Reactivity of Coordinated Ligands in Some Cobalt(III) Dipeptide Complexes*

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In the reaction of [Co(glygly)NO_2en] (glygly-H_2 = glycylglycine, en = ethylenediamine) with acetaldehyde in aqueous solution at pH 11 four condensation products have been obtained. Their separation has been performed by means of adsorption chromatography on a cationic exchanger, in hydrogen form. On the basis of data obtained from electronic, ^1H and ^{13}C n.m.r. spectra, and also from the results of elemental analysis and paper chromatography, the reaction products obtained have been identified as: a) a mixture of [Co(threogly)NO_2 en] (threogly H_2 = threonylglycine) and [Co(allothreogly)NO_2 en] (allothreoglyH$_2$ = allothreonylglycine); b) [Co(glygly)-NO_2(CH_3CH=en)] (CH_3CH=en = N-ethylideneethylenediamine) and c) [Co(CH_3CH=glygly)NO_2en] (CH_3CH=glygly H_2 = N-ethylideneglycylglycine).

Introduction

It is known [2] that amino acid residues are selectively activated on coordination of dipeptides with metal ions; on account of that, in alkaline media, H–D exchange in bis(dipeptidato)cobalt(III) complexes [3, 4], as well as in monodipeptidato complexes of the type $[Co(a_1 - a_2)(NH_3)_3]^+$** and $[Co(a_1 - a_2)(dien)]^+$ [5], respectively, takes place only at the C-terminal CH_2 (or CH) group. As additional proof of dipeptide activation at the C-terminal residue, the same authors [4, 5] have cited the formation of coordinated glycylthreonine in base-catalyzed condensation of coordinated glycylglycine with acetaldehyde.

For the formation of serylglycine instead of glycylserine in the reaction of glycylglycine with formaldehyde in the presence of Cu(II) ion [6], Uyama and coworkers [7] assumed an intermediate formation of Schiff base of coordinated glycylglycine. In fact, their experiments have shown that in Ni(II) and Co(III) complexes of Schiff bases, derived from salicylaldehyde and dipeptides, the isotopic exchange at pD 11 takes place exclusively at the N-terminal CH_2 (or CH) group. Accordingly, in reactions of coordinated dipeptide Schiff bases with aldehydes the alkylation occurs at the N-terminal and not at the C-terminal residue [7, 8], indicating that the former residue is considerably more activated in these complexes.

The results relating to the course of the reaction of coordinated dipeptides with aldehydes are thus controversial, since in complexes of the type $[Co(a_1 - a_2)_2]^-$, $[Co(a_1 - a_2)(NH_3)_3]^+$ and $[Co(a_1 - a_2)(dien)]^+$ [4, 5], respectively, the condensation reaction takes place at the C-terminal methylene group of the dipeptide ligand, whereas in the case of glycylglycine activated by Cu(II) ion [6] the reaction occurs at the N-terminal methylene group.

Therefore we decided to continue the investigations of the reaction of coordinated dipeptides with aldehydes in order to attempt to clarify its mechanism. In this paper the investigations were carried out with a dipeptide cobalt(III) complex, previously prepared in our laboratory [9].

Experimental

Reaction of [Co(glygly)NO_2 en] with Acetaldehyde

To a solution of [Co(glygly)NO_2en] [9] (2.06 g, 0.007 mol, in 200 cm^3 of water) was added acetaldehyde (3.16 cm^3, 0.056 mol) and the corresponding volume of sodium hydroxide solution (C_{NaOH} = 0.1 mol dm^{-3}) to adjust the pH of the reaction mixture to 11. The solution obtained was allowed to stand for 4 hours at room temperature. It was then neutralized with hydrochloric acid (C_{HCl} = 0.1 mol dm^{-3}), concentrated in a rotary evaporator (40 °C) to a volume of 10 cm^3, and left to stand at room temperature for 12 hours. By this time the greatest part of the starting complex had crystallized out. The separated crystals were filtered off by a vacuum filter. The filtrate obtained was concentrated on a rotary evaporator (40 °C) to a volume of about 5 cm^3, and poured on a cationic Dowex 50 W × 8 (200–400 mesh) column (8 cm length and ϕ 3 cm), in hydrogen form. The elution was carried out with distilled water (at a rate of 3 cm^3 per minute), whereby four zones were formed on the column. The first zone, which contained [Co(threogly)NO_2en] and [Co(allothreogly)

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**$a_1 - a_2$ represents coordinated dipeptide anion, and dien stands for diethylenetriamine.

