Redox-active tyrosine residues: role for the peptide bond in electron transfer

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Supporting Information

Electrospray mass spectrometry (MS) was used to assess isotope incorporation into chlorophyll *a* (chl *a*). The cyanobacterium, *Synechocystis* sp. 6803, was cultured in the presence either of natural abundance (control) or $^2H_4$ labeled tyrosine.[1] Chl *a* was extracted from isolated photosystem I,[2] using a seven-fold dilution into 100% methanol. Denatured protein was then pelleted by centrifugation for 1 min in an Eppendorf centrifuge at 6.5 rpm. This procedure leaves extracted chl *a* in the supernatant. For MS analysis, the extracted chl *a* was again diluted seven-fold into an aqueous solution, containing 80% methanol and 1% acetic acid. Final chl concentrations ranged from 0.014 to 0.017 mg chl/ml.

The chl *a* sample was infused directly into a ThermoFinnigan (San Jose, CA) LCQ ion trap mass spectrometer. The LCQ heated capillary temperature was 230°C, and the spray voltage was 4.7 kV. Full scans were collected for a range of 300 to 1200 *m/z* (data not shown) and were followed by zoom scans with a center at 894 ± 10 *m/z* (Fig. 1). Other default settings from ThermoFinnigan were used for the full and zoom scans. Positive ions were detected.

Fig. 1 presents electrospray ionization MS data acquired from chl *a*. The parent $[M+H^+]$ ion corresponds to the most abundant ion at 893.5 *m/z*.[3] In Fig 1A, chl *a* was extracted from cultures grown on control tyrosine. In Fig. 1B, chl *a* was extracted from cultures grown on labeled tyrosine. Incorporation of deuterium into chl *a* would result in a change in the isotope distribution in Fig. 1B, giving increased abundance at 894.5, 895.5 and 896.5 *m/z* relative to the $[M+H]^+$ ion. Upon comparison of Fig. 1A and B, no statistically significant difference in the relative isotope distribution was observed (Table 1). These results indicate that there is no detectable deuterium incorporation into chl *a* from $^2H_4$ tyrosine.

Table 1. Electrospray MS analysis of chl *a*.

Chl sample	*893.5 m/z*	*894.5 m/z*	*895.5 m/z*	*896.5 m/z*
Control	100	60.62 ± 1.54	20.16 ± 0 .67	5.02 ± 2.08
$^2H_4$- labeled	100	59.45 ± 1.16	19.59 ± 0.46	5.12 ± 0.33

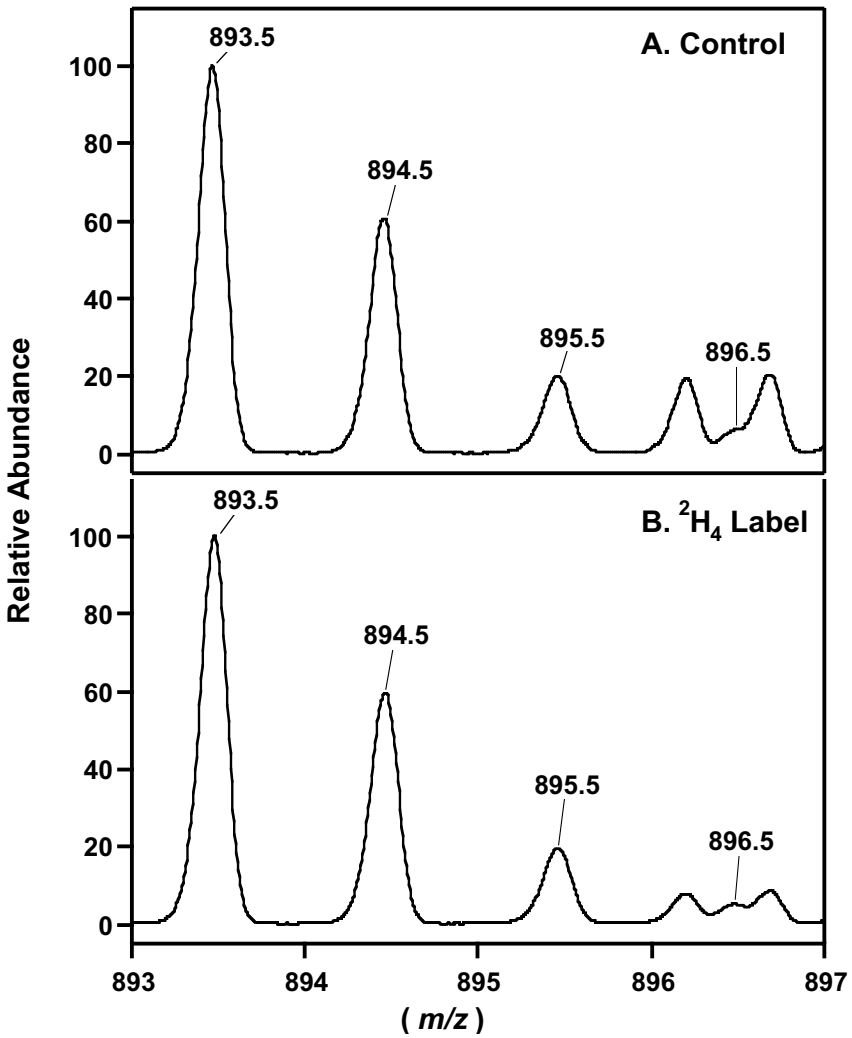

Figure 1. Representative electrospray mass spectra of chl *a*. (A) Chl *a* was extracted from cyanobacteria cultured on control tyrosine (1164 scans). (B) Chl *a* was extracted from cyanobacteria cultured on $^2H_4$ labeled tyrosine (1364 scans). In (A) and (B), the spectra have been normalized to the abundance of the $[M+H]^+$ peak at 893.5 *m/z*.

References

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