

Photostability of a Recombinant Monoclonal Antibody

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INTRODUCTION

Evaluation of photostability of new biopharmaceutical drug substances and products is an integral part of drug development process. ICH has established guidelines for photostability studies which states that a drug substance should be exposed to not less than 1.2 million Lux-hours of overall illumination. Protein substances exposed to light are predisposed to certain chemical reaction on amino acid residues, most commonly methionine oxidation. A recombinant monoclonal antibody, MAb1, was subjected to light stress and the resulting changes were determined by various functional and analytical methods. A number of oxidation sites were identified by LC/MS/MS analysis of tryptic peptide map, including two methionine residues in the Fc region that were oxidized to an extent of ~30-40%. Two tryptophan residues within the CDR regions were also identified to be oxidized to a smaller extent.

MATERIALS & METHODS

MAb1 samples and light treatment: Humanized monoclonal antibody, MAb1, was produced at Genentech, Inc. from a CHO cell line. Total three samples were prepared for photostability studies: 1) control sample, vial wrapped with aluminum foil; 2) test sample, vial light exposed (unwrapped); 3) Time Zero (T0), untreated. Both control & light exposed samples were kept in the light box for 24hrs. Light exposure following ICH1 guideline with following setting for a Atlas SUNTEST CPS+ Light Box: Irradiance level = 250 watts/square meter; Time set for 24 hours; Total UV dose = 538 watt-hours/square meter; Total visible dose = 1,320,000 lux-hours. **SEC:** The molecular size distribution of MAb1 samples was determined using isocratic conditions on a TosohHaas TSK G3000SWXL column (7.8 X 300 mm). The separation was conducted at ambient temperature with a flow rate of 0.5 mL/min. The column effluent was monitored at 280 nm.

CE-SDS-LIF: The MAb1 samples were analyzed by CE-SDS using a Beckman PA 800 CE system after labeled with a fluorescent dye. Separation was accomplished using SDS-MW gel buffer (Beckman Coulter) as the sieving matrix, with the capillary maintained at a temperature of 40°C throughout the separation. The migration of labeled components was monitored by laser-induced fluorescence (LIF).

Imaged capillary IEF: The distribution of charge variants in MAb1 samples was assessed by imaged cIEF using an ICE280 analyzer (Convergent Bioscience) with a fluorocarbon-coated capillary cartridge (100 µm X 5 cm). MAb1 samples were diluted, mixed with the ampholyte solution, and then focused by a potential of 3000 volts for 10 minutes. An image of the focused MAb1 charge variants was obtained by passing 280 nm UV light through the capillary and into the lens of a CCD digital camera.

LC/MS of intact & reduce MAb1: Both intact and reduced MAb1 samples were analyzed with Agilent 6210 ESI-TOF mass spectrometer coupled with a nano-Chip-LC system. The MAb1 samples, at about 5 ng antibodies per injection, were desalted by RP-HPLC for direct online MS analysis. Molecular masses were derived from multiple charged ions and deconvoluted using the Agilent MassHunter maximum entropy algorithms.

LC/MS/MS of tryptic MAb1 peptide map: MAb1 samples were digested with trypsin after reduction and alkylation. The resulting peptides were analyzed with a Thermo LTQ-Orbitrap mass spectrometer coupled with an Agilent 1200 capillary HPLC system. HPLC column: Jupiter Sum C18 250 X 1.0 mm; flow rate: 70 µL/min; column oven temperature: 55 °C; Solvent A: 0.1% TFA in water; B: 0.09% TFA in 90% ACN. Orbitrap setting: MS resolution 60000, MS/MS collected in data dependent mode, using LTO.

RESULTS & DISCUSSION

I. Turbidity & pH

MAb1 sample	pH	Turbidity	
		360nm	450nm
T0	5.8	0.0026	0.001
Control (foiled)	5.8	0.0026	0.001
light exposed	5.8	0.0085	0.0013

Table 1. Turbidity & pH results of light stressed MAb1 compared with control samples.

II. Potency: Antigen Binding Assay

Sample Description	Reportable Value (Mean)		n = 2
	(% Specific Activity)	%DIH*	
MAb1 T0 - Photostability	99	4	
MAb1 Control Photostability	104	6	
MAb1 Light-Exposed Photostability	72	14	

Table 2. Potency (antigen binding assay) results of light stressed MAb1 compared with control samples.

III. Purity: SEC & CE-SDS (non-reduced)

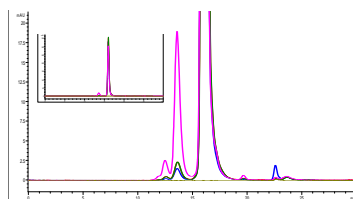


Figure 1: Overlay of SEC chromatograms of light stressed MAb1 and control samples. Significant increase in high molecular weight species was observed.

MAb1 Samples	%HMWS	% Main	%LMWS
Reference	0.8	99.1	0.1
Control	1.2	98.7	0.1
T0	1.2	98.7	0.1
Light Exposed	8.8	90.6	0.6

Table 3. SEC results of light stressed MAb1 compared with control samples.

