L-Histidine-containing Peptides as Models for the Interaction of Copper (II) and Nickel (II) Ions with Sperm Whale Apomyoglobin*

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SUMMARY

1. The association and ionization constants for nickel (II) and a selection of L-histidine-containing peptides have been computed together with the constants for copper (II) and acetylglycylglycyl-L-histidylglycine.

2. A close parallel between the titration behavior of copper (II) apomyoglobin complexes and model peptides has been obtained on the assumption that 1 of the 4 bound metal ions is situated at the NH₂-terminal portion of the polypeptide chain.

3. Visible absorption spectra of nickel (II) and copper (II) apomyoglobin complexes containing 4 metal ions per mole have been measured and found to be consistent with the binding of 1 metal ion at the NH₂-terminal locus and 3-metal ions in the interior of the peptide chain at histidine loci.

4. Optical rotatory dispersion and circular dichroism spectra in the visible region have been measured for both the protein and the peptide complexes. Strict similarities were not, in general, found and the various explanations for this failure are discussed.

Nickel (II) ions have been shown to promote the ionization of protons from peptide bonds in complexes of simple dipeptides and polypeptides (2) and from complexes of histidine-containing dipeptides (3). Previous communications (4, 5) demonstrated the adequacy of peptides such as acetylglycylglycyl-L-histidine as models for the interaction of copper (II) ions with sperm whale metmyoglobin and apomyoglobin as judged by titration studies. The present communication deals with comparisons of the visible absorption spectra, optical rotatory dispersion, and circular dichroism spectra of complexes of copper (II) and nickel (II) ions with sperm whale apomyoglobin and selected L-histidine-containing peptides. In particular, it deals with acetylglycylglycyl-L-histidylglycine, which may be regarded as perhaps a better model than those considered hitherto for the histidine environment in a polypeptide chain. As an adjunct to the present study, the titration behavior of the complexes of nickel (II) and L-histidine-containing peptides has been investigated together with the copper (II) complex of acetylglycylglycyl-L-histidylglycine which extends work of this type already reported (4, 5).

EXPERIMENTAL PROCEDURE

Physical Chemical Measurements—Titrations were carried out as previously described (4, 6). The spectropolarimetry with the Bendix Polarmatic instrument and spectrophotometry with the Cary model 14 spectrophotometer were normally applied to the same solution under conditions described previously (5, 6). Results are reported in terms of molar absorbance e and molecular rotation

\[ [M] = \left( \frac{\alpha}{100} \right) \times \frac{M}{d} \]

in which \( \alpha \) = (observed rotation in degrees/°), \( d \) is the path length in decimeters, \( c \) is the concentration in grams per ml and \( M \) is the molecular weight. A light path of 10 mm or 1 mm was generally used with concentrations of the complexes in the 0.01 M range. Rotations were measured on samples with an absorbance in the visible region of generally less than 0.2. Measurements of circular dichroism spectra were made on the RousSEL-Jouan instrument kindly made available by Dr. T. S. Piper of the University of Illinois. Results are quoted in terms of \( e_L - e_R \), the difference in molar absorbance between the left and right circularly polarized beams.

The apomyoglobin complexes were studied at about 4 × 10⁻⁴ M metal ion concentration in a 100-mm cell for the spectrophotometry, a 50-mm cell for the circular dichroism, and a 20-mm cell for the spectropolarimetry.

Preparation of Sperm Whale Apomyoglobin—The protein was prepared from metmyoglobin by the modified method of Breslow (7). A further modification was introduced by diluting the metmyoglobin solution to a concentration of about 2 mg per
ml during the extraction with 2-butanone. The resulting protein solution was completely colorless after two extractions.

Preparation of Apomyoglobin Complexes—Copper (II) and nickel (II) complexes (4:1) of apomyoglobin were prepared by taking the protein solution to above pH 11 and adding the metal ion as the chloride. The pH was then readjusted to 11 over a period of a few hours. No added electrolyte was present. The final protein concentration was 0.107 mM.

