Commentary

Resonance Raman study on pentacoordinated and hexacoordinated ferrous nitrosyl myoglobin

The influence of pH

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Received 25 May 1983

The resonance Raman (RR) spectra of the hexacoordinated ferrous nitrosyl sperm whale myoglobin are independent of pH for both the 200–700 cm and 1350–1650 cm^{-1} regions. In agreement with earlier ESR [Eur. J. Biochem. (1972) 31, 578–584] and contrary to recent RR [Biochemistry (1982) 21, 6989–6995] results the RR spectra do not indicate a transition to the pentacoordinated ferrous nitrosyl derivative at low pH. However, interaction of the protein with sodium dodecylsulfate, leads to the formation of a pure pentacoordinated state with a typical RR spectrum. Replacement of ^{14}NO for ^{15}NO in this pentacoordinated ferrous nitrosyl derivative does not exhibit isotope effects using 413.1 excitation.

Sperm whale myoglobin *Resonance Raman spectra* *Penta- and hexa-coordinated Nitrosyl derivative*

1. INTRODUCTION

Resonance Raman spectra of nitrosyl myoglobin (NO–Mb) for two pH values using 413.1 nm excitation were reported in [1]. They concluded that a pH-induced spectral change occurs upon lowering the pH from 8.4 to 5.8 and that this change is due to hexacoordinated ferrous nitrosyl myoglobin being converted to pentacoordinated ferrous nitrosyl myoglobin. This spectral change is said to be due to the protonation of the proximal imidazole at low pH which causes breakage of the iron–imidazole bond.

However, this assumption of a broken iron–base bond at low pH is contradictory to earlier ESR results [2] which clearly demonstrated a hyperfine pattern characteristic for the hexacoordinated stage of ferrous NO-Mb, also at low pH, thus excluding any pH-induced change of the coordination state under these conditions. We, therefore, have repeated the resonance Raman study of ferrous myoglobin at different pH values. However, we have not found any pH dependence in the spectra. Comparing these spectra with ESR spectra obtained under corresponding conditions we can assign the resonance Raman spectra to hexacoordinated ferrous NO-Mb.

Again, ESR experiments have clearly demonstrated that a monomeric hexacoordinated ferrous nitrosyl haemoglobin could be reversibly converted to the pentacoordinated ferrous state by binding of detergent anions to the protein at alkaline pH [3,4]. We also present the resonance Raman spectra of the pure pentacoordinated ferrous NO-Mb, formed upon sodium dodecylsulfate–protein interaction at alkaline pH.

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Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

2. MATERIALS AND METHODS

The nitrosyl myoglobin samples were prepared from completely deaerated met-myoglobin solutions (sperm whale myoglobin; Serva, Heidelberg). Buffers used were 0.2 M Tris–HCl (pH 9.2) and 0.05 M citrate–phosphate (pH 5.8). After repeated evacuation and flushing of the met-myoglobin solution with prepurified nitrogen, nitric oxide gas was introduced. The nitric oxide gas was cleaned by washing with sodium hydroxide solution. After about 7 h, the hexacoordinated ferric nitrosyl myoglobin (an ESR-silent species), which is the first species formed, undergoes complete autoreduction to hexacoordinated ferrous nitrosyl myoglobin [5,6].

The pentacoordinated ferrous NO-Mb was formed by addition of a 30 M excess of sodium dodecylsulfate to the hexacoordinated NO-Mb at pH 9.2. Alkaline pH is necessary to prevent micelle formation of the detergent.

Alternatively, the nitrosyl derivatives of Mb have been prepared as in [3,4] by releasing NO in the deaerated met-Mb solution by reduction of sodium nitrite with ascorbic acid.

The resonance Raman spectra were obtained from a sensitive multichannel Raman system [7], using the 413.1 nm line from a Spectra Physics Model 171-01 Krypton-ion laser, with a 90° scattering geometry. All spectra were obtained at room temperature.

3. RESULTS AND DISCUSSION

The resonance Raman spectra of ferrous nitrosyl myoglobin at pH 5.8 and 9.2 are presented in fig. 1. It is evident that there are no differences in the spectra due to pH change. We have found this to be true regardless of the method of preparation. For example, we have prepared NO-Mb at pH 5.2 with $NaNO_2$ which is reduced by sodium ascorbate and also at pH 7.2 starting from CO-Mb and then adding nitric oxide gas for replacement of CO at the already reduced haem iron.

