Volume 228, number 2, 310-316 FEB 05576 February 1988

A resonance Raman study of the binding of ethanol and methanol to ferrihemoglobin

B.B. Muhoberac, J.A. Shelnutt° and M.R. Ondrias†

Department of Chemistry, Purdue University School of Science, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46223, °Process Research Division, Sandia National Laboratories, Albuquerque, NM 87185 and *†Department of Chemistry, University of New Mexico, Albuquerque, NM 87131, USA*

Received 10 December 1987

The interactions of ethanol and methanol with ferrihemoglobin were examined using resonance Raman spectroscopy. After binding either alcohol, the low-frequency resonance Raman spectra of human ferrihemoglobin are almost identical to the unperturbed spectrum except for shifts in the 309 cm^{-1} band to higher frequency by as much as 8 cm^{-1}. The ethanol-induced shift is greater than that with methanol even though complex formation was less for ethanol than methanol. The spectral changes imply a site-specific, similar binding of these alcohols to ferrihemoglobin which may involve steric interactions. Possible assignments of the 309 cm^{-1} band to structural features as well as potential mechanisms of the alcohol-induced spectral changes are discussed.

Ferrihemoglobin; Ethanol; Methanol; Resonance Raman spectroscopy; Ligand binding; (Methemoglobin)

1. INTRODUCTION

The structure and function of hemoglobin can be altered by alcohols, however their mechanism(s) of interaction is(are) not altogether clear. Alcohols exert divergent effects on hemoglobin structure including enhanced stability at lower but denaturation at higher concentrations [1]. Alcohols decrease O_2 affinity [2,3], decrease the extent of polymerization of hemoglobin S [4], and alter drug-mediated iron oxidation rates [5]. Furthermore, these divergent effects depend systematically upon alkyl chain length and branching [1,4–6]. Both changes in solvent dielectric constant and generalized hydrophobic destabilization have been used to explain the above-mentioned effects [3,4,7]. However, it has been proposed [8] that ethanol and methanol bind to ferrihemoglobin in a site-specific manner analogous to that observed with small ligands such as H_2O, N_3^- and OCN^-. Moreover, X-ray diffraction shows that the more bulky dichloromethane also binds to hemoglobin in a site-specific manner [9] and similar binding is suggested by more indirect methods for butane, pentane and neopentane by Wishnia [10].

The titration of ferrihemoglobin with ethanol and methanol induces modest changes in both the optical absorption and frozen solution EPR spectra [11] which are different from those induced either by addition of strong field ligands or by perturbants that cause the distal histidine to replace water at the iron [12,13]. Using the recent band assignments of Makinen and Churg [14] along with previously quantitated alcohol-induced spectral shifts [8], it is now clear that ethanol binding increases the energy of both the porphyrin-to-metal charge transfer transitions ($a_{2u} \longrightarrow d_{xz}, d_{yz}$) near 15.9 kK and the vibronically coupled band (Q_v) near 20.0 kK similarly. These changes suggest perturbation of not only energy levels associated with axial ligation, but also those involving the porphyrin macrocycle. Brill et al. [15] showed that these two alcohols alter the high-spin EPR spec-

Correspondence address: B.B. Muhoberac, Department of Chemistry, Purdue University School of Science, Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46223, USA

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

trum of ferrihemoglobin increasing both the heme rhombicity (in plane directional inequivalence) and the spread (distribution) of conformations producing this rhombicity. The results of both spectral techniques are consistent with conservative ligand replacement at the iron (such as alcohol for water) or a perturbation that enhances the interaction between the heme and some element of lower symmetry in the heme pocket.

