THERMAL AND PHOTOCHEMICAL REACTIONS OF CHROMIUM (IV) — DIPEROXO COMPLEXES WITH ORGANIC SUBSTRATES–EVIDENCE FOR HYDROXYLATION OF PROLINE AND PHENOL.

C. K. RANGANATHAN, T. RAMASAMI, D. RAMASWAMY & M. SANTAPPA

Central Leather Research Institute, Madras

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The diperoxo complexes of chromium in an unusual oxidation number *viz.* 4, react with organic substrates such as proline and phenol to give hydroxyproline and *o*-benzoquinone as products respectively. The organic products were identified by electronic spectra as well as by their characteristic R_f values in thin layer chromatography using n-propanol–water (1:1 v/v) mixture and chloroform as solvents for proline and phenol reaction products respectively. The studies reveal that the hydroperoxide radicals formed during the decomposition of Cr(IV)–diperoxo complexes give rise to the hydroxylation processes observed. The hydroxylation reactions are facilitated by light and yields of hydroxyproline upto 9% (from proline) have been obtained. The study presents one of the first examples of the use of Cr(IV) systems for biologically relevant processes such as hydroxylation.

Introduction

It is known that iron (II)-hydrogen peroxide system involving the formation of radical intermediates finds both biochemical and industrial applications.[1-3] Recently, chromium (IV) complexes of the formulation $Cr(O_2)_2L(H_2O)_n$ where L = ethylenediamine or diethylenetriamine and n = 0 or 1, have been prepared and their reactions studied.[4] It has been reported that the acid decomposition of Cr(IV)–diperoxo complexes (omitting water ligands) is attended by the formation of radical intermediates like hydroperoxide as in (1).[4]

$$Cr^{IV}(O_2)_2L + 2H^+ \rightleftharpoons Cr(O_2H)_2L^{2+} \xrightarrow{H^+} LCr^{III} + O_2H_2 + O_2H \quad \ldots\ldots(1)$$

In the biosynthesis of the leather making protein – collagen – the hydroxylation of proline involving molecular oxygen, iron(II) and hydrogen sources such as ascorbic acid is believed to proceed through the formation of radical intermediates.[5,6] The recent development in the mechanism of the hydroxylation of proline is the suggested implication of O_2^- ion which may be considerd the conjugate base of O_2H radical.[7] Similar radical intermediates are visualised also in the action of copper proteins which have a role in the biosynthesis of vegetable tannins.[8,9] While in all these biologically relevant processes, photo-excitation may well alter the redox properties of the intermediates involved, there are ambiguities in the mechanism of hydroxylation of either proline

or phenol in biochemical pathways. An examination has now been made to make use of Cr(IV)-diperoxo complexes as a source of hydroperoxide radicals and assess their reactions with organic substrates such as proline and phenol both under thermal and photo-chemical reaction conditions.

Materials and methods

The chromium(IV) complexes, Diperoxo-aquoethylenediamine chromium(IV), $Cr(O_2)_2$-en (H_2O) and Diperoxodiethylenetriamine chromium(IV), $Cr(O_2)_2$ dien were prepared and characterised according to standard methods.[10-13] Proline (reagent grade, BDH) and freshly steam distilled phenol (BDH, Analar) were used. The other materials employed were of reagent grade and no further purification was carried out.

Photolysis studies

Photolysis studies were carried out on Applied Physics Photolysis device fitted with a 250 w medium pressure mercury lamp and a f/1.8 adjustable quartz lens for focussing the lamp output. The lamp had the rated emission power of 5×10^{18} photons/second/steradian. An excitation band of 335-450nm was selected using a chemical filter consisting of 1.0 M $CoSO_4$, 0.023 M $CuSO_4$ and 0.004 M KVO_3 in a three compartment cell or using an interference filter. The sample to be photolysed was contained in a beaker fitted with a quartz window and was thermostated at 28° C. The irradiation time was registered in an automatic timer interfaced to the source power supply. The products of the photolysis of Cr(IV)-diperoxo complexes in the presence of organic substrates like proline were studied after chromatographic separation.

