Biuret Reaction of Transfusion Gelation. II

Influence of some Bivalent Metal Ions on the Absorption Spectrum of Nickel-Transfusion Gelation Complex

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With 1 Figures

Summary

Spectrophotometric studies of nickel-transfusion gelatin complex in presence of increasing amounts of various metal ions viz., Cu^{++}, Co^{++}, Zn^{++}, Cd^{++} and Pb^{++} were undertaken to know the binding of these metal ions to the protein under biuret conditions. Studies carried out at pH 12.0 indicated that these metal ions combined with nitrogen of the peptide linkage replacing nickel from its complex. The amount with which a particular metal ions lower the extinction coefficient of the nickel complex may be taken as a measure of the extent of its combination to the protein, and the relative decrease in the extinction coefficient by equal amounts of various metal ions has been interpreted in terms of their order of affinities for the nitrogen of the peptide linkage.

During the course of our studies on the interaction of copper and nickel with transfusion gelatin[3]) (under biuret condition), it occurred to us that, the bivalent metal ions should replace nickel from its transfusion gelation complex if they have got sufficient degree of affinity either equal to or greater than nickel for the nitrogen of the peptide linkage. The replacement may be observed by measuring the decrease in the extinction coefficient of nickel-transfusion gelatin complex in presence of increasing amounts of a particular metal ion. Such method would provide an easy way to estimate the binding capacity of metal ions which are transparent to the visible region, and direct spectrophotometric method fails to give any information. A

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[3]) WAHID U. MALIK and M. MUZAFFARUDDIN (Part I of this series), J. prakt. Chemie [4] **30**, 140 (1965).

method similar to this was employed by KLOTZ and co-workers[4]), and they have demonstrated that 375 mμ band, characteristics of Cusulphahydryl linkage could be used as an indicator for the detection of other protein mercaptides such as zinc, cadmium and lead-albumin mercaptides. To our surprise this method gave results of far reaching importance and the binding of Zn, Cd, Co and Pb with the nitrogen of peptide linkage could be detected easily, using 430 mμ band (absorption beak of nickel-transfusion gelatin complex) as an indicator.

Experimental

Apparatus: Absorption measurements were carried out by means of BAUSCH and LOMB Spectronic 20, and BECKMAN Model G-PH-meter was employed for pH measurements.

Solutions and reagents: Nickel-transfusion gelatin biuret complex was prepared by mixing equal valumes of nickel chloride (dilute solution) and transfusion gelatin (3% concentration). The pH of the mixture was brought to 12.3 and heated slightly (35 to 40 °C) for about half an hour. The resulting mixture was filtered off. The nickel hydroxide precipitate was washed repeatedly with warm dilute alkali (pH 12.0) until the washings were free from protein. The complex was stored in pyrex glass bottles. The nickel content of the complex was estimated by taking an accurately measured volume of the complex, the pH was brought to 5.0 when the pink colour of the complex disappeared, and complexometric titration[5]) was done using EDTA and Meruxide as titrant and indicator respectively. It is assumed that amount of nickel bound to the protein (probably to carboxylate side chain) may exchange the ligand and undergo complex formation preferentially with EDTA.

Chemically pure samples (A.R.) of cupric chloride, zinc sulphate, cadmium sulphate, cobalt chloride and lead nitrate were used as source of metal ions. Dilute potassium hydroxide and potassium chloride were employed to maintain the constant pH and ionic strength respectively. In case of lead salt, potassium nitrate was used to adjust the ionic strength.

Procedure: 2 ml. of nickel complex was mixed with varying amounts of copper, zinc, cadmium, cobalt and lead in different 5 sets of pyrex test tubes. The pH of all the mixtures were adjusted to 12.0 and ionic strength to 0.15 by the addition of requisite amounts of potassium hydroxide and potassium chloride solution respectively. The total volume made upto 10 ml. The optical densities of all the mixtures were measured along with the blank solution which contained only 2 ml. of complex in a total volume of 10 ml. under identical conditions.

The extinction coefficient of the nickel ion was calculated by means of the expression

$$\log \frac{I_0}{I} = E\,cd.$$

where $\log \frac{I_0}{I}$ is the observed optical density, E is the molar extinction coefficient, c is the molar concentration of nickel, and "d" is the depth of cell. The same equation was employed when observation were made in presence of different metal ions. The results are summarised in Table 1.

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[4]) I. M. KLOTZ, J. M. URQUHART and H. A. FIERS, J. Amer. chem. Soc. **74**, 5537 (1952).

[5]) G. SCHWARZENBACH and H. IRVING, „Complexometric titrations" Interscience Publishers Inc., New York, 1957.

