Dependence of Substrate-Water Binding on Protein and Inorganic Cofactors of Photosystem II

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Except where otherwise acknowledged, the work presented in this thesis is my own and was performed under the supervision of Dr Tom Wydrzynski.

Garth S. Hendry, September 2002

αριστου μευ υδωρ

‘The noblest of elements is water’

Pindar

Acknowledgments

When the All Blacks take to the paddock and thump the Wallabies in the semi-final of the RWC 2003 at Stadium Australia late next year, the boys will know that the ensuing success of world cup victory is all about commitment, team work and knowing that everyone, on and off the field, gave it 110%.

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Abstract

The photosynthetic water oxidation reaction is catalyzed by an inorganic $Mn_4O_xCaCl_yHCO_3^-{}_z$ cluster at the heart of the oxygen evolving complex (OEC) in photosystem II. In the absence of an atomic resolution crystal structure, the precise molecular organization of the OEC remains unresolved. Accordingly, the role of the protein and inorganic cofactors of PSII (Ca^{2+}, HCO_3^- and Cl^-) in the mechanism of O_2-evolution await clarification. In this study, rapid ^{18}O-isotope exchange measurements were applied to monitor the substrate-water binding kinetics as a function of the intermediate S-states of the catalytic site (i.e. S_3, S_2 and S_1) in Triton X-100 solubilized membrane preparations that are enriched in photosystem II activity and are routinely used to evaluate cofactor requirements. Consistent with the previous determinations of the ^{18}O exchange behavior in thylakoids, the initial ^{18}O exchange measurements of native PSII membranes at *m/e* = 34 (which is sensitive to the $^{16}O^{18}O$ product) show that the 'fast' and 'slowly' exchanging substrate-waters are bound to the catalytic site in the S_3 state, immediately prior to O_2 release. Although the slowly exchanging water is bound throughout the entire S-state cycle, the kinetics of the fast exchanging water remains too fast in the S_2, S_1 [and S_0] states to be resolved using the current instrumentation, and left open the possibility that the second substrate-water only binds to the active site after the formation of the S_3 state. Presented is the first direct evidence to show that fast exchanging water is already bound to the OEC in the S_2 state. Rapid ^{18}O-isotope exchange measurements for Ex-depleted PSII (depleted of the 17- and 23-kDa extrinsic proteins) in the S_2 state reveals a resolvable fast kinetic component of $^{34}k_2$ = 120 ± 14 s^{-1}. The slowing down of the fast phase kinetics is discussed in terms of increased water permeation and the effect on the local dielectric following removal of the extrinsic subunits. In addition, the first direct evidence to show the involvement of calcium in substrate-water binding is also presented. Strontium replacement of the OEC Ca^{2+}-site reveals a factor of ~3-4 increase in the ^{18}O exchange of the slowly exchanging water across the S_3, S_2 and S_1 states while the kinetics of the fast exchanging water remain unchanged. Finally, a re-investigation of the proposed role for bicarbonate as an oxidizable electron donor to photosystem II was unable to discern any ^{18}O enrichment of the photosynthetically evolved O_2 in the presence of ^{18}O-bicarbonate. A working model for O_2-evolution in terms of these results is presented.

Publications

Arising from this work:

Hendry, G., and Wydrzynski, T. (2002) Substrate-water exchange kinetics in photosystem II reveal S-state dependent interactions with calcium. In preparation.

Hendry, G., and Wydrzynski, T. (2002) The two substrate-water molecules are already bound to the oxygen evolving complex in the S_2 state of photosystem II. *Biochemistry* in press.

Hillier, W., **Hendry, G**., Burnap, R. L., and Wydrzynski, T. (2001) Substrate water exchange in photosystem II depends on the peripheral proteins. *J. Biol. Chem.* **276,** 46917-46924.

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Abbreviations

α	miss parameter
β	double hit parameter
^oC	degrees Celsius
μF	microFaraday
μg	microgram
μL	microliter
μM	micromolar
mA	milliampere
mL	milliliter
mM	millimolar
ADP	adenosine di-phosphate
ATP	adenosine tri-phosphate
bRC	bacterial photosynthetic reaction center
CA	carbonic anhydrase
Chl	chlorophyll
CW-EPR	continuous wave EPR
DCBQ	2,6-dichloro-p-benzoquinone
EGTA	ethyleneglycol-bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid
ENDOR	electron nuclear double resonance
EPR	electron paramagnetic resonance
ESEEM	electron spin echo envelope modulation
EXAFS	extended X-ray absorption fine edge structure
Ex-depleted PSII	PSII depleted of the 17- and 23-kDa extrinsic proteins
FTIR	Fourier transform infrared spectroscopy
fwhh	full width half height
GlAc	glacial acetic acid
K	Kelvin
kDa	kiloDalton
kJ	kiloJoules
kV	kilovolt
LED	light emitting diode
LHCII	light harvesting complex, type II protein

LMW	low molecular weight protein
m/e	mass per charge
MeOH	methanol
MES	4-morpholinoethanesulfonic acid
MIL	0.001 inches
MLS	S_2 state EPR multiline signal
NADP	nicotinamide adenine di-phosphate
NMR	nuclear magnetic resonance
OEC	oxygen evolving complex
PAGE	polyacrylamide gel electrophoresis
PCET	proton coupled electron transfer
pH	$-\log_{10}[H_3O^+]$
Pheo	primary electron acceptor molecule, pheophytin
P_i	inorganic phosphate
PSI	photosystem I
PSII	photosystem II
PPBQ	phenyl-p-benzoquinone
P_{680}	primary electron donor in PSII
Q_A	primary quinone electron acceptor molecule in PSII
Q_B	secondary quinone electron acceptor molecule in PSII
SDS	sodium dodecyl-sulfate
TL	thermoluminescence
UV	ultra-violet
(*v/v*)	volume per volume
(*w/v*)	weight per volume
WOC	water oxidizing complex
XANES	X-ray absorption near edge spectroscopy
XAS	X-ray absorption spectroscopy
XES	X-ray emission spectroscopy
Y_D	redox active tyrosine-161 of the D_2 protein
Y_{inj}	oxygen yield from the $H_2{}^{18}O$ injection
Y_Z	redox active tyrosine-161 of the D_1 protein
Y_{2x}	oxygen yield due to a double hit
Y_{3N}	normalized oxygen yield on the third flash
Y_{3C}	corrected oxygen yield on the third flash