N-tert-Butoxy carbonyl glycyl-L-iminobenzylhistidylglycine Benzyl Ester (Compound I)—A suspension of L-iminobenzylhistidylglycine benzyl ester dihydrobromide (4) (2.34 g) in chloroform (50 ml) was treated with triethylamine (1.2 ml) and N-tert-butoxycarbonylglycine p-nitrophenyl ester (4) (1.24 g) and allowed to stand at room temperature overnight. The solution was diluted with chloroform (300 ml), washed with cold 0.1 N sodium hydroxide solution (4 × 5 ml), and dried over sodium sulfate. Evaporation of the solvent in a vacuum left a syrup which crystallized from ethanol water. After two recrystallizations, the product (1.39 g, 60%) melted at 132.5–134.5°. \( R_F \) 0.75 in Solvent B and 0.50 in Solvent A.

\[
\begin{align*}
\text{C}_{25} \text{H}_{24} \text{N}_{20} \text{O}_{4} & \\
\text{Calculated: C 61.30, H 5.88, N 15.32} & \\
\text{Found: C 61.04, H 5.97, N 15.02} & \\
\end{align*}
\]

Acetylglycylglycyl-L-histidylglycine (Compound II)—A solution of Compound II (600 mg) in liquid ammonia (150 ml) was treated with small pieces of sodium until a deep blue color persisted for 5 min. Dowex 50W-X2 on the ammonium cycle (6 g) was added, and the mixture was stirred for 30 min and allowed to evaporate. The residue was extracted with deionized water (2 × 50 ml), and the pH (7.5) was adjusted to 4.8 by the addition of a few drops of 1 N HBr. The solution was lyophilized and washed with pH 0 carbonate buffer (50 ml), then with water (20 ml), and allowed to dry in the air. After three recrystallizations from 95% ethanol, the product (0.68 g, 30%) melted at 193–195°. \( R_F \) 0.60 in Solvent B.

\[
\begin{align*}
\text{C}_{25} \text{H}_{24} \text{N}_{20} \text{O}_{4} & \\
\text{Calculated: C 61.30, H 5.88, N 15.32} & \\
\text{Found: C 61.04, H 5.97, N 15.02} & \\
\end{align*}
\]

Results and Discussion

Titration Studies—Table I lists the formation and ionization constants for 1:1 complexes of nickel (II) and selected L-histidine-containing peptides together with the results for copper (II)

<table>
<thead>
<tr>
<th>Peptide</th>
<th>( pK_a )</th>
<th>( pK_{a\text{dissoc}} )</th>
<th>( pK_{a\text{H+}} )</th>
<th>( pK_{a\text{H+}} )</th>
<th>( pK_{a\text{H+}} )</th>
<th>( pK_{a\text{H+}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycyl-L-histidine...</td>
<td>2.66</td>
<td>6.77</td>
<td>8.24</td>
<td>ND</td>
<td>ND</td>
<td>6.10</td>
</tr>
<tr>
<td>Acetylglycyl-L-histidine...</td>
<td>2.84</td>
<td>6.87</td>
<td>8.22</td>
<td>ND</td>
<td>ND</td>
<td>6.20</td>
</tr>
<tr>
<td>Acetylglycyl-L-histidine...</td>
<td>3.02</td>
<td>6.85</td>
<td>8.11</td>
<td>ND</td>
<td>ND</td>
<td>8.30</td>
</tr>
<tr>
<td>Acetylglycyl-L-histidylglycine</td>
<td>3.08</td>
<td>7.18</td>
<td>2.94</td>
<td>2.30</td>
<td>8.65</td>
<td>8.95</td>
</tr>
<tr>
<td>Acetylglycyl-L-histidyglycine</td>
<td>3.29</td>
<td>6.90</td>
<td>2.93</td>
<td>8.40</td>
<td>8.50</td>
<td>8.60</td>
</tr>
</tbody>
</table>

