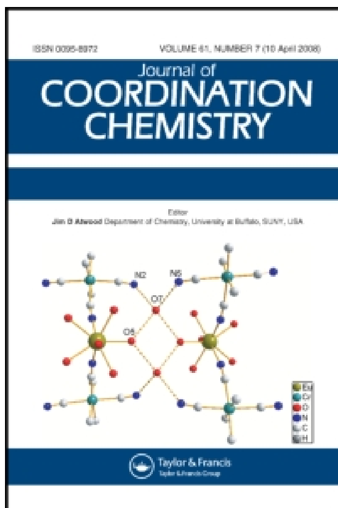
This article was downloaded by:
On: *23 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

IRREVERSIBLE REDOX REARRANGEMENT OF DIOXYGEN COMPLEXES. I. SELECTIVE OXIDATION OF DIPEPTIDES COORDINATED TO COBALT(II)

Wesley R. Harris[a]; Arthur E. Martell[a]
[a] Department of Chemistry, Texas A & M University, College Station, Texas

To cite this Article Harris, Wesley R. and Martell, Arthur E.(1980) 'IRREVERSIBLE REDOX REARRANGEMENT OF DIOXYGEN COMPLEXES. I. SELECTIVE OXIDATION OF DIPEPTIDES COORDINATED TO COBALT(II)', Journal of Coordination Chemistry, 10: 1, 107 — 113

To link to this Article: DOI: 10.1080/00958978008079857

URL: http://dx.doi.org/10.1080/00958978008079857

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. Coord. Chem., 1980, Vol. 10, pp. 107–113
0095-8972/80/1001-0107 $04.50/0

© 1980 Gordon and Breach Science Publishers, Inc.
Printed in Great Britain

IRREVERSIBLE REDOX REARRANGEMENT OF DIOXYGEN COMPLEXES. I. SELECTIVE OXIDATION OF DIPEPTIDES COORDINATED TO COBALT(II)

WESLEY R. HARRIS and ARTHUR E. MARTELL*

Department of Chemistry, Texas A & M University, College Station, Texas 77843

(Received August 13, 1979)

The decomposition of the cobalt dioxygen complexes of three dipeptides has been investigated by removal of the metal from the final reaction mixtures, followed by gas chromatographic and mass spectrometric analyses of the organic reaction products. The results indicate that the reaction involves the oxidation of coordinated dipeptide, and that the ligands are oxidized exclusively at the N-terminal amino acid residue.

Downloaded At: 20:33 23 January 2011

INTRODUCTION

Cobalt dioxygen complexes have received considerable attention in recent years, and several reviews of the field are available.[1-3] A common characteristic of such complexes is their irreversible rearrangement to oxygen inert monomeric cobalt (III) complexes. This facet of oxygen complex chemistry has received relatively little attention, however,[4-6] even though this decomposition to cobalt(III) is one of the principal limitations to the large scale use of cobalt(II) complexes for oxygen transport and storage in biological systems as well as for commercial applications. Recent work in this laboratory has shown that cobalt dioxygen complexes of dipeptides decompose to yield cobalt(III) complexes and water, not peroxide.[7] Complete reduction of the original bridging dioxygen strongly suggests that coordinated ligand is being oxidized to supply the needed electrons. Up to the present time, however, the nature of ligand oxidation reactions in dioxygen complexes and the site of oxidation have not been investigated. As a first step in a general study of this problem, the products of the irreversible rearrangement of the μ-peroxobridged cobalt complexes of glycylglycine (gly-gly), L-alanylglycine (ala-gly), and glycyl-L-alanine (gly-ala) have been studied by gas chromatographic and mass spectrometric analyses. The results obtained indicate that ligand oxidation involves only the N-terminal amino group of the dipeptide.

EXPERIMENTAL

Oxygenation and Oxidation

Approximately 100 mg of $Co(NO_3)_2 \cdot 6H_2O$ and a four-fold excess of dipeptide were dissolved in 50 ml of water. Concentrated NaOH was added with vigorous aeration to bring the pH to 9, which resulted in the formation of the dark brown dioxygen complex. Upon standing, the solution changed from brown to a deep red color characteristic of simple cobalt(III) complexes. The reaction was allowed to go to completion; the process required from 1–4 hr, depending on the ligand.

