cant export in the CPIZ despite low productivities in the water column.

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10 June 1992; accepted 17 September 1992

X-ray Detection of the Period-Four Cycling of the Manganese Cluster in Photosynthetic Water Oxidizing Enzyme

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X-ray absorption near-edge structure spectra of the manganese (Mn) cluster in physiologically native intermediate states of photosynthetic water oxidation induced by short laser flash were measured with a compact heat-insulated chamber equipped with an x-ray detector near the sample surface. The half-height energy of the Mn K-edge showed a period-four oscillation dependent on cycling of the Joliot-Kok's oxygen clock. The flash number–dependent shift in the Mn K-edge suggests that the Mn cluster is oxidized by one electron upon the S_0-to-S_1, S_1-to-S_2, and S_2-to-S_3 transitions and then reduced upon the S_3-to-S_0 transition that releases molecular oxygen.

Atmospheric oxygen is a result of water cleavage by oxygenic photosynthesis in plants. The fundamental view of the mechanism of photosynthetic water oxidation was introduced by Joliot *et al.*, who first found the period-four oscillation of oxygen evolution under flashing light (*1*). The oscillation suggested that the water-oxidizing machinery becomes sequentially oxidized through five different intermediate states, denoted as S_i (i = 0 to 4), to evolve molecular oxygen through the cumulative use of the energy of four photons (*2*). Much circumstantial evidence has suggested that the chemical entity of the machinery is a tetranuclear Mn cluster (*3, 4*). However, this generally accepted concept still lacks conclusive evidence because of the difficulty in monitoring the chemical or electronic state of Mn at each S-state by a direct detection method. We report an entire set of x-ray absorption near-edge structure (XANES) spectra of the Mn cluster at respective S-states, generated under physiological conditions by a series of laser flashes, that reveals a characteristic period-four oscillation of the Mn K-edge energy. These spectra are indicative of a period-four change in the oxidation state or electronic environment, or both, of the Mn cluster. The results demonstrate that every reaction step of the S-state turnovers involves a direct reaction of Mn.

X-ray absorption spectroscopy has been successfully applied to detect oxidation of Mn upon S_2 formation (*5–9*). In these experiments, the S_2-state was accumulated in a dense photosystem II (PSII) sample by continuous light illumination at a cryogenic temperature. In flashing light experiments, however, the sample concentration must be low enough to ensure light saturation by a short flash. In order to measure a high-quality XANES spectra of dilute PSII samples, we designed a compact heat-insulation chamber equipped with an x-ray detector that was 1.5 cm from the sample surface, which enabled us to collect the fluorescent x-ray from Mn at high efficiency. The processed XANES spectra of PSII membranes after zero to five flashes are presented in Fig. 1A along with the original data points. The K-edge of the spectrum changed with the flash number, as is shown more clearly by Fig. 1B, in which all five spectra are displayed on the same expanded energy scale after correction of background baseline (*8*). The half-height energy of the Mn K-edge was estimated to be 6551.7 ± 0.2 eV for dark-adapted PSII (S_1-state) but was upshifted by 0.7 eV after one flash (S_2-state). Both of these two K-edge energy values and the K-edge upshift upon the S_1-to-S_2 transition are consistent with those reported previously (*6, 8, 9*). The relatively small extent of the upshift compared with the reported value (~1 eV) is due to both dissipation of the S_2-state by reduction by tyrosin D and incompleteness of the S_1-to-S_2 transition by misses (see legend to Fig. 2). The upshift of Mn K-edge energy has been interpreted to mean that Mn(III) is oxidized to Mn(IV) as the S_2-state is accumulated by continuous illumination at a cryogenic temperature (*5–9*). Thus our results indicate that oxidation of Mn occurs upon the formation of physiologically native S_2-state by a single flash given at room temperature. The half-height energies of our spectra were determined to be 6552.4 ± 0.2, 6553.4 ± 0.2, 6551.5 ± 0.2, 6551.7 ± 0.2, and 6552.0 ± 0.2 eV after 1, 2, 3, 4, and 5 flashes, respectively.

