Stoichiometric determination of pheophytin in photosystem II of oxygenic photosynthesis

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(Received 24 September 1985)

Key words: chlorophyll, pheophytin, photochemical reaction center II, photosystem II

Abstract. Pheophytin and chlorophyll extracted from oxygen-evolving photosystem II particles, chloroplast thylakoids and cyanobacterial cells were separated by column chromatography with DEAE-Toyopearl, and quantitatively determined by spectrophotometry. The molecular ratio of chlorophyll a + b to pheophytin a was about 100 in spinach photosystem II particles and about 140 in spinach thylakoids. Using flash spectrophotometry of P680 and measurement of flash-induced oxygen yield, the molecular ratio of the chlorophyll to the photochemical reaction center II was determined to be about 200 in the photosystem II particles. These findings suggest that the stoichiometry in photosystem II particles is one reaction center II and two pheophytin a molecules per about 200 chlorophyll molecules. The same stoichiometry for pheophytin to the reaction center II was obtained in the cyanobacteria, Anacystis nidulans and Synechocystis PCC 6714. A quantitative determination of pheophytin a and the electron donor P700 in stroma thylakoids from pokeweed suggests that photosystem I does not contain pheophytin.

Introduction

Photosystem (PS) II particles prepared from thylakoids with Triton X-100 [9] have enabled us to establish that the stoichiometry of components in the oxygen-evolving complex is one molecule each of the 33-kDa, 24-kDa and 18-kDa extrinsic proteins, four Mn atoms and two cytochrome b-559 per about 220 chlorophyll (Chl) molecules [12, 13, 14]. The molecular ratio of Chl to the reaction center II in PS II particles has been estimated to be about 220:1 by co-electrophoresis of the intrinsic polypeptides from the particles and purified reaction center II complex [14]. To confirm this stoichiometry, a further study is necessary to quantitatively determine the reaction center II by different methods, such as spectrophotometry of P680 and measurement of flash-induced oxygen yield.

Klimov et al. [7] demonstrated that pheophytin (Pheo)a is the intermediate
electron acceptor between P680 and the primary quinone acceptor of the 
photochemical reaction in PS II. However, determination of nonphotoreducible 
Pheo had been difficult until a simple technique to separate Pheo from Chl, 
such as ion-exchange column chromatography [16], was developed, since 
the presence of a much greater amount of Chl made it impossible to determine 
Pheo by simple spectrophotometry. By a combined use of ion-exchange 
column chromatography and spectrophotometry [17], we determined the 
Pheo a content in a purified reaction center II preparation and suggested that 
there are two Pheo a molecules per reaction center II.

In order to establish the stoichiometry of Pheo a, the photochemical 
reaction center II and Chl, we determined the molecular ratios of Pheo a 
and the reaction center II to Chl in various samples from organisms that 
carry out oxygenic photosynthesis.

Materials and methods

Spinach (Spinacia oleracea) was purchased from a local market. Chloroplast 
thylakoids were prepared by grinding the leaves in a medium containing 
400 mM sucrose, 10 mM NaCl and 50 mM Na/phosphate buffer (pH 7.8), 
followed by differential centrifugation. PS II particles were prepared from the 
thylakoids with Triton X-100 as described previously [9]. Stroma thylakoids 
and grana thylakoids (corresponding to SD-26 and SD-41, respectively) were 
prepared from pokeweed (Phytolacca americana) growing in the campus 
of Saitama University according to the method described previously [22].

Anacystis nidulans (TX 20) was obtained from the Algal Collection in 
the Institute of Applied Microbiology, University of Tokyo. The cells were 
grown in the medium of Kratz and Myers [8] under aeration with 1% CO2 in 
air. Synechocystis PCC 6714 (Aphanocapsa 6714), kindly provided by Dr. 
C. Astier (Laboratoire de Photosynthèse, CNRS, Gif-sur-Yvette), was grown 
in the medium of Herdman et al. [4] as modified by Astier et al. [1] under 
aeration with 1% CO2 in air. Both cultures were grown at 28°C under con-
tinuous incandescent illumination at an intensity of 3,000 lux. Cell at the 
middle-logarithmic phase were harvested for use in the experiments.

Pigments were extracted from the thylakoids and PS II particles of spinach 
and from the stroma and grana thylakoids of pokeweed with 80% acetone, 
and from the cyanobacterial cells with 90% methanol. Pigments were also 
extracted from spinach leaves by disrupting the leaves in nine volumes of 
methanol with a blender. In all cases, extraction was repeated with the same 
solvent mixtures, and the extracts were combined. The extracted pigments, 
corresponding to about one mg Chl, were transferred to diethylether [20], 
and the solvent was evaporated to complete dryness under reduced pressure.

The resultant crude pigment preparation was dissolved in 10 ml dry 
acetone, and one ml of the solution was applied to a DEAE-Toyopearl 
column. Pheo a and carotenoids were eluted with 7 ml dry acetone, after