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Isolation of PS II Reaction Centre and Its Relationship to the Minor Chlorophyll-Protein Complexes

Edith L. Camm and Beverley R. Green

Botany Department, University of British Columbia, Vancouver, BC, Canada

Evidence is presented for the identification of the chlorophyll- protein complex CPa-1 (CP 47) as the reaction centre of photosystem II (PS II). We have developed a simple, rapid method using octyl glucoside solubilization to obtain preparations from spinach and barley that are highly enriched in PS II reaction centre activity (measured as the light-driven reduction of diphenylcarbazide by 2,6-dichlorophenolindophenol). These preparations contain only the two minor chlorophyll-protein complexes CPa-1 and CPa-2. During centrifugation on a sucrose density gradient, there is a partial separation of the two CPa complexes from each other, and a complete separation from other chlorophyll-protein complexes. The PS II activity comigrates with CPa-1 but not CPa-2, strongly suggesting that the former is the reaction centre complex of PS II. Reaction centre preparations are sensitive to the herbicide 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), but only at much higher concentrations than those required to inhibit intact thylakoid membranes.

A model of PS II incorporating our current knowledge of the chlorophyll-protein complexes is presented. It is proposed that CPa-2 and the chlorophyll $a+b$ complex CP 29 may function as internal antenna complexes surrounding the reaction centre, with the addition of variable amounts of the major chlorophyll $a+b$ light-harvesting complex.

Key words: photosystem II, reaction centre, octyl glucoside, spinach, CPa -1, CP 47, chlorophyll-protein complexes

One major aim of chloroplast researchers is to purify and characterize functional components of the photosynthetic apparatus. Since detergents must be used to isolate and separate the membrane components, the risk of damaging their biological activity must be balanced against the requirement for purified entities. The usual procedure is to disrupt the membrane with a moderately high concentration of detergent, dilute the detergent, and separate the components by various methods. One method, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), when performed under relatively nondenaturing conditions, results in the separation of a number of chlorophyll-protein (CP) complexes [1–3]. The nomenclature of these bands is full of complexities, partly because various laboratories have slightly different techniques,

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and it is not certain if the observed CP complexes are, in fact, exactly comparable. Most importantly, it is not always clear how these complexes are related to the functional activities of the two photosystems.

Some progress has been made in the characterization of the components of photosystem I [4,5]. Most authors report a major, relatively slow running CP complex containing only chlorophyll *a*, termed CP I. It is generally accepted that CP I represents the reaction centre of photosystem I and is surrounded in the thylakoid by a number of antenna chlorophyll molecules and several additional polypeptides to make up the larger PS I unit.

Progress has also been made in understanding the major antenna complex of photosystem II, the light-harvesting chlorophyll *a*+*b* complex (LHCP), which has also been well characterized from preparations involving Triton extraction and sucrose density gradient centrifugation [6]. The LHCP is believed to be related to a series of CP complexes observed as bands in SDS gels [1,7–9]. These complexes contain both chlorophylls *a* and *b* and occur as a series of oligomers. The oligomers of increasing molecular mass, as seen on SDS gels, are variously referred to as LHCP 3, LHCP 2, and LHCP 1 [1]; as Chl *a*/*b*-P_2, Chl *a*/*b*-P_2*, and Chl *a*/*b*-P^{**}[3]; and as CP II and CP II^* in our system [8]. However, it is not completely clear how the Triton preparation of LHCP [6] is related to the above-mentioned series of CP complexes seen on gels.

As well as the LHCP, gel electrophoresis reveals another *a*+*b* complex, CP 29, with its own distinctive polypeptide [2,10], which is not found in the Triton LHCP preparations.

Little is yet known about the reaction centre of photosystem II (PS II). We have recently characterized two additional chlorophyll-proteins, which appear to be associated with PS II rather than PS I [11,12]. These complexes, CPa-1 and CPa-2, contain only chlorophyll *a*. Either or both have been suggested to be the reaction centre of PS II, based on their absence from PS II defective mutants [3,13–15], and because spectroscopic evidence indicates that the reaction centre should contain only chlorophyll *a* [16].

