+1 respectively. None of the oxidants are physiological partners for cytochrome *c*. Eight cytochrome *c* derivatives have been investigated with modifications at lysines 7, 13, 25, 27, 60, 72, 86 and 87. First-order rate constants were obtained at I = 0.10 *M* (NaCl) with the oxidant always in large excess. The influence of the modified residues on the bimolecular rate constants defines the protein molecular surface involved.

With the inorganic 3– and 3+ oxidants at pH 7.2 the site for electron exchange is the solvent accessible edge of the heme prosthetic group or a closely related structure on the front surface of the molecule. The reaction with $Fe(CN)_6^{3-}$ is most strongly influenced by modification of lysine 72 (3.6-fold decrease in rate constant), a residue to the left of the exposed heme edge. This same locality has been defined from NMR studies as a region where interaction of cytochrome *c* with $Fe(CN)_6^{3-}$ occurs [8]. However, it is the region around lysine 27, to the right of the heme edge, which is most influential in the reaction with $Co(phen)_3^{3+}$ (7.3-fold increase compared to native).

With the blue Cu proteins, the interacting domain on cytochrome *c* is again located at the front surface of the protein and encompasses the solvent accessible exposed edge of the heme group. The rate constant for the reaction with parsley plastocyanin (1.5×10^6 M^{-1} s^{-1}) decreases by half (0.75×10^6 M^{-1} s^{-1}) for the lysine 13 derivative, which is the most influential modification. Conversely the reaction with azurin (–1 charge), which was studied at pH 6.1 and 8.6 (at either extremity of a pH transition), gave rate constants 6.6×10^3 M^{-1} s^{-1} at pH 6.1 and 4.0×10^3 M^{-1} s^{-1} at pH 8.6, which increase on modification to 4.1×10^4 M^{-1} s^{-1} (pH 6.1) and 2.7×10^4 M^{-1} s^{-1} (pH 8.6) for the most influential modification at lysine 72. The reaction with cytochrome *c* occurs at a negative site on plastocyanin, whereas azurin behaves as a positively charged reactant, and displays very similar characteristics to stellacyanin (+7) and *A. variabilis* plastocyanin (+1). Thus with the latter at pH 7.0 the rate constant of 2.5×10^3 M^{-1} s^{-1} for native increases on modification at lysine 72 to 2.2×10^4 M^{-1} s^{-1} for stellacyanin and at pH 7.5 a value of 7.3×10^4 M^{-1} s^{-1} for *A. variabilis* plastocyanin increases to 2.6×10^5 M^{-1} s^{-1} for the lysine 72 modification.

The differences in reactivity patterns for the blue Cu proteins probably reflect the different distributions of charged and uncharged residues on the surface of the proteins, which result in different charge as well as other secondary interactions. They do not necessarily indicate the use of different sites on cytochrome *c* as is implied with the inorganic oxidants. Indeed the reactions of the blue Cu proteins with cytochrome *c* are non-physiological, and these proteins have not been subjected to selective evolutionary forces that would have resulted in favourable co-adaptation of surface domains relevant to electron transfer. Thus the bimolecular rate constants for these reactions are small compared to those involving electron exchange with the natural redox partners for cytochrome *c*. Also we note that the lysine modification effects reported here are small compared with the massive effects observed for the physiological partners [9].

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A Comparative Study of Free and Polymer Immobilized Hemin Catalysts of Oxidation of Cystein

V. K. IMSHENNIK, I. P. SUZDALEV

Institute of Chemical Physics, Academy of Sciences, Moscow, U.S.S.R.

A. N. ASTANINA, V. D. KOPYLOVA, P. Yu. TRUSOV

Moscow State University, Moscow, U.S.S.R.

Iron porphyrins attached to side ionizable groups of ion exchange polymers (styrene–divinyl benzene matrix) may be considered as model compounds of heme proteins.

We studied the conditions of immobilization of hemin (iron(III) protoporphyrin IX chloride) on different ion exchange polymers and the catalytic activity of bound and free hemin species in the oxidation reaction of cystein (RSH):

$$4RSH + O_2 \rightarrow 2RS{-}SR + 2H_2O$$

TABLE I.