* Equilibrium constants are defined as follows (4):

\[
\begin{align*}
\text{Cu}^2+ + L & \rightleftharpoons \text{CuL}^+ & K_1 &= \frac{[\text{CuL}^+]}{[\text{Cu}^2+] [L^-]} \\
\text{CuL}^+ + \text{H}^+ & \rightleftharpoons \text{CuL}^2+ & K_2 &= \frac{[\text{CuL}^2+]}{[\text{CuL}^+] [\text{H}^+]} \\
\text{Cu}^2+ + L & \rightleftharpoons \text{CuL}^+ & K_c &= \frac{[\text{CuL}^+]}{[\text{Cu}^2+] [L^-]} \\
\text{Cu}^2+ + \text{H}^+ & \rightleftharpoons \text{CuL}^2+ & K_c' &= \frac{[\text{CuL}^2+]}{[\text{Cu}^2+] [\text{H}^+]} \\
\text{CuL}^+ + \text{H}^+ & \rightleftharpoons \text{CuL}^2+ & K_c'' &= \frac{[\text{CuL}^2+]}{[\text{CuL}^+] [\text{H}^+]} \\
\text{Cu}^2+ & \rightleftharpoons \text{CuL}^2+ & K_c''' &= \frac{[\text{CuL}^2+]}{[\text{Cu}^2+]} \\
\end{align*}
\]

† Taken from Martin and Eedsall (3).

\( \text{Cu}_2\text{H}_2\text{N}_2\text{O}_4 \cdot \text{H}_2\text{O} \)

Calculated: C 43.52, H 5.74, N 21.75
Found: C 43.83, H 5.85, N 21.13

Table I

Formation and ionization constants for 1:1 complexes of nickel (II) and L-histidine-containing peptides at 25° and 0.16 ionic strength.
and acetylglucylglycyl-L-histidylglycine. No attempt was made to compute formation constants for peptides containing a free α-amino group. For the acetylated peptides, formation constants were computed by methods outlined previously (1). The pKₐ values for nickel (II) complexes were computed by the method used for the analysis of nickel (II) and triglycylglycine (2) and should be regarded with a degree of uncertainty.

The log K₁ values for nickel (II) and acetylglycylglycyl-L-histidine and acetylglycylglycyl-L-histidyglycine are comparable to those found for the formation constants for nickel (II)imidazole complexes (9). The log K₁ value for copper (II) and acetylglycylglycyl-L-histidyglycine of 3.95 agrees favorably with the value of 3.89, predicted from the correlation with the pK of the imidazole group found from previous studies (4).

The pKₐ values of nickel (II) and glycylglycyl-L-histidine exhibit the same extensive overlapping that was found for the copper (II) complexes resulting from the cooperative effect of initial association at both the α-amino group and imidazole nitrogen atom. However, the pKₐ values for the interaction of nickel (II) with the acetylated peptides and glycylglycylglycyl-L-histidine also show this overlapping, which can be attributed in this case to a change in stereochemistry from octahedral to square planar, the latter form being characterized by an intense yellow color (2, 3).

For the copper (II) complex of acetylglycylglycyl-L-histidylglycine, the predicted pKₐ value is 6.35 (4), which is significantly larger than the observed value of 5.95. The pKₐ' value of 6.45 is also about 0.7 log unit larger than the expected value, but the pKₐ value of 9.00 is comparable to others found in similar peptides (4). It is not entirely clear from structural considerations why these pKₐ values should differ by such an amount. Substitution of α-glycine or L-histidine on the L-histidine carbonyl group would not be expected to affect the chelation of the copper (II) to the peptide bonds on the NH₂-terminal side of the L-histidine residue.

In general, the pKₐ values for the nickel (II) complexes were about 2 log units higher than for the copper (II) complexes. A comparison of the titration curves of nickel (II) and copper (II) with acetylglycylglycyl-L-histidyglycine is shown in Fig. 1. The overlapping pKₐ values, noted in Table I, are reflected in the extreme flatness of the titration curve of the nickel (II) complexes between 1 and 4 equivalents of NaOH added.

