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SHORT COMMUNICATION

Dichloromethane and Myoglobin Function

A. C. NUNES AND B. P. SCHOENBORN

Biology Department, Brookhaven National Laboratory, Upton, New York 11973

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Stewart *et al.* (1) observed an increase in carbon monoxide–hemoglobin in humans exposed to dichloromethane and suggested that the increased CO level was due to the metabolism of dichloromethane to CO. An alternative hypothesis is put forth in Settle's work on the effect of xenon (2) and cyclopropane[1] on the binding affinity of carbon monoxide to myoglobin. In these studies he showed that xenon and, to a much larger degree, cyclopropane, which both bind to myoglobin, alter the CO affinity.

Xenon and cyclopropane bind to myoglobin (3–6) at a specific site located in the interior of the molecule about equidistant from the heme-linked histidine and one of the pyrrole rings of the heme group. These myoglobin complexes are stabilized purely by van der Waals forces (3–6). The binding sites of $HgBr_2^-$ and I_3^- (7, 8) also involve this internal cavity, but it is questionable that these ions bind solely because of van der Waals interactions. Since the shape, size, and van der Waals radii of dichloromethane (Table 1) are similar to those of xenon and cyclopropane, it is likely that similar molecular interaction with these heme proteins occur. It is therefore suggested that dichloromethane, also an anesthetic, may act similarly and increase the CO-hemoglobin affinity sufficiently to explain the results of Stewart *et al.* As a first step in the elucidation of the phenomena observed by Stewart *et al.* (1), we undertook this X-ray diffraction analysis (9) to determine whether dichloromethane binds to myoglobin as do xenon and cyclopropane.

Sperm whale metmyoglobin crystals prepared by the method of Kendrew and Parrish (10) were mounted in quartz capillaries containing, apart from mother liquor, some dichloromethane with a partial pressure of approximately 35 cm Hg. Crystals so prepared showed increased susceptibility to radiation damage compared to normal metmyoglobin crystals. X-ray intensities of the hk0, h01, and 0k1 reflections were collected to 2.7-A resolution with multiple film precession photos. From these data, electron density difference maps between metmyoglobin exposed to dichloromethane and native metmyoglobin were calculated, using the phase angles of the native metmyoglobin.[2] These difference electron density maps are somewhat complicated but depict major features in the region corresponding to the xenon and cyclopropane binding site. This binding site is in the interior of the protein molecule, approximately equidistant from one of the pyrrole rings of the heme group and the ring of the heme-linked histidine. Region 1 in Fig. 1 represents the addition of dichloromethane to metmyoglobin

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[1] W. Settle, personal communication.

[2] H. C. Watson and J. C. Kendrew, unpublished observations.

TABLE 1

Some physical properties of anesthetics whose myoglobin-binding properties have been studied

Molecule	Formula	Profile	Van der Waal's Radius (Å)	Polarizability ($Å^3$)	Ionization Energy (ev)	Binds to Myoglobin?
Krypton	Kr		1.96	2.5	14.00	no
Nitrous Oxide	N_2O		2.02	3.4	12.89	no
Acetylene	C_2H_2		2.13	3.5	11.40	no
Xenon	Xe		2.13	4.2	12.13	yes
Ethylene	C_2H_4		2.21	3.9	10.50	no
Ethane	C_2H_6		2.29	4.1	11.50	no
Cyclopropane	C_3H_6		2.43	5.5	10.09	yes
Dichloromethane	CH_2Cl_2		2.54	4.1	11.35	yes

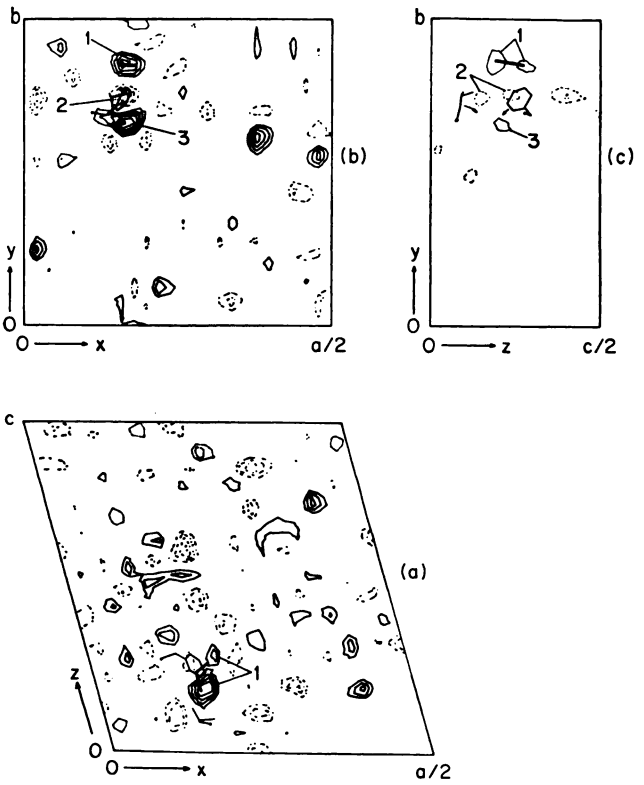

FIG. 1. *Difference electron density maps: h01 (a), hk0 (b), and 0k1 (c)*

Contours are at arbitrary intervals. ——, positive contours; -----, negative contours. The zero and first contours have been omitted. The positions of phenylalanine (H14) and leucine (G5) are depicted as in the native structure. The arrows (c) indicate the most likely reorientations of these groups in the derivative. The probable location of the dichloromethane is also shown.