A combined spectral and thermodynamic analysis of the alcohol-induced optical absorption changes in ferrihemoglobin and comparison with those induced in ferricytochrome *c* lead Muhoberac and Brill [8] to propose that ethanol and methanol bind directly to the iron of ferrihemoglobin, whereas, in contrast, 1-propanol binds to ferricytochrome *c* at a hydrophobic crevice. Unfortunately, 1-propanol binding to ferricytochrome *c* was the only example found of a contrasting alcohol-ferrihemeprotein interaction. The 1-propanol-ferrihemoglobin interaction could not be studied because this alcohol denatures ferrihemoglobin even at low concentrations. Furthermore, the spin state of the ferrihemoglobin complexes differed from that of the ferricytochrome *c* complexes throughout the above-mentioned study. These complications suggest that a more direct probe of the interaction of ethanol and methanol with ferrihemoglobin that has the potential to differentiate between alcohol interaction with the iron, its axial ligands and the porphyrin macrocycle would be useful.

This letter reports the effects of ethanol and methanol on the resonance Raman spectra of human and horse ferrihemoglobin, and documents a prominent ethanol-induced change in the 309 cm^{-1} resonance Raman band. Possible assignments of this band to structural features as well as the potential mechanisms of the alcohol-induced changes are discussed. Direct binding of ethanol and methanol to the ferric iron is considered along with the orientation of the alcohols within the heme pocket.

2. MATERIALS AND METHODS

Human ferrihemoglobin was prepared from freshly drawn blood without the use of nitrite. The purification procedure included the following steps: (i) DEAE column chromatography at pH 8.0 in 0.01 M Tris-HCl with elution in 0.10 M buffer; (ii) $K_3Fe(CN)_6$ oxidation; (iii) Sephadex G-25 column chromatography with 0.033 M phosphate buffer, pH 6.4; and (iv) overnight dialysis against this same buffer. Complete conversion to ferrihemoglobin was verified by optical absorption spectroscopy. Horse heart hemoglobin was purchased as a lyophilized powder from Sigma and prepared using the last three steps outlined above. All chemicals were of reagent grade and the labeled methanol was purchased from Aldrich as CD_3OD, 99.5 atom% D.

Resonance Raman spectra were recorded at ambient temperature (22°C) with a rotating cell difference spectrometer described in detail in [16]. This spectrometer can detect very small frequency shifts (approx. 0.1 cm^{-1}) by simultaneously collecting data from the alcohol-perturbed and unperturbed samples. The laser excitation frequency employed was 413.1 nm, and data acquisition routinely required from 1.5 to 3.0 h. Difference spectra were calculated by computer subtraction of the two simultaneously acquired resonance Raman spectra after appropriate scaling such as to minimize the scattering differences of several peaks. The extent of complex formation between ferrihemoglobin and the alcohols was calculated to be 80 and 97% for ethanol and methanol, respectively, using the K_d values given in [11].

No alcohol Raman bands are evident in the spectra reported herein except for those near 1452 and 1481 cm^{-1}. Ethanol Raman bands are not resonance enhanced, and therefore do not appear in the low-frequency (100–800 cm^{-1}) region of the ferrihemoglobin spectra. Similarly, methanol has no Raman bands in the low-frequency region.

3. RESULTS AND DISCUSSION

Fig.1 (a–c) shows the effects of binding ethanol and methanol on the low-frequency resonance Raman spectrum of human ferrihemoglobin. Before binding the spectrum exhibits Raman bands at 262, 309, 349, 374 (shoulder), 386, 412 and 436 (shoulder) cm^{-1}, and is in agreement with band frequencies and relative intensities of ferrihemoglobin spectra reported elsewhere [17]. After binding ethanol or methanol the spectra are almost identical to the unperturbed ferrihemoglobin spectrum except for similar changes near 309 cm^{-1}. The ethanol versus water and methanol versus water difference spectra are also included in fig.1 (d and e). A difference band centered at 313 cm^{-1} dominates the spectra of both alcohol complexes with an intensity approx. 7–10-times greater than any of the other difference features. This difference band apparently originates when a portion of the 309 cm^{-1} Raman band shifts by as much as 8 cm^{-1} to higher frequency with alcohol binding. The Raman intensity changes producing this shift are seen more clearly in fig.2 (a and b) with the ethanol complex of horse

