Resonance Raman evidence of chloride binding to the heme iron in myeloperoxidase

Masao Ikeda-Saito+, Pramod V. Argade and Denis L. Rousseau

+*Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104*, and *AT&T Bell Laboratories, Murray Hill, NJ 07974, USA*

Received 4 February 1985

The resonance Raman spectra of ferric derivatives of myeloperoxidase at pH 8 show ligand-dependent differences. The data are consistent with the resting enzyme and the chloride and fluoride derivatives all having 6-coordinated high-spin configurations. At pH 4 we find that the resting enzyme is susceptible to photodegradation from our low power incident laser beam. Chloride binding inhibits this denaturation. Our data support direct binding of chloride to the enzyme under physiological conditions.

Myeloperoxidase *Raman spectrum*

1. INTRODUCTION

Myeloperoxidase (EC 1.1.11.7), a green heme enzyme [1], is one of the essential components of the antimicrobial systems of the polymorphonuclear leukocytes [2]. The unique property of myeloperoxidase is its ability to catalyze the formation of hypochlorous acid from hydrogen peroxide and chloride ion [3]. The enzyme is a tetramer, which consists of two heavy (M_r about 55000) and light (M_r about 15000) subunits [4]. Each heavy subunit contains one iron-containing prosthetic group, the structure of which is yet to be determined. The net reaction catalyzed by the enzyme seems simple but understanding of the mechanism of enzyme action of myeloperoxidase on a molecular basis has been hindered by the lack of knowledge of the structure of the active center of the enzyme and by uncertainty over the chloride binding site. Determination of the chloride binding site is of prime importance to understand the functional mechanism of myeloperoxidase. Some authors have proposed that the chloride binds to the iron atom and others that it binds at the periphery of the heme [5–7].

In recent resonance Raman scattering studies from three independent research groups it has been proposed that the chromophore of the enzyme is a chlorin since the many lines in its high frequency region are not observed for porphyrins and are consistent with the Raman spectra of chlorins [8–10]. The absence of lines in reduced forms of the enzyme with frequencies higher than 1610 cm^{-1} indicates either that there is no formyl or vinyl group present or if present such groups are not resonance enhanced by coupling with the chlorin macrocycle. Here, we report the resonance Raman spectra of the ferric enzyme and the effect of chloride binding to the enzyme in its ferric state. We find substantial changes in the resonance Raman spectrum upon chloride binding. We interpret these changes as evidence that chloride binds to the central iron atom of the chlorin. However, the iron chlorin retains its high-spin configuration upon chloride binding. On lowering pH to 4.0, the ferric enzyme is partially converted by the laser beam to a low-spin configuration. Chloride addition blocks this conversion so that the spectra of the chloride-bound chlorin are the same at pH 4.0 and 8.0.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

2. MATERIALS AND METHODS

Myeloperoxidase was isolated from out-dated leukophoresis preparations, which were supplied from the Penn-Jersey Blood Program of the American Red Cross, Philadelphia, PA. Remaining erythrocytes in the leukophoretic preparations were removed by the method of Rothstein et al. [11]. Homogeneous preparation of myeloperoxidase was obtained by the method of Yamada et al. [12] with modification, details of which will be published elsewhere. All solutions used for preparation of the enzyme contained 1 mM phenylmethanesulfonyl fluoride and 1 μM pepstatin A to prevent proteolytic degradation [13]. A SDS gel electrophoresis was carried out by the method of Weber and Osborn [14]. The electrophoretic pattern of the purified enzyme was similar to the one reported by Anderson et al. [12]. The ratio of absorbance at 430 and 280 nm (A_{430}/A_{280}) of the enzyme used in this study was 0.85. Resonance Raman spectra were recorded by the Raman difference instrument described by Rousseau [15] at room temperature. 30–90 min was required to record a spectrum with a reasonably good signal to noise ratio. The enzyme concentration was about 0.2 mM. The buffers used were 0.1 M citrate-phosphate (pH 4.0), 0.1 M phosphate buffer (pH 8.0). The excitation frequency used was 413.1 nm.

