References

Dale, R. E., and Eisinger, J. (1974), *Biopolymers 13, 1573.*
Förster, Th. (1949), *Z. Naturforsch. A 4,* 321.
Förster, Th. (1959), *Discuss. Faraday Soc. 27,* 7.
Haxo, F. T., Kycia, J. H., Siegelman, H. W., and Somers, G. F. (1972), *Int. Symp. Carotenoids, 3rd (Cluj),* Abstr. p 73.
Haxo, F. T., Kycia, J. H., Somers, G. F., Bennett, A., and Siegelman, H. W. (1976), *Plant Physiol. 57,* 297.
Kasha, M. (1963), *Rad. Resiat. 20,* 55.
Kasha, M., Rawls, H., and El-Bayoumi, M. (1965), *Pure Appl. Chem. 11,* 371.
Mantulin, W. W., and Song, P. S. (1973) *J. Am. Chem. Soc. 95,* 5122.
Moore, T. A. (1975), Ph.D. Dissertation, Texas Tech University, Lubbock.
Moore, T. A., and Song, P. S. (1973), *Chem. Phys. Lett. 19,* 128.
Olson, J. M., Philipson, K. D., and Sauer, K. (1973), *Biochim. Biophys. Acta 292,* 206.
Philipson, K. D., and Sauer, K. (1972), *Biochemistry 11,* 1880.
Prézelin, B. B. (1975), Ph.D. Dissertation, University of California, San Diego.
Prézelin, B. B., and Haxo, F. T. (1974), *J. Phycol. Suppl. 10,* 11.
Prézelin, B. B., and Haxo, F. T. (1976), *Planta* (in press).
Sineshchekov, V. A., Litvin, F. F., and Das, M. (1972), *Photochem. Photobiol. 15,* 187.
Song, P. S., and Moore, T. A. (1974), *Photochem. Photobiol. 19,* 435.
Song, P. S., Chae, Q., Fujita, M. and Baba, H. (1976), *J. Am. Chem. Soc. 98,* 819.
Strain, H. H., and Sherma, J. (1969), *J. Chem. Educ. 46,* 476.
Strauss, G., and T. H. Tien (1973), *Photochem. Photobiol. 17,* 425.

Equilibrium between Six- and Five-Coordinated Hemes in Nitrosylhemoglobin: Interpretation of Electron Spin Resonance Spectra†

Attila Szabo* and Max F. Perutz*

ABSTRACT: Nitrosylhemoglobin without inositol hexaphosphate, which has the quaternary oxy structure, shows an electron spin resonance (ESR) spectrum similar to that of a synthetic nitrosylheme with piperidine in the sixth coordination position. Nitrosylhemoglobin with inositol hexaphosphate, which has the quaternary deoxy structure, shows an ESR spectrum which is a composite of that of the nitrosylheme with piperidine and of a synthetic nitrosylheme in which the sixth coordination position is empty.

In the absence of organic phosphates, the allosteric equilibrium of nitrosylhemoglobin A, as of other low spin compounds of hemoglobin A, is biased strongly toward the quaternary oxy structure. However, nitrosylhemoglobin A is unique among these compounds in allowing inositol hexaphosphate (IHP[1]) to switch the equilibrium to the deoxy structure (Cassoly, 1974; Salhany, 1974; Perutz et al., 1976). The transition is accompanied by marked changes in electronic, infrared, and ESR spectra; the latter were first demonstrated by Rein et al. (1972), but could not then be interpreted. The clue to their interpretation came from Maxwell and Caughey's (1976) study of the infrared NO stretching frequencies in nitrosylhemoglobin. They found that without IHP nitrosylhemoglobin exhibits a single ^{14}NO stretching frequency at 1615 cm^{-1}, similar to that of the six-coordinated 1-methylimidazole Fe(II)-NO protohemedimethyl ester. Addition of IHP caused the intensity of this band to be halved and a new band at 1668 cm^{-1} to appear. This frequency corresponds to that given by the five-coordinated Fe(II)-NO protohemedimethyl ester. These results indicate the coexistence of two distinct chemical species: one in which the iron is linked to N_ϵ of the proximal histidine and to the NO nitrogen and another in which the bond to the proximal histidine is either broken or severely stretched.

Figures 1a and 1b show the ESR spectra of nitrosylhemoglobin with and without IHP. They are rhombic. Without IHP strong resonances appear at $g = 1.97$, 2.03, and 2.06; in addition there are indications of hyperfine splitting. On addition of IHP, strong hyperfine splitting at g_z is superimposed on this spectrum. The spectra of [^{15}N]nitrosylhemoglobin with IHP published by Maxwell and Caughey (1976) show marked hyperfine splitting also at g_x and g_y.