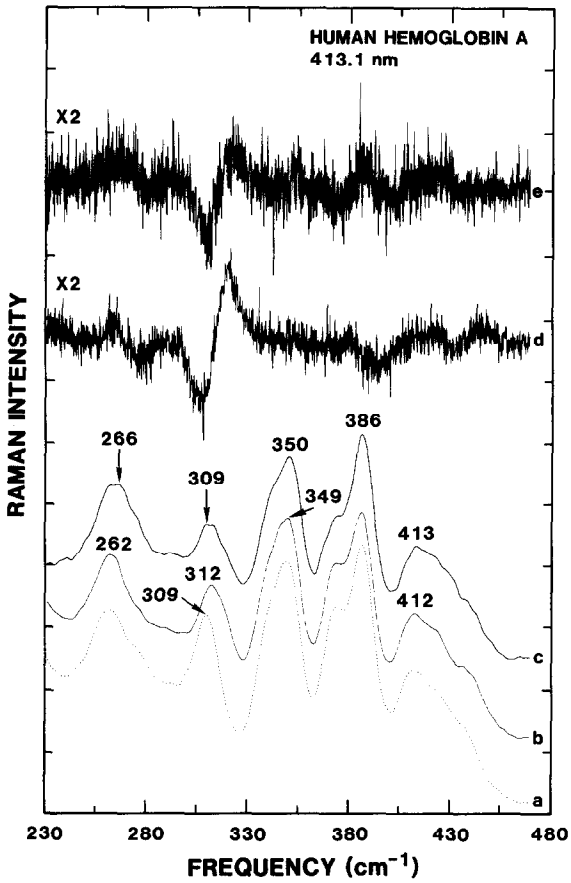

Fig.1. Low-frequency resonance Raman spectra and difference spectra describing the interaction of ethanol and methanol with human ferrihemoglobin. Raman spectra of ferrihemoglobin were recorded in the absence (a) and in the presence of 0.86 M ethanol (b) and 1.68 M methanol (c) in 0.04 M phosphate buffer, pH 7.0. Computer-subtracted difference spectra were calculated for ethanol (d) and methanol (e). Spectra were shifted vertically for clarity.

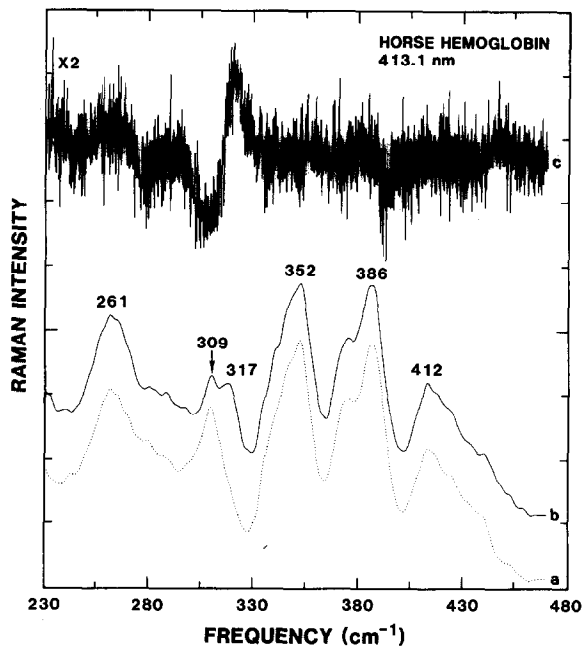

Fig.2. Low-frequency resonance Raman spectra and difference spectrum describing the interaction of ethanol with horse ferrihemoglobin. Raman spectra of ferrihemoglobin were recorded in the absence (a) and presence of 0.86 M ethanol (b) in 0.04 M phosphate buffer, pH 7.0. The computer-subtracted difference spectrum (c) is also included.

ferrihemoglobin in which the two resulting Raman bands are resolved. In addition, figs 1 and 2 together show that the ethanol-induced changes in horse and human ferrihemoglobin differ minimally which suggests the alcohol effect is not species specific.