3. RESULTS AND DISCUSSION

The resonance Raman spectra of the ferric myeloperoxidase at pH 8 and its complexes with fluoride, chloride, and cyanide, as well as the difference Raman spectrum between the chloride complex and the resting enzyme are presented in fig.1. In a comparison between these spectra and those of typical porphyrin-containing heme proteins, there are more lines in the high frequency region of the spectrum. The presence of these lines has been interpreted as evidence that the chromophore is a chlorin with reduced molecular symmetry in comparison to heme proteins in which the chromophore is a porphyrin [8–10]. In spite of the lowered symmetry many Raman lines appear to have the same structural dependencies in chlorins as in porphyrins. For example ν_4, the electron density marker line in the 1350–1380 cm^{-1} region [16],

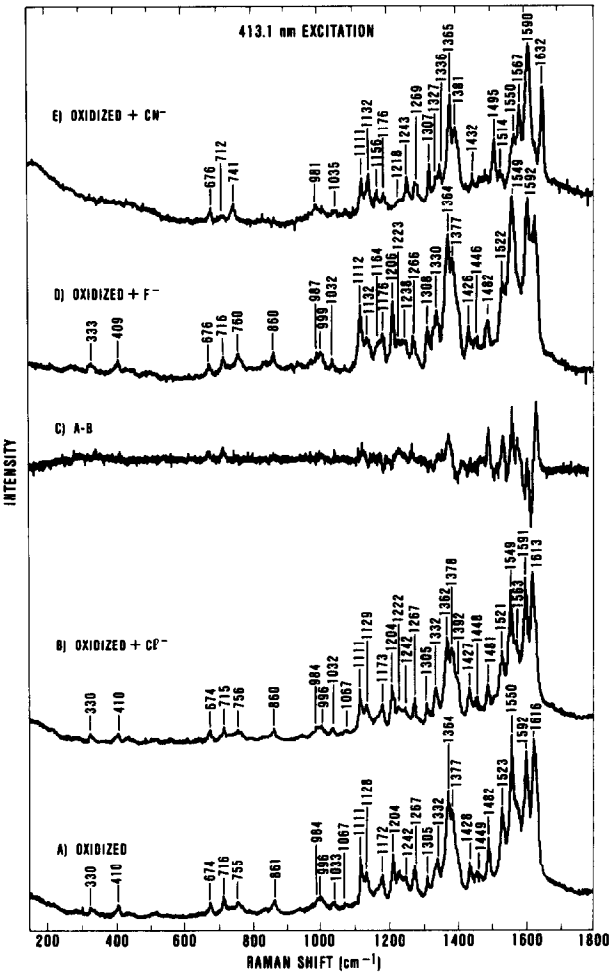

Fig.1. Resonance Raman spectra of oxidized derivatives of myeloperoxidase with 413.4 nm excitation. A, resting enzyme (0.1 mM per iron). B, addition of chloride (0.2 M) to the resting enzyme. C, difference spectrum between the oxidized and oxidized plus chloride preparations. D, addition of fluoride (0.2 M) to a resting preparation. E, addition of cyanide (4 mM) to the resting enzyme. All the samples were buffered in 0.1 M phosphate and were at approx. pH 8.

shift by several wavenumbers to higher frequency upon going from the reduced to the oxidized states of the enzyme, just as it does in model compounds and heme proteins in which the chromophore is a porphyrin moiety [17].

The highest frequency line, ν_{10} (following the assignments of Abe et al. [16]) in the porphyrin spectrum has been proposed to have its analogue in chlorins also [18,19] (Ozaki, Y., personal com-

munication). In porphyrin this mode has been demonstrated to be sensitive to the coordination of the central iron atom and its spin state [20] as may be seen in table 1 where the characteristic frequencies for the various spin and coordination states are listed. As is also evident in the table, the model chlorin compounds studied by Ozaki and coworkers [18,19] have the same structural dependencies as the porphyrins. Of particular note is the variation in this frequency for the most common spin and coordination states. For example, the frequency for the 6-coordinated high-spin state is low (1614–1618 cm^{-1}) in the chlorins and the frequency for the 6-coordinated low-spin state is high (1641 cm^{-1}).