Reaction Work-up

To facilitate removal of the metal ion, the cobalt was reduced to the divalent state using two alternative methods. In one case, the reaction mixture was acidified to pH 0.8 and degassed 15 m with nitrogen. The solution was then poured over amalgamated zinc and stirred under nitrogen for an additional 30 m. The zinc was then removed, and the nitrogen was replaced by hydrogen sulfide. The pH was raised to 8–9 by the addition of NaOH, which resulted in the precipitation of CoS. The precipitate was removed by filtration to give a colorless solution which was acidified and rotovapped to dryness, yielding a colorless solid.

Constant potential electrolysis was also employed as a method for reducing the cobalt. The initial

reaction mixture was acidified to pH 1 by the addition of HCl, followed by electrolysis at −1.0 v. versus the saturated calomel electrode using mercury pool and platinum wire electrodes. The cobalt(II) was then removed as described above.

Derivatization

The colorless solid obtained from the reaction mixture was dissolved in methanol and rotovapped to dryness twice, then dissolved in 0.3 M HCl/methanol and stirred for thirty minutes to esterify all free carboxylate groups. This solution was evaporated to dryness, and the trifluoroacetyl derivatives were prepared by the room temperature method described by Gherke.[8] Pure samples of the dipeptides, as well as a number of carboxylic and amino acids were also derivatized for use as chromatographic standards. Because the work-up conditions were rather harsh, blanks were run to determine the effects of the experimental procedures themselves on the dipeptides. Ligand samples were put through the same procedures: aeration at high pH, exposure to the reductants and to H_2S, and derivatization as described above.

Gas Chromatography

Gas chromatograms were recorded on a Hewlett Packard Model 5803A chromatograph equipped with an fid detector and a stainless steel column (8′ x 1/8″ id) prepacked by Hewlett Packard with 3% OV-17 adsorbed onto 80/100 mesh Chromosorb W. The injection port and detector were heated to 250°C. The fid detector was operated at 28 and 15 psi air and hydrogen, respectively. The flow rate of the helium carrier gas was 35 ml/min at an oven temperature of 90°C.

The instrument was controlled by a microcomputer terminal, which automatically detected the peaks, recorded the retention times, and integrated the peak area, in addition to accepting the necessary input for temperature programing. Two programs were used. In one the temperature was held at 90° for 8 m, and then increased at 5°/min up to 200°. In the second program, the initial temperature was held at 70° for 5 m, followed by an increase of 5°/min up to 120°. The rate was then dropped to 3°/min up to a final temperature of 200°. Peak intensities are reported as area percent excluding the signal due to solvent. The trifluoroacetic anhydride used to derivatize the samples gradually degraded the column, so that retention times change over a period of months. Therefore, peaks are identified by their relative retention time q, where q is defined as the retention time of the peak divided by the current retention time of the unreacted dipeptide. Thus in every reaction mixture the dipeptide has a q value of 1.00.