The alcohol-induced change in only one resonance Raman band implies a site-specific interaction of alcohol with the protein in contrast to the more numerous spectral changes that would be expected for multiple alcohol binding sites, for multiple orientations of alcohol at a single binding site, or for an alcohol-induced protein conformational change. For example, IHP induces shifts in not one but several low-frequency Raman bands in ferrihemoglobin [17]. Furthermore, a change in only one Raman band would not be expected if the alcohol concentrations used were denaturing in agreement with previous stability studies [11].

The ethanol- and methanol-induced resonance Raman difference spectra of ferrihemoglobin given in fig.1 are strikingly similar. This similarity, along with the common structural features of the alcohols argues for either the same or similar sites of interaction of both alcohols with ferrihemoglobin as well as the same or similar mechanisms of interaction with the heme. In addition, even though the degree of complex formation is lower in the ferrihemoglobin·ethanol than the ferrihemoglobin·methanol complex (80 versus 97%), the ethanol-induced spectral change is larger. This effect is probably related to the increased size of ethanol over methanol coupled with steric constraints and will be examined in more detail below.

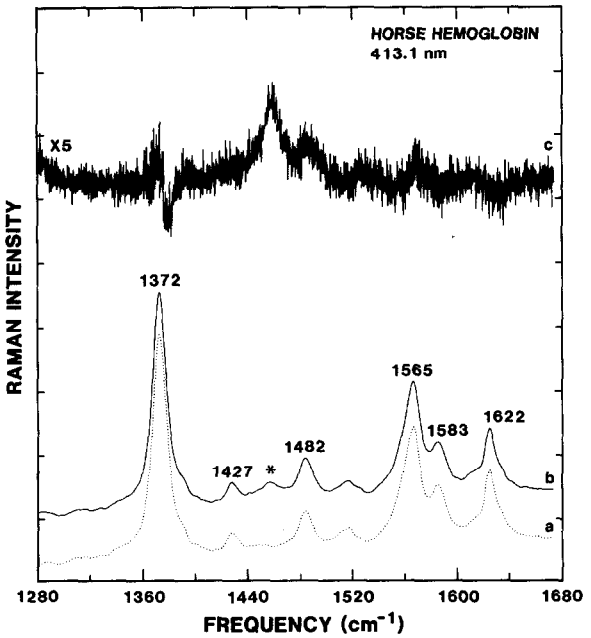

Fig.3. High-frequency resonance Raman spectra and difference spectrum describing the interaction of ethanol with horse ferrihemoglobin. Raman spectra of ferrihemoglobin were recorded in the absence (a) and presence of 0.86 M ethanol (b) in 0.04 M phosphate buffer, pH 7.0. The ethanol Raman band is marked (*) for clarity. The computer-subtracted difference spectrum (c) is also included.

Fig.3 shows the effect of ethanol on the high-frequency resonance Raman spectrum of horse ferrihemoglobin. Ethanol causes almost no change in the positions or relative intensities of the core-size and spin-state marker bands at 1622, 1565, and 1482 cm^{-1}. This behavior implies that ethanol binding perturbs the iron spin-state only minimally [18,19] as might be expected for the conservative substitution of an ethanol for a water bound at the iron or for ethanol binding in a hydrophobic crevice minimally perturbing the iron. Ethanol does, however, induce a modest decrease in frequency of the oxidation-state marker band at 1372 cm^{-1} of about 0.3 cm^{-1}. Changes in the position of this band in the same direction and of slightly larger magnitude (-0.5 to -1.0 cm^{-1}) have been linked to IHP-induced changes in the tertiary and quaternary structure of high-spin ferrihemoglobin [20]. The possible origins of these effects include the alteration of non-bonded interactions between the porphyrin macrocycle and the protein which may indirectly perturb the iron-histidine bond [20]. Ethanol binding within the heme pocket would probably perturb such non-bonded interactions. Along these lines, the alcohol-induced optical difference spectra are more complex than those induced by binding of ligands that perturb only the iron spin-state.