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NO_2en], came off the column about one hour after the beginning of the elution, and was eluted out within 45 minutes. The eluate obtained was concentrated on a rotary evaporator (40 °C) to a volume of 2 cm^3, and was left to stand at room temperature for 20 hours. By this time there crystallized out from the solution 0.04 g (1.7%) of the aforementioned mixture of diastereoisomers, in the form of orange crystals. *Anal.* Calcd. for $CoC_8H_{18}N_5O_6$: C, 28,32; H, 5.31; N, 20.65%. Found: C, 28.29; H, 5.29; N, 20.87%.

Ninety minutes after completion of the first zone elution the second zone came off the column, and this was found to contain [Co(glygly)NO_2(CH_3CH=en)]. This zone was eluted within about 75 minutes. The eluate obtained was concentrated on a rotary evaporator (40 °C) to a volume of about 1 cm^3, to which 4 cm^3 of methanol were added. The precipitated orange crystals (0.15 g, 6.7%) of the aforementioned complex were separated by vacuum filtration. *Anal.* Calcd. for $CoC_8H_{16}N_5O_5$: C, 29.92; H, 5.02 N, 21.81%. Found: C, 29.74; H, 5.44; N, 22.13%.

The third zone appeared off the column 100 minutes after completion of the second zone elution; it contained [Co(CH_3CH=glygly)NO_2en], which was eluted within 90 minutes. The eluate obtained was concentrated on a rotary evaporator (40 °C) to a volume of about 2 cm^3, and allowed to stand at room temperature for 24 hours. By then there had crystallized out from the solution 0.08 g (3.6%) of the above complex, in the form of orange crystals. *Anal.* Calcd. for $CoC_8H_{16}N_5O_5$: C, 29.92; H, 5.02; N, 21.81%. Found: C, 30.06; H, 4.93; N, 22.09%.

The last zone contained the starting complex.

Decomposition of [Co(allothreogly)NO_2en] and [Co(threogly)NO_2en], Hydrolysis of the Dipeptides Liberated and Identification of the Amino Acids Obtained

The decomposition of complexes, isolated as a mixture of diastereoisomeric forms, as well as the hydrolysis of the dipeptides liberated, were performed as described in a previously published paper [4]. The identification of the amino acids obtained was carried out by descending paper chromatography [10].

Electronic Absorption Spectra

Electronic absorption spectra in the visible and ultraviolet regions were recorded on a Varian UV-visible Super Scan 3 spectrophotometer. Visible spectra were taken in aqueous solution (2×10^{-3} mol dm^{-3}), and in the ultraviolet region with aqueous solutions having concentrations of 2×10^{-4} mol dm^{-3}. The length of the cell used was 0.5 cm. Absorption maxima and logϵ are given in Table I.

Proton Magnetic Resonance Spectra

These spectra were recorded on a Varian HA 60 spectrometer in D_2O, with DSS as the internal standard.

TABLE I. Absorption Maxima (nm) and logϵ of the Synthesized Complex Compounds*.

No	Complex	λ_1	logϵ_1	λ_2	logϵ_2
I	[Co(glygly)NO_2en]	476	2.40	329	2.95
II	[Co(threogly)NO_2en]	476	–	329	–
III	[Co(allothreogly)NO_2en]				
IV	[Co(glygly)NO_2(CH_3CH=en)]	473	2.46	332	3.06
V	[Co(CH_3CH=glygly)NO_2en]	471	2.46	332	3.11

*Complexes II and III were isolated as a mixture

^{13}C Nuclear Magnetic Resonance Spectra

^{13}C n.m.r. spectra were run on a Brucker SXP-100 spectrometer, in D_2O. Chemical shifts are given with reference to TMS, using dioxane as the internal standard.

