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Short Communications

Artificial Peroxidases

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A reinvestigation of the activities of some artificial peroxidases has given results somewhat at variance with those previously [1-3] reported. The reasons are at present unknown, and the results are presented without comment.

The horse radish peroxidase was prepared, split, and combined with various hemins as previously described [4,5]. The activities were measured by the mesidine method [6] which is well fitted even for the determination of weak activities since no appreciable decolourization occurs. For convenience the activities were expressed as the increase in absorbancy (A) at 490 mμ per min for a micromolar protein concentra-

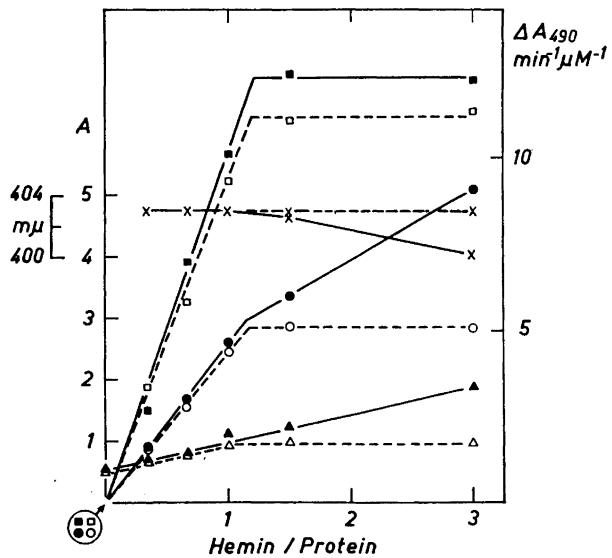

Fig. 1. Effect of the ratio between protohemin and apoprotein on enzymatic activity and light absorption in recombination experiments. All solutions were 28 μM in apo-HRP with 50 mM phosphate of pH 7.3 as buffer. Activities and spectra were determined after 40 h at 4°C (full drawn curves and filled symbols). Aliquots were chromatographed on Dowex 2, previously washed with the buffer until A< 0.01. Five retention volumes were collected and examined (dashed lines and open symbols). The next fifteen volumes gave no light absorption. Right scale and squares: Enzymatic activity of apoprotein with varying proportions of hemin. Left scale: Absorbances at the top of the Soret band (circles) and at 280 mμ (triangles) for 28 μM protein. Short scale and crosses: Wavelength for the maximum of the Soret band.

tion. The ratio of two for hemin to protein was used, thus ensuring that the native apoprotein was saturated with the prosthetic group. All protein concentrations were based upon micro-Kjeldahl determinations.

The activity of unsplit HRP has been determined for four preparations and ΔA_{490} min^{-1} μM^{-1} was found to be 10.6, 11.4, 12.1, and 11.8, average 11.5.

Fig. 1 confirms the observation that the hemin that is nonspecifically attached to the apoprotein is removed by an anion exchanger. The hemin bound as the prosthetic group of HRP and the hemin present in excess of the protein moiety differ in their effects on the light absorption in the Soret band region. At 280 mμ, however, no such difference can be seen. The activity of the recombined HRP in Fig. 1 was found to be 12.4 min^{-1} μM^{-1}.

Table 1. Enzymatic activities of some artifical peroxidases.

Hemin	Positions of the carboxyl groups in the prophyrin ring	Activity ΔA_{490} min^{-1} μM^{-1}	Positions of the α-band of pyridine hemochromogen, mμ
Protohemin IX	6,7	11.5	557
Hematohemin IX	6,7	15.5	548
Mesohemin IX	6,7	15.7	547
Deuterohemin IX	6,7	6.1	545
Diacetyl-deuterohemin IX	6,7	0.3	582
Mesohemin I	6,8	0.4	547
Coprohemin I	2,4,6,8	0.1	548
Coprohemin III	2,4,6,7	0.1	548

When seven different preparations of protohemin were used in recombination experiments with the same batch of apoprotein, the activities of the holoenzymes were found as 9.9 ± 0.8 (S.D.). Since all activity values are based on nitrogen determinations, the somewhat low value may be caused by a partial inactivation of the apoprotein.

Some results with various hemins are given in Table 1. Mesohemin IX peroxidase and hematohemin IX peroxidase were 1.3 times more active than the unsplit peroxidase, whereas deuterohemin IX peroxidase was only 0.5 times as active. Diacetyldeuterohemin IX does combine with the apoenzyme, as reported previously [5], but the resulting hemoprotein is inactive in this assay. The hemins with propionic acid residues in the six and eight positions are inactive, probably because of inability to react in an adequate way with the apoprotein. In the case of the four-carboxylic hemins the lack of activity may depend upon the nature of the hemin polymer.

There seems to be some correlation between the spectrum and the enzymatic activities of the meso-, hemato-, proto-, and diacetyldeuterohemin IX peroxidases.

A full report will be published.

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Deuterohäminperoxydase

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In früheren Untersuchungen wurde festgestellt, dass Meerrettichperoxydase — Apoeiweiss (Apo-MRP) sich mit gewissen Häminen kuppeln lässt, wobei peroxydatisch aktive Verbindungen entstehen [1]. So geben Proto-, Meso- und Hämatohämin IX, die alle Propionsäuren in den Stellungen 6 und 7 besitzen, aktive Peroxydasen, während Diacetyldeuterohämin IX zwar gekuppelt wird, die Verbindung ist aber peroxydatisch inaktiv [2]. Deuterohä-