ENDOGENOUS CATALASE AND SUPEROXIDE DISMUTASE ACTIVITIES IN PHOTOSYNTHETIC MEMBRANES

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1. Introduction

The photochemical activity of chlorophyll–protein complexes isolated from chloroplast membranes is of considerable interest for the eventual understanding of the mechanism of photosynthesis. Complexes, highly enriched in the photosystem I reaction center chlorophyll, P-700, are being investigated in a number of laboratories [1–3]. The photoreduction of oxygen with electrons from ascorbate has been an often studied photosystem I reaction [4–6]. These studies have indicated the functional need for catalase [7] and superoxide dismutase (SOD) [8] activities close to photosystem I in order to protect the membranes from damage by free radicals which could be formed from superoxide and hydrogen peroxide [9,10].

Therefore, we tested a P-700-chlorophyll–protein complex purified according to [2] for catalase and SOD and found both of these enzymic activities in our preparations. These results posed the question as to whether these enzymes are integral components of photosystem I, or whether catalase, at least, is entirely peroxisomal in origin but adhered to chloroplast membranes during their isolation [11]. The catalase question was addressed [12] by carefully preparing intact chloroplasts and washing them 5 times to remove most of this enzyme. The results showed not only that the surface catalase was removed by the washings, but also that the catalase activity of osmotically-shocked, washed chloroplasts decreased down to the same low level as before shocking (~ 2 μmol O_2 evolved mg $chl^{-1} \cdot min^{-1}$ from an initial value of 30). It was concluded that this low level of activity was insignificant, and that catalase is not an endogenous chloroplast enzyme.

Because the final low level of activity observed in [12] was about the same as we had found in our purified chlorophyll–protein complex, we prepared our complex from chloroplasts which had been washed 12 times according to the procedure in [12]. Our objective was to remove as much of the peroxisomal catalase as possible from isolated intact chloroplasts and then to determine the partition of the remaining catalase activity among chloroplast fractions. As will be shown below, even though on a chlorophyll basis the level of catalase activity was very low in these washed plastids, the specific activity was > 3-times higher in a purified photosystem I reaction center-chlorophyll *a*–protein complex. In another experiment the catalase activity in a suspension of chloroplast fragments broken in a French pressure cell was compared with centrifugal fractions of these fragments enriched either in photosystem I or II. Again it was found that the specific activity was ~ 4-times higher in the system I particles than in the original suspension or system II particles. These results strongly suggest that catalase activity is an integral part of photosystem I. As in [13] we found SOD enriched in our photosystem I preparations.

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2. Experimental

Chloroplasts were isolated in 0.33 M sorbitol, 50 mM Tris (pH 7.8) from about 5 kg market spinach. The deveined leaves were first chopped, then blended for 60–90 s with a polytron, and finally filtered 3 times through 2 layers of Miracloth and 8 layers of cheesecloth each time. The chloroplasts were sedimented by bringing the centrifuge rotor to 2000 × *g* and down again within 75 s. The sediment was washed 12 times with the same isotonic buffer, including 2 times (washes 6 and 7) with the addition of EDTA (final 2 mM) and the same centrifugation regime. Each washing suspension contained about 8 mg chlorophyll in 40 ml buffer.

A P-700-chlorophyll *a*–protein complex was purified as in [2] from the washed chloroplast pellet (17 mg chlorophyll) by homogenization in 128 ml 1% Triton X-100 and chromatography on hydroxylapatite. During this procedure ~80% of the chlorophyll passed through the column (Triton eluate). Most of the remaining chlorophyll was removed from the column by further washing with 1% Triton, and an aliquot of this latter eluate was labeled 'Triton wash'. After washing the column with sufficient 10 mM phosphate buffer (pH 7.8) to remove most of the Triton, a chlorophyll–protein complex containing < 1% of the original chlorophyll *a* was eluted in 0.2 M phosphate. The photooxidation of P-700 was measured in a Perkin Elmer Dual Beam spectrophotometer according to [2] and the ratio of chlorophyll *a* to P-700 found to be ~40; an ~10-fold enrichment of the reaction center in the complexes.

In a second experiment, spinach chloroplast fragments which had been prepared in 0.15 M KCl, 50 mM Tris (pH 8) and washed twice in 50 mM Tris, but without the precautions described above to keep them intact, were forced through a French pressure cell twice. The homogenate was centrifuged at 10 000 × *g* for 30 min (10 K-sed), and the resulting supernatant was spun at 144 000 × *g* for 60 min (144 K-sed). The 144 K-sed was washed twice by resuspension in 50 mM Tris and repeated centrifugation.

