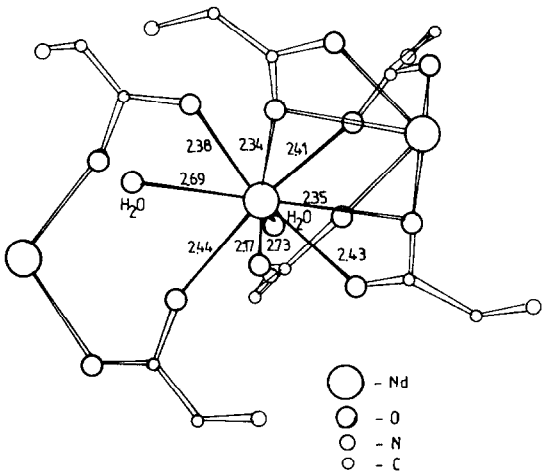

Fig. 1. X-ray structure of $[Nd(Gly)_3][ClO_4]_3 \cdot 5H_2O$.

ion complexes with aminoacids dimeric and polymeric structures may be observed.

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Cu(II) as a Probe in Protein Chemistry-Binding Sites of Cu(II) in Collagen as Investigated by EPR and Electronic Spectroscopies

M. MARZONA

Istituto Chimica Organica Industriale, Università di Torino, Turin, Italy

R. P. FERRARI and P. M. LAUSAROT

Istituto Chimica Generale ed Inorganica, Università di Torino, Turin, Italy

Several authors [1] have found a correlation between the copper content of plasma and a chronic disease of the connective tissue usually known as rheumatoid arthritis. The copper present in the plasma is distributed between two types of complexes, labile and stable bonded ones respectively. Arthritis diseases seem to be associated with an increase in the concentration of the stably linked copper. On this basis one can surmise that some copper must be present within tendon or muscles.

In this framework we have taken into consideration the interactions of copper(II) with collagen as the major protein constituent of the tendon. The techniques used for these studies were EPR and Electronic Spectroscopy. The copper(II) is well known as a very good probe to perform EPR and electronic structural investigations on the natural and artificial copper proteins [2]. As part of our work on the protein-metal ions interactions we have examined the collagen–Cu(II) complexes (molar ratio 1:1) in the pH range 3.1–13.0.

Soluble rat collagen was obtained according to the Gallop method [3]. The analysis of the EPR spectra of freeze-dried compounds below pH = 5.4 shows the presence of the hyperfine and superhyperfine structures which are evidence for the coordination of Cu(II) to collagen at acid pH. In particular the three nitrogen superhyperfine lines suggest the one nitrogen coordination. In the pH range 5.4–10.0, collagen–Cu(II) complexes were isolated as gels, while in the pH interval 11.0–13.0 we have freeze-dried compounds. The EPR parameters of these complexes are reported in Table I: $g_{//}$ varies from 2.32 to 2.16 and $A_{//}$ varies from 140 gauss to 210 gauss. We further notice the dominance of one type

TABLE I. EPR Parameters of Collagen–Cu(II) Complexes, at 77 K.

pH	$g_{//}$	$A_{//}$
5.4	2.32	140
6.6	2.28	160
7.2	2.25	180
8.8	2.26	180
9.6	2.25	180
10.0	2.25	180
12.1	2.25	180
	2.18	200
13.0	2.16	210

of collagen–Cu(II) complex at each pH value. Hyperfine and superhyperfine structures appear in the whole pH range; specifically at pH = 12.0 nine nitrogen superhyperfine lines (A_N = 15 gauss), agree with copper coordination to four nitrogen. The EPR results are supported by further electronic spectroscopical investigations, which shows the existence of two d–d transitions, one at $\bar{\nu}$ = 12000 cm^{-1} and the other one in the $\bar{\nu}$ interval 15000–19000 cm^{-1}. Oxygenated complexes are dominant at lower pH ($\bar{\nu}$ = 12000 cm^{-1}) while with increasing pH nitrogen sites become available and progressively replace oxygenated ligands at the highest pH ($\bar{\nu}$ = 19000 cm^{-1}). Such behaviour closely resembles that observed for Cu(II)-oligopeptide models [4] and Cu(II)-β-lactoglobulin [5] complexes. However the presence of a Cu(II)–collagen complex with $g_{//}$ = 2.18 and $A_{//}$ = 200 gauss can be observed at higher pH with respect to the sequence: oligopeptides, insulin [6], β-lactoglobulin. Therefore we can suppose that nitrogen peptides become available for Cu(II)

coordination to collagen, at higher pH. This is in agreement with the collagen distinctive conformation arising from specific aggregation and cross-linking.