Visible Spectral Properties—Table II lists the visible absorption characteristics of nickel (II) complexes of L-histidine-containing peptides together with the copper (II) complex of acetylglucylglycyl-L-histidyglycine, all at pH 11. In effect these represent the spectra of the ML⁻ species for all except acetylglucyl-L-histidine, where the NiL⁻ species is represented, according to the nomenclature used previously (5). Also included are the results for the copper (II) and nickel (II) complexes of apomyoglobin and tetracycylglycine at the same pH. Lₐ max refers to the position of a second absorption band on the long wavelength side of the major peak in the nickel (II) complexes. For the complex of glycyl-L-histidine, this band is absent. For glycylglycyl-L-histidine and glycylglycylglycyl-L-histidine, it is a poorly defined shoulder; hence, the corresponding εₐ max values must be regarded as only approximate. But for the acetylated peptides the spectra are readily resolved into contributions from two absorption bands of comparable strength. As discussed further below, this latter observation is true to a slightly lesser extent for the apomyoglobin complex.

Resolution of the spectra of intermediate nickel (II) complexes was not attempted. Only the square planar NiL⁻ species possesses any significant color, whereas the octahedral species are pale blue and would be difficult to resolve accurately because of the uncertainty in the ionization constants.

Copper (II) and Nickel (II)-Apomyoglobin Complexes—The visible absorption spectrum of a 4:1 copper (II)-apomyoglobin complex at pH 11 is shown in Fig. 2, Curve 1; ε is expressed per mol ion. The single peak has a λₐ max of 530 μm which is exactly similar to the λₐ max found for the complex at pH 10 by Breslow (10). However, the higher εₐ max value of 112 compared to a value of 98 found by Breslow (10) can be accounted for partially by the narrower peak. Fig. 2 also includes the spectrum of the CuL⁻ species of acetylglucylglycyl-L-histidyglycine with a λₐ max of 555 μm and εₐ max of 90 (Curve 2). If one considers
Fig. 2. Visible absorption spectra of: Curve 1, a 4:1 complex of copper (II) and apomyoglobin at pH 11.0; Curve 2, the CuL\textsuperscript{2-} species of the copper (II) acetylglycylglycyl-L-histidylglycine complex; Curve 3, the computed spectrum of a mixture of 1 mole of the CuL\textsuperscript{2-} species of the copper (II)-tetraglycylglycine complex and 3 moles of the CuL\textsuperscript{2-} species of the copper (II) acetylglycylglycyl-L-histidylglycine complex. ε values are expressed per copper (II).

The available evidence is that strong binding of copper (II) ions to metmyoglobin levels off at 7 per molecule which should apply approximately to apomyoglobin (10, 11). Hence, eteris paribus, with 4 metal ions bound, the probability of there being one at the NH\textsubscript{2}-terminal site is only one-third of that for binding at histidine loci. However, at this pH there is no element of competition by protons for the basic form of the free α-amino group by virtue of a higher association constant. Log \( K_1 \) for copper (II) triglycylglycine is 4.93 (12), while the value for acetylated L-histidine peptides is about 4.0. Thus, it is not unreasonable to consider the copper (II) complex of apomyoglobin as made up of a population of molecules with the predominant species carrying 1 metal ion at the NH\textsubscript{2} terminus and 3 metal ions somewhere in the interior of the peptide chain at histidine loci.