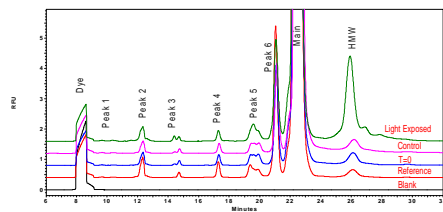


Figure 2: Overlay of CE-SDS-LIF (non-reduced) electropherograms of light stressed MAb1 and control samples. Again, significant increase in high molecular weight species was observed.

MAb1 Sample	Peak 1		Peak 2		Peak 3		Peak 4		Peak 5		Peak 6		Main	HMW
	% Corrected Area	% Corrected Area	% Corrected Area	% Corrected Area	% Corrected Area	% Corrected Area	% Corrected Area	% Corrected Area	% Corrected Area	% Corrected Area	% Corrected Area			
Ref	0.05	0.96	0.16	0.47	0.85	4.11	92.9	0.56						
Control	0.07	0.71	0.18	0.34	0.96	2.94	93.3	1.59						
T0	0.07	0.72	0.18	0.36	0.96	2.98	93.7	1.04						
Light Exposed	0.12	0.97	0.30	0.39	1.24	3.65	86.6	6.70						

Table 4. CE-SDS-LIF (non-reduced) results of light stressed MAb1 compared with control samples.

IV. Charge Heterogeneity Profile: imaged cIEF

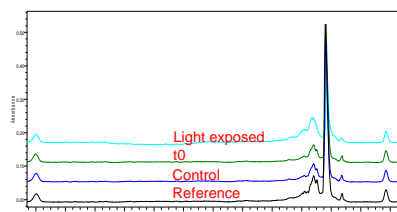


Figure 3: Overlay of imaged capillary IEF electropherograms of light stressed MAb1 and control samples. Significant increase in acidic species was observed.

MAb1 Sample	%Acidic	% Main	%Basic
REF	30.7	66.7	2.7
CONTROL	29.2	67.7	3.2
T0	27.9	69.1	3.1
Light Exposed	59.0	38.2	2.9

Table 5. Imaged capillary IEF results of light stressed MAb1 compared with control samples.

V. Mass determination: LC/MS of Intact & Reduced MAB

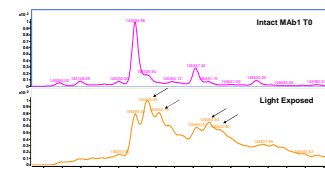


Figure 4: Deconvoluted MS spectra of intact MAb1 samples. Potential oxidized products were observed in light exposed sample (with arrow).

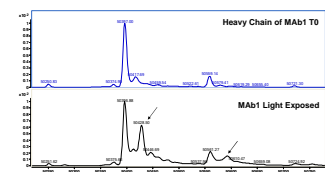


Figure 5: Deconvoluted MS spectra of reduced MAb1 samples (heavy chain). Potential oxidized products were observed in light exposed sample (with arrow).

VI. LC/MS/MS of Tryptic Peptide Map

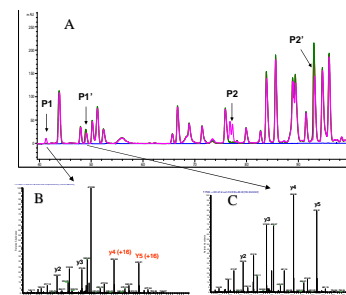


Figure 6: Identification of methionine oxidation in MAb1: A) Overlay of RP-LC UV profiles of tryptic peptide map of light stressed MAb1 and control samples. P1 & P1' were Mox and native forms of peptide DTLMISR respectively, P2 & P2' were Mox and native forms of another peptide. B) MS/MS spectrum of DTLMISR with methionine oxidation. C) MS/MS spectrum of native DTLMISR.

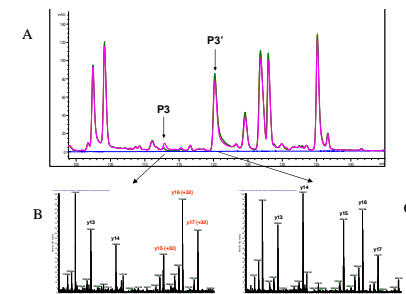


Figure 7: Identification of tryptophan oxidation (+32) in MAb1: A) Overlay of RP-LC UV profiles of tryptic peptide map of light stressed MAb1 and control samples. P3 & P3' were Wox2 and native forms of peptide ...XXASWDAXX... respectively. B) Partial MS/MS spectrum of ...XXASWDAXX... with tryptophan oxidation (+32). C) Partial MS/MS spectrum of native ...XXASWDAXX...

MAb1 Peptide	Control % Oxidation	Light Exposed % Oxidation
DTLMoxISR	2	40
...XXSVWoxHEXX...	1.5	30
...XXASWox2DAXX...	0	10
...XXNAWox2MSXX...	0	1

Table 6: Summary of oxidation level of several methionine and tryptophan residues in MAb1 light stressed and control samples.

CONCLUSIONS

1. Monoclonal antibody, MAb1, was subjected to light stress and the resulting samples were comprehensively characterized by various functional and analytical methods.
2. Reduced antigen binding affinity was observed in the light stressed MAb1.
3. Higher amount of aggregates, acidic species and oxidation products were observed in the light stressed material.
4. Two methionine sulfoxide oxidation sites in the Fc region and two tryptophan oxidation (+32) sites in the CDRs were identified by LC/MS/MS analysis of tryptic peptide maps.

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