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Infrared Spectra of Carbonyl Lactoperoxidase

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Lactoperoxidase (LP) [1] is a remarkable enzyme, being an efficient scavenger of peroxides, an oxidant of usual peroxidase substrates and halide ions [2] while also catalyzing the disproportionation of dithionite [3]. Like other peroxidases LP has a low redox potential and a protoheme prosthetic group, but a mixed spin type spectrum and a strong heme–protein attachment single out LP as being unique. For this reason we have studied the CO stretch of LP•CO at pH 5–9.

The CO stretch of carbonyl horseradish peroxidases (HRP) A and C is pH dependent in terms of number and positions of ir bands [4]. This was interpreted as indicating the presence of a titratable group near the active center influencing the carbonyl resonator at low pH by H-bonding to the oxygen atom in CO. The ir spectrum of LP•CO exhibits CO stretches at 1940, 1955, and 1961 cm^{-1} (Fig. 1 Spectra at intermediate pH values omitted). The 1940 cm^{-1} band predominates, and its position is independent of pH and buffer species. The 1955 cm^{-1} band is intense at pH 5, gives a shoulder at pH 7, and has vanished at pH 9. The 1961 cm^{-1} band changes in the reverse manner. The low intensity and essentially pH-independent band at ~1913 cm^{-1} may not be related to bound CO.

The ir spectra of LP and HRP A, C have in common *a*) a pH-independent stretch at 1937 ± 3 cm^{-1} with a half-band width of 12 cm^{-1}; *b*) a broader band, sensitive to protons or dipoles. Stretch *a*) is attributed to CO in a narrow, apolar environment, whereas in *b*) CO may dwell in a more open site. The balance between the 1955 and 1961 cm^{-1} stretches

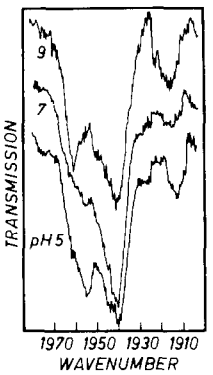

Fig. 1. CO lactoperoxidase (2–2.5 m*M* heme) reduced with dithionite at pH 9 (50 m*M* borate, 1 m*M* EDTA); pH 7 (50 m*M* 3,3-dimethylglutarate, 1 m*M* EDTA); pH 5 (50 m*M* citrate, 1 m*M* EDTA). Digilab Interferometer FT-14C.

may depend upon a change in polarity due to a proton or, more likely, a polar group. The properties *a*) and *b*) seem to be unique to peroxidases, although at present there is no explanation of why stretch *b*) is located on opposite sides of *a*) in HRP and LP. The active site of lactoperoxidase may be different from that of other peroxidases, either lacking the distal base, or the ligand binding site being rigid and preventing carbonyl interaction with the distal base [5].

The redox potentials of LP and HRP A, C (–180, –212, and –265 mV) and ν_{CO} (1940, 1938, and 1933 cm^{-1}) of the *a*) stretch follow the correlation found for other heme proteins [4].

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