Inorganica Chimica Acta, 20 (1976) L17 - L19
©Elsevier Sequoia S.A., Lausanne – Printed in Switzerland

Raman Resonant Effect on a Cobalt Histidine Complex, a Model of a Metalloprotein

G. CHOTTARD* and J. BOLARD

Départment de Recherches Physiques, Laboratoire associé au C.N.R.S. no 71, Université Pierre et Marie Curie, 75230, Paris Cedex 05, France

(Received July 12, 1976)

Interesting structural information has been obtained from the Raman Resonant Effect (RRE) on proteins absorbing strongly in the visible, owing to the presence of either a prosthetic group with delocalized π electrons, or a metal ion allowing charge transfers of low energy [1]. We think that labelling of non absorbing proteins by a transition metal ion could extend the field of the RRE. We report here the results of a study of $[Co(his)_2]^{2-}$ (his = $NH_2{-}\underset{\displaystyle R}{\underset{|}{C}}{-}COO^-$, R = $CH_2{-}C_3H_3N_2^-$), a tetrahedral cobalt(II) complex, which may approximate the metal environment of a metalloenzyme, if we focus on the nitrogen donor atoms and the geometry of their arrangement.

This complex has been already studied by Yoshida *et al.* [2], who observed only a slight resonance on internal vibrations of the ligand. This report showing a marked discrepancy with our previous results on CoX_4 complexes [3], we considered it worthwhile to reinvestigate the RRE of $[Co(his)_2]^{2-}$.

Experimental

$[Co(his)_2]^{2-}$ has been prepared in strongly basic medium, as described by P. M. Morris *et al.* [4]. Under these conditions, the imidazole ring is deprotonated, and the histidinate dianion is bound to the cobalt by the α amino nitrogen and by the negatively charged pyrrolic nitrogen of the imidazole ring. The complex has been studied in solution, under nitrogen atmosphere, in presence of an excess of histidinate ion. The spectra have been obtained on a Coderg double monochromator PHO, using exciting lines from Ar^+ and Kr^+ lasers. Intensities have been evaluated from peak heights, and ratioed to the 930 cm^{-1} band of the perchlorate ion, present in the solution. Corrections for reabsorption and spectral response of the instrument have been made.

Results and Discussion

As expected, the Raman spectrum (Fig. 1) is very similar to that of the free histidinate ion, which is present in excess, though some extra bands of low intensity and changes in the relative intensities of complex bands are noticeable. Contrary to Yoshida's report [2], bands are observed below 1000 cm^{-1}.

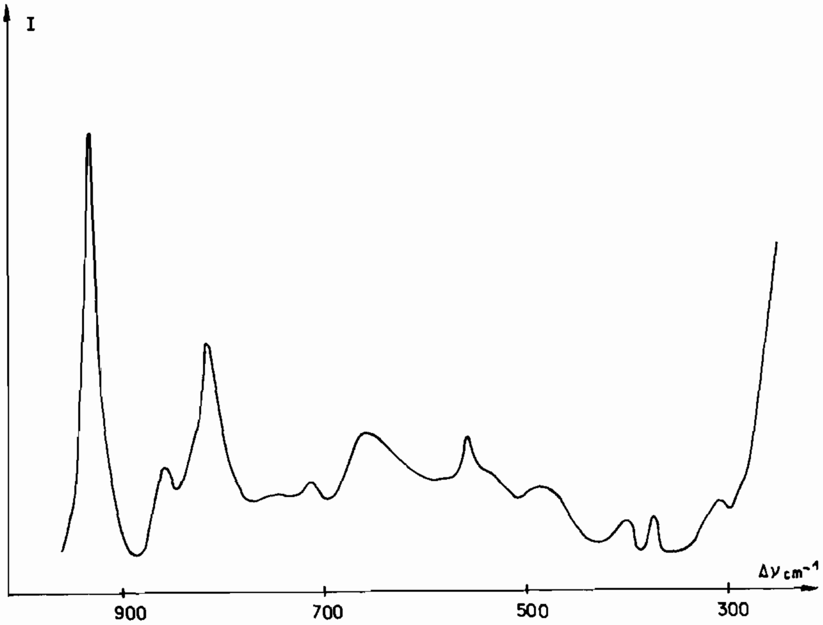

Fig. 1. Raman spectrum of a $[Co(his)_2]^{2-}$ solution. ν_0 = 476.2 nm; power 100 mW; spectra slitwidth 10 cm^{-1}; conc. 0.04 *M*; pH 14; excess $(his)^{2-}$ 5 times.