Further evidence of this type is presented in Fig. 3, which shows the analogous spectra for nickel (II) complexes. The spectra in this case have the added advantage for interpretation of a second absorption band, albeit overlapping with the major peak. This second, long wave length absorption is not present in the NiL\textsuperscript{2-} species of tetracylglycylglycine. Hence, the presence of this type of complex in the protein would be expected to reduce the absorbance at 485 \( \text{m}_{\lambda} \) compared to that at 425 \( \text{m}_{\lambda} \). This is found to be the case. Examination of Fig. 3 reveals that the computed spectrum closely parallels the protein complex spectrum in profile. All three spectra have maxima at 425 \( \text{m}_{\lambda} \) and, as closely as can be determined, also at 485 \( \text{m}_{\lambda} \). However, the ratios of absorbance at these two wave lengths are not the same. For the protein complex, Curve 1, and the computed spectrum, Curve 3, the ratios of absorbance at 425 \( \text{m}_{\lambda} \) to absorbance at 485 \( \text{m}_{\lambda} \) are 1.61 and 1.64, respectively. The ratio of 1.13 for the acetylglycylglycyl-L-histidylglycine complex, Curve 2, rules out this peptide as being typical of all the binding sites.

Fig. 3. Visible absorption spectra of: Curve 1, a 4:1 complex of nickel (II) and apomyoglobin at pH 11.0; Curve 2, the NiL\textsuperscript{2-} species of the nickel (II)-acetylglycylglycyl-L-histidylglycine complex; Curve 3, the computed spectrum analogous to Curve 3 in Fig. 2.

1 G. F. Bryce, unpublished observation.
The anionic form of the peptide is taken as the starting point.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Value of $(-\Delta F/M)$ at pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.50</td>
</tr>
<tr>
<td>Triglycylglycine</td>
<td>1.12</td>
</tr>
<tr>
<td>Tetraglycylglycine</td>
<td>1.16</td>
</tr>
<tr>
<td>Acetylglycylglycyl-L-histidine</td>
<td>0.56</td>
</tr>
<tr>
<td>Acetylglycylglycyl-L-histidylglycine</td>
<td>0.50</td>
</tr>
<tr>
<td>Tetraglycylglycine + three complexes of acetylglycylglycyl-L-histidylglycine</td>
<td>1.16</td>
</tr>
<tr>
<td>Tetraglycylglycine + three complexes of acetylglycylglycyl-L-histidylglycine</td>
<td>0.71</td>
</tr>
<tr>
<td>Triglycylglycine + three complexes of acetylglycylglycyl-L-histidylglycine</td>
<td>1.16</td>
</tr>
<tr>
<td>Triglycylglycine + three complexes of acetylglycylglycyl-L-histidylglycine</td>
<td>0.70</td>
</tr>
<tr>
<td>Triglycylglycine + three complexes of acetylglycylglycyl-L-histidylglycine</td>
<td>1.15</td>
</tr>
<tr>
<td>Tetraglycylglycine + three complexes of acetylglycylglycyl-L-histidine</td>
<td>0.86</td>
</tr>
<tr>
<td>Metmyoglobin</td>
<td>1.25</td>
</tr>
<tr>
<td>Apomyoglobin</td>
<td>1.38</td>
</tr>
</tbody>
</table>

These two sets of evidence provide strong indications for the nature of the chelation loci for copper (II) and nickel (II) in apomyoglobin.

Comparison of Titration Behavior of Complexes of Copper (II) with Apomyoglobin and Model Peptides—It was noted in an earlier communication (4) that the titration behavior of the copper (II) complex of apomyoglobin could be paralleled by complexes of acetylated histidine peptides as judged by variation with pH of $(-\Delta F/M)$, the average number of protons liberated per metal ion bound. The agreement was good above pH 7.5, but there was a deficit of protons displaced from the model peptide complexes at lower pH values. Now, if account is taken of binding to the NH₂-terminus portion of the molecule then the most accurate model should contain the contribution of one peptide not containing histidine. Data are available for triglycylglycine (12), tetraglycylglycine, and a selection of acetylated L-histidine peptides described here or in a previous study (4). The values of $(-\Delta F/M)$ computed for "mixtures" representing 1 copper (II) at the NH₂ terminus and 3 at histidine loci are presented in Table III in comparison with the observed values for metmyoglobin and apomyoglobin. Fig. 4 is a graphical representation of the data for the three best fitting models and for apomyoglobin. It is evident that a close parallel throughout the entire pH range is not obtained from any of the models. However, for reasons discussed elsewhere (4), exact similarity is not to be expected because of difficulties in defining the environment in the protein. The discrepancy is partially alleviated by inclusion of either a triglycylglycine (Curve 2)
or tetraglycylglycine (Curves 3) complex but is nevertheless significant. The unusual titration properties of the complex of acetylglycylglycyl-L-histidylglycine (Curves 4) almost match the protein at pH 6.80, but an overcompensation at pH 7 to 8 results in excessively large values for \(-\Delta R/\Delta \alpha\). The best over-all fit is obtained from three complexes of the acetylglycylglycyl-L-histidine type and one of either triglycylglycine or tetraglycylglycine (Curves 2 and 3).