Catalase activity was determined in a Clarke-type oxygen electrode attached to an E and K recorder by measuring the rate of O_2 evolution following addition of 4 μl 1% H_2O_2 to 4 ml sample in the electrode chamber at 25°C. SOD was measured by its inhibition of the rate of nitro blue tetrazolium (NBT) reduction by O_2^- generated when xanthine oxidase was added to xanthine [14]. The rate of dye reduction was followed at 560 nm in a Cary 17 spectrophotometer. All chemicals for the assay were purchased from Sigma Biochemical Co. One unit of SOD has been defined as the amount that causes a 50% decrease in the rate of NBT reduction. Incidentally, we observed that the control rate of NBT reduction by O_2^- was so rapid in 0.5% Triton that the assay could not be used for measuring SOD activity in the presence of this detergent.

3. Results and discussion

Values in table 1 show that our isolated chloroplasts before or even without extensive washing exhibited a very low level of catalase activity; at least 40-times less than that reported in [12]. However, the significant point is that the P-700-chlorophyll–protein complex which contained < 1% of the chlorophyll had a 2–4-fold higher specific catalase activity than had the original washed chloroplasts. All this activity was inhibited by 1 mM azide.

It is well established that the French press fractionation procedure used here [15] produces particles in the 144 K-sed which are enriched in photosystem I. We measured the photooxidation of

Table 1
Catalase activity of chloroplasts, Triton homogenate and chlorophyll-containing fractions

Exp. sample	Catalase activity (μmol O_2 mg $chl^{-1} \cdot min^{-1}$)
1. Chloroplasts (first isolated)	0.50
Chloroplasts (washed 12 times)	0.30
Triton–chloroplast homogenate	0.40
Triton eluate	0.12
Triton wash	0.04
Chlorophyll–protein complex	1.07
2. French press homogenate	0.57
10 K-sed	0.62
144 K-sed	2.75
144 K-sed, washed 2 times	2.00

P-700 in the fractions and found > 2-times more of this photosystem I reaction center chlorophyll in the 144 K-sed as in the 10 K-sed or original homogenate. We cannot measure P-700 quantitatively in chloroplast membrane particles because the reduction of photooxidized P-700 by endogenous electron donors is rapid and makes the distinction between light-induced absorption and fluorescence changes technically difficult to determine with our equipment. However, it is again significant that catalase activity was enriched in the system I fraction (table 1) even after washing this fraction twice.

It was shown [13] that 80% of the chloroplast SOD activity was associated with isolated photosystem I particles. They found about 35 SOD units/mg chlorophyll in these particles from spinach. Since the P-700-chlorophyll–protein complex is more enriched in photosystem I reaction centers than the particulate centrifugal fractions, it is noteworthy that SOD activity is also enhanced in the complex on a chlorophyll basis (table 2). The SOD activity we observed in our P-700-chlorophyll *a*–protein preparations was blocked completely by 1 mM KCN. This may indicate that this activity is caused by the Cu–Zn-containing enzyme described [16]. However, other compounds may also show cyanide-sensitive SOD activities [17]. Cytochromes *f*, b_6, and plastocyanin are close to photosystem I in vivo and will co-chromatograph with the P-700-chlorophyll–protein on hydroxylapatite [2,18]. We can remove these electron carriers by pretreating the chloroplasts with digitonin [18]. However, even after this treatment, a high SOD activity remained with the chlorophyll–protein.

It will be of interest to investigate P-700-chlorophyll–proteins isolated and purified by different procedures to determine how closely both of these enzymic activities are associated with the reaction center. It seems likely to us that the catalase activity discussed here is not caused by an enzyme identical to peroxisome catalase, but rather to a chloroplast component with catalase activity. Nevertheless, we are led to the conclusion that a small amount of an enzyme with catalase activity (see also [19]) is an integral part of photosystem I and can function along with superoxide dismutase to prevent singlet oxygen and hydroxyl radicals [8–10] from causing membrane damage during light-induced O_2 reduction.

Table 2
Superoxide dismutase activity in a chloroplast homogenate and purified chlorophyll–protein complexes

Exp. sample	SOD units/mg chl
French press homogenate	19
Chlorophyll–protein complexes (3 preparations)	88, 81, 60
Chlorophyll–protein complexes (digitonin pretreated)	62

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