At this point it is instructive to discuss how alcohols might specifically interact with the heme in ferrihemoglobin in a manner consistent with the observed changes in the 309 cm^{-1} resonance Raman band. More explicitly, four possible sources of the 309 cm^{-1} Raman band will be considered as will five associate mechanisms of the alcohol-induced shift to higher energy. As mentioned previously, a general perturbation of the protein conformation by alcohol would probably lead to changes in several Raman bands at different frequencies simultaneously, not just a change at 309 cm^{-1}, and is therefore not considered. Furthermore, the unperturbed Raman band at 309 cm^{-1} may originate as the sum of contributions from different sources such that alcohol perturbation of one source may result in only a partial shift in intensity.

The first possibility is that the 309 cm^{-1} resonance Raman band originates with one or both porphyrin vinyl groups and that ethanol and methanol bind along the heme periphery perturbing this group. This possibility follows from a previous Raman band assignment of the $\delta C_b C_\alpha C_\beta(2)$ out-of-phase bending mode of a vinyl group to a band near 310–320 cm^{-1} [18,21]. Alcohol perturbation of the globin-vinyl interaction might shift the 309 cm^{-1} band, and the up to 8 cm^{-1} increase in frequency with the alcohols is of the same magnitude as that found [21] with the potentially more extreme vinyl group perturbation in ferrimyoglobin·imidazole (309 cm^{-1}) versus the bisimidazole complex of ferriprotoporphyrin (312 cm^{-1}). However, this out-of-phase vinyl group bending mode has an associate in-phase mode with a Raman band at 420 cm^{-1} [21], and no significant change was observed at 420 cm^{-1} with alcohol binding to ferrihemoglobin. Furthermore, no change near 1560 cm^{-1} (ν_2) was observed with the ferrihemoglobin·ethanol complex and this high-frequency band is the band most strongly coupled to the vinyl bend [18]. These results, along with the deeply buried position of the vinyl groups

[22] make alteration of this group by alcohol binding unlikely, even though much of the 309 cm^{-1} band intensity in ferrihemoglobin may originate with such vibrations.

The second possibility is that the resonance Raman band at 309 cm^{-1} is the iron-nitrogen(histidine) stretch, and that alcohol binds to ferrihemoglobin in the proximal heme pocket altering its frequency. This stretch has been assigned to a 220 cm^{-1} band in ferrohemoglobin [23] and one at 248 cm^{-1} in ferrimyoglobin [24], however the latter band is absent in ferrihemoglobin. Alcohol perturbation might change protein-imposed steric constraints on the proximal histidine and/or change the strength of the proximal histidine N_δH-carboxyl hydrogen bond. Both perturbations have been implicated in control of O_2 binding [25–27]. Furthermore, this mechanism has been used to explain a pH-dependent shift in the iron-nitrogen(histidine) band of peroxidase [28]. In model bisimidazole ferroheme complexes, the iron-nitrogen(imidazole) stretch frequency increases 15 to 25 cm^{-1} when the imidazole becomes unhindered [29] and the iron-nitrogen(imidazole) bond strength increases. When model complexes are deprotonated, the iron-nitrogen(imidazole) stretch frequency increases as much as 25 cm^{-1} [25] again reflecting a stronger bond to iron. If the observed alcohol-induced increase in frequency of the 309 cm^{-1} band were attributed to these mechanisms, this would imply that alcohol diminishes steric hindrance of or globin-induced tension on the proximal histidine, or increases the strength of the histidine N_δH-carboxylate hydrogen bond. Although the magnitude of the alcohol-induced change is in line with these iron-nitrogen bond perturbations, it is unclear as to whether they actually occur in ferrihemoglobin.