and corresponds to the xenon or cyclopropane site. Model building and approach distance calculations show that regions 2 and 3, best seen in Fig. 1b, are most likely caused by reorientations of the side chains of phenylalanine H14 and leucine G5. Figure 2a depicts this region of the native metmyoglobin structure superimposed on the hk0 difference map, while Fig. 2b shows the same area with the addition of dichloromethane and the reoriented side chains of H14 and G5. Possible interference with a few other groups, such as leucine F4 and isoleucine FG4, cannot be ruled out. The observations by Settle suggest two minor sites in addition to the major binding site. Such low-occupancy minor sites, however, are not obvious in these complicated difference maps, where not all minor features are interpretable. The loss of a negatively charged ion (possibly sulfate) bound to histidines E7 and G17 has been previously observed in azide (11), deoxy (12), and xenon myoglobin (3). Careful examination of the present maps (Fig. 1) indicates only a minor negative region at that location.

These X-ray difference analyses show that xenon, cyclopropane, and dichloromethane

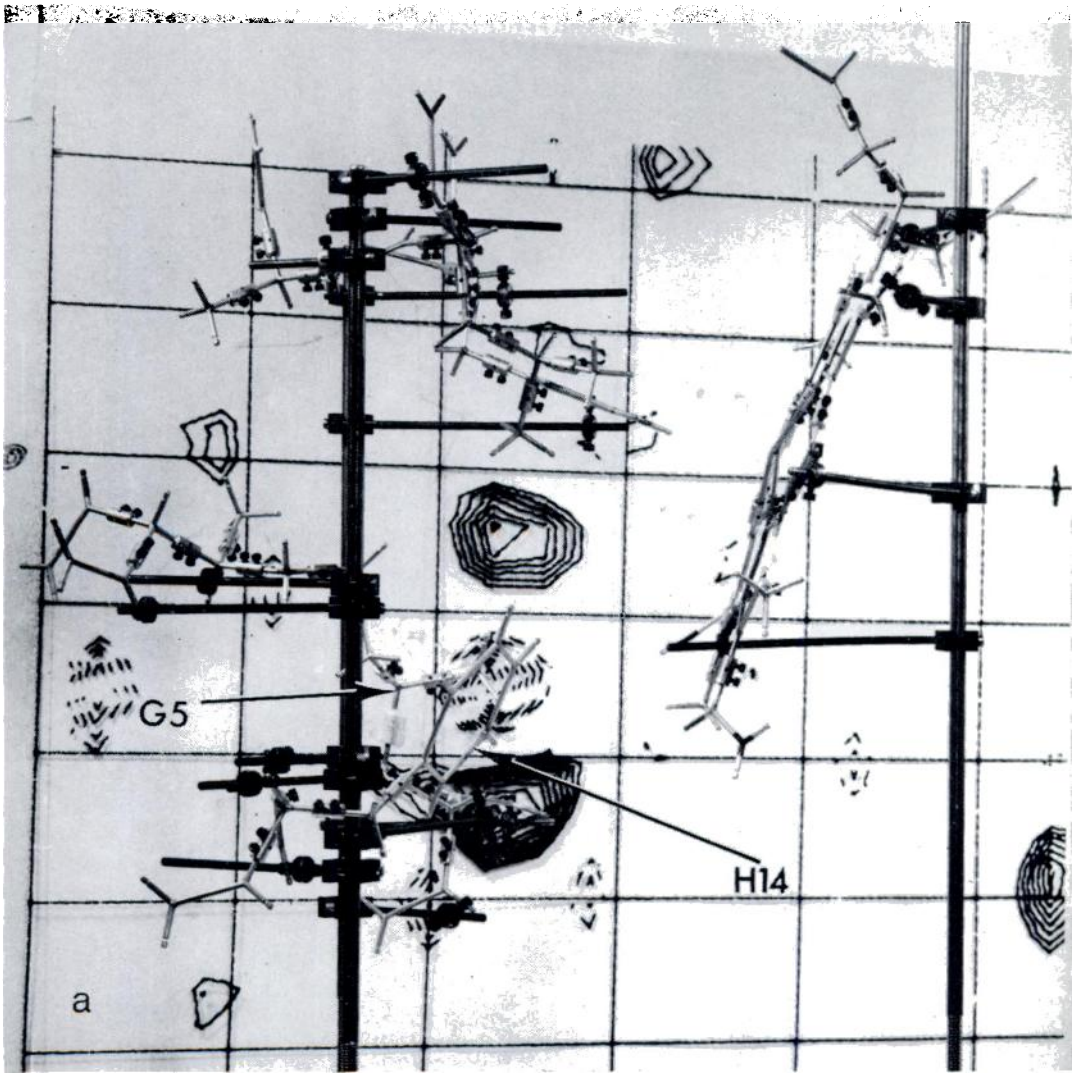

FIG. 2a. *Skeletal model of native metmyoglobin region involved in binding of dichloromethane superimposed on the hk0 difference electron density map*

FIG. 2b. *Probable structure of the derivative, depicting addition of dichloromethane and likely positions of side chains of phenylalanine H14 and leucine G5*

molecules of increasing size cause increasing deformation at the same binding site. Ethane, ethylene, acetylene, krypton, and nitrous oxide, with similar van der Waals radii and polarizability (Table 1), do not, however, bind to myoglobin.[3]

Previous observations have established that xenon and cyclopropane bind at the same location in myoglobin and that these compounds affect the CO affinity with increasing magnitude. It is now suggested that the still larger dichloromethane, which binds at the same site, increases the CO affinity still further. This may account for the observations of Stewart et al. (1).

[3] H. T. Magnussen and B. P. Schoenborn, unpublished observations.

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