Spectrophotometric Studies on the Binding of Cr(iii) to Transfusion Gelatin

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With 3 figures

Sumary

Spectrophotometric studies of mixtures containing Cr(iii) and transfusion gelatin were undertaken to know the binding of chromic ion to the protein. Experiments carried out between pH 3.7 and 5.8 had indicated the binding of the metal through the carboxyl groups of the protein. The extent of metal binding was found to be dependent upon metal-protein ratio, maximum binding taking place in the ratio metal to protein as 42:1.

The nature of metal-protein binding has been successfully worked out by a number of workers[1–10]), using spectrophotometric method. Investigations in this direction have, however, been less extensive with chromium as the metal bound. References worth mentioning are (i) KÜNTZEL's[11–12]) work on the chromium glycine complex to demonstrate the role of chromic ions in chrometanning, (ii) GUSTAVSON's[13])[14]) researches on chromium-pro-

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tein interaction leading to the conclusion that the metal ion got bound through carboxylic groups of proteins. Since the above mentioned studies were limited to gelatin, it was thought worthwhile to reinvestigate the problem systematically employing more simpler species. In the present communication the influences of factors, viz. concentration of the reactants, pH and ionic strength on the extent of binding of chromium to transfusion gelatin are discussed.

Experimental

Reagents

Transfusion gelatin (6 per cent Con.) was used throughout the investigation. Chromic chloride (BAKER, A. R.) potassium chloride (A. R.) were used for preparing the solutions, chromium concentration was determined colorimetrically[15]). Walpole acetate buffers were prepared from 0.2 M solutions of acetic acid and sodium acetate and their pH's were checked by BACKMAN pH meter Model G using glass electrode.

Apparatus

Light absorption measurements were carried out by Model DU spectrophotometer using tungsten lamp as the light source and corex cell (1 cm. depth).

Procedure

The following sets were analysed.

(i) Chromic chloride (1×10^{-2} M) and gelatin (1.8 per cent) were mixed in a number of pyrex boiling tubes. Their pHs were adjusted to 3.7, 4.4, 4.8, 5.2, 5.57 and 5.8 by the addition of buffers. The ionic strength was maintained at 0.4 by adding requisite amount of 1 M KCl. Another set in which the pHs were adjusted by the addition of dilute KOH instead of the buffers was also analysed spectrophotometrically.

(ii) At a fixed pH (5.57) and ionic strength (0.4) two sets of mixtures were prepared. In one the chromium concentration (1×10^{-2} M) was kept fixed and the concentration of the protein was varied while in the other there was fixed amount of protein and the concentration of the metal ion was changed.

(iii) Mixtures were analysed spectrophotometrically at three different ionic strength Viz. 0.5, 0.4, and 0.2 taking varying concentrations of metal ion with a fixed amount of protein (1.8%).

The molar extinction coefficient E of chromic ion was calculated by means of the expression.

$$\text{Log}\,\frac{I_0}{I} = E\,Cd$$

where C is the molar concentration of chromium, d is the depth of the cell (1 cm.) and Log I_0/I is the observed optical density at $\lambda_{max} = 580$ mμ. The results are given in Table 1 and 2.

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Table 1

Effect of pH, metal and protein concentration on chromium transfusion gelatin interaction

(a) Protein con. 1.8%, $[Cr^{+++}] = 1 \times 10^{-2}$ M, $\mu = 0.40$						
(i) pH (with acetate buffer)	3.7	4.4	4.8	5.2	5.57	5.8
E-values (at 580 mμ)	24.2	25.4	27.7	29.0	30.00	30.4
(ii) pH (with KOH)	3.6	4.2	4.4	4.7	4.9	5.2
E-values (at 580 mμ)	32.5	33.5	34.5	35.4	36.5	37.2
(b) Protein conc. 1.5%, pH 5.57, $\mu = 0.40$						
Chromium con. ($\times 10^{-2}$ M)	0.25	0.50	0.75	1.0	1.5	2.0	3.0
E-values (at 580 mμ)	56.0	33.0	26.8	24.0	21.0	19.8	18.1
(c) Chromium con. 1×10^{-2} M, pH 5.57, $\mu = 0.40$							
Protein con. (%)	0.6	0.9	1.2	1.5	1.8	2.1	3.5	4.0
E-values (at 580 mμ)	16.2	17.7	20.8	24.0	26.0	30.2	33.0	35.2

Table 2

Effect of ionic strength on Chromium transfusion gelatin interaction

(a) Protein con. 1.8%, $\lambda_{max} = 580$ mμ

Chromium con. 1×10^{-2} M	E-values at ionic strenght		
	$\mu = 0.5$	$\mu = 0.4$	$\mu = 0.2$
0.5	48.0	41.2	39.6
1.0	32.5	31.6	31.5
2.5	—	18.6	18.3
3.0	20.1	20.3	20.5
4.0	19.0	19.2	19.5

Discussion

The absorption studies carried out in the pH range 3.7 to 5.8 at different wave lengths give a maximum at 580 mμ (Fig. 1) showing thereby the binding of the metal through the carboxyl groups of the protein[16] (pK = 4.8) only. Moreover, since no shift in maximum is observed even at pH 5.8, it may be concluded that the imidazole groups of the protein are not involved in binding chromic ions (expected λ_{max} 540 mμ. GREEN and ANG[15]) on Cr(iii) alanine complex).

