Supporting Information

A Snapshot of Enzyme Catalysis Using Electrospray Ionization Mass Spectrometry

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Preparation of sample solutions.

1 mL arabinose-5-phosphate (A5P) (5 mM) (cat# A2013, Sigma-Aldrich Com.) in 10 mM ammonium acetate (cat# 43,131-1, Sigma-Aldrich Co.) buffer (pH 7.8) was passed through IC-H Maxi-Clean Cartridge (Part # 30256, Alltech Corp.) to remove metal ions, and then the pH of A5P solution was adjusted to 7.6 by ammonium hydroxide (Cat # 9721-01m J. T. Baker, Phillipsburg, NJ). The concentrated A5P solution was diluted to 60 μM by addition of 10 mM ammonium acetate buffer (pH 7.8). The solution of the enzyme 3-deoxy-D-*manno*-2-octulosonate-8-phosphate synthase (KDO8PS) was prepared by overnight dialysis against 20 mM ammonium acetate buffer, pH 7.8. The concentrated KDO8PS was diluted to 80 μM by 10 mM ammonium acetate buffer (pH 7.8).

ESI-MS experiments.

The method is based on the continuous flow setup (Figure 1). Two syringes were advanced simultaneously by a syringe pump (model PHD 2000 infusion, Harvard Apparatus, South Natick, MA) at several total flow rates of 220, 160, 100, and 10 μL/min, respectively. Syringe 1 (volume of 1000 μL) contained the solution of the KDO8P synthase in 10 mM NH_4OAc buffer, pH 7.8; syringe 2 (volume of 1000 μL) contained the substrate, A5P, in the same buffer. Both syringes were connected to a 'reaction' fused silica capillary (TSP030150, i.d. of 30 ± 3 μm, Polymicro Technologies, Phoenix, AZ) by a zero dead volume-mixing tee (MY1XCS6, Valco Instrument Co. Inc., Houston, TX). The reaction time is controlled by the total flow rate for the same length of the 'reaction' capillary between the mixing point and other end of the fused silica capillary located in electrospray source. The length of the 'reaction' fused silica capillary and flow rates allowed reaction time points 7, 10, 16, and 160 ms, corresponding to the total flow rates 220, 160, 100, and 10 μL/min, respectively. The intensities of KDO8PS were kept at the constant at the different total flow rates by adjusting the position of electrospray ionization probe. The length for the electrospray ionization probe is 3.7 cm and was custom designed and built by Analytica of Branford (Branford, CT).

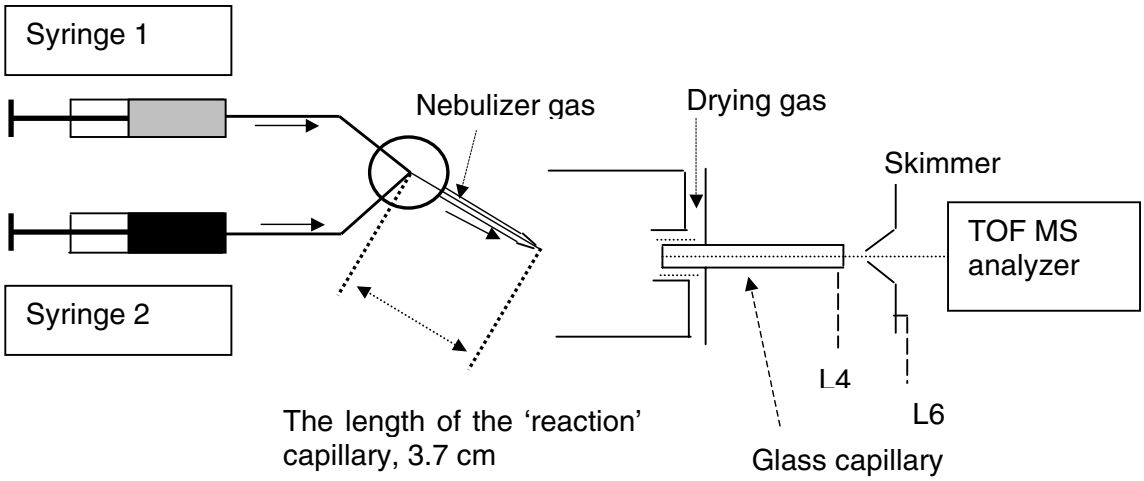

Figure 1. Experimental apparatus for ESI mass spectrometric experiments carried out in this work. Solid arrow indicates the flow directions of the solution from two syringes to ESI source. Collision energy was applied between L4 and L6.