We shall now compare these spectra with those of synthetic heme derivatives published by Wayland and Olson (1974). The six-coordinated Fe(II)-NO tetraphenylporphinepiperidine shows a rhombic ESR spectrum with strong resonances similar

† From the Department of Chemistry, Indiana University, Bloomington, Indiana 47401 (A.S.), and the MRC Laboratory of Molecular Biology, Cambridge, England (M.F.P.). *Received May 25, 1976.* One of us (A.S.) acknowledges the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

[1] Abbreviations used: ESR, electron spin resonance; IHP, inositol hexaphosphate.

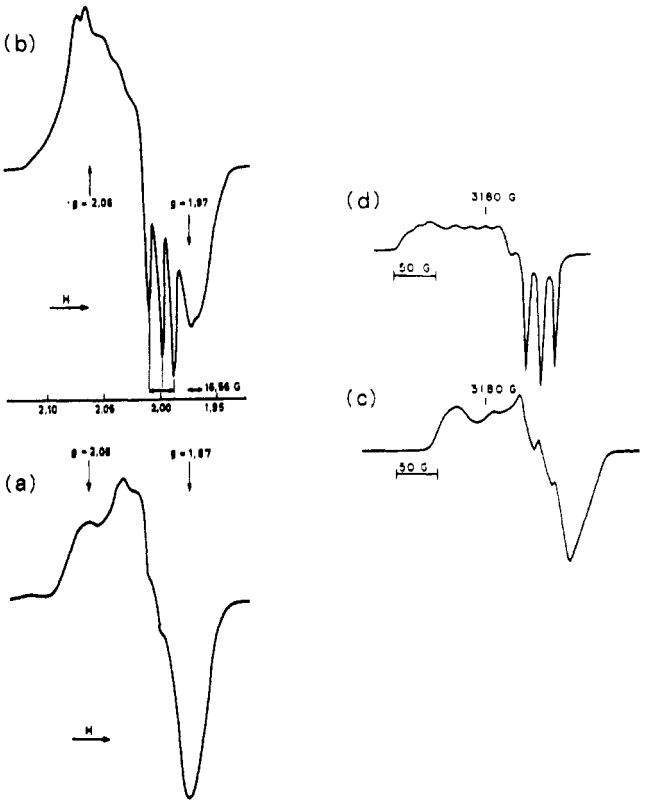

FIGURE 1: ESR spectra of nitrosylhemoglobin and synthetic nitrosylhemes. (a) Nitrosylhemoglobin without IHP; (b) nitrosylhemoglobin with IHP, both at 77 K; (c) Fe(II)-NO tetraphenylporphinepiperidine; (d) Fe(II)-NO tetraphenylporphine, both in toluene glass at 120 K. a and b are reproduced, with permission, from Rein et al. (1972); c and d are from Wayland and Olson (1974).

to those of nitrosylhemoglobin without IHP (Figure 1c). The five-coordinated Fe(II)-NO tetraphenylporphine lacks these strong resonances but shows instead strong hyperfine splitting at g_z and weak hyperfine splitting at g_x and g_y (Figure 1d). Figure 1b shows that the spectrum of nitrosylhemoglobin with IHP is a composite of Figures 1c and 1d: it shows the prominent resonances of the six-coordinated nitrosylheme superimposed on the strong hyperfine splitting of the five-coordinated one, thus confirming the coexistence of five- and six-coordinated hemes. Experiments with hybrid hemoglobins led Perutz et al. (1976) to conclude that the rupture of the Fe-N_ϵ bond takes place mostly in the α subunits; a more accurate estimate by Nishikura and Sugita (1976) attributes about 80% of the spectral changes to the α subunits. It is remarkable that combination of the β subunits with IHP should break the iron atoms from the proximal histidines at a site in the α subunits 34 Å away from the IHP-combining site.

References

Cassoly, R. (1974), *C.R. Hebd. Seances Acad. Sci., Ser. D 278,* 1417-1420.

Maxwell, J. C., and Caughey, W. S. (1976), *Biochemistry 15,* 388-396.

Nishikura, K., and Sugita, Y. (1976), *J. Mol. Biol.* (in press).

Perutz, . F., Kilmartin, J. V., Nagai, K., Szabo, A., and Simon, S. R. (1976), *Biochemistry 15,* 378-387.

Rein, H., Ristau, O., and Scheler, W. (1972), *FEBS Lett. 24,* 24-26.

Salhany, J. M. (1974), *FEBS Lett. 49,* 84-86.

Wayland, B. B., and Olson, L. W. (1974), *J. Am. Chem. Soc. 96,* 6037-6041.