BIOCHEMISTRY and PHYSIOLOGY

Peroxidases from Wheat Germ

A PREVIOUS communication[1] described the preparation of two crystalline hæmoproteins (556) and (566), which possess strong peroxidatic activity. It was found in later investigations[2] that both hæmoproteins form complexes with hydrogen peroxide and are not reduced with diphosphopyridine nucleotide or succinate in the presence of an active homogenate or particles from wheat germ. This indicates that both hæmoproteins (556) and (566) are peroxidases and thus can be called peroxidase (556) and (566), respectively.

In the previous work, peroxidase (566) was obtained in very low yield. When the first ammonium sulphate precipitate cake from wheat germ extract was kept for several months, only peroxidase (556) and no peroxidase (566) was obtained. However, in the direct spectroscopic examination of wheat germ after reduction with sodium dithionite the predominant band is at 560–570 mμ, and that at 556 mμ is very weak.

We have therefore adopted a new method for the preparation of peroxidase (566), as follows. Wheat germ, without pretreatment by organic solvents, was suspended in five volumes of 0·1 *M* phosphate buffer of *p*H 7·0 and squeezed by pressing through a cloth. The fraction of the filtrate precipitated between 20 and 60 per cent saturation with ammonium sulphate was dialysed. It was then adsorbed on 'Amberlite' *CG*–50 equilibrated at *p*H 6·5 with 0·02 *N* ammonium phosphate buffer. After elution with 0·25 *N* buffer of *p*H 7·0, the eluate—in which peroxidase (566) was present in relatively larger amount but which contained no peroxidase (556)—was again adsorbed on 'Amberlite' *XE*–64 and eluted successively with 0·15 *N* and 0·25 *N* buffer of *p*H 7·0. Both fractions were separately chromatographed on 'Amberlite' *XE*–64 resin columns using 0·075 *N* and 0·10 *N* buffer of *p*H 7·0, respectively. Crystalline peroxidase (566), which has an octahedronal form, was obtained from both main effluents in the same way as in the earlier methods[1] (Fig. 1).

Peroxidase (566) thus obtained forms a stable complex with hydrogen peroxide which cannot be identified with any of the established complexes. This complex has absorption peaks at 415 mμ, 540 mμ and 570 mμ (Fig. 2. The Soret peak was determined by means of a Beckman spectrophotometer). Peroxidase (556), however, forms a very labile complex similar to complex II. Peroxidase (556) and (566) may be identified with peroxidase II and I, respectively, prepared from horse radish by Theorell[3]. According to Keilin and Hartree[4] peroxidase I is a modified form of peroxidase II.

Fig. 1. Crystalline peroxidase (566). (Octahedron, × 200)

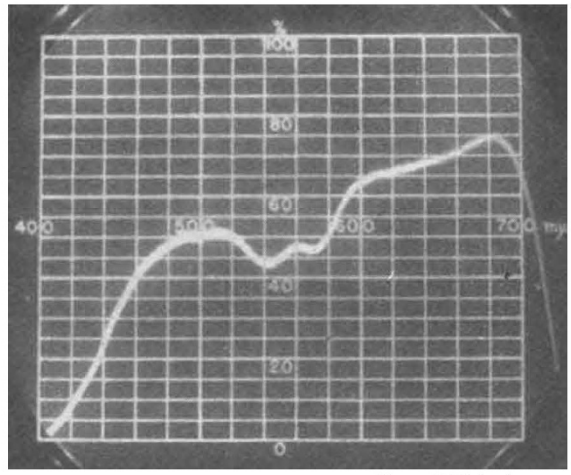

Fig. 2. Absorption spectrum of peroxidase (566)–hydrogen peroxide complex obtained with a rapid-scanning spectrophotometer. Reaction mixture contained about 0·5 per cent peroxidase (566), 0·005 *M* hydrogen peroxide and 0·06 *M* phosphate buffer of *p*H 6·0. The figure shows the spectrum of the complex in the visible region 5 min. after addition of hydrogen peroxide

Recently, Lundegårdh[5] obtained a peroxidase similar to peroxidase (556) from wheat roots treated with cold acetone. However, from the properties and behaviour during the preparation of the peroxidases, it seems that peroxidase (566) is the native protein, whereas peroxidase (556) is a modified form. Other properties of peroxidases (556) and (566) are being studied.

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[2] Tagawa, K., and Shin, M. (unpublished work).
[3] Theorell, H., *Ark. Kemi Min. Geol.*, **15**, B, No. 24 (1941).
[4] Keilin, D., and Hartree, E. F., *Biochem. J.*, **49**, 88 (1951).
[5] Lundegårdh, H., *Nature*, **181**, 28 (1958).

Factors affecting the Rate of Bilirubin Conjugation *in vitro*

THE conjugation of bilirubin to its glucuronides in rat liver homogenates was studied, using boiled liver extract[1], uridine diphosphoglucuronic acid (UDPGA), uridine diphosphoglucose (UDPG) with diphosphopyridine nucleotide (DPN+), or the system consisting of uridine triphosphate (UTP) with glucose-1-phosphate (G–1–P), and diphosphopyridine nucleotide as sources of the glucuronosyl radical. The effects of various substances on the formation of bilirubin glucuronides from bilirubin are listed in Table 1. Under the conditions used in these experiments, liver homogenates hydrolysed 5·7 per cent of added phenolphthalein glucuronide (0·42 m*M*) in 30 min., this hydrolysis being inhibited (44·5 per cent) by 5·5 m*M* potassium glucuronate, and even more