Results and Discussion

As seen from the Experimental, in the reaction of [Co(glygly)NO_2en] with acetaldehyde four products were obtained in small yields (Table I, II–V). Two of them (II and III) were isolated as a mixture, which has not been separated so far. The eluate of the first zone of the chromatographic separation of the reaction products consists of two substances (II and III), as established by hydrolysis and paper chromatographic identification of the amino acids obtained.

Electronic spectra of the products are characteristic of cobalt(III) chromophores of the type CoN_5O. These complexes exhibit d–d electronic transitions in the regions in which the absorption maxima of the starting substance (I) also appear (Table I). It may thus be concluded that the chromophore remains unchanged in the course of the reaction. In addition, from Table I it is seen that the first absorption maximum of product V is shifted 5 nm towards shorter wavelengths with respect to the starting substance, whereas in case of product IV the shifting amounts to about 3 nm. The absorption maxima of the starting substance (and of the mixture of the products II and III) however are all found at the same wavelength. On this basis it might be assumed that in the first two cases the condensation involves hydrogen atoms linked directly to the ligator, *i.e.*, to the nitrogen atom. However, in case of the products II and III it might be presumed that the reaction occurs at some of the methylene groups (not linked directly to the metal ion), so that the introduced group is expected to produce a smaller influence than when linked directly to the ligator. This assumption is in accordance with the p.m.r. spectra of the isolated complex compounds (Fig. 1). In Fig. 1 it is seen that the spectra of the products IV and V have doublets at 2.30 and 2.25 ppm, arising from methyl group protons, as

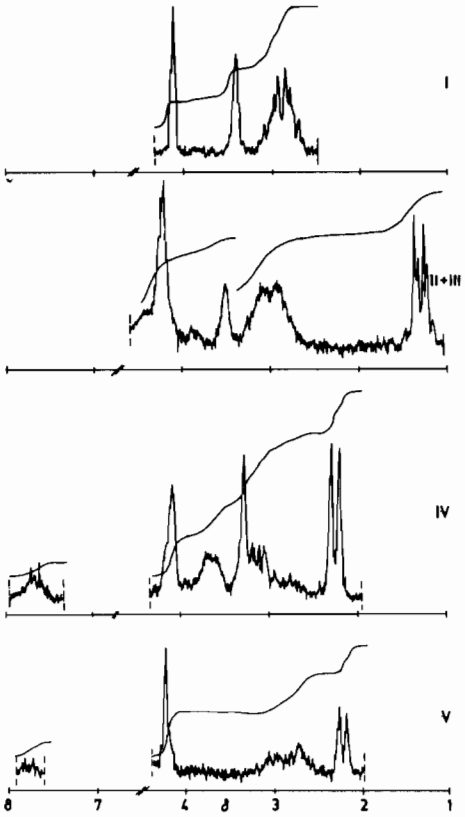

Fig 1. ^{1}H n.m.r. spectra of [Co(glygly)NO_2en] (I), [Co-(threogly)NO_2en] and [Co(allothreogly)NO_2en] (II + III), [Co(glygly)NO_2(CH_3CH=en)] (IV), [Co(CH_3CH=glygly) NO_2en] (V).

well as signals at 7.8 and 7.7 ppm, arising from one proton. The positions of these resonances correspond to the positions of the resonances of the N-ethylidene group protons, which confirms the aforesaid assumption that these products derive from the condensation of the aldehyde with amino group. One of them (V) is the complex which contains Schiff base of glycylglycinato ligand, since its p.m.r. spectrum shows that the imino group formed shifts the signal of the N-terminal CH_2 group by 0.85 ppm towards lower magnetic field (with respect to the position of this group signal in the p.m.r. spectrum of the starting complex). This conclusion is supported by the fact that the shift of the C-terminal CH_2 group signal is negligible. An analogous shift of the signals of methylene groups of coordinated ethylenediamine appears in the spectrum of the product IV, which contains the Schiff base of this ligand. On the basis of general features of its p.m.r. spectrum, product IV was taken to be a single substance and not a mixture of two possible mono-condensation products. In the case of the formation of a mixture of products, the spectrum would be expected to be more complex, since one product would contain N-ethylidene group in the *trans*-position, and the other in *cis*-position with respect to the nitro group. This assumption is supported by the appearance of only one, relatively narrow zone in the separation of this product by ion exchange. This product behaves in an analogous way in the course of thin-layer chromatography on silica gel. Finally, also in agreement with the above assumption is the ^{13}C n.m.r. spectrum of this product (Fig.