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Silver–Gelatin Complex

TARASANKAR PAL

Department of Chemistry, Calcutta University Postgraduate Centre, Agartala-799004, Tripura, India

A new chemical process for binding silver(I) with gelatin in alkaline medium is described. The complex is susceptible to reducing substances and produces an intense red coloured, very stable silver sol. Oxygen-dependent dissolution of silver sol is discussed.

Introduction

Silver(I) complexed by ligands containing both oxygen and nitrogen donor atoms is easily reduced to metallic sol [1]; some amino acids, sodium sulphamoyl benzoate [2], albumin, and gelatin serve well for the complexation of silver(I) ion. Amongst all the reagents, an aqueous solution of gelatin and silver nitrate forms an alkali-soluble silver compound. Carbon monoxide, ascorbic acid and formaldehyde reduce the alkaline solution of silver gelatin complex, yielding a silver sol, which is intensely coloured, finely powdered, very stable, and follows Beer's law in a wide concentration range [3]. Here we report the quantitative redissolution of silver sol in the presence of oxygen and a suitable complexing ion.

Results and Discussion

Gelatin is well known but has had few analytical applications as a complexing reagent even though it has proved to be a sol stabiliser. Other protein molecules such as egg albumin or serum albumin give the same results as gelatin. Here the complexation is very effective. Even precipitated silver chloride becomes soluble in gelatin solution in 2 *M* sodium hydroxide. It is very likely that the free silver ions are reduced to metal in accordance with the formulation for the silver compound of *p*-sulphamoyl benzoic acid adopted by Cinhandu [4]:

$$2\,Ag^{+} + R + 4OH' \rightleftharpoons 2\,Ag^{\circ} + Re + 2\,H_2O$$

R = Reducing agent, Re = Reduced state of R

The reducing solute rapidly reduces the silver ions to metallic silver while the gelatin is involved in the mechanism supplying free silver ions and probably contributing to the exceptional stability of the colloidal particle. The protein molecule (0.5%) stabilizes the sol in a temperature range of 10–30 °C; indoor light causes no harm. If the temperature is high enough (above 30 °C) the decay in the absorbance value of the exposed solution indicates very slow deposition of metallic silver. The unexposed solution remains unaltered over a wider range of temperature. The reduced silver looks like a very stable colloidal solution with an intense absorption.

Hence it can be a very good selective method [5] for the determination of traces of Ag(I) in biological samples in the presence of various ions. Silver gelatin complex on exposure to carbon monoxide, formaldehyde and ascorbic acid [6] *etc.* quantitatively forms metallic sol. The colour intensity of the exposed solution depends on the solutes added to the silver gelatin complex. If the solute neutralizes the alkaline solution to a greater extent (less than 1.5 *M*) destabilisation of sol occurs. Most of the metal ions are not reduced under the same conditions. Only Hg(I) breaks into a black precipitate, thereby not hindering photometry.

Aqueous solution of cyanide reacts with the sol in the presence of oxygen according to the reaction:

$$4\,Ag + 8\,NaCN + O_2 + 2H_2O \rightleftharpoons 4\,Na[Ag(CN)_2] + 4NaOH$$

The reaction for the silver sol is instantaneous and the end point is red to colourless. Thus silver sol in gelatin is not only a very good method for determining traces of carbon monoxide, formaldehyde and ascorbic acid but is also selective for Ag(I) determination in protein solution using both photometric and volumetric procedures. The complexation of silver sol above pH 6.5 in the presence of cyanide enables one to determine dissolved oxygen in water by photometric means at pH above 6.5 using a wavelength of 410 nm. The method is comparable to that of Duncan [7].

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