This paper reports the isolation of highly enriched photosystem II preparations from spinach, which contain only the two CPa complexes. During the isolation of these reaction centre preparations, there is a partial separation of the two chlorophyll *a* complexes on a sucrose gradient. The photosystem II activity comigrates with one of these complexes, CPa-1 (CP 47 in our earlier work [2,12,17,18]), indicating that it probably carries the reaction centre chlorophyll P680. A preliminary report of this work has been published [19].

METHODS

Chloroplasts were isolated from market spinach or greenhouse-grown chlorina f2 barley and washed to remove stromal proteins [2].Thylakoids were first suspended in 100 mM sorbitol, 50 mM tricine-NaOH (pH 7.6), 10 mM NaCl, 0.5 mM $MgCl_2$; 1 incubated for 15 min; and pelleted at 10,000 g. The pellet was extracted twice within 30 mM octyl glucoside in 2 mM Tris-maleate, pH 8.0. The second extract (typically 20–30% of total chlorophyll), was loaded on a 10–30% sucrose gradient containing 30 mM octyl glucoside, 0.75 mM EDTA, and 2 mM Tris-maleate (pH 8.0), and centrifuged at 110,000 g for 16 h at 4°C.

Fractions from the gradient were assayed for chlorophyll according to Arnon [20], and PS II reaction centre activity using the photoreduction of 2,6-dichlorophenolindophenol (DCPIP) with diphenylcarbazide [21]. These preparations cannot reduce DCPIP using water as an electron donor. Part of each fraction was electrophoresed on 10% polyacrylamide gels containing 0.1% SDS at 4°C as previously described. The unstained gels were scanned for chlorophyll at 680 nm using a Helena R & D densitometer. The amount of chlorophyll in each chlorophyll-protein complex was estimated from the relative area under each peak and the amount of chlorophyll applied to the gel.

RESULTS

The Reaction Centre Core Contains Only CPa-1 and CPa-2 (CP 47 and CP 43)

When the chlorophyll-containing octyl glucoside extract was centrifuged on a sucrose gradient, there was a large chlorophyll peak at about 13% sucrose with a shoulder on the higher density side (Fig. 1, top). The main band had a low *a*/*b* ratio, characteristic of the LHCP, while the *a*/*b* ratio of the shoulder was dramatically higher. When photosystem II reaction centre activity was measured using the light-driven reduction of DCPIP by diphenylcarbazide, it was found that the specific activity was indeed highest in the shoulder fractions (ranging from 70–120 μmol DCPIP reduced/mg chlorophyll/hr). This is what would be expected if the chlorophyll *a*- containing reaction centre core were separating from the LHCP on the gradient.

Electrophoresis of individual fractions revealed that the distribution of chlorophyll-protein complexes varied along the gradient (Fig. 1, bottom). CP II and CP II*, the monomer and oligomer forms of the LHCP [8], were associated with the dark green chlorophyll *b*-containing main band. The minor *a*+*b* complex CP 29 was enriched at the extreme upper part of the gradient. The fractions with high PS II activity contained only the two chlorophyll *a* complexes, CPa-1 and CPa-2. (These complexes were called CP 47 and CP 43 in our earlier work [2,12,17,18]). This shows that either or both of these complexes comprise the reaction centre core of PS II.

It should be noted that the fractions rich in LHCP have little or no activity in the PS II assay, even though they are high in chlorophyll. This shows that the assay is indeed specific for PS II, and is not a non-specific reaction catalyzed by traces of free chlorophyll.

Evidence That CPa-1 Is the Reaction Centre Core

It can be seen in Figure 1 that the distributions of CPa-1 and CPa-2 across the gradient are not identical. When the amount of chlorophyll in each complex is plotted across the gradient and compared with the PS II activity in each fraction (Fig. 2), the peak of activity coincides with the peak of CPa-1 distribution and not that of CPa-2. The chlorophyll *b*-containing complexes are not associated with any photochemical activity. This experiment has been repeated a number of times with the same result: total activity parallels the distribution of CPa-1 rather than CPa-2. This strongly suggests that CPa-1 (CP 47) carries the reaction centre of Photosystem II.

