 215 nm (ε ~9000 L mol^{-1} cm^{-1}) and 280 nm (ε ~12500 L mol^{-1} cm^{-1}). The addition of NaOH led to a slight increase in absorption. However, subsequent addition of $MgCl_2$ resulted in an additional increase in the value of ε by 2000—3000 L mol^{-1} cm^{-1} over 20—60 min, while the positions of the maxima remained unchanged, which is apparently indicative of an increase in the polar conjugation upon coordination of the >C=O...Mg^{2+} type in the complexes formed. It is known that this type of conjugation and additional polarization of C=O bonds upon complexation with Mg^{2+} and Ca^{2+} ions promote nucleophilic addition to carbonyl groups.[11,12]

A low rate of formation of complex particles was also manifested in experiments on hydrogenation. For example, if hydrogenation of complex **2** with $MgSO_4$ started within 10 min after the reagents had been mixed, the diastereomeric excess of the corresponding *N*-acetyl-*S,S*-dipeptide was 30%, while if the reaction mixture was kept before hydrogenation for 3 h, the diastereomeric excess was 60%.

The data obtained allow the conclusion that the number of ligands in the complex particle decreases as the relative content of the metal salt increases. As a result, interactions of the metal ion with COOH groups are strengthened, their acidity increases, and the probability of formation of chelate structures increases due to coordination with the carbonyl groups. Consequently, the conformational rigidity and shielding of C=C bonds subjected to hydrogenation increase, which lead to a decrease in the reaction rate and to the enhancement of its stereoselectivity.

This work was financially supported by the Russian Foundation for Basic Research (Project No. 98-03-33034) and by the Russian Foundation for Basic Research in cooperation with the German Research Society (Deutsche Forschung Gemeinschaft, Project No. 96-03-00102).

References

1. V. M. Belikov, I. N. Lisichkina, and A. I. Vinogradova, *Zh. Org. Khim.*, 1995, **31**, 220 [*Russ. J. Org. Chem.*, 1995, **31**, 194 (Engl. Transl.)].
2. I. N. Lisichkina, A. I. Vinogradova, T. Yu. Vasil'eva, L. N. Kurkovskaya, M. B. Saporovskaya, and V. M. Belikov, *Izv. Akad. Nauk, Ser. Khim.*, 1996, 1964 [*Russ. Chem. Bull.*, 1996, **45**, 1862 (Engl. Transl.)].
3. I. V. Blagodatskikh, O. I. Shchegolikhina, T. A. Larina, A. A. Zhdanov, and V. G. Vasil'ev, *Vysokomol. Soedin., Ser. A*, 1996, **38**, 1876 [*Polym. Sci. Russ., Ser. A*, 1996, **38**, 1239 (Engl. Transl.)].
4. J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, J. Wiley and Sons, New York—London, 1961.
5. M. B. Saporovskaya, L. M. Volkova, and V. A. Pavlov, *Zh. Anal. Khim.*, 1989, **44**, 525 [*Sov. J. Anal. Chem.*, 1989, **44** (Engl. Transl.)].
6. H. Schmidbaur, J. Back, D. L. Wilkinson, and G. Muller, *Chem. Ber.*, 1989, **122**, 1433.
7. H. Schmidbaur, P. Kiprof, O. Kumberger, and J. Riede, *Chem. Ber.*, 1991, **124**, 1083.
8. E. E. Kim and K. Eriks, *Struct. Chem.*, 1990, **1**, 281; *Chem. Abstrs.*, 1991, **114**, No. 1, 7112.
9. D. E. Fenton, *Alkali Metals and Group IIA Metals*, in *Comprehensive Coordination Chemistry*, Ed. G. Wilkinson, Pergamon Press, New York, 1987, **3**, 9.
10. I. N. Lisichkina, A. I. Vinogradova, N. B. Sukhorukova, E. V. Tselyapina, M. B. Saporovskaya, and V. M. Belikov, *Izv. Akad. Nauk, Ser. Khim.*, 1993, 601 [*Russ. Chem. Bull.*, 1993, **42**, 569 (Engl. Transl.)].
11. R. Fett, J. M. Brighente, J. Costa, and R. A. Yunes, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 1639.
12. H. Saimoto, K. Yoshida, T. Murakami, M. Morimoto, H. Sashiwa, and Y. Shigemasa, *J. Org. Chem.*, 1996, **61**, 6768.

Received March 17, 1998;
in revised form September 7, 1998