One very important factor so far has been omitted from consideration, the influence of the side chains on the ionization equilibria. The bulky aliphatic side chains of valine and leucine cause an increase in the pK\(_a\) value in copper (II) complexes of glycyl-L-valine (13) and glycyl-L-leucine (14) to 4.75 and 4.80, respectively, compared to 4.25 for glycylglycine. This could be the result of the expenditure of energy in the reorganization of the hydrotactoid environment of these side chains in free solution. No data are available for similar effects on pK\(_a\) values in larger peptides. It is to be anticipated that the same process would operate for aliphatic residues on the NH\(_2\)-terminal side of an L-histidine residue to raise the pK\(_a\) values. Little can be said concerning the effect of other side chains but, as noted previously (4), structural factors could be present in the protein which would promote or favor apposition of the first peptide bond to the copper (II), thereby lowering the pK\(_a\) value. A much greater "proximity factor" would be required to cause the same reduction in pK\(_a\)'s. A more definitive description must await a study of L-histidine peptides representing sequences of myoglobin.

**Optical Rotatory Dispersion and Circular Dichroism Spectra—**

Complexes such as the CuL\(^2-\) species of acetylglycylglycyl-L-histidine exhibit well defined Cotton effects around the visible absorption band (5), and it would not be unreasonable to expect the chelation loci in copper (II) apomyoglobin complexes to behave in a similar manner. However, from the foregoing discussion, it is apparent that account must be taken of the rotatory properties of a complex representing the NH\(_2\)-terminus. A suitable model would be tri-L-alanyl-L-alanine. We have employed here di-L-alanyl-L-alanine, the properties of which should not be too dissimilar and from which it should be fairly easy to extrapolate to the more representative peptide. Anomalous dispersion in the NH\(_2\)-terminal complex of apomyoglobin should be greater in magnitude and displaced towards shorter wave lengths than those of di-L-alanyl-L-alanine complexes.\(^2\)

Fig. 5 illustrates the ORD\(^3\) spectra of the CuL\(^-\) species of di-L-alanyl-L-alanine and the CuL\(^2-\) species of acetylglycylglycyl-L-histidine (A) and the CD spectra of the same (B). The CuL\(^2-\) species of the latter peptide complex is the form existing at pH 11, and the CuL\(^-\) species of di-L-alanyl-L-alanine represents maximum involvement of peptide nitrogen atoms without the complication of ionizations from coordinated water molecules. The negative ORD Cotton effect of this complex centered at 570 \(\mu\)m is reproduced as a negative CD Cotton effect at 555 \(\mu\)m. The absorption spectrum (Table II) consisted of a single peak at 555 \(\mu\)m. By extrapolation to tri-L-alanyl-L-alanine, we may predict that the Cotton effect for this complex would also be negative, centered at about 520 to 530 \(\mu\)m and perhaps of twice the magnitude. It was suggested earlier (5) that the ORD spectrum of the CuL\(^2-\) species of acetylglycylglycyl-L-histidine was the resultant of two Cotton effects of opposite sign located so that the positive extrema overlapped. The CD spectrum in Fig. 5B confirms this interpretation where it is seen that there is a negative Cotton effect centered at 590 \(\mu\)m and a positive one at 500 \(\mu\)m. The crossover points in the ORD spectrum are 600 \(\mu\)m and 490 \(\mu\)m, respectively. The slightly skew shape of this CD curve indicates partial overlap so that the exact wave lengths of the transitions cannot be determined. It is apparent that they would not differ by more than 5 \(\mu\)m from the wave lengths quoted above. It should be mentioned here that the CD spectrum of copper (II)-acetylglycylglycyl-L-histidine was identical with the above in the \(\lambda_{max}\) values and only slightly different in the magnitudes of \(\epsilon_L - \epsilon_R\).