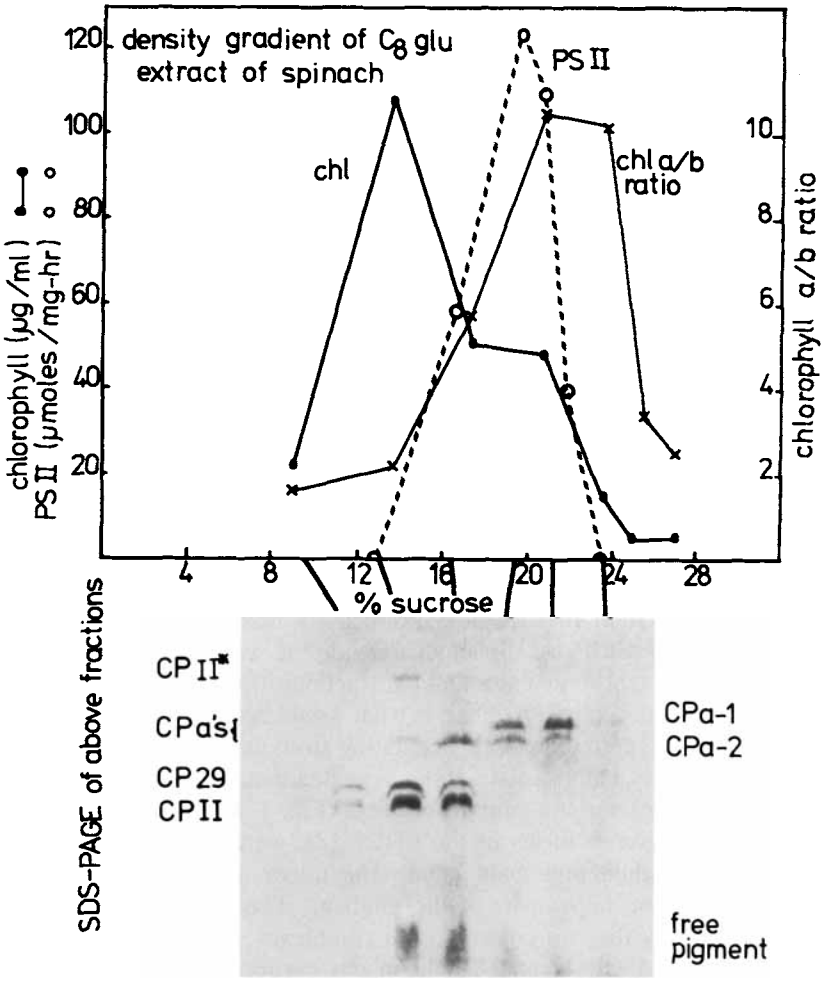

Fig. 1. Top: Distribution of chlorophyll and PS II activity along a 10–30% sucrose density gradient of an octyl glucoside extract of spinach. The gradient was centrifuged for 16 hr at 110,000 g and 4°C in a Beckman SW50.1 rotor. ●—●, chlorophyll (µg/ml): ○--○, PS II activity (µmol DCPIP reduced per mg chlorophyll/hr); ×—×, chlorophyll *a*/*b* ratio. Bottom: Fractions from the above gradient electrophoresed on 10% acrylamide in the presence of 0.1% SDS. This gel is unstained. Since the CPa-containing fractions were very dilute, no attempt was made to load equal volumes or equal amounts of chlorophyll in each slot.

Chlorophyll *b*-less Barley

We attempted to sidestep the problem of large amounts of LHCP encountered in spinach by using the barley chlorina f2 mutant, which does not synthesize chlorophyll *b* and therefore does not assemble the LHCP [22]. The results are shown in Figure 3. In this case, the PS II-active shoulder is much more prominent, and as in spinach, the most highly active fraction contains only CPa-1 and CPa-2. This confirms the connection between these two complexes and the PS II reaction centre. In contrast to the case with spinach, however, the peaks of CPa-1 and CPa-2 are separated by