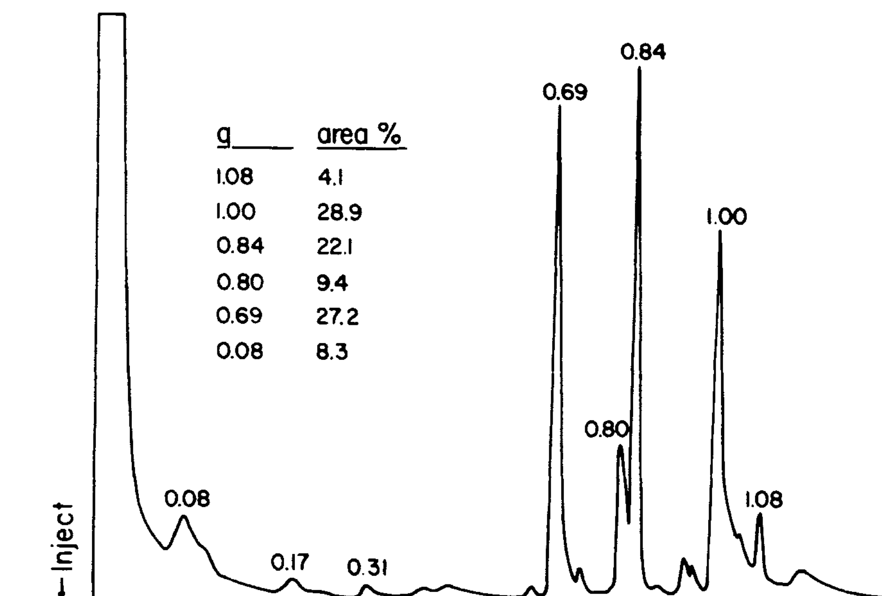


FIGURE 1 Gas chromatogram of the derivatized reaction products of the irreversible redox rearrangement of the cobalt dioxygen complex of alanylglycine; temperature program: 90°C for 8 min, then increased at 5°/min to 200°C; He flow rate = 35 ml/min. Retention time for q = 1.00 is 28.6 min.

Downloaded At: 20:33 23 January 2011

Combined gas chromatrographic-mass spectrometric analyses and high resolution mass spectra were obtained through the Center for Trace Characterization of Texas A & M University. To insure consistency, the column described above was transferred to their instrument for the gc-ms analysis.

RESULTS

The gas chromatogram of the derivatized reaction products from the redox rearrangement of the dioxygen complex of ala-gly is shown in Figure 1. The peak assigned a q of 1.00 is conclusively identified as ala-gly by comparison of its retention time to that measured for known ala-gly standards and from its mass spectrum, which is shown in Figure 2. Previous work by Stenhagen has shown that the pair of intense peaks at m/e values of 140 and 141 are characteristic of N-terminal alanyl dipeptides.[9] He also showed that the mass fragment at 69 due to CF_3 is characteristic of trifluoroacetyl derivatives of dipeptides. The empirical formulas of the 140, 72 and 70 m/e peaks have been determined by high resolution mass spectrometry and confirm the assignments made in Figure 2.

The chromatogram shown in Figure 1 shows two main reaction products at q values of 0.94 and 0.69. The mass spectrum of the 0.69 peak is shown in Figure 3. The set of peaks at m/e 140 and 141 have been replaced by a single peak at 141. The fragments of mass 198, 116, and 88 have been analyzed by high resolution mass spectrometry to obtain the empirical formulas shown in Table 1. Loss of the ester group, $CO{-}O{-}CH_3$, from ala-gly would result in a fragment with the formula $C_6H_8N_2O_2F_3$ and a mass of 197. Thus the $C_6H_7NO_3F_3$ fragment of mass 198 appears to result from the substitution of an O atom for an NH group. The loss of the 140, 141 pair in the mass spectrum indicates that the substitution is at the N-terminal alanine residue. The fragments of mass 88 and 116 can be assigned on the basis of the high resolution data to the unaltered C-terminal groups $NH{-}CH_2{-}CO{-}OCH_3$ and $CO{-}NH{-}CH_2{-}CO{-}OCH_3$. Thus the peak at $q = 0.69$ represents the compound $CF_3{-}CO{-}O{-}CH(CH_3){-}CO{-}NH{-}CH_2{-}CO{-}OCH_3$.

The mass spectrum of the compound appearing at $q = 0.81$ is shown in Figure 4. It is significant that there is no mass 69 peak due to CF_3, indicating that the material is not a trifluoroacetyl derivative. The presence of the 116 and 88 peaks indicates that the C-terminal glycine residue has not been altered. That the m/e 179 peak represents the molecular ion is indicated by the presence of M-31 (loss of $-OCH_3$) and M-59 (loss of $CO{-}OCH_3$) peaks. A molecular weight of 179 corresponds to the compound $Cl{-}CH(CH_3){-}CO{-}NH{-}CH_2{-}CO{-}OCH_3$. Such an assignment is strongly supported by the appearance of M + 2 peaks for all the chloride containing fragments, with an M/M + 2 intensity ratio of 3 : 1, which corresponds to the relative abundances of Cl^{35} and Cl^{37}.