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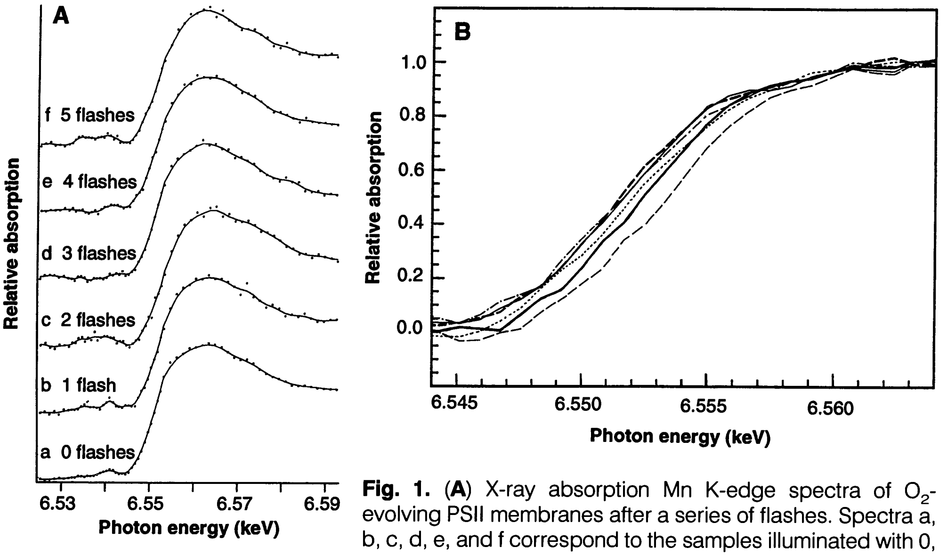

Fig. 1. (**A**) X-ray absorption Mn K-edge spectra of O_2-evolving PSII membranes after a series of flashes. Spectra a, b, c, d, e, and f correspond to the samples illuminated with 0, 1, 2, 3, 4, and 5 flashes, respectively. Original data points were processed by the smoothing algorithm of Marchand and Marmet (*19*). (**B**) Effect of flash number on Mn K-edge spectra. Nonilluminated PSII (—·—); PSII after illumination with 1 (——), 2 (— —), 3 (— —), 4 (– – –), and 5 (– – – – – –) flashes. Spinach PSII membranes capable of O_2 evolution were prepared as described (*20*), suspended in 0.4 M sucrose, 20 mM NaCl, 40 mM Mes-NaOH (pH 6.5), and relaxed in darkness for 6 hours. A 15.0-μl aliquot of the sample membranes (4 mg/ml) was mounted on a membrane filter (Toyo Roshi, TM-300, 7 by 4 mm) placed on a Cu sample holder, excited with a saturating Nd-YAG laser flash (~7 ns, 23 mJ/cm^2, and 532 nm) at 10°C for an interval of 1 s, and quickly cooled to 77 K in liquid N_2. The x-ray Mn K-edge spectra were measured at the Photon Factory of the National Laboratory for High Energy Physics, Tsukuba, with a sagittally shaped Si(111) crystal monochromator by fluorescence detection mode with an EG&G modified Si(Li) solid-state detector as described (*8*). Samples were kept in darkness at 80 K during data collection. Energy calibration was done based on the pre-edge peak of $KMnO_4$ at 6543.3 eV. For each spectrum, seven to nine scans were averaged.

As shown in Fig. 2, the half-height energy of the Mn K-edge exhibits a clear period-four oscillation with minima after the zeroth and third flashes (open circles). An appreciable upshift of the K-edge energy was detected after the second flash as well as after the first flash, suggesting a direct involvement of the Mn cluster in this transition as well. Since one molecule of oxygen is known to be formed and released after the third flash (S_3-to-S_0 transition) (*1*), we may reasonably expect a large change in valence or structure or both of the Mn cluster after the third flash if this step involves the Mn cluster as the direct reaction device. In fact, the K-edge energy upshifted stepwise upon the first and second flashes by ~1 eV each, but was significantly downshifted by 1.9 eV after the third flash. The K-edge energy did not appreciably change after the fourth flash but upshifted slightly again after the fifth flash. Almost the same oscillation pattern was obtained in an independent experiment in which the PSII membranes were excited by saturating Xe flashes instead of laser flashes. Closed triangles show the oscillation pattern obtained from a simulation in which K-edge energies for individual S-states and miss hits were assumed to be fitted to the measured K-edge data by taking into account the S-state distributions after each flash. The best fit was obtained when the following set of K-edge energies was used: 6550.7, 6551.7, 6552.5, and 6553.7 eV for S_0-, S_1-, S_2-, and S_3-states, respectively.