A third possibility is that the 309 cm^{-1} resonance Raman band is an iron-oxygen(water) stretch, and that alcohol binds in the distal pocket either perturbing this stretch or replacing it with an iron-oxygen(alcohol) stretch. A band that represents the iron-oxygen(water) axial stretch has not yet been assigned. However, distal ligand stretches in other high- and low-spin ferrihemeprotein complexes fall in the range of about 400–500 cm^{-1} [18,30], and are apparently specific to both the electronic effects of and steric constraints on each ligand. Since water is uncharged, its interaction with iron is likely to be weaker than other ferric ligands, and thus it should have a lower stretch frequency. A careful examination of the low-frequency Raman spectra of both weak and strong field ligand complexes of human and carp ferrihemoglobin [17] as well as ferrimyoglobin [31] shows that the Raman band near 309 cm^{-1} is both of largest relative amplitude and most well defined in the water complexes. Furthermore, when ferrimyoglobin is raised from pH 4.8 to 11.7, the pronounced band near 309 cm^{-1} decreases to only a fraction of its original intensity [24]. Taken together, these data are consistent with the iron-oxygen(water) stretch contributing some fraction of the intensity of the 309 cm^{-1} band.

The iron-ligand stretch assignments in the F^-, OH^-, N_3^- and CN^- complexes of ferrihemeproteins have been verified by isotopic substitution [18,30]. This technique was applied to the ferrihemoglobin · methanol complex, and fig.4 gives the low-frequency resonance Raman spectra (a and b) and difference spectrum (c) for D_3COH versus H_3COH bound to horse ferrihemoglobin. If

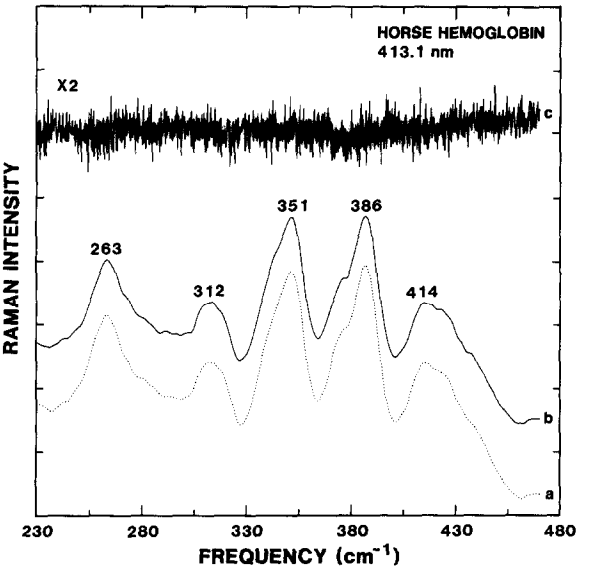

Fig.4. Low-frequency resonance Raman spectra and difference spectrum describing the interaction of unlabeled and deuterium labeled methanol with horse ferrihemoglobin. Raman spectra of ferrihemoglobin were recorded in the presence of either CH_3OH (a) or CD_3OD (b) at 1.68 M in 0.04 M phosphate buffer, pH 7.0. The computer-subtracted difference spectrum (c) is also included.

methanolate is treated as a point mass bound to iron, the calculation for replacement of hydrogen by deuterium gives a 10 cm^{-1} decrease in frequency. The actual substitution, however, does not cause a clear change near 309 cm^{-1} nor at any other part of the difference spectrum. This absence of an isotope-induced difference spectrum is equivocal and can be interpreted in two ways: (a) alcohols do not bind at the iron but somewhere in the distal heme pocket and from there perturb the iron-oxygen(water) stretch centered at 309 cm^{-1} or (b) alcohols bind at the iron replacing water but neither the iron-oxygen(water) nor the iron-oxygen(methanol) stretches are Raman active. It would follow from (b) that the assignment of the 309 cm^{-1} band to an iron-oxygen(water) stretch is incorrect, and its origin and perturbation are through another mechanism.