The gas chromatogram of a derivatized reaction mixture of glycylglycine is shown in Figure 5. The peaks at $q = 1.00$ and 0.30 were identified as gly-gly and glycine by comparison of their retention times

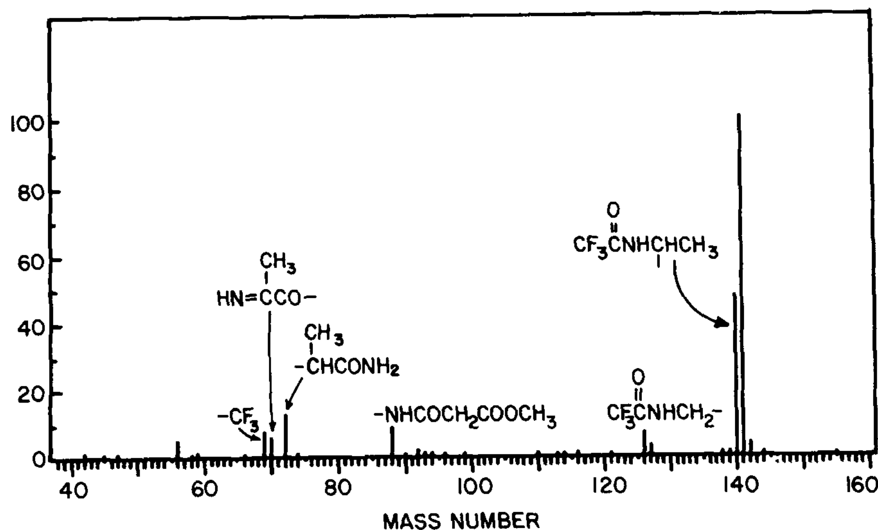


FIGURE 2 Mass spectrum of trifluoroacetylalanylglycine methyl ester.

Downloaded At: 20:33 23 January 2011

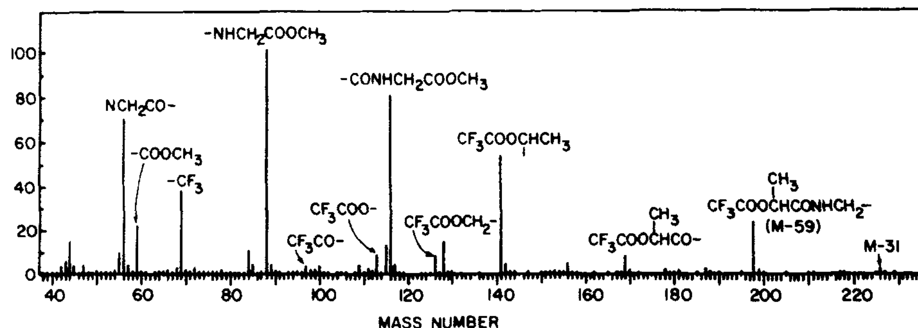


FIGURE 3 Mass spectrum of the q = 0.69 ala-gly reaction product, 2-trifluoroacetoxypropionylglycine methyl ester.

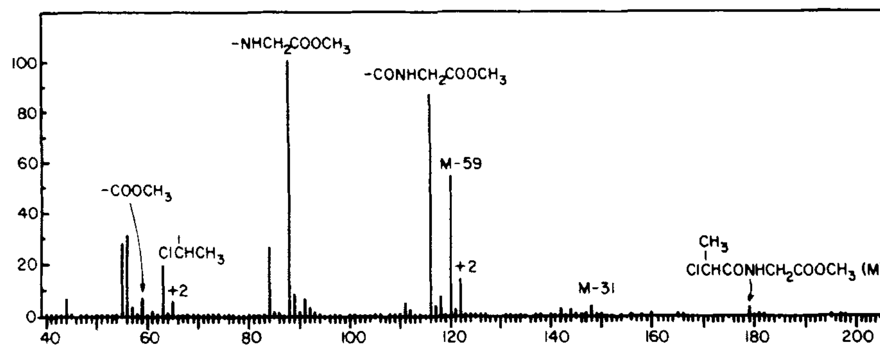


FIGURE 4 Mass spectrum of the q = 0.81 ala-gly reaction product, 2-chloropropionylglycine methyl ester.