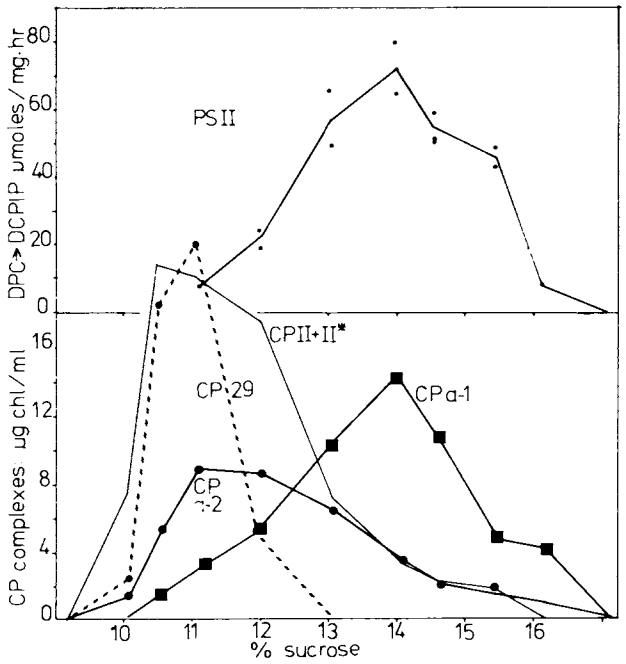

Fig. 2. Distribution of spinach CP complexes and total PS II activity along a 10–25% sucrose gradient. The gradient was centrifuged for 16 hr at 81,500 g and 4°C in a Beckman SW27 rotor. ●—●, PS II activity (μmoles DCPIP reduced per mg chlorophyll/hr). Content of chlorophyll-protein complexes in each fraction (μg chlorophyll/ml):—, CP II plus CPII*; ●----●, CP 29; ●—●, CPa-2; ■—■, CPa-1.

only one gradient fraction, so it was not possible to associate the photochemical activity exclusively with one or the other of the complexes.

It is interesting to note in Figure 3 that the fractions where LHCP would have been found in a normal plant still contain some chlorophyll. However, when these fractions were electrophoresed, all that could be seen was free chlorophyll (eg fraction 8%). This suggested that an unstable form of the LHCP might be partly assembled but fall apart when the sucrose gradient fraction was applied to the gel [23]. This preparation also contained some CP I, probably as a result of its increased content in the mutant [23].

Herbicide Sensitivity

The herbicide-binding protein is known to be closely associated with the PS II core and may also bind the secondary electron acceptor "B" [24]. DCPIP reduction by pooled CPa-rich fractions and by unfractionated octyl glucoside extracts was measured in the presence and absence of DCMU. Figure 4 shows that both of them were much less sensitive to DCMU than were whole thylakoids. Under the conditions of assay, about 1,000 times more DCMU is required for total inhibition of octyl glucoside-treated material. These results agree with those obtained by Mullet and co-workers using octyl glucoside-treated Triton PS II preparations [25]. This could mean that the secondary acceptor is still present but has an altered binding constant; but the inhibition could also be due to nonspecific binding of DCMU at some other site.