The simulated data indicate that the K-edge energy upshifts by 1.2 eV after the S_2-to-S_3 transition. The simplest interpretation for this upshift would be an oxidation of one more Mn(III) atom upon the S_2-to-S_3 transition. This interpretation consistently explains the disappearance of S_2 multiline electron paramagnetic resonance (EPR) signal upon formation of the S_3-state (*10*) as well as the observation that the S_2-to-S_3 transition involves a near-ultraviolet absorption change that is essentially the same as observed upon the S_1-to-S_2 transition (*11, 12*). It is, however, noted that the present data do not necessarily exclude the possibility of partial oxidation of the ligand of the Mn cluster upon the S_2-to-S_3 transition. An alternative interpretation for this upshift is that the K-edge energy changes because of some configurational rearrangement of the Mn cluster (*13*). As suggested by chemical models variously proposed for water oxidation, the Mn cluster may undergo a configurational rearrangement upon the formation of an O–O bond between the two water molecules (*14*) or upon binding of a substrate water molecule at the S_3-state (*15*). Contrary to our observations, however, it was reported that no appreciable change took place in XANES spectrum when the S_3-state was partially accumulated by continuous light at −20°C (*16*). A possible cause for this discrepancy may be the different conditions used for accumulation of the S_3-state. Presumably, the state generated at a cryogenic temperature is not identical with the native S_3-state generated at room temperature.

The simulated data also indicate a marked downshift of Mn K-edge energy upon the S_3-to-S_0 transition. This change may be interpreted as indicating a reduction of Mn in this transition, although no such reduction has ever been experimentally verified. We note that this interpretation is relevant to the flash-induced proton release pattern (*17, 18*). The relatively large K-edge downshift by 3.0 eV leads us to further assume that a two- or three-electron

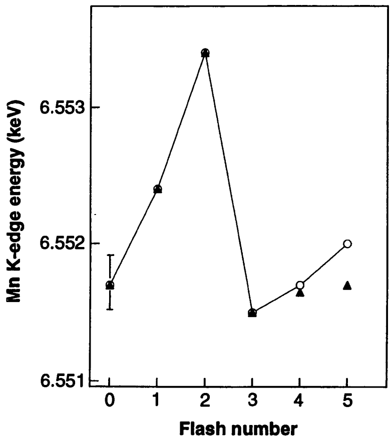

Fig. 2. Oscillation of Mn K-edge energy of PSII membranes under single-turnover flashes. The half-height energies of Mn K-edge spectra after zero to five flashes (○) were compared with the simulated values (▲). For the simulation, the most probable S-state distributions after illumination with a series of flashes were calculated by assuming that the dark-adapted sample consisted of 100% S_1-state and 25% of tyrosin D as reduced form (*21, 22*) and that there were no double hits. One-half of the reduced tyrosin D that remained was assumed to be oxidized by higher S-states during the flash interval, as the half recombination time of the reduced tyrosin D with the higher S-states has been reported to be ~1 s (*22, 23*). The K-edge energies of individual S-states and miss hits were appropriated to account for the measured K-edge data. The best fit was obtained when a 9% miss hits and the set of K-edge energies were assumed to be 6550.7, 6551.7, 6552.5, and 6553.7 eV for the S_0-, S_1-, S_2-, and S_3-states, respectively.

reduction occurs in the Mn cluster upon the S_3-to-S_0 transition, presuming that the valence change of Mn is the main cause for this K-edge energy downshift. The simulated data also indicate that the K-edge energy upshifts by 1.0 eV upon the S_0-to-S_1 transition. The extent of the upshift of the simulated K-edge energy is coincident with those upon the S_1-to-S_2 and S_2-to-S_3 transitions, suggesting that an oxidation of Mn(III) to Mn(IV) might be involved in the S_0-to-S_1 transition as well.