Table 1
Effect of different bivalent metal ions on extinction coefficient of nickel-transfusion gelatin complex
Total concentration of nickel in mixture = 1.0×10^{-3} M. λ max 430 mμ

Concentration of added metal ions $\times 10^{-3}$ M	Extinction coefficient in presence of				
	Cu^{++}	Co^{++}	Zn^{++}	Cd^{++}	Pb^{++}
0	172.0	172.0	172.0	172.0	172.0
0.25	168.0	170.0	172.0	171.2	172.0
0.50	160.0	164.0	168.0	168.0	169.2
1.00	136.0	160.0	164.0	164.0	168.0
1.50	124.0	152.0	160.0	160.0	166.4
2.00	120.8	144.0	152.0	152.0	160.0
% Reduction in E-values	29.7	16.2	11.6	11.6	7.0

Discussion

With the establishment of 560 mμ and 430 mμ bands as the characteristics of the copper-nitrogen and nickel-nitrogen linkage respectively during the biuret reaction of transfusion gelatin[3]), experiments were conducted so as to see whether these bands could be used as indicators for the detection of other metal-protein complexes. Addition of increasing amounts of Co, Ni, Zn, Cd and Pb to the copper complex did not give any encouraging results, except that the added metal ions preceipitated out as hydroxides at such high pH (12). The centrifuged solution when subjected to spectrophotometric analysis neither the λ max showed any detectable shift nor the optical density of the complex itself changed significantly. The observations indicated that the added metal ions are unable to replace the copper ions from its protein complex. Hence, copper must have strongest affinity for the nitrogen of the peptide linkage. The experiments were repeated with nickel complex as described in the experimental part and the results of considerable interest were obtained.

It is evident from Table 1 that 430 mμ band of nickel complex could be used as an indicator for the detection of the degree of affinity of the different bivalent metal ions for the nitrogen of the peptide linkage. The effect of copper on absorption spectrum may well be seen from Fig. 1. Addition of an incresing amounts of copper to nickel complex diminishes 430 mμ band so much so at high copper concentration, this band has been completely abolished and in fact a new band at 560 mμ makes its appearance. Simultaneously the colour of the complex changes from pink to violet and a green precipitate of nickel hydroxide is formed, showing thereby that copper easily replaces the nickel ions from its protein complex.

Table 1 demonstrates the relative effect of Cu, Co, Zn, Cd and Pb on the extinction coefficient of the nickel complex. When the metal ions are added one fourth of the total nickel concentration (i.e. 0.25×10^{-3} M) present in the complex, copper produces a reduction of 2.3% and the other metal ions exert negligible effect. When half the amount (0.5×10^{-3} M) of total nickel

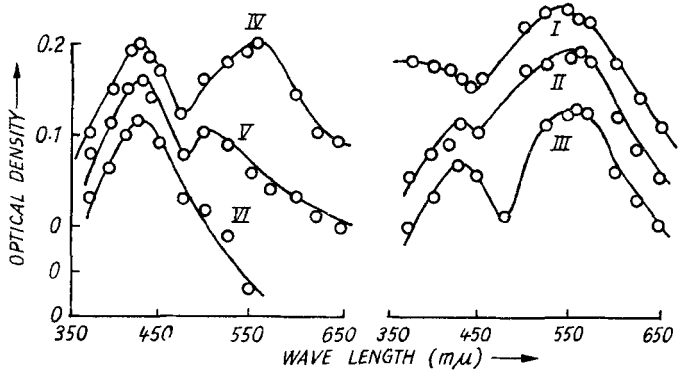

Fig. 1. Absorption spectra of nickel-transfusion gelatin complex in presence of cupric ions at pH 12.0. Curves I to V for 2.0, 1.5, 1.0, 0.5 and 0.2×10^{-3} M Cu^{++} and curve VI for nickel-transfusion gelatin complex without cupric ions

concentration (1×10^{-3} M) was added the percentage reduction in extinction coefficient goes upto 7.0 in case of copper and other metal ions still have very insignificant effect. When equivalent amounts of metals (1×10^{-3} M) and the nickel (1×10^{-3} M) present in the mixture the extinction coefficient is greatly decreased in case of copper (21.0%) and Co, Zn, Cd and Pb produce 7.0%, 4.6%, 4.6% and 2.3% reduction respectively. The percentage reduction in extinction coefficient when various metal ions and nickel present in a ratio of 2:1 in the mixture is shown in Table 1. Such data reveal that, all the metal ions do not replace nickel from its protein complex to the same extent. It is, therefore, assumed that the amount with which different bivalent metal ions reduce the extinction coefficient of nickel complex may be taken as a measure of their degree of affinities for the nitrogen of the peptide linkage. The following order is seems to be pertinent.

$$Pb < Zn = cd < Co < Cu$$

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