Downloaded At: 20:33 23 January 2011

TABLE I
High Resolution Mass Spectrometry of Alanylglycine and Its Degradation Products

Formula	Calc. Mass	Obs. Mass	Proposed Molecular Fragment
$C_6H_7NO_3F_3$	198.0378	198.0385	$CF_3-C(=O)-O-CH(CH_3)-C(=O)-NH-CH_2$
$C_4H_5NOF_3$	140.0317	140.0323	$CF_3-C(=O)-NH-CH(CH_3)$
$C_3H_3NOF_3$	126.0165	126.0167	$CF_3-C(=O)-NH-CH_2$
$C_4H_6NO_3$	116.0350	116.0348	$C(=O)-NH-CH_2-C(=O)-O-CH_3$
$C_3H_6NO_2$	88.0395	88.0398	$NH-CH_2-C(=O)-O-CH_3$
C_3H_6NO	72.0448	72.0449	$CH(CH_3)-C(=O)-NH_2$
C_3H_4NO	70.0294	70.0293	$NH=C(CH_3)-C(=O)$

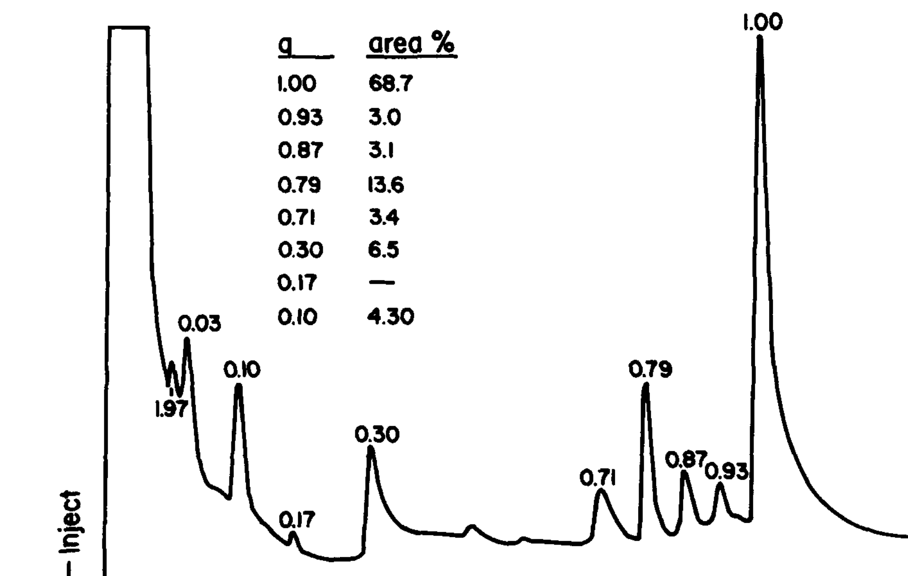


FIGURE 5 Gas chromatogram of the derivatized reaction products of the irreversible redox rearrangement of the cobalt dioxygen complex of glycylglycine; temperature program: 90°C for 8 min, then increased at 5°/min up to 200°C; He flow rate = 35 ml/min. Retention time for q = 1.00 is 32.4 min.