Three arguments support (b). First, the isotopic substitution of $H_2{}^{18}O$ for $H_2{}^{16}O$ at the iron in ferrimyoglobin is not revealed by changes in the low-frequency resonance Raman spectrum even though a 20 cm^{-1} decrease in frequency of the band at 497 cm^{-1} is observed with $^{18}OH^-$ substitution for $^{16}OH^-$ [30]. Second, the steric constraints on an iron-oxygen(alcohol) bond may be similar to those on an iron-oxygen(water) bond such that the former bond is not Raman active. Third, the binding of an alcohol in the heme crevice in addition to water might cause crowding and the perturbation of more than one Raman band, and this was not found. Thus, replacement of an iron-oxygen(water) by an iron-oxygen(alcohol) bond cannot be ruled out. However, a simple reduced mass calculation supports (a) because replacement of water by ethanol or methanol at the iron should lead to a frequency decrease, and not an increase from 309 to 317 cm^{-1} as observed.

A fourth possibility is that the alcohol-dependent 309 cm^{-1} band originates with the out-of-plane methine deformation ($\gamma C_m C_a$) and alcohol binding shifts this peak to higher energy. Studies using X-ray crystallography suggests there are two places in the distal pocket that may be large enough to accommodate ethanol. These are (i) the N_3^- (and OCN^-) binding site which extends from the iron radially outward to a point midway between the methine carbon (across from the propionic acid side chains) and pyrrole ring II [32], and (ii) the dichloromethane binding site over pyrrole ring III [9]. Damping of a methine out-of-plane vibration would more likely occur with the alcohol in the former rather than the latter position. The damping of the methine deformation by crowding induced by ethanol would be expected to raise the frequency of the 309 cm^{-1} band as is observed. Furthermore, with this conformation the ethanol might, because of its size, interact more strongly than methanol and cause a greater frequency shift. Greater alcohol-induced spectral changes were observed with ethanol than methanol in this study, even though the percent of methanol-bound protein was greater than that which was ethanol bound. Furthermore, the positioning of alcohol in the N_3^- binding site suggests that the hydroxyl would probably be oriented for coordination to the iron. In this manner both iron-binding and nonbonded interactions could be combined within a single coordinating geometry, and case (b) would apply. Alcohol-induced changes in both types of interactions are supported by other spectroscopic evidence (i.e. optical absorption in combination with EPR spectroscopy).

In summary, this letter reports that the alcohol-induced resonance Raman difference spectra of both human and horse ferrihemoglobin consist mainly of a single, prominent band centered near 313 cm^{-1}. The magnitude of the difference spectrum induced with ethanol is greater than that with methanol, but the shapes and band centers are almost identical. These data imply a site-specific, similar binding of both alcohols to ferrihemoglobin, which appears to involve a contribution from steric interactions. The difference spectrum is caused by an alcohol-induced increase in frequency of the 309 cm^{-1} resonance Raman band, which emphasizes the importance of understanding its structural origin. Further studies along these lines are presently underway.

Acknowledgements: This work was supported by NIAAA Grant AA06935 (to B.B.M.), US Department of Energy Contract DE-AC04-76-DP00789 (to J.A.S.), Gas Research Institute Contract 2082-260-0767 (to J.A.S.), and NSF Grant DMB8604435 (to M.R.O.).

REFERENCES

[1] Asakura, T., Adachi, K. and Schwartz, E. (1978) J. Biol. Chem. 253, 6423–6425.