Ionizable ligands	Content hemin ($mg/g_{polym.}$)	Kinetic $W \times 10^4$ M RSH L.s.$g_{pol.}$
NHCH:NCH:CH[a] (imidazole)	5.0	12.1
=NH; ≡N; ≡N–	7.5	9.2
=NH; $-NH_2$	2.4	7.5
$-NH_2$; =NH; ≡N	2.0	7.3
$C_6H_5NCH_3$	2.3	4.5
COOH[b]	<0.1	4.6
$Po(OH)_2$	<0.1	0
$SO_2(OH)$	0	0
C_6H_5NCOOH[c]	<0.1	3.7
$C_6H_5NCH_3$; $PO(OH)_2$	<0.1	3.0

[a]Anion. [b]Cation. [c]Ampholyte type ligands.

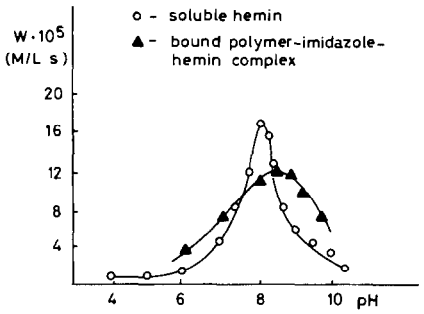

Fig. 1. Catalytic activity.

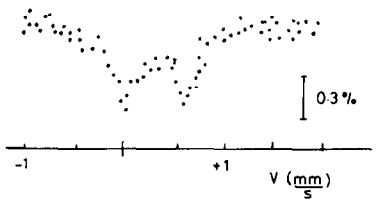

Fig. 2. Mössbauer spectrum of polymer–imidazole–hemin complex at 77 K.

It was found that the adsorption of hemin was higher for polymers with anion type ligands compared with polymers with cation type ones. The ESR spectra of bound species were similar to free hemin ones ($g \simeq 6$, $g \simeq 2$) and were used with optical spectra for controlling the binding process.

The oxidation reaction was obtained at 40 °C in 10 mL water solution containing 2×10^{-3} *M* RSH and 0.1 g polymer-bound hemin species. The highest catalytic activity was observed on polymer–imidazole–hemin complex (1st line of Table I, Fig. 1.)

The reaction for free hemin is first order in RSH, hem, O_2: $W = kC_{RSH}C_{hem.}C_{O_2}$ with concentration of RSH ($5 \times 10^{-2} \div 3 \times 10^{-1}$ *M*); hemin ($3.2 \times 10^{-6} \div 4 \times 10^{-4}$ *M*); O_2($0.9 \times 10^{-3} \div 8.1 \times 10^{-3}$ *M*). The pH dependence of W of bound and free hemin is shown in Fig. 1.

The shift of the peak of the Soret band up to 388 nm of soluble hemin shows some aggregation of hemin in solution. The Mössbauer spectra of polymer-bound hemin species at 300 K and 77 K yield resolved quadrupole doublets with nearly the same values for ΔE_Q (0.7–0.8 mm/s) and δ_{Fe}(0.4–0.5 mm/s) that correspond to high spin ferric states. The differences between these spectra and the unresolved doublet of free hemin arise from Fe–ligands binding and changes in electronic spin relaxation rate due to the distribution of hemin through the polymer matrix. A more detailed Mössbauer study will be carried out with ^{57}Fe enriched samples and measurements at helium temperatures.

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Energetic Correlation of Oxygen Affinities and Soret Absorption Maxima of Deoxyhemeproteins

R. J. KASSNER

Department of Chemistry, University of Illinois, Chicago, Ill. 60680, U.S.A.

The oxygen affinities of hemeproteins have been shown to exhibit a wide range of values [1]. The structural perturbations which influence the oxygen affinity and other properties of these proteins have not been fully elucidated. It has previously been observed that a shift in the positions of the Soret absorption maxima of the α and β chains of hemoglobin is associated with a change in oxygen affinity [2]. A comparison of the physical chemical properties of various monohemeproteins has revealed that a correlation exists between the oxygen affinities and the Soret absorption maxima of the deoxyhemeproteins. Table I shows the $P_{1/2}$ values, corresponding

TABLE I. O_2 Affinities and Soret Absorption Maxima of Deoxyhemeproteins.

Hemeprotein	$P_{1/2}$[a] Torr	λ nm	Ref.
Aplysia Mb	2.7	438	1, 3
Horse Mb	0.70	435	4, 5
Sperm Whale Mb	0.51	434	6, 7
Human Hb α chain	0.46	430	8, 2
Human Hb β chain	0.40	428.5	8, 2
Soybean Lb	0.05	427	9, 10

[a]Pressure of oxygen for one-half oxygenation at pH 7.0 and 20 °C.