Downloaded At: 20:33 23 January 2011

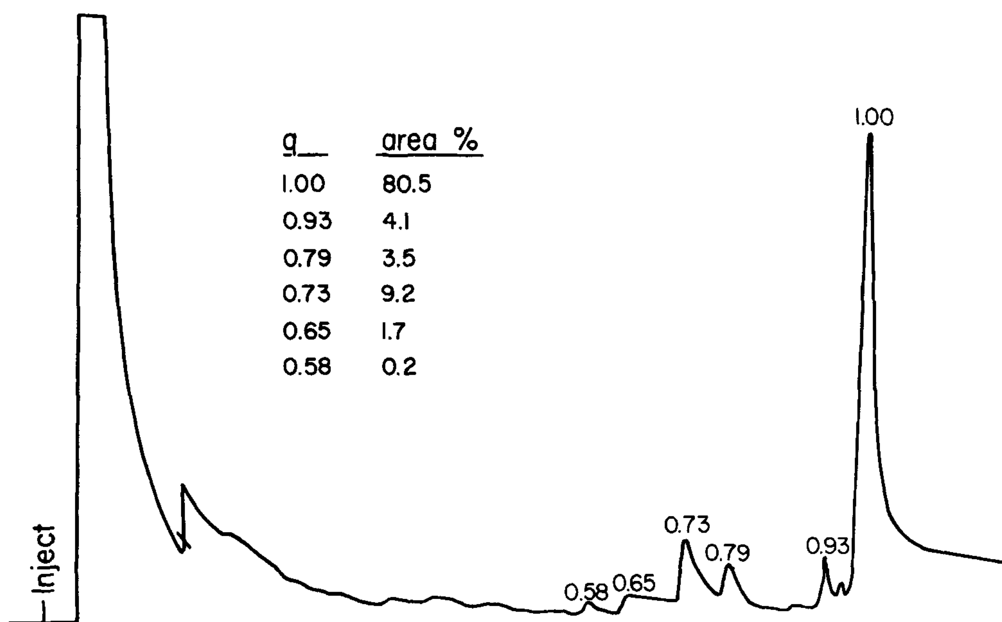


FIGURE 6 Gas chromatogram of the derivatized reaction products of the irreversible redox rearrangement of the cobalt dioxygen complex of glycylalanine; temperature program; 70°C for 5 min, then increased at 5°/min up to 125°C, then increased at 3°/min up to 200°C; He flow rate = 35 ml/min. Retention time for q = 1.00 is 36.1 min.

to those of standard samples. These assignments were later confirmed by mass spectral analysis. The glycine peak and the small peak at $q = 0.93$ are both present in roughly the same proportion in the chromatograms of gly-gly blank runs (ligand samples put through the complete work-up and derivatization procedures in the absence of cobalt), thus these species are not considered relevant to the cobalt(III) promoted oxidation of the ligand.

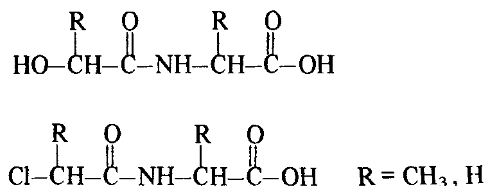
The compounds with q values of 0.71 and 0.79 are shown by their mass spectra to be the trifluoroacetyl methyl esters of glycolylglycine ($CF_3-CO-O-CH_2-CO-NH-CH_2-CO-OCH_3$) and chloroacetylglycine ($Cl-CH_2-CO-NH-CH_2-CO-OCH_3$), and are thus directly analogous to the products observed in the ala-gly system. However, in contrast to that of the ala-gly reaction mixture, the gly-gly chromatogram also contains significant amounts of other compounds, which appear at q values of 0.87, 0.10, and 0.07. The retention times of the latter two match those of chloroacetic and glycolic acid, indicating some hydrolysis of the two reaction products described above.

The compound giving the third peak at $q = 0.87$ has not been identified. However, its mass spectrum contains the familiar intense peaks at 88 and 116, indicating that the C-terminal glycine residue is still intact. In addition, there is no m/e 69 peak, suggesting that the N-terminal glycine has been altered to the point that no trifluoroacetyl derivative can be formed. Thus, even though all the reaction products have not been conclusively identified, it is still clear that the metal promoted oxidation of the coordinated gly-gly involves only the N-terminal amino acid.

The chromatogram of a reaction mixture of gly-ala is shown in Figure 6. As for the two previous systems, the peak assigned a q of 1.00 has been identified by its retention time and mass spectrum as unreacted gly-ala. The two major products in this system appear at q values of 0.73 and 0.79. These have been identified from their mass spectra as glycolylalanine and chloroacetylalanine. The peak at 0.93 is present only in samples prepared by electrolysis, and thus is not a reliable indication of metal promoted reactions.