The present study demonstrates that the valence or structure or both of the Mn cluster oscillates period-four during the cycling of the Joliot-Kok's oxygen clock, showing directly that the so-called S-states reflect mainly the differences in redox states or configurations or both of the Mn cluster during the four-electron cyclic reaction.

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22 June 1992; accepted 29 September 1992

Linear Metal Nanostructures and Size Effects of Supported Metal Catalysts

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Nickel metal catalysts composed of nanometer by micrometer strips have been produced with solid-state microfabrication techniques. The strips are actually the edges of nickel-catalyst thin films, which are sandwiched between separating support layers, which are also nanometers thick. These linear nanostructures constitute well-defined and well-controlled catalytic entities that reproduce the size of traditional supported metal clusters in one dimension, thus separating size from total number of atoms in the catalyst. Examination of their catalytic activity showed that they duplicate the behavior of conventional supported clusters. A specific rate maximum was observed for the hydrogenolysis of ethane at a nanoscale dimension similar to the cluster size at which the rate is maximum in the case of the supported cluster studies, whereas the hydrogenation of ethylene shows no such size dependency. The results suggest that the surface-to-volume ratio or the number of atoms in the catalytic entity cannot be the source of these size effects and that either support effects or nonequilibrium surface structures are the determining factors.

The need for maximizing the effective surface area of catalytically active metals in heterogeneous systems led to the extensive use of highly dispersed metals in the form of clusters supported on a variety of carrier materials. The rates of certain reactions, when normalized to unit surface area of the clusters, as well as the selectivities of some of them, depend on the overall metal cluster size under otherwise similar reaction conditions (*1, 2*). If one characterizes the size of the supported metal cluster by an equivalent average diameter, clusters smaller than ~10 nm exhibit such size dependence. The phenomenon is of considerable technological and scientific importance: (i) it suggests that one could produce catalyst structures that show greater normalized (specific) reaction rates or affect the selectivity in a favorable way or both (*2*), (ii) it furnishes data for testing electronic and geometrical models of catalysis (*2*), and (iii) it can be used to probe chemically the transition from atoms to bulk solids (*3*).

Traditionally, supported metal catalysts are produced through chemical routes (such as impregnation and coprecipitation). The catalytic activity of the resulting supported clusters is examined, and the reaction results are correlated with theoretical calculations that provide the structural and electronic features of idealized and unsupported metal clusters as a function of their total number of atoms. This number is taken to be representative of the measured average size of the produced clusters and serves as a metaphor for structure. The problems with using supported clusters (nonuniform shapes and sizes, possible existence of adventitious surface species, very difficult direct characterization of their surfaces, and failure to distinguish between size and total number of atoms in the cluster) have led to studies with simpler model systems: single crystals and unsupported metal clusters. However, the results from these studies are at best indicative of what may happen on supported clusters. They address only certain issues of the whole problem; for example, the single crystal studies investigate the role of specific surface structures on the origin of the phenomenon. There is a need for developing a scheme that allows the investigation of the size effects without so many limitations. We describe a new class of supported metal catalysts that are much more carefully defined, illustrate their use in addressing the origin of the size effects, and demonstrate the inappropriateness of the metaphor of the "number of atoms."

By vacuum-depositing alternating layers of metal catalyst and support material on a suitable substrate, one can fabricate a layered synthetic microstructure (LSM) whose edge exhibits a compositionally modulated surface that consists of catalyst and support (Fig. 1). The topmost layer is made out of support material so that the only exposed metal catalyst is in the form of strips between those of the support. The thickness of the metal layers can be controlled, is in the nanometer scale, and is comparable to the size of supported metal clusters. Although the width of the catalyst strips is of the order of nanometers, the length of the strips is macroscopic (micrometer scale or larger). In effect, the catalyst is created as a linear microstructure that preserves only one dimension in the nanometer range. As a result, the number of connected metal atoms in this linear structure is orders of magnitude greater than in the typical cluster and separation of dimension from the

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