From the data shown in fig.1 for myeloperoxidase at pH 8.0, the resting enzyme, the fluoride derivative, and the chloride derivative all have frequencies for ν_{10} in the 1613–1616 cm^{-1} range. This frequency is consistent with a 6-coordinated high-spin configuration for each of these preparations. In the resting enzyme at pH 8, coordination of a water molecule at the sixth position is likely resulting in the 6-coordinated high-spin form of the enzyme. The addition of chloride or fluoride also yields the characteristic 6-coordinated high-spin spectrum. The changes in the chlorin spectrum which occur on binding these ligands are accounted for as being induced by slight modification of the iron d-orbitals due to coordination of fluoride or chloride to the iron atom replacing the water molecule. The high-spin configuration of the resting enzyme as well as the fluoride and chloride complexes are also indicated by spectroscopic and magnetic measurements on the enzyme [21,22].

Table 1

Raman frequencies (cm^{-1}) of ν_{10} for different coordination states (5c, 5-coordinate; 6c, 6-coordinate) and spin states (hs, is, ls: high, intermediate, and low spin, respectively) of ferric porphyrin and chlorins compared to myeloperoxidase (pH 8.0)

	Porphyrins	Chlorins	Myeloperoxidase
5c, hs	1629–31	1627–32	
5c, is	1645		
6c, hs	1615–25	1614–18	1613–1616 (resting, F^-, Cl^-)
6c, is	1634–36		
6c, ls	1639–41	1641	1632 (CN^-)

The porphyrin data are taken from [20] and the chlorin data from [18,19]. The myeloperoxidase data are from the present studies

The cyanide-bound complex is known to have low-spin configuration [21,22]. We find that ν_{10} for the complex has a frequency of 1632 cm^{-1}, as seen by Sibbett and Hurst [9]. This is somewhat low for a low-spin complex (see table 1). Evidently, the protein interactions with the chlorin prevent full structural relaxation as was attained in the bisimidazole low-spin 6-coordinate model compound which has a frequency of 1641 cm^{-1} for ν_{10}. Possibly, protein restraint does not allow the iron atom to go all the way into plane yielding the lower frequency.

We have also obtained spectra of ferric myeloperoxidase and its chloride complex at pH 4.0. At this pH the chloride-bound form has the same spectrum as it has at pH 8.0. However, we find that the spectrum of the resting enzyme at pH 4.0 is quite different and varied from experiment to experiment. With time, this spectrum changed from one that had characteristic high-spin frequencies to one that has low-spin frequencies similar to those we found in the cyanide-bound complex. We conclude that our incident laser beam (power < 50 mW) denatures the resting enzyme at pH 4 such that the iron is partially converted from high spin to low spin during the measurements of the spectrum. Photo-degradation of the ferric enzyme by incident laser beam was also observed by Shelnutt et al. (personal communication) both in acidic and neutral pH regions; however, we could not detect photo-degradation of the resting enzyme at pH 8 during the measurements of the spectrum. This discrepancy may be due to other differences in the experimental conditions. Since the spectrum for the chloride complex of the enzyme at pH 4.0 is the same as that at pH 8.0, chloride binding to the enzyme apparently inhibits this conversion to the low-spin state. We propose that the 6-coordinated low-spin configuration in the resting enzyme at pH 4 results from a direct coordination of a distal residue to the sixth coordination position of the iron, i.e., the formation of a hemeichrome. Prevention of this conversion in the chloride complex is a further indication that the chloride ion occupies the sixth coordination position of the iron.

In the data reported here significant changes are detected in the skeletal modes of the chlorin

chromophore upon chloride binding. For example, ν_4 (1364 cm^{-1}) shifts by 2 cm^{-1} and ν_{10} (1616 cm^{-1}) shifts by 3 cm^{-1}. Such changes are most consistent with the chloride binding directly to the heme iron atom. If, instead, the chloride ion interacted with the periphery of the chlorin, large changes would be expected in the low frequency modes, such as those seen in the comparison between deoxy and photodissociated hemoglobins where differences in the peripheral environments occur [23]. Direct binding of the chloride to the iron atom is supported by low pH (pH 4) data in which chloride blocks hemichrome formation. Observations of significant changes in optical properties [5] upon chloride binding and our conclusion that chloride binds to the iron atom are contrary to some previous studies which have indicated essentially no effect of chloride on the EPR spectral properties of myeloperoxidase at neutral pH [6]. The present Raman data, however, are in good agreement with our new optical and EPR results [8] in which it was found that binding of chloride to the enzyme at neutral pH yields EPR and optical changes consistent with the formation of another high-spin state. The intraphagosomal pH is between 4.5 and 5 [24] and chloride concentration is 0.1 M [25]. Therefore, myeloperoxidase is complexed with chloride in phagosomes, and the chloride complex formation appears to enhance the stability of the enzyme at low pH. Although the present study strongly suggests the direct coordination of chloride to the iron atom in enzyme-chloride complex, more study is required to determine how this chloride complex of myeloperoxidase plays an essential role in its catalytic activity.