Fig. 6A and B illustrates the ORD and CD spectra of the analogous nickel (II) complexes. The CD Cotton effects of the nickel (II)-acetylglycylglycyl-L-histidine complex appear at 420 \(\mu\)m and 485 \(\mu\)m, which are very close to the estimated positions of the resolved partial absorption bands (Table II) and not very different from the crossover points in the ORD spectra.

\(^2\) Based on a trend observed with other simple peptides (T. P. Bryce and F. R. N. Gurd, in preparation).

\(^3\) The abbreviations used are: ORD, optical rotatory dispersion; and CD, circular dichroism.
or tetraglycylglycine (Curve 3) complex but is nevertheless significant. The unusual titration properties of the complex of acetylglycylglycyl-L-histidylglycine (Curve 4) almost match the protein at pH 6.80, but an overcompensation at pH 7 to 8 results in excessively large values for \( -\Delta E_{c} / \Delta \varepsilon_{m} \). The best over-all fit is obtained from three complexes of the acetylglycylglycyl-L-histidine type and one of either triglycylglycine or tetraglycylglycine (Curves 2 and 3).

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**Optical Rotatory Dispersion and Circular Dichroism Spectra—**

Complexes such as the CuL\(^{2-}\) species of acetylglycylglycyl-L-histidine exhibit well defined Cotton effects around the visible absorption band (5), and it would not be unreasonable to expect the chelation loci in copper (II) apomyoglobin complexes to behave in a similar manner. However, from the foregoing discussion, it is apparent that account must be taken of the rotatory properties of a complex representing the NH\(_{2}\) terminus. A suitable model would be tri-L-alanyl-L-alanine. We have employed here di-L-alanyl-L-alanine, the properties of which should not be too dissimilar and from which it should be fairly easy to extrapolate to the more representative peptide. Anomalous dispersion in the NH\(_{2}\)-terminal complex of apomyoglobin should be greater in magnitude and displaced towards shorter wave lengths than those of di-L-alanyl-L-alanine complexes.

Fig. 5 illustrates the ORD spectra of the CuL\(^{2-}\) species of di-L-alanyl-L-alanine and the CuL\(^{2-}\) species of acetylglycylglycyl-L-histidine (A) and the CD spectra of the same (B). The CuL\(^{2-}\) species of the latter peptide complex is the form existing at pH 11, and the CuL\(^{2-}\) species of di-L-alanyl-L-alanine represents maximum involvement of peptide nitrogen atoms without the complication of ionizations from coordinated water molecules. The negative ORD Cotton effect of this complex centered at 570 m\(\mu\) is reproduced as a negative CD Cotton effect at 565 m\(\mu\). The absorption spectrum (Table II) consisted of a single peak at 555 m\(\mu\). By extrapolation to tri-L-alanyl-L-alanine, we may predict that the Cotton effect for this complex would also be negative, centered at about 520 to 530 m\(\mu\) and perhaps of twice the magnitude. It was suggested earlier (5) that the ORD spectrum of the CuL\(^{2-}\) species of acetylglycylglycyl-L-histidine was the resultant of two Cotton effects of opposite sign located so that the positive extrema overlapped. The CD spectrum in Fig. 5B confirms this interpretation where it is seen that there is a negative Cotton effect centered at 590 m\(\mu\) and a positive one at 500 m\(\mu\). The crossover points in the ORD spectrum are 600 m\(\mu\) and 490 m\(\mu\), respectively. The slightly skew shape of this CD curve indicates partial overlap so that the exact wave lengths of the transitions cannot be determined. It is apparent that they would not differ by more than 5 m\(\mu\) from the wave lengths quoted above. It should be mentioned here that the CD spectrum of copper (II)-acetylglycylglycyl-L-histidine was identical with the above in the \( \lambda_{\max} \) values and only slightly different in the magnitudes of \( \epsilon_{L} - \epsilon_{R} \).