Product analyses

The products of the reaction of Cr(IV)-diperoxo complexes with proline and phenol in the presence of and absence of light were analysed. The chromium products were separated using ion-exchange columns. Unreacted proline and its reaction products with Cr(IV) complexes were obtained in non-ionic portions of the ion-exchange eluate and they were identified by thin layer chromatographic behaviour. The products of the phenol reaction were separated either by means of thin layer chromatography (using $CHCl_3$ as the solvent) or by an extraction of the aqueous mixture with hexane.

Typically, solutions of Cr(IV)-diperoxo complexes $[Cr(IV)] = 0.2 \times 10^{-3}$ M, [proline] $= 1 \times 10^{-3}$ M and $[H^+] = 0.005$ or 0.05 M were irradiated for 0, 30, 60, and 90 min. Aliquots of the reaction mixture (50 ml) (with and without irradiation) were passed through columns of cation exchanger (Dowex 50W-X8) and anion exchanger successively. The non-ionic products were obtained by washing the anion exchanger with 0.05 M NaCl and the non-ionic products were chromatographed on a silica gel G thin layer plates. The solvents used for TLC were n-propanol-water (1:1 v/v) mixture for proline and chloroform for phenol reaction products.[13,14] The u.v.-visible spectra of the non-ionic products formed in the dark as well as photochemical reactions with phenol as a substrate were recorded in hexane along with those of the authentic samples of *o*- and *p*- benzoquinone in the same solvent. The non-ionic products in the case of proline were examined for hydroxyproline by TLC using standard methods.

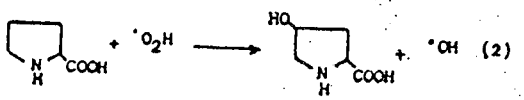
Analyses

The chromium concentrations were estimated by chromate analyses[15] while the hydroxyproline content was estimated by standard methods.[16]

Results and discussion

The reaction of phenol with Cr(IV)-diperoxo complexes in acidic media yielded an intense brown reaction mixture. The coloured product was extractable in hexane and the molar absorption coefficient at the absorption maximum of 385 nm *viz.* 2.5×10^3 $M^{-1}cm^{-1}$ compares favourably with those of the data already reported for o-benzoquinone.[17] The u.v.-visible spectra are known to provide useful means to distinquish between *o*- and *p*-benzoquinone.[17] The formation of *o*-benzoquinone as the product is also inferred by the comparison of the R_f value with that of an authentic sample (prepared freshly according to standard procedures) on a TLC,*viz.*,0.17 with chloroform as the solvent.[14] The *para* derivative is known to have an R_f value of 0.4.[14] Similarly the formation of hydroxyproline on the reaction of proline may be inferred from a TLC pattern with characteristic R_f value of 0.63 (as against 0.48 for proline) with n-propanol-water (1:1 v/v) mixture for development.[18]

The hydroperoxide radicals formed from Cr (IV) diperoxo complexes may react with proline according to (2) to give hydroxy-proline and .OH



(2)

The hydroxy radicals generated may, in turn, undergo reactions with proline. The quantitative estimations of hydroxyproline formed revealed that the photo catalysis of Cr (IV)-diperoxo-proline reaction gave rise to higher efficiency of hydroxylation as in Table-1. The photo-chemical pathways for the reaction of Cr(IV)-diperoxo complexes with phenol gave intractable aromatic products. In spite of the low yields (*ca.*1.0-9-0%) of hydroxyproline, the thermal and photo-chemical reactions of Cr(IV)-diperoxo species in the presence of proline does indeed highlight the possibility of O_2H or O_2^- being involved in the mechanism of hydroxylation of collagen.

TABLE 1

The percentage conversion of proline into hydroxy proline (HP) on the reactions with $Cr(O_2)_2en(H_2O)$. Irradiation time = 30 min.

Reaction	10^3[*Cr(IV)*] *m mols*	*Proline used m mols*	$[H^+]$/ *M*	$\frac{100 \times HP}{Cr(IV)}$
Without irradiation	3.1	1.24	0.05	1.0
	4.2	1.31	0.20	5.2
	3.8	1.50	0.40	5.4
With irradiation	3.1	1.24	0.05	3.1
	4.2	1.31	0.20	7.9
	3.8	1.50	0.40	8.3

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