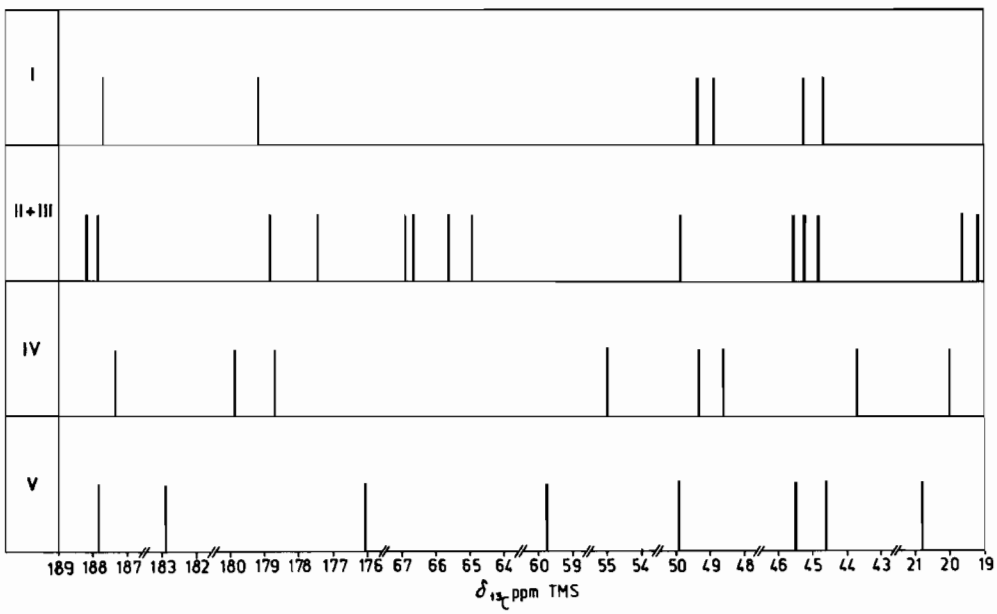

Fig. 2. The ^{13}C chemical shifts of the starting complex (I) and of the obtained condensation products (II + III, IV and V).

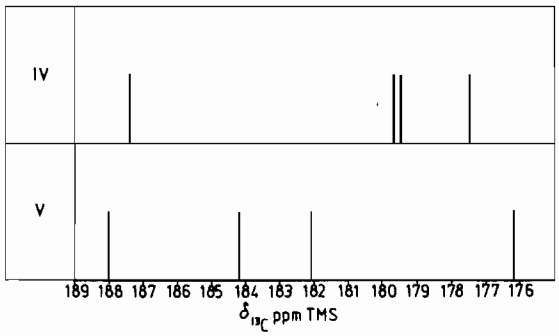

Fig. 3. The chemical shifts of the carboxylic, carbonyl and imino carbons in ^{13}C off-resonance decoupled spectra of [Co(glygly)NO_2 (CH_3CH=en)] (IV) and [Co(CH_3CH=glygly) NO_2en] (V).

2), in which for each C-atom there appears only one signal.

Figure 2 shows also that the spectra of the products IV and V have the signals for imino group C-atoms in the region in which there also appear signals for carboxylic and carbonyl C-atoms, respectively. Their positions at 182.9 ppm in the spectrum of the product V, and at 178.8 ppm in the spectrum of the product IV, have been determined on the basis of signal shapes, namely doublets to which these atoms give rise in the ^{13}C off-resonance decoupled spectrum shown in Fig. 3. From ^{13}C n.m.r. spectra of these substances it might be seen that the resonances for C-atoms of methylene groups in the α-position to the introduced N-ethylidene group are shifted by about 10 ppm with respect to resonances of these atoms when present in the α-position to the amino group. In the p.m.r. spectrum of the mixture of products II and III methyl resonances at 1.28 and 1.33 ppm, as well as the position and the ratio of the integrals of signals at 3.53 and 4.17 ppm, show that this mixture consists of complexes in which dipeptidato ligand contains as N-terminal amino acid threonine and/or allothreonine, and not glycine. This means that these complexes derive from the condensation of acetaldehyde with N-terminal CH_2 group of coordinated glycylglycine. The above mentioned dilemma has been cleared up by performing chromatogrphic analysis of the amino acids obtained upon hydrolysis of the liberated dipeptides. In this way it has been established that the reaction of coordinated glycylglycine with acetaldehyde affords a mixture of complexes, some of which contain coordinated threonylglycine, and some allothreonylglycine. Therefore, as expected, the products II and III exhibit most pronounced differences in those regions of the ^{13}C n.m.r. spectrum in which there appear the signals for N-terminal amino acid residue of the dipeptide ligand.