The molar extinction coefficient values at 580 mμ when plotted against pH give an S-shaped curve (Fig. 2, A and B). The inflexion occuring near about pH 5.0. This result again indicates that a large number of carboxyl

[16]) M. MUZAFFARUDIN, SALAHUDDIN and WAHID U. MALIK, J. Ind. chem. Soc. **40**, 467 (1963).

groups are made available in this pH range (84 groups)[17]) and offer the maximum number of sites for the binding of chromium. A comparison of extinction coefficient values measured in acetate buffers and KOH also gives a few interesting results. It is found that E-values are larger in KOH medium than in the acetate buffers (Table I, a). From these results it may be

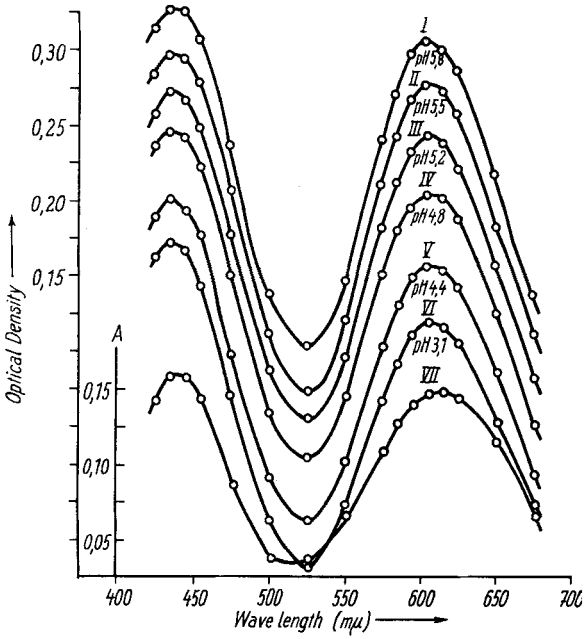

Fig. 1. Absorption spectra of Chromium-gelatin complex. Cuvers; VII for 1×10^{-2} M Cr^{3+}; I to VI for Chromium-gelatin mixtmes at different pHs. Scale A for curve VII

concluded that acetate ions exert a negative influence on metal protein interaction. Our results may be interpreted in the light of GUSTAVSON's[18]) assumption who visualises a lesser reactivity of the chromium ions in presence of the acetate solution due to the formation of uncharged chloro-species $(Cr_2(OH)_5Cl)^\circ$ of the chromic ions.

Metal protein ratio appears to exert a large influence on the binding of chromium to the protein. It is evident from the results on the spectrophotometric titrations carried out with metal ions in presence of a fixed amount of protein and vice versa (Table 1, b and c). From the results it may be conc-

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luded that the presence of a large proportion of metal in the reaction mixture brings about a decrease in the binding capacity. On the other hand, presence of larger amounts of protein in the reaction mixture invariably brings about an increase in the binding of the metal to protein. On plotting the difference of optical density of the metal ion and the complex against

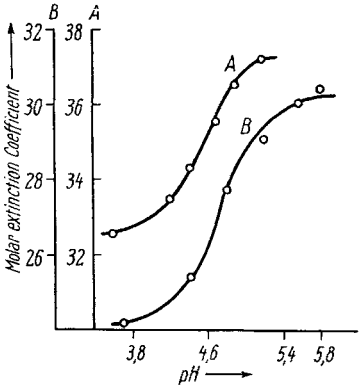

Fig. 2. Effect of pH on molar extinction coefficent. Curve A with KOH and Curve B with acetate buffer; Protein concentration 1 · 8% and concentration of Cr^{3+}, 1×10^{-2} M

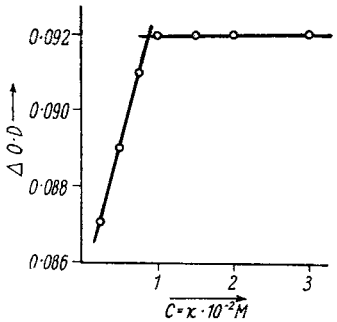

Fig. 3. Plot of △ O · D (difference in optical densities of chromium-gelatin mixture and that of chromium alone) against C (chromium concentration)

metal ion concentration an inflexion is observed at metal concentration equal to 0.85×10^{-2} M (Fig. 3). This interesting observation leads us to conclude that the maximum binding takes place at the metal protein ratio $0.85 \times 10^{-2} : 0.2 \times 10^{-3}$ that is 42 : 1.

Experiments performed at three ionic strengths, viz. 0.2, 0.4 and 0.5 go to show that the extent of metal protein interaction increases with increase in ionic strength. The effect is most pronounced in dilute solution rather than in concentrated one where almost no change in extinction coefficient values is observed with increase in the ionic strength (Table 2).

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