The voltage applied between L4 and L6 called CID energy is very important to detect the non-covalent complexes, so we optimized all parameters in order to observe the clear peak of intermediate complex. The voltage 120 between L4 and L6 is optimum value for us to detect the intermediate complex.

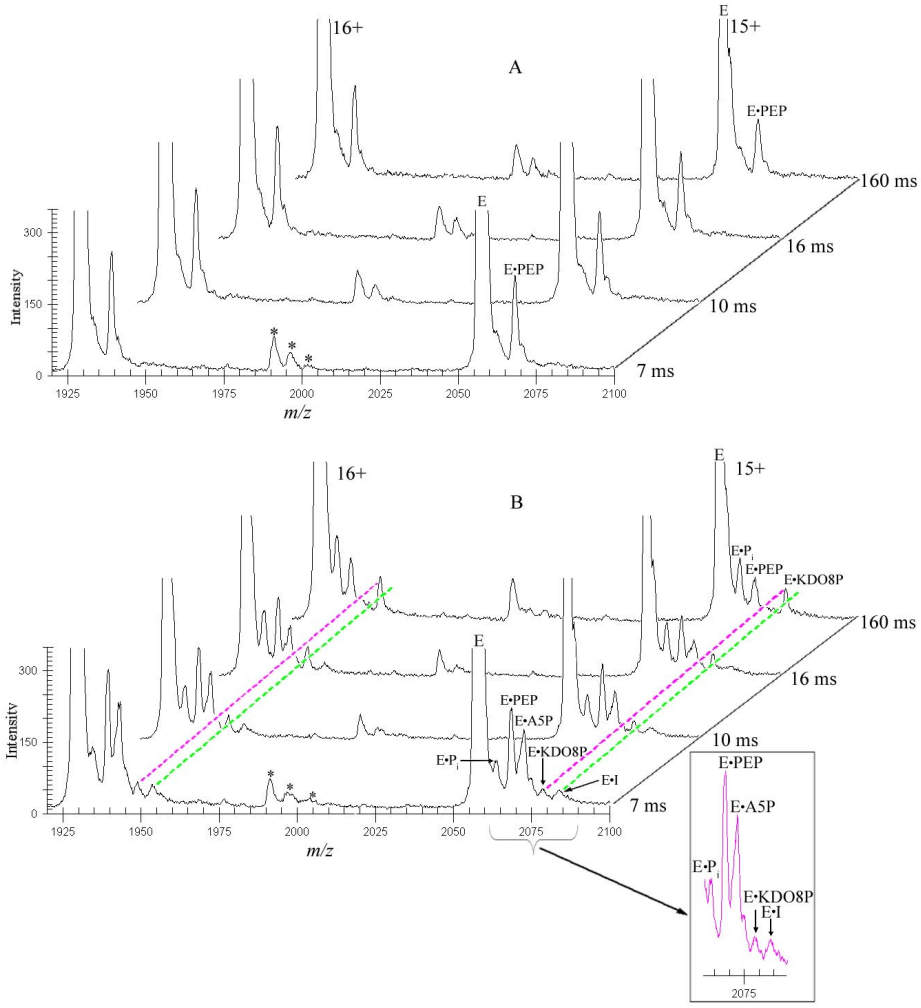

Figure 2. ESI-TOF mass spectra of KDO8PS during catalysis. **(A)** ESI-TOF mass spectra of KDO8PS containing the first substrate PEP, in the positive ion mode. Each trace represents the average of 15 mass spectra recorded in Trap Pulse Mode with 60000 pulses per mass spectrum. The peaks marked E and E•PEP correspond to the KDO8PS and KDO8PS with PEP bound, respectively, at 15+ and 16+ charge states at the reaction times: 7 ms, 10 ms, 16 