[2] Cordone, L., Cupane, A., San Biagio, P.L. and Vitrano, E. (1979) Biopolymers 18, 1975–1988.
[3] Cordone, L., Cupane, A., San Biagio, P.L. and Vitrano, E. (1981) Biopolymers 20, 39–51.
[4] Poillon, W.N. (1980) Biochemistry 19, 3194–3199.
[5] MacDonald, V.W. and Charache, S. (1982) Biochim. Biophys. Acta 705, 48–54.
[6] Cassatt, J.C. and Steinhardt, J. (1971) Biochemistry 10, 3738–3742.
[7] Cordone, L., Cupane, A., D'Alia, F. and De Stefano, M.G. (1982) Faraday Symp. Chem. Soc. 17, 161–174.
[8] Muhoberac, B.B. and Brill, A.S. (1980) Biochemistry 19, 5157–5167.
[9] Schoenborn, B.P. (1976) Proc. Natl. Acad. Sci. USA 73, 4195–4199.
[10] Wishnia, A. (1969) Biochemistry 8, 5064–5069.
[11] Brill, A.S., Castleman, B.W. and McKnight, M.E. (1976) Biochemistry 15, 2309–2316.
[12] Blumberg, W.E. and Peisach, J. (1971) in: Probes of Structure and Function of Macromolecules and Membranes (Chance, B. et al. eds) vol.2, pp.215–228, Academic Press, New York.
[13] Yonetani, T., Iizuka, T. and Waterman, M.R. (1971) J. Biol. Chem. 246, 7683–7689.
[14] Makinen, M.W. and Churg, A.K. (1983) in: Iron Porphyrins Part I (Lever, A.B.P. and Gray, H.B. eds) pp.141–235, Addison-Wesley, Reading, MA.
[15] Brill, A.S., Fiamingo, F.G. and Hampton, D.A. (1986) J. Inorg. Biochem. 28, 137–143.
[16] Shelnutt, J.A. (1983) J. Phys. Chem. 87, 605–616.
[17] Henry, E.R., Rousseau, D.L., Hopfield, J.J., Noble, R.W. and Simon, S.R. (1985) Biochemistry 24, 5907–5918.
[18] Spiro, T.G. (1983) in: Iron Porphyrins Part II (Lever, A.B.P. and Gray, H.B. eds) pp.89–159, Addison-Wesley, Reading, MA.
[19] Felton, R.H. and Yu, N.-T. (1978) in: The Porphyrins (Dolphin, D. ed.) vol.III, pp. 347–393, Academic Press, New York.
[20] Rousseau, D.L., Shelnutt, J.A., Henry, E.R. and Simon, S.R. (1980) Nature 285, 49–51.
[21] Choi, S. and Spiro, T.G. (1983) J. Am. Chem. Soc. 105, 3683–3692.
[22] Bunn, H.F. and Forget, B.G. (1986) Hemoglobin: Molecular, Genetic and Clinical Aspects, pp.13–36, Saunders, Philadelphia, PA.
[23] Argade, P.V., Sassaroli, M., Rousseau, D.L., Inubushi, T., Ikeda-Saito, M. and Lapidot, A. (1984) J. Am. Chem. Soc. 106, 6593–6596.
[24] Teraoka, J. and Kitagawa, T. (1981) J. Biol. Chem. 256, 3969–3977.
[25] Stein, P., Mitchell, M. and Spiro, T.G. (1980) J. Am. Chem. Soc. 102, 7794–7797.
[26] Stanford, M.A., Swartz, J.C., Phillips, T.E. and Hoffman, B.M. (1980) J. Am. Chem. Soc. 102, 4492–4499.
[27] Valentine, J.S., Sheridan, R.P., Allen, L.C. and Kahn, P.C. (1979) Proc. Natl. Acad. Sci. USA 76, 1009–1013.
[28] Teraoka, J. and Kitagawa, T. (1980) Biochem. Biophys. Res. Commun. 93, 694–700.
[29] Hori, H. and Kitagawa, T. (1980) J. Am. Chem. Soc. 102, 3608–3613.
[30] Asher, S.A., Vickery, L.E., Schuster, T.M. and Sauer, K. (1977) Biochemistry 16, 5849–5856.
[31] Desbois, A., Lutz, M. and Baneryee, R. (1979) Biochemistry 18, 1510–1518.
[32] Deatherage, J.F., Obendorf, S.K. and Moffat, K. (1979) J. Mol. Biol. 134, 419–429.