The resonance Raman study of the conversion of ferric NO-Mb to ferrous NO-Mb has been done in [6]. A spectrum that is due to a mixture of ferric and ferrous NO-Mb, taken about 40 min after introduction of nitrix oxide gas to the met Mb solution (fig. 2). It is nearly identical the pH 5.8 spectrum (in fig. 2 of [1], supposedly the pentacoordinated ferrous NO-Mb. We suggest that this pH 5.8 spectrum is due to a mixture of ferric NO-Mb and ferrous NO-Mb. Although these authors started with CO-Mb, it is possible that their sample was not fully reduced. At low pH and reduced temperature, the ferric form of NO-Mb may be somewhat stabilized.

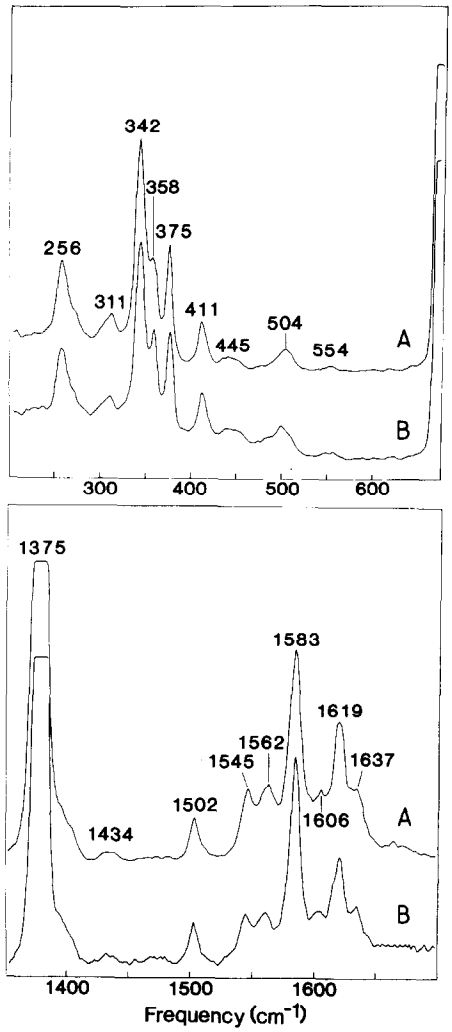

Fig. 1. Resonance Raman spectra of ferrous nitrosyl myoglobin at pH 5.8 (A) and pH 9.2 (B). Experimental conditions: Excitation wavelength, 413.1 nm; laser power, 20 mW; slit width, 100 μm; slit height, 0.2 cm; delay, 10000 (303 s); buffers, 0.05 M citrate–phosphate and 0.2 M Tris–HCl; haem, 80 μM.

According to [8–10], the stoichiometric interac-

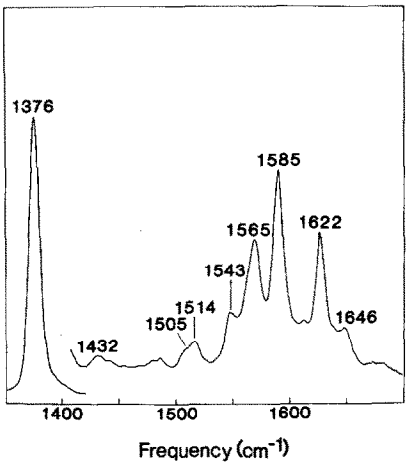

Fig. 2. Resonance Raman spectrum of a mixture of ferric and ferrous nitrosyl myoglobin at pH 9.2; experimental conditions as in fig. 1; spectrum taken 40 min after exposure of met-myoglobin solution to nitric oxide gas.

tion of a detergent such as sodium dodecylsulfate ($NaDodSO_4$) with the protein leads to a conversion of the haemoglobin structure resulting in a stretching of the iron–base bond. For a monomeric insect nitrosyl haemoglobin a reversible transition from the hexacoordinated to pentacoordinated complex has been described by ESR [3,4,11]. Resonance Raman spectra of NO-Mb plus $NaDodSO_4$ are shown in fig. 3. These spectra are nearly identical to those obtained from the pentacoordinated protoporphyrin IX iron (II)·NO complex in 3% $NaDodSO_4$ [12]. Both spectra show the disappearance of the 1620 cm^{-1} line and the appearance of the 1646 cm^{-1} line. This is consistent with the observation that pentacoordinated nitrosyl haems give rise to a 1645 cm^{-1} line as first observed in [13]. Coincidentally this line also appears in ferric NO-Mb (see fig. 2). Also, the 1502 cm^{-1} line shifts to 1512 cm^{-1} in both the pentacoordinated and the ferric NO-Mb. However, since the exact nature of the porphyrin ring modes is not known and it is difficult to predict what effect oxidation or pentacoordination will have on the porphyrin, it is not hard to believe that these two lines (~1646 cm^{-1} and ~1512 cm^{-1}) may arise in both cases.

