Role of Water in the Stability of Oxyhemoglobin

Abstract. Electron exchange involving the valence states of iron occurs via water bridges. Molecular oxygen reversibly displaces the sole coordinated water of the ferrous iron in hemoglobin, and, in the absence of this ready path for electron transfer, the oxygen is transported without oxidation of the ferrous iron of hemoglobin.

The heme in hemoglobin combines reversibly with molecular oxygen without oxidation of the ferrous iron (*1*)

$$Fe_p^{II}(H_2O) + O_2 \rightleftharpoons Fe_p^{II}(O_2) + H_2O \quad (1)$$

while the ferrous iron of free heme is readily oxidized by oxygen. Because of the physiological importance of oxygen transport by hemoglobin, these reactions have attracted considerable attention, yet the causes of these differences in behavior have not yet been explained in an entirely satisfactory manner.

The hemes in hemoglobin are evidently buried within the protein molecule, and, as a consequence, the situation is complicated by the details of protein structure. Wang *et al.* (*2*) have suggested that the globin in hemoglobin partially screens the hemes with hydrophobic groups, thus tending to exclude water and altering the effective dielectric constant at the bonding sites. A hemoglobin model has been synthesized, and its properties have been studied in order to substantiate these views.

I have pointed out that processes in solution involving iron(III), and, by implication, iron(II), may be more dependent on specific solvation effects than on changes in the macroscopic dielectric constant (*3*). Furthermore, explanations which invoke the notion of the microscopic dielectric constant are inherently ambiguous since this concept is not amenable to direct experimental investigation.

Studies of the kinetics of the iron (II)-iron(III) electron-exchange reaction (*4*), especially in mixed solvent media (*5*), have yielded one conclusion relevant to the problem of the stability of oxyhemoglobin: that the 9- to 10-kcal/mole iron(II)-iron(III) oxidation-reduction process involves transfer of a single electron through waters of solvation. Electron transfer by alternative mechanisms is less favored energetically. This conclusion is supported by the observations that the iron(II)-iron(III) exchange does not occur in the absence of water (*5*), that the specific reaction rate constant goes through a maximum with increasing concentration of catalyzing, strongly-complexing anions (*6, 7*), that there is a pronounced deuterium effect (*8*), and that a cyanide ligand must be replaced by water in $Fe(CN)_6^{-4}$ before that species can be oxidized by hydroperoxides (*9*). Also, work on the iron(II)-iron(III) exchange, especially that of Hudis and Wahl (*6*) together with work on a multitude of other electron-exchange reactions, has clearly shown the absence of any systematic reactant charge-product dependency, and thus, presumably, the comparative unimportance of dielectric constant effects.

Now, in hemoglobin, four of the six coordination positions in iron(II) are occupied with the near-planar porphyrin system, one is occupied by the globin, and the sixth only is occupied by a water molecule (*1, 10*). This single water molecule, on the basis of the above arguments, must play a crucial role in oxidation processes, and when it is displaced, even by oxygen, no path will remain for the ready oxidation of iron(II). Oxyhemoglobin, therefore, should be quite resistant to oxidation. Any ligand, such as CO, F^-, CN^-, or even alcohols [assuming behavior similar to that of iron(III) (*3*)] which can displace the water of solvation of iron(II) should impair oxygen transport by hemoglobin and produce unfortunate physiological effects. In the instance of free heme, on the other hand, with presumably two waters of solvation, the situation is less critical, and even when one of the water molecules is replaced by oxygen or some ligand the remaining water will still provide a path for electron transfer.

Oxygenated heme ("oxyheme"), if formed, would be unstable in aqueous solution and would be oxidized immediately to the ferric state, just as certain synthetic, reversible oxygen-addition complexes of iron(II) that have been reported (*11*) are unstable in the presence of water.

$Mo(CN)_8^{-3}$ and $IrCl_6^{-2}$ oxidize hemoglobin and its derivatives (*12*). Possibly the species $Mo(H_2O)(CN)_7^{-2}$ and $Ir(H_2O)Cl_5^-$, carrying their own water bridges, are responsible for the oxidation. Dissociation of the derivative, as in the case of the oxidation of carbonmonoxyhemoglobin by $Fe(CN)_6^{-3}$, or displacement of the ligand of iron(II) by the anionic oxidant, may be a preliminary step. It is significant to notice that cationic oxidizing agents, such as $Fe(phenanthroline)_3^{+3}$ and $Ru(dipyridyl)_3^{+3}$, of equal or even greater oxidizing power, are ineffective (*12*), although size rather than coulombic repulsion may be critical in these cases.

Consideration of the role of water, together with specific protein influences, may well provide an explanation for the behavior of hemoglobin and related compounds without recourse to speculation concerning the microscopic dielectric constant at reaction sites.

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9 February 1959

Inheritance of Diego Blood Group in Mexican Indians

Abstract. Diego blood factor is characteristic of Mongoloid populations. A study of 152 samples from Mexican Indians (Tlaxcaltecans) revealed this factor in 20.39 percent of the cases. Analysis of 30 Indian matings with 62 children support the hypothesis that the Diego antigen is transmitted through a gene capable of expressing itself in a single or double dose.

The studies of the Diego blood factor (Di^a) were begun in 1954, when Levine, Koch, McGee, and Hill (*1*) demonstrated its presence in the serum of a Venezuelan mother whose baby had hemolytic disease. This antibody, besides having pathological importance, has proved to be of considerable anthropological interest, for it exists only exceptionally in the blood of the Caucasoid (*2*) and has not been found in Negroes (*3*), in Australian aborigines, or in Polynesians (*4*), while it has been detected in Mongoloid bloods in percentages ranging from 5 (in Chinese from Canton) (*5*) to 45.8 (in Kainganges Indians, in Brazil). According to our studies of a sample of 152 bloods taken from not-closely-related Tlaxcaltecan Indians with some slight Spanish mix-

Table 1. Inheritance of Diego blood factor (Exp., expected; Obs., observed).

Matings (Obs.)	Children			
	Di(a+)		Di(a–)	
	Exp.	Obs.	Exp.	Obs.
D × D (0)	0	0	0	0
D × d (11)	11.1	8	9.9	13
d × d (19)	0	0	41	41