---

*To whom correspondence should be addressed.

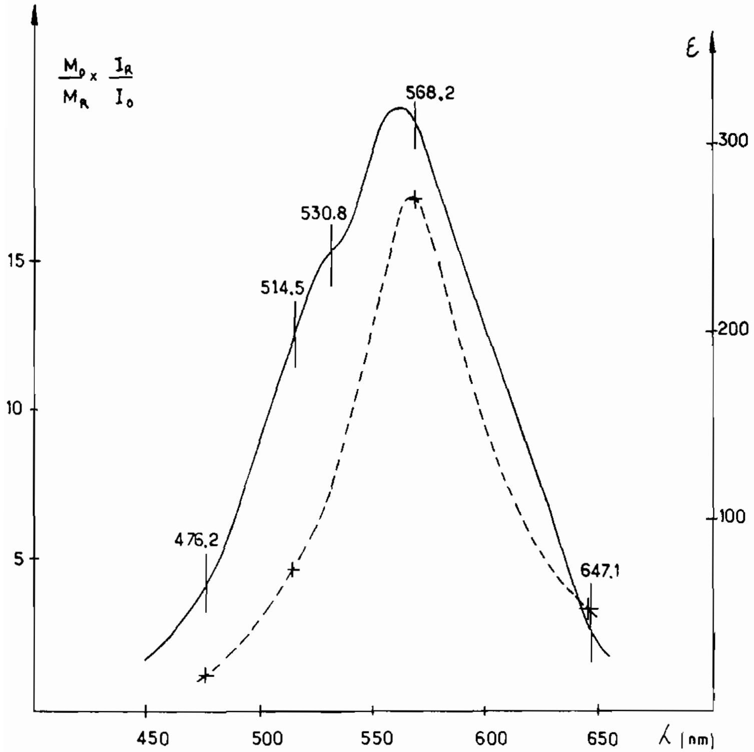

Fig. 2. Visible absorption (———) of $[Co(his)_2]^{2-}$ and excitation profile (– – – –) of the 305 cm^{-1} vibration. M_0 and M_R are the molarities of the complex and of ClO_4^- respectively, I_0 and I_R the scattered intensities of the 305 cm^{-1} mode of the complex and of the 930 cm^{-1} mode of ClO_4^-.

The frequencies of the main bands are: 305 (w); 365 (w); 396 (w); 540 (w); 660 (w, broad); 812 (S); 863 (m); 980 (m), 1002 (m); 1031 (w); 1097 (m); 1154 (m); 1197 (w); 1228 (m); 1255 (S); 1276 (S); 1317 (m); 1347 (m); 1404 (sh); 1434 (m); 1479 (w); 1520 (w); 1552 (S); 1575 (sh); 1630 (m).

The 305 cm^{-1} band is a unique feature of the spectrum of the complex. Its frequency compares well with other Co–N stretching frequencies (for example νCo–N = 306 cm^{-1} for $[Co(NCS)_4]^{2-}$. The depolarization ratio ρ measured in the green is 0.5 ± 0.1. For a regular tetrahedron one expects two Raman active stretching vibrations: a A_1 mode (ν_1) and a T_2 mode (ν_3) with ρ equal to 0 and 3/4 respectively, either in Raman Effect (RE) or in RRE. The measured value is therefore indicative of a decrease in symmetry. If one calculates, from McClain's tables [5]. the ρ values for vibrations deriving from the A_1 and T_2 modes of the tetrahedron, it is only starting from subgroup S_4 that one gets $\rho \neq 0$ for the A mode, deriving from A_1; the B mode ($\leftarrow T_2$) being characterized by $\rho = 3/4$ (RE and RRE) and the E mode ($\leftarrow T_2$) being characterized by $\rho = 3/4$ (RE) and $\rho > 3/4$ (RRE). Therefore we assign the observed vibration to the A Co–N stretching mode of the complex, of actual S_4 symmetry.

The intensity of the 305 cm^{-1} vibration has been measured, relatively to the intensity of the 930 cm^{-1} vibration of the ClO_4^- ion, for different exciting frequencies. The corrected values are listed in Table I, and the derived excitation profile is plotted in Fig. 2, with the visible absorption cuvre. These intensity changes can be rationalized within the framework of Albrecht's Vibronic Theory of Raman Intensity [6]. According to Albrecht the scattered intensity may be either of type A, *i.e.* originating from vibrational interactions with a single electronic excited state, or of type B, *i.e.* originating from vibronic coupling of two excited states.