Also, it should be noted that the pentacoordinated NO-Mb if ^{15}NO is replaced by ^{14}NO does not give rise to any isotope sensitive lines in the lower frequency region that could be assigned to the Fe(II)–NO stretching or Fe(II)–NO bending vibration. This is consistent with the observations that the axial NO ligand vibrations of pentacoordinated haems are not resonance enhanced with this laser excitation, as observed in NO–HbA plus IHP and protoporphyrin iron(II)–NO [12].

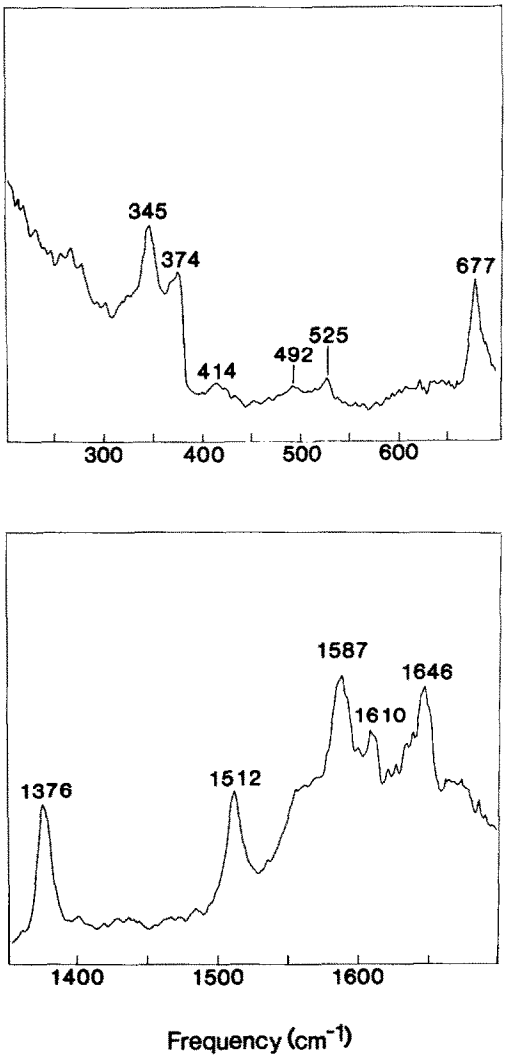

Fig. 3. Resonance Raman spectra of ferrous nitrosyl myoglobin at pH 9.2 in the presence of 30 molar excess of sodium dodecylsulfate, experimental conditions as in fig. 1.

ACKNOWLEDGEMENTS

This research was supported by Grants from the National Institutes of Health to N.-T.Y., the Deutsche Forschungsgemeinschaft to K.G. and the Medizinische Biophysik e.V. to K.G.

REFERENCES

[1] Walters, M.A. and Spiro, T.G. (1982) Biochemistry 21, 6989–6995.
[2] Trittelvitz, E., Sick, H. and Gersonde, K. (1972) Eur. J. Biochem. 31, 578–584.
[3] Overkamp, M., Twilfer, H. and Gersonde, K. (1976) Z. Naturforsch. 31c, 524–533.
[4] Christahl, M. and Gersonde, K. (1982) Biophys. Struct. Mech. 8, 271–288.
[5] Dickinson, L.C. and Chien, J.C.W. (1971) J. Am. Chem. Soc. 93, 5036–5040.
[6] Benko, B. and Yu, N.-T. (1982) unpublished.
[7] Yu, N.-T. and Srivastava, R.B. (1980) J. Raman Spectrosc. 9, 166–168.
[8] Gersonde, K. and Netter, H. (1964) J. Mol. Biol. 10, 475–487.
[9] Gersonde, K., Seidel, A. and Netter, H. (1965) J. Mol. Biol. 14, 37–47.
[10] Gersonde, K. (1969) J. Mol. Biol. 42, 358–300.
[11] Christahl, M., Twilfer, H. and Gersonde, K. (1982) Biophys. Struct. Mech. 9, 61–72.
[12] Tsubaki, M. and Yu, N.-T. (1982) Biochemistry 21, 1140–1144.
[13] Szabo, A. and Barron, L.D. (1975) J. Amer. Chem. Soc. 97, 660–662.