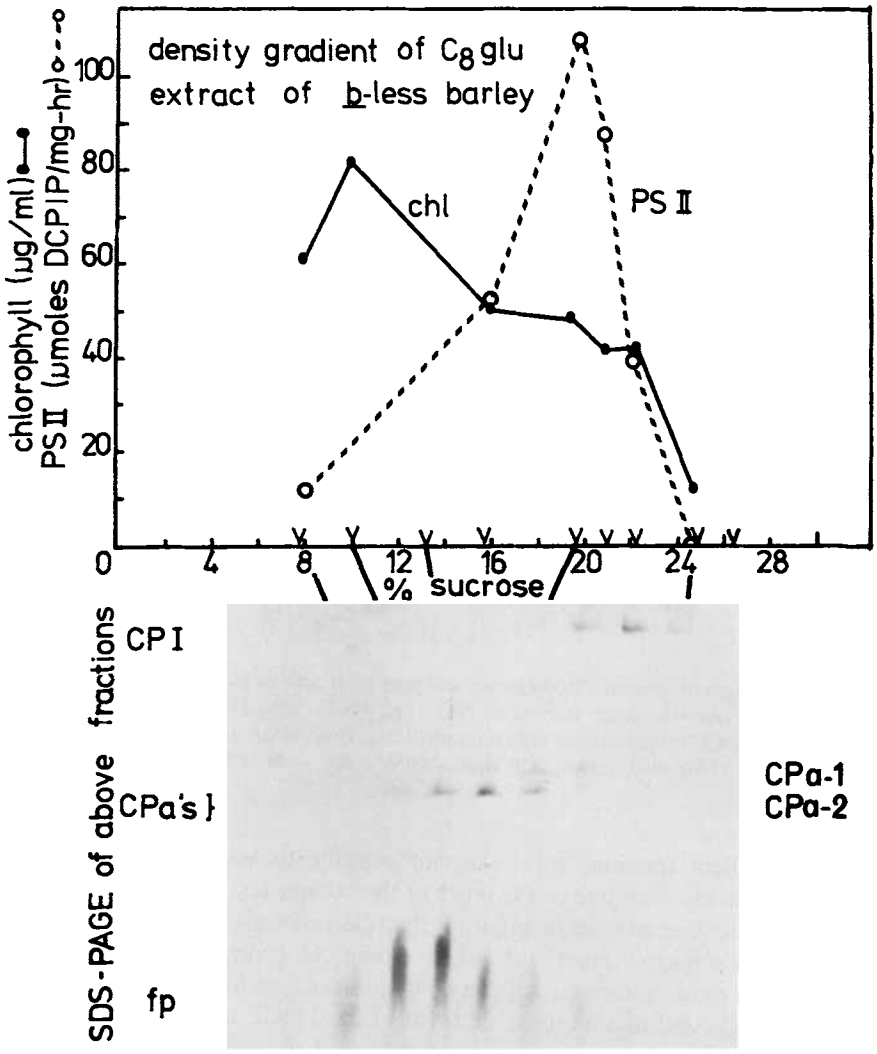

Fig. 3. Top: Distribution of chlorophyll and PS II specific activity along a 10–30% sucrose density gradient of an octyl glucoside extract of chlorophyll *b*-less barley. The gradient was centrifuged for 16 hr at 110,000 g and 4°C in a Beckman SW50.1 rotor. Symbols as in Figure 1. Bottom: Fractions from the gradient electrophoresed as in Figure 1.

DISCUSSION

This is the first direct confirmation that the minor chlorophyll *a* complexes as seen on gels of octyl glucoside extracts are involved with PS II. This simple procedure allows the preparation of a fraction that retains PS II electron transfer activity and contains no CP I, CP II, or CP II*, or CP 29 detectable by electrophoresis. This shows that the PS II activity must be associated with either CPa-1 or CPa-2. Plots of total activity versus amounts of each complex across the gradient show that the peak

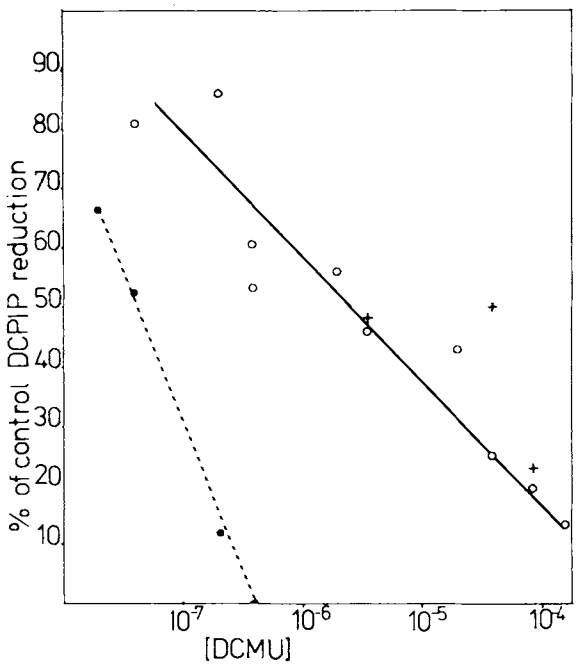

Fig. 4. Effect of DCMU on PS II activity in whole thylakoids and octyl glucoside preparations. ●—●, thylakoids: 1.86 μg chlorophyll/ml; ○—○, octyl glucoside extract: 4.0 μg chlorophyll/ml: +—+, PS II-rich fraction from a sucrose gradient: 0.96 μg/ml.

of PS II activity coincides with the peak of CPa-1. This strongly suggests that CPa-1 is the reaction centre of PS II. H. Nakatani has recently detected a transient absorption change at about 680 nm on irradiation of the complex CPa-1 isolated from SDS gels [Nakatani, personal communication], and it would be interesting to see if this change also follows the distribution of PS II activity on the gradient.