It might be concluded from the above that the formation of coordinated threonylglycine and allothreonylglycine respectively, as well as the formation

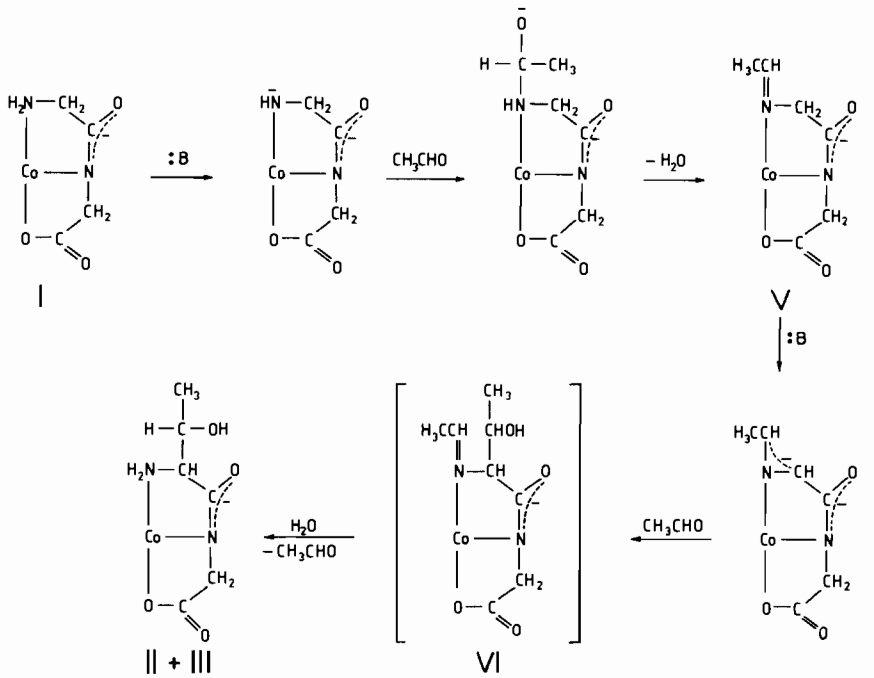

Fig. 4. Assumed mechanism for the formation of [Co(threogly)NO_2en] and [Co(allothreogly)NO_2en] by condensation of [Co(glygly)NO_2en] with acetaldehyde.

of N-ethylideneglycylglycine, indicate that in the investigated base-catalyzed condensation of coordinated dipeptide with acetaldehyde, the first phase of the reaction takes place at the amino group giving rise to the corresponding complex of the dipeptide Schiff base (V) (Fig. 4). The formed N-ethylidene group facilitates ionization of the C–H bond of the N-terminal methylene group and stabilizes by resonance the formed carbanion, so that in the second phase of the process acetaldehyde reacts with the N-terminal CH_2 group of the compound V, and not with the C-terminal CH_2 group, as should be expected on the basis of previous results in regard to the degree of activation of methylene groups in dipeptidato Co(III) complexes [4, 5]. This phase is most probably followed by the hydrolysis of the imino group which gives rise to products II and III. Namely, since the compound V is very easily hydrolyzed giving the complex I, it might be assumed that the CH_3CHOH group, when introduced in the α-position relative to the imino group, facilitates the hydrolysis of the latter. This offers an explanation why the intermediate VI could not be isolated.

The proposed mechanism is supported by the fact that a mixture of products II and III was also obtained by the reaction of the isolated complex V with acetaldehyde.

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