Moss oxalic acid oxidase — A flavoprotein

After its discovery by HOUGET, MAYER AND PLANTEFOL[1,2], moss oxalic acid oxidase has been studied by FRANKE *et al.*[3,4] and NIEKERK-BLOM[5]. However, the nature of this peculiar enzyme was not elucidated. FRANKE *et al.*[3,4], on the basis of the fact that their enzyme solutions did not exhibit fluorescence, were critical of the idea that the oxidase could be a flavoprotein. In the following, evidence will be given which permits the conclusion that this oxidase, contrary to FRANKE's belief, is indeed such a protein. The active group probably is riboflavin or riboflavin phosphate (Flavine mononucleotide, FMN), not flavine adenine dinucleotide (FAD).

A dozen species belonging to the *Bryales* were tested and all of them were found to be active, including two species normally growing in water (namely, a *Fontinalis* species and an unidentified aquatic moss). The material was collected at various locations in the State of Washington, in the spring of 1955. The best results were obtained with *Hylocomium splendens, Rhytidiadelphus squarrosus* and *Hylocomium loreum*. The thoroughly cleaned moss, dried at 35 °C, was stored in the form of a powder which kept its activity for a long time. Even vigorous boiling of the powder in water for a 15 minute period did not destroy this activity. However, it did change the respiratory quotient observed in the oxalate oxidation by the powder in Warburg vessels; whereas the nonheated powder exhibited an R.Q. definitely higher than 2 and sometime approaching 4, the boiled powder shows one very close to 2. The explanation for this phenomenon, forwarded by NIEKERK-BLOM[5], is that the oxalate decomposition in unheated moss or moss powder is due to the combined activities of oxalic oxidase and catalase, as follows:

$$\text{I. } (COOH)_2 + O_2 \longrightarrow 2\,CO_2 + H_2O_2 \text{ (oxidase) R.Q. } = 2$$
$$\text{II. } H_2O_2 \longrightarrow H_2O + \tfrac{1}{2}\,O_2 \text{ (catalase).}$$

$$(COOH)_2 + \tfrac{1}{2}\,O_2 \longrightarrow 2\,CO_2 + H_2O \quad \text{R.Q. } = 4$$

Heating destroys the catalase without appreciably damaging the oxidase, so that hydrogen peroxide will accumulate and the R.Q. must drop to 2. Final proof that this concept is correct was given by the present writers by "reconstructing" the system, out of boiled moss powder and crystalline beef catalase (Fig. 1).

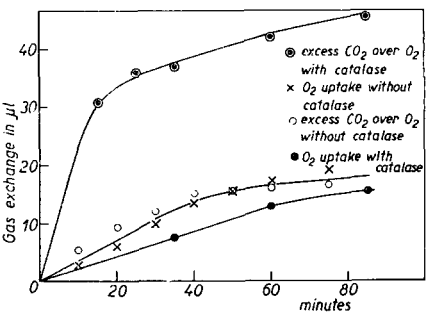

Fig. 1. Oxalate decomposition by boiled moss powder and by boiled moss powder with beef catalase. Warburg experiment, temperature 20° C. Main compartment: 50 mg of boiled and dried moss powder, 1.8 ml *M*/15 phosphate buffer pH 5.0, 0.8 ml water, 0.2 ml solution of crystalline beef catalase (or water). Side arm: 0.2 ml *M*/10 oxalate pH 5.0. Center well: 0.2 ml water or 0.2 ml KOH solution. Plotted: oxygen-consumption and excess CO_2-production over oxygen-consumption. The R.Q.'s derived from this graph, for a one hour period, are 4 for the experiment with and 2 for the experiment without catalase.