DISCUSSION

The three systems included in this study have essentially identical reaction products, which are shown in the structural formulas below:



A previous kinetic study on the decomposition of dipeptide–cobalt–dioxygen complexes has shown that both the rate and pH dependence of this reaction vary considerably with changes in the peptide R groups.[7] There are even significant kinetic variations between the reactions of the dioxygen complexes of linkage isomers such as ala-gly and gly-ala. It was suspected that the presence of alkyl substituents on the dipeptide might shift the site of ligand oxidation and thus result in a variation in reaction rates. The results presented here show that this is not the case. The presence of methyl substituents at either of the α carbons makes no difference in the observed reaction products.

Even though the amide group is not altered during the reaction of the dioxygen complexes, it must have a significant effect on the nature of this process, since complexes of simple polyamines such as triethylenetetraamine react much more slowly than those of the dipeptides. Since bis(gly-gly)cobalt(III) has a reduction potential of nearly −0.5 V[7], it is doubtful that the differences in rate are caused by a more strongly oxidizing metal center in the dipeptide complexes. Thus, the amide functionality may be influencing the intrinsic reactivity of the ligand amino group.

Although previous data definitely indicate that the decomposition of the peptide dioxygen complexes involves the oxidation of coordination ligand,[7] none of the products recovered from these reaction mixtures is a direct oxidation product of the original dipeptide. One would presume that oxidation of the N-terminal residue of a dipeptide would result in formation of an imine, NH=C(R)–CO–NH–CH(R)–COOH. However, the dissociation of such a compound into strongly acidic solution during sample preparation would result in hydrolysis to the corresponding aldehyde or ketone. The results presented here indicate that the reduction of the metal is accompanied by the partial reduction of the product as well. Amalgamated zinc is, in fact, a known reagent for the reduction of carbonyl compounds (Clemmensen reduction).[10] This appears to be followed by partial conversion to the halide. There seems to be ample opportunity for halogenation, which may occur in both the reduction and subsequent esterification in 0.3 M HCl/methanol.

Downloaded At: 20:33 23 January 2011

This reaction scheme is certainly speculative, but the appearance of these products in reaction mixtures of the dioxygen complexes only, and not in solutions of dipeptide standards and more significantly not in blank reaction mixtures which contained no cobalt, is strong evidence that the reaction of the dioxygen complexes to form inert monomeric cobalt(III) chelates involves a metal promoted alteration of the ligand. In addition, it is now clear that the dipeptide ligands react solely at the N-terminal residue. There is no indication of decarboxylation or oxidation of the amide nitrogen-α carbon bond. These results may now be used in the design of reversible dioxygen complexes which will be more resistant to inactivation due to the gradual formation of mononuclear, inert cobalt(III) complexes.

ACKNOWLEDGEMENT

This work was supported by a grant. No. A–259, from The Robert A. Welch Foundation.

REFERENCES

1. G. McLendon and A. E. Martell, *Coord. Chem. Revs.*, **19**, 1 (1976).
2. F. Basolo, B. M. Hoffman and J. A. Ibers, *Acc. Chem. Res.*, **8**, 384 (1974).
3. R. G. Wilkins, *Adv. Chem. Ser.*, No. **100**, 111 (1971).
4. M. S. Michailidis and R. B. Martin, *J. Amer. Chem. Soc.*, **91**, 4683 (1969).
5. R. D. Gillard and A. Spencer, *J. Chem. Soc., A*, 2718 (1969).
6. E. D. McKenzie, *ibid*, 1655 (1969).
7. W. R. Harris, R. C. Bess, A. E. Martell and T. H. Ridgway, *J. Amer. Chem. Soc.*, **99**, 2958 (1977).
8. C. W. Gherke and D. L. Stalling, *Sep. Science*, **2**, 101 (1967).
9. E. Stenhagen, *Z. Anal. Chem.*, **181**, 462 (1961).
10. J. Roberts and M. Caserio, "Basic Principles of Organic Chemistry", p. 458, W. A. Benjamin, Inc., New York, N.Y., 1964.

Downloaded At: 20:33 23 January 2011