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 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. The activities were measured by the mesidine method which is well fitted even for the determination of weak activities since no appreciable decolorization occurs. For convenience the activities were expressed as the increase in absorbancy (A) at 490 m\(\mu\) per min for a micromolar protein concentra-

![Graph](image)

*Fig. 1.* Effect of the ratio between protohemin and apoprotein on enzymatic activity and light absorption in recombination experiments. All solutions were 28 \(\mu\)M 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 m\(\mu\) (triangles) for 28 \(\mu\)M 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 $\Delta A_{405}$ min$^{-1}$ $\mu$M$^{-1}$ 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 m$\mu$, however, no such difference can be seen. The activity of the recombined HRP in Fig. 1 was found to be 12.4 min$^{-1}$ $\mu$M$^{-1}$.

Table 1. Enzymatic activities of some artificial peroxidases.

<table>
<thead>
<tr>
<th>Hemin</th>
<th>Positions of the carboxyl groups</th>
<th>Activity of pyridine, $\Delta A_{405}$ min$^{-1}$</th>
<th>Positions of the $\alpha$-band of the prophyrin ring</th>
<th>Activity of pyridine, $\Delta A_{405}$ min$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protohemin IX</td>
<td>6,7</td>
<td>11.5</td>
<td>557</td>
<td></td>
</tr>
<tr>
<td>Hematohemin IX</td>
<td>6,7</td>
<td>15.5</td>
<td>548</td>
<td></td>
</tr>
<tr>
<td>Mosohein IX</td>
<td>6,7</td>
<td>15.7</td>
<td>547</td>
<td></td>
</tr>
<tr>
<td>Deuterohein IX</td>
<td>6,7</td>
<td>6.1</td>
<td>545</td>
<td></td>
</tr>
<tr>
<td>Diacetyl-deuterohein IX</td>
<td>6,7</td>
<td>0.3</td>
<td>582</td>
<td></td>
</tr>
<tr>
<td>Mosohein I</td>
<td>6,8</td>
<td>0.4</td>
<td>547</td>
<td></td>
</tr>
<tr>
<td>Coprohemin I</td>
<td>2,4,6,8</td>
<td>0.1</td>
<td>548</td>
<td></td>
</tr>
<tr>
<td>Coprohemin III</td>
<td>2,4,6,7</td>
<td>0.1</td>
<td>548</td>
<td></td>
</tr>
</tbody>
</table>

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. Mosohein IX peroxidase and hematohemin IX peroxidase were 1.3 times more active than the unsplit peroxidase, whereas deuterohein IX peroxidase was only 0.5 times as active. Diacetyldeuterohein IX does combine with the apoenzyme, as reported previously, 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 diacetyldeuterohein IX peroxidases.

A full report will be published.


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Deuterohäminperoxidase

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In früheren Untersuchungen wurde festgestellt, dass Meerrettichperoxidase — Apoeweiß (Apo-MRP) sich mit gewissen Häminen kuppelt; wobei perox dytisch aktive Verbindungen entstehen 4. So geben Proto-, Meso- und Hamatohämin IX, die alle Propionsäuren in den Stellungen 6 und 7 besitzen, aktive Peroxidasen, während Diacetyldeuterohämin IX zwar gekuppelt wird, die Verbindung ist aber peroxydatisch inaktiv 5. Deuterohäminperoxidase:

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