Fig. 6A and B illustrates the ORD and CD spectra of the analogous nickel (II) complexes. The CD Cotton effects of the nickel (II)-acetylglycylglycyl-L-histidine complex appear at 420 m\(\mu\) and 485 m\(\mu\), which are very close to the estimated positions of the resolved partial absorption bands (Table II) and not very different from the crossover points in the ORD

**Footnotes:**

1. Using the nomenclature of A.

2. Based on a trend observed with other simple peptides (G. F. Bryce and F. R. N. Gurd, in preparation).

3. The abbreviations used are: ORD, optical rotatory dispersion; and CD, circular dichroism.

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plex curves shown in Fig. 6B provided allowance is made for a much larger contribution from the di-L-alanyl-L-alanine complex, based on the premise that the tri-L-alanyl-L-alanine would have higher $\epsilon_L \neq \epsilon_R$ values.

Configuration about Copper (II) and Nickel (II) Ions in Apomyoglobin Complexes—It is pertinent to the discussion at this stage to examine certain properties of the model peptides to establish just what can be expected from the ORD and CD of the protein complexes. In a previous communication (5) it, was suggested that the origin of the optical activity in the visible region of copper (II) complexes of acetylated L-histidine peptides was due to asymmetry induced around the metal ion by a partially tetrahedral array of the ligand nitrogen atoms. The 4 nearest ligand atoms in several related crystalline copper (II) complexes have been found to be not exactly coplanar (15).

Now, since this configuration appeared to be controlled by the conformation of the chelate ring containing the L-histidine residue, it was necessary to postulate an interaction between the hydroxyl oxygen of the L-histidine carboxyl group (or of the peptide bond linking the carboxyl-terminal glycine as in acetylglucylglycyl-L-histidylglycine) and the metal ion. From a study of molecular models, the interaction can occur with the carboxyl group in an axial orientation. An equatorial orientation leads to the alternative conformation of the six membered ring and an effectively inverted configuration around the metal ion. The extent of distortion of the square plane into a flattened tetrahedral arrangement need not be exactly comparable in the two alternative configurations. In the interior of a peptide chain, the multitude of inter-side chain interactions and other factors stabilizing the protein configuration probably impose severe constraint on the mobility of the polypeptide components and may limit the extent of formation of each of the puckered forms of the ring.

If we are to entertain the possibility of an NH$_2$-terminal binding site, an explanation for the optical activity in copper (II) and nickel (II) di-L-alanyl-L-alanine complexes must be sought. In this case, no simple interaction is present in the complex which would produce configurational dissymmetry. Preliminary studies on tripeptides made up of glycine with one L-alanyl residue in each of the three positions indicates that the magnitudes of the Cotton effects are dependent on which chelate nitrogen bonds to the copper in an apical position.4 The relative importance of such forms could well differ between the small peptides and the protein. There is some crystallographic evidence that in copper (II) complexes with the square planar array somewhat distorted, the apical bond in the square pyramidal arrangement need not be normal to the approximate plane of the square base (16).

There are undoubtedly many other factors which preclude strict parallels between the optical rotatory behavior of the protein complexes and the peptide models considered here. Paramount among these is the influence of side chains. It is premature at this stage to assess the influence of side chains on the optical rotatory properties of model peptides, and a more definitive answer must await the study of small segments of the myoglobin polypeptide chain.

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l-Histidine-containing Peptides as Models for the Interaction of Copper (II) and Nickel (II) Ions with Sperm Whale Apomyoglobin
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