What is the role of CPa-2? In view of the demonstrated heterogeneity of PS II [26], it was tempting to suggest that it might correspond to a less active form of PS II, such as the beta centres of Melis and Homann [27]. However, a Triton-generated PS II-active fraction which contained only alpha centres had the same proportions of CPa-1 and CPa-2 as whole membranes [Green, in preparation]. It is likely that this type of PS II heterogeneity is more a reflection of the association of LHCP with PS II cores than any difference in the cores themselves [28]. However, one other possibility should still be considered: that another polypeptide might be required for the detection of PS II electron transport, and that this polypeptide is separated from CPa-2 but not CPa-1 on the gradient.

In the absence of any demonstrated photochemical activity, it is tempting to suggest that CPa-2 may be an antenna complex, since it is generally considered that there must be an antenna chlorophyll *a* closely associated with the PS II reaction centre [29]. Further characterization will have to await the separation of CPa-1 and a-2 in larger quantities. It is likely that CP 29, with an *a*/*b* ratio of 3–4, is also an antenna complex, since spectroscopic studies indicate that all the chlorophyll *b* in the thylakoid membrane transfers its energy to chlorophyll *a* [26].

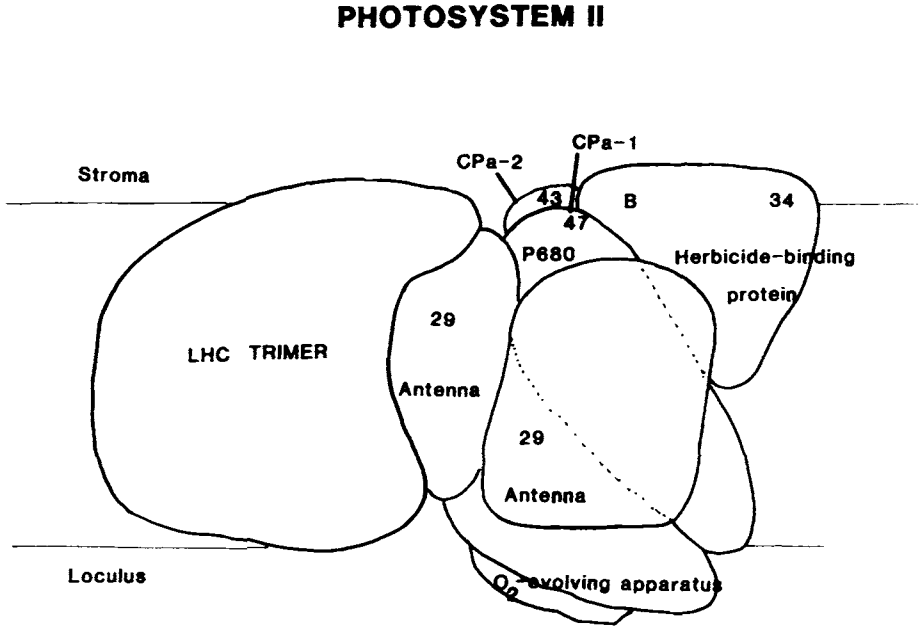

Fig. 5. Model of a possible arrangement of chlorophyll-protein complexes in photosystem II. Details of the isolation and characterization of the complexes are given in [2,8,11,12, and 17]. CP 29 and CPa-2 are assigned roles as internal antenna complexes, as discussed in the text.

The above considerations can be assembled in a coherent model for the arrangement of chlorophyll-protein complexes in photosystem II (Fig. 5). Two copies of CP 29 are included because it accounts for about twice as much of the total chlorophyll as CPA-1 and CPa-2, and in addition can be isolated as a dimer [12,30]. Various workers have evidence for the surface location of the 32-kilodalton polypeptide, based on susceptibility to trypsin [24]. The CPa's seem somewhat resistent to trypsin, and we have suggested that they may be partly shielded by other membrane proteins [18]. The LHCP is postulated to consist of trimers of its two constituent chlorophyll-proteins based on the relative amounts of these complexes [8]. The LHCP units are deliberately not arranged symmetrically because there is no requirement for symmetry, and to emphasize the fact that variable numbers of units of LHCP can be associated with a reaction centre core [31].

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