The fact that the enzyme shows hardly any KCN-sensitivity and that it produces hydrogen peroxide, can be interpreted as evidence for the flavoprotein nature of the enzyme. Better evidence was obtained by the present writers by the use of a special, cell-free, water-clear solution prepared from moss extracts, which contained the enzymic protein in a somewhat purified form. This fluid alone did not show great activity when allowed to act on oxalic acid, but it could be stimulated very markedly by additions of riboflavin or riboflavin phosphate (Figs. 2 and 3); flavine adenine dinucleotide gave only slight stimulation. In control experiments, the flavin compounds alone (without the protein fraction obtained from moss) gave a negligible oxidation of the oxalic acid. Equimolecular concentrations of riboflavin and riboflavin phosphate gave the same degree of activation (Fig. 3). Since this happened even when strongly dialyzed enzyme was used in a malate buffer, the possibility that the riboflavin is phosphorylated seems remote. Also, additions of sodium arsenate did not have any inhibiting effect on the activation of the protein fraction by riboflavin. This point and the possible role of the enzyme in the metabolism of the moss plant, form the subject of continued investigations.

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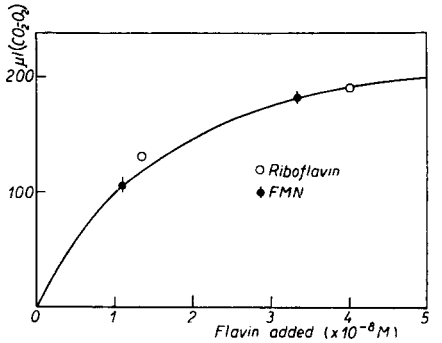

Fig. 2. Activation of the protein component of moss oxalic acid oxidase by various flavin compounds. Warburg experiment, temperature 21° C. Main compartment: 1 ml of apo-enzyme, 1 ml *M*/15 phosphate buffer pH 4.0, 1 ml of aqeuous flavin solution. Side arm: 0.2 ml of *M*/10 oxalate pH. 3.9. No KOH in center well. Measured: excess CO_2-production over O_2-consumption. The blank, without any addition of flavin (not plotted), was very close to zero.

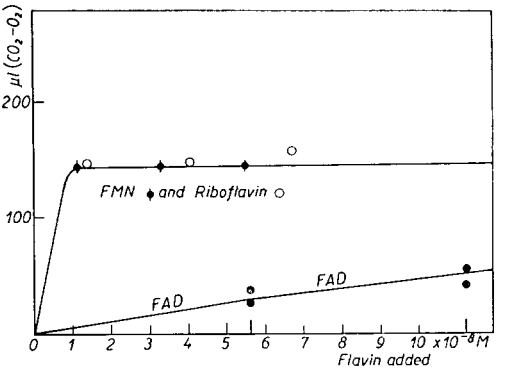

Fig. 3. Comparison of the activating effect of riboflavin and riboflavin phosphate on the apo-enzyme of moss oxalic acid oxidase, in *M*/15 malate buffer pH 4.0. Otherwise conditions as in Fig. 2.

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Particle size and chemical composition of the crystallites in bone and synthetic apatites

The form and size of the crystallites in bone has been determined by several methods. From the broadening of the reflections in the high-angle X-ray diffraction patterns it was soon clear that the particles were elongated in the direction of the *c*-axis and their length has been measured to 200–290 A[1,2,3,4]. The width is much less and cannot be settled with any accuracy from line-broadening measurements. However, the diffuse particle scatter in the low-angle X-ray region has revealed that the rod-shaped crystallites have a length of 210–220 A and a width of 65–75 A[5,6]. Electron microscopy of bone on the other hand, has shown that the crystallites are tabular with dimensions of about 350 × 400 × 25–50 A[7]. Tabular crystals, only a few unit cells thick, having a large extension in two dimensions have been observed in some apatite-like precipitates[4,8], but long needle-shaped crystallites, apparently hexagonal prisms, are also found in such synthetic samples[9].

It is a well-known fact that bone as well as precipitates in the CaO-P_2O_5-H_2O-system show a wide variation in chemical composition although their X-ray diffraction patterns are identical. This discrepancy has been interpreted in various ways and several types of calcium phosphates having an apatite structure and molar Ca to P-ratios ranging from 1.3 to 2.0 have been proposed.