TABLE I. Measured Intensities of the 305 cm^{-1} Vibration and Calculated F_A^2 and F_B^2 Terms, *versus* the Exciting Frequency ν_0.

ν_0 (nm)	Measured Intensities	F_A^2/ν_S^4	F_B^2/ν_S^4 ν_f = 360 nm	F_B^2/ν_S^4 ν_f = 200 nm
476.2	1	1	1	1
514.5	4.6	236	27.7	3.6
530.8	7.5	1733	66	10.3
568.2	17	117700	424	80
647.1	3	119	0.5	1.3

The A and B processes show different frequency dependence, the scattered intensities being proportional respectively to F_A^2 and F_B^2, with

$$F_A = \nu_S^2 \frac{\nu_e^2 + \nu_0^2}{(\nu_e^2 - \nu_0^2)^2}$$

$$F_B = 2\nu_S^2 \frac{\nu_e\nu_f + \nu_0^2}{(\nu_e^2 - \nu_0^2)(\nu_f^2 - \nu_0^2)}$$

where ν_0 is the exciting frequency, ν_S is the frequency of the scattered light, and ν_e and ν_f are the frequencies of the excited electronic states. The A term contributes to the intensity of the A_1 mode only, whereas the B term contributes to the intensity of any vibration whose symmetry is contained in the direct product of the representations of the e and f excited states.

The visible spectrum of $[Co(his)_2]^{2-}$ is fairly typical of tetrahedral Co(II) complexes; the two bands at 1125 and 558 nm being assigned respectively to the $^4T_1(F) \leftarrow {}^4A_2$ and $^4T_1(P) \leftarrow {}^4A_2$ transitions [4]. In the UV region, there is an intense band whose maximum is *ca.* 200 nm, and a shoulder at 370 nm (ϵ *ca.* 70). Keeping the tetrahedral geometry, we assign the intense band to a charge transfer transition to a $^4T_1$ excited state, and the 370 nm shoulder to a d–d transition to a doublet state, maybe $^2T_1$ by analogy with $CoCl_4$ [7]. But if one considers that the actual symmetry is at most S_4, the T_1 states are split in A + E. Considerations of direct products of the representations of these sublevels show that the vibrations active in vibronic coupling are of A, B and E symmetry.

As the A mode can be enhanced via a A or B process, we have calculated the frequency dependence of the scattered intensity and compared it to the measured intensities (corrected for the ν_S^4 factor and normalized to 1 for ν_0 = 476.2 nm). The results are given in Table I: the frequencies used in the calculation are ν_e = 558 nm, ν_f = 360 nm (column 4) and ν_f = 200 nm (column 5). The F_B^2 term calculated from ν_f = 200 nm gives the best fit with the experimental values. There is a discrepancy for strict resonance, which is not surprising, as a damping term has been neglected in the F_A^2 and F_B^2 denominators, and this term becomes predominant under resonance.

Conclusion

We have observed a preferential intensity enhancement of a metal–ligand vibration, using an exciting line whose frequency is close to the maximum of the d–d absorption band. Depolarization ratio and intensity measurements allow us to assign this vibration to the A stretching mode of the $Co(N)_4$ group, of actual S_4 symmetry, and to precise that it is enhanced *via* vibronic coupling of d–d and C.T. excited states.

We hope to extend this result to a transition metal–protein complex, chosen as to present partially allowed d–d transitions in the visible. Owing to the ϵ values implied by such a choice, the concentration range for the complex will be such that protein vibrations will probably be present in the spectrum. Nevertheless, consideration of the excitation profile should allow a distinction between metal–ligand and protein low frequency modes.

References

1 T. G. Spiro, *Acc. Chem. Res., 7,* 339 (1974).
2 C. M. Yoshida, T. B. Freedman and T. M. Loehr, *J. Am. Chem. Soc., 97,* 1028 (1975).
3 G. Chottard and J. Bolard, *Chem. Phys. Lett., 33,* 309 (1975).
4 P. J. Morris and R. B. Martin, *J. Am. Chem. Soc., 92,* 1543 (1970).
5 W. M. McClain, *J. Chem. Phys., 55,* 2789 (1971).
6 J. Tang and C. A. Albrecht, "Raman Spectroscopy", Vol. 2, Chap. 2, H. A. Szymansky ed., Plenum Press, N. Y. (1970).
7 J. Ferguson, *J. Chem. Phys., 39,* 116 (1963).