ACKNOWLEDGEMENTS

We thank the Penn-Jersey Regional Red Cross Blood Services, Philadelphia for their supply of the out-dated granulocyte concentrates, Dr T. Yonetani for his support, and Dr D. Safer for electrophoresis. M.I.-S. wishes to thank Dr K.-G. Paul of the University of Umea, Umea, Sweden for his kind support at the initial stage of this investigation. We thank Dr Y. Ozaki for sending us his unpublished results. The portion of this work carried out at the University of Pennsylvania was supported by research grant AI-20463 from the National Institute for Allergy and Infectious Diseases.

REFERENCES

[1] Agner, K. (1941) Acta Physiol. Scand. 2, suppl.8, 1–62.
[2] Klebanoff, S.J. and Clark, R.A. (1978) The Neutrophil: Function and Clinical Disorders, North-Holland Publishing Co., Amsterdam.
[3] Harrison, J.E. and Schultz, J. (1976) J. Biol. Chem. 251, 1371–1374.
[4] Andrews, P.C. and Krinsky, N.I. (1981) J. Biol. Chem. 256, 4211–4218.
[5] Stelmaszynska, T. and Zglinczynski, J.M. (1974) Eur. J. Biochem. 45, 305–312.
[6] Wever, R. and Bakkenist, A.R.J. (1980) Biochim. Biophys. Acta 612, 178–184.
[7] Andrews, P.C. and Krinsky, N.I. (1982) J. Biol. Chem. 257, 13240–13245.
[8] Ikeda-Saito, M., Prince, R.C., Argade, P.V. and Rousseau, D.L. (1984) Fed. Proc. 43, 1561.
[9] Sibbett, S.S. and Hurst, J.K. (1984) Biochemistry 23, 3007–3013.
[10] Babcock, G.T., Ingle, R.T., Oertling, W.A., Davis, J.S., Averil, B.A., Hulse, C.L., Stufkens, D.J., Bolscher, B.G.J.M. and Wever, R. (1985) Biochim. Biophys. Acta, in press.
[11] Rothstein, G., Bishop, C.R., Athens, J.W. and Ashenbrucker, H.E. (1971) Blood 38, 302–311.
[12] Yamada, M., Mori, M. and Sugimura, T. (1981) Biochemistry 20, 766–771.
[13] Anderson, M.R., Atkin, C.L. and Eyre, H.J. (1982) Arch. Biochem. Biophys. 214, 273–283.
[14] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[15] Rousseau, D.L. (1981) J. Raman Spectrosc. 10, 94–99.
[16] Abe, M., Kitagawa, T. and Kyogoku, Y. (1978) J. Chem. Phys. 69, 4526–4534.
[17] Spiro, T.G. and Burke, M.J. (1976) J. Am. Chem. Soc. 98, 5482–5489.
[18] Ozaki, Y. (1978) PhD. dissertation, Osaka University, Osaka, Japan.
[19] Ozaki, Y., Kitagawa, T. and Ogoshi, H. (1979) Inorg. Chem. 18, 1772–1776.
[20] Teraoka, J. and Kitagawa, T. (1980) J. Phys. Chem. 84, 1928–1935.
[21] Ehreberg, A. (1962) Arki. Kemi 19, 119–128.
[22] Wever, R. and Platt, H. (1981) Biochim. Biophys. Acta 661, 215–239.
[23] Ondrias, M.R., Rousseau, D.L. and Simon, S.R. (1983) J. Biol. Chem. 258, 5638–5642.
[24] Jense, M.S. and Bainton, D.F. (1973) J. Cell. Biol. 56, 379–388.
[25] Page, L.B. (1961) in: Syllabus of Laboratory Examinations in Clinical Diagonosis (Page, L.B. and Culver, P.J. eds) p.5, Harvard Univ. Press, Cambridge, MA.