Inorganica Chimica Acta, 33 (1979) L181–L182

Infrared Frequency Lowering of the C–O Stretching Mode of Carbon Monoxyhemoglobin Kansas Associated with the R → T Switch

J. R. KINCAID, T. G. SPIRO

Chemistry Department, Princeton University, Princeton, New Jersey 08540, U.S.A.

J. S. VALENTINE

Department of Chemistry, Douglass College, Rutgers, The State University, New Brunswick, New Jersey 08903, U.S.A.

D. D. SAPERSTEIN and A. J. REIN

Merck, Sharp and Dohme Research Laboratories, Rahway, New Jersey 07065, U.S.A.

Received January 19, 1979

In the continuing effort to understand the structural basis of hemoglobin allostery [1, 2], attention focuses on the molecular pathway [3] between ligand binding and the change in quaternary structure [4, 5]. While the designation T ('tense') for the deoxy quaternary structure implies a tension at the heme group to account for the lowered oxygen affinity, it has been recognized [1, 2] that no significant force need be exerted on the heme group in the deoxy form per se, and indeed none has so far been detected [6–8]. Rather, tension may be generated upon binding of ligands in the T quaternary state. A key molecule for examining this question is hemoglobin Kansas, a low affinity Hb mutant [9], which can be switched in its ligated forms from the R to the T quaternary state by addition of inositol hexaphosphate (IHP) [10].

The infrared stretching frequency of carbon monoxide bound to heme proteins has been a useful probe of the heme environment [11–14]. The frequency is sensitive to the extent of π-back donation from the iron atom, which in turn depends on the electron withdrawing or donating properties of the peripheral heme constituents, and of the fifth (axial) ligand [12]. In addition the frequency is sensitive to distal interactions; hydrogen bonding and electron donor interactions have been implicated in peroxidase [13] and in hemoglobin [14, 15] respectively. Thus it is of interest to examine the change in the CO frequency, if any, between R and T quaternary states.

We have recorded the infrared spectrum of COHb Kansas; the C–O frequency was the same, 1951 cm^{-1}, as that of COHb A, run under the same conditions. Addition of IHP had no detectable effect on the Hb A spectrum, but it did produce a 0.7 cm^{-1} decrease in the C–O frequency for Hb Kansas, as shown in the Figure. This implies that a definite, though small, change in the CO bond energy is induced by the quaternary structure change. In this regard, the infrared frequency is apparently more

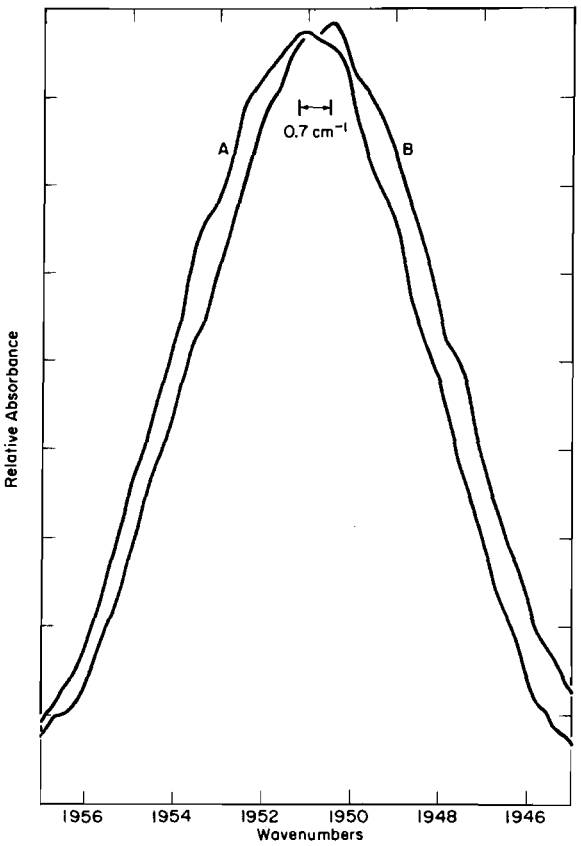

Figure. Fourier transform infrared spectrum of the C–O stretching band of COHb Kansas without (A) and with (B) addition of excess IHP.

sensitive than the ^{13}C nmr chemical shift, which shows no change upon IHP addition to COHb Kansas [16]. Recently Brunori and coworkers [17] have observed a similar effect for trout COHb IV. Lowering of the pH from 7.8 to 6.2, which is accompanied by conversion to a low affinity form of the protein, produced a 0.4 cm^{-1} lowering of the C–O frequency. The direction of the shift is the same for trout as for Kansas, but the magnitude appears to be somewhat lower for trout.

If the low ligand affinity of T state hemoglobin is connected with stabilization of the out-of-plane structure characteristic of deoxy heme [2], then forcing the R → T switch in ligated hemoglobin may be expected to weaken the bond between the iron atom and the proximal imidazole. This effect is rather well established in nitroxyl hemoglobin, for which infrared [15], esr [18] and resonance Raman [19, 20] data are all consistent with substantial weakening or breaking of the proximal imidazole bond in half the Hb subunits upon R → T conversion with IHP. Addition of IHP to NOHb produces a ~ 50 cm^{-1} increase in the N–O stretching frequency for half the hemes, a shift similar to that observed upon loss

of N-methylimidazole from its complex with nitrosyl protoheme dimethyl ester in chloroform [15]. The shift is consistent with reduction in π-back bonding from Fe to NO upon loss of the electron donor axial ligand. A similar effect would be expected for carbonyl hemes, although smaller in extent, NO being a stronger π acceptor than CO. Indeed, addition of N-methylimidazole to the carbonyl adduct of reduced hemin in aqueous phosphate buffer, pH 10.5, decreases ν_{CO} from 1967 to 1948 cm^{-1} [21]. The increase expected if the proximal imidazole bonds were broken in COHb might be somewhat larger than this 19 cm^{-1} difference, since there may be a weak axial interaction between OH^- and the carbonyl heme in the aqueous buffer.

The observed shift upon IHP addition to COHb Kansas is small, and is in the direction *opposite* to that expected for weakening of the proximal imidazole bond. The decrease in frequency is, however, consistent with an enhancement of the distal imidazole interaction which has been inferred to exist in COHb [14] and NOHb [15]. It is possible that the R → T switch in COHb Kansas and trout COHb IV induces both a weakening of the proximal imidazole bond and a strengthening of the distal imidazole interaction, with compensating effects on ν_{CO}, resulting in the small observed shifts.

Experimental

Hemoglobins were separated on CM-cellulose (Whatman CM-52) as the carbon monoxide complexes as previously described [9]. The hemoglobin rich fractions were concentrated by ultrafiltration (Amicon PM-10) and buffer exchanged by chromatography on Sephadex Co-25 equilibrated with CO saturated 0.1 *M* Bis-Tris (Sigma #B-9754), pH 7.0. The CO saturated solutions were transferred to sealed CaF_2 cells (0.05 cm path length) with a gas tight syringe. Spectra recorded at room temperature with a Nicolet 7199 Fourier-transform infrared spectrometer. The digitized spectrum contained 65,000 data points, corresponding to ~ 0.24 cm^{-1} resolution. The spectra were ratioed to that of the buffer alone, taken in the same cell.

Acknowledgements

We thank Drs. R. G. Shulman and S. Ogawa of Bell Laboratories for assistance with protein purification procedures, Mr. David Boyce for the generous gift of his hemoglobin, and Professor W. S. Caughey of Colorado State University for communicating unpublished results. This work was supported by NIH Grants HL 12526 (to TGS) and GM 24759 (to JSV). JSV is the recipient of a Research Career Development Award from the USPHS.

References

1 R. G. Shulman, J. J. Hopfield and S. Ogawa, *Quart. Rev. Biophys., 8,* 325 (1975).
2 M. F. Perutz, *Brit. Med. Bull., 32,* 195 (1976).
3 B. R. Gelin and M. Karplus, *Proc. Natl. Acad. Sci., 74,* 801 (1977).
4 M. F. Perutz, *Nature, 228,* 726 (1970).
5 M. F. Perutz, *Nature, 237,* 495 (1972).
6 P. Eisenberger, R. G. Shulman, B. M. Kincaid, G. S. Brown and S. Ogawa, *Nature, 274,* 30 (1978).
7 T. G. Spiro and J. M. Burke, *J. Amer. Chem. Soc., 98,* 5482 (1976).
8 J. Kincaid, P. Stein and T. G. Spiro, (submitted for publication) (1978).
9 J. Bonaventura and A. Riggs, *J. Biol. Chem., 243,* 980 (1968).
10 S. Ogawa, A. Mayer and R. G. Shulman, *Biochem. Biophys. Res. Commun., 49,* 1485 (1972).
11 a) J. O. Alben and W. S. Caughey, *Biochem., 7,* 175 (1968). b) W. S. Caughey, C. H. Barlow, D. H. O'Keeffe and M. C. O'Toole, *Ann. N.Y. Acad. Sci., 206,* 296 (1973).
12 C. H. Barlow, P.-I. Ohlssen and K.-G. Paul, *Biochem., 15,* 2225 (1976).
13 J. P. Collman, J. J. Brauman, T. R. Halbert and K. S. Suslick, *Proc. Nat. Acad. Sci. U.S., 73,* 3333 (1976).
14 J. D. Satterlee, M. Teintze and J. H. Richards, *Biochem., 17,* 1456 (1978).
15 J. C. Maxwell and W. S. Caughey, *Biochem., 15,* 388 (1976).
16 R. G. Shulman, S. Ogawa, A. Mayer and C. L. Castillo, *Ann. N.Y. Acad. Sci., 222,* 9 (1973).
17 F. Ascoli, E. Gratton, F. Riva, P. Fasella and M. Brunori, *Biochim. Biophys. Acta, 533,* 534 (1978).
18 A. Szabo and M. F. Perutz, *Biochem., 15,* 4427 (1976).
19 A. Szabo and L. D. Barron, *J. Am. Chem. Soc., 97,* 17 (1975).
20 J. M. Burke, P. Daly, P. Wright and T. G. Spiro (submitted for publication).
21 W. S. Caughey, (private communication).
22 R. S. Drago, T. Bergelstijk, J. A. Breese and J. P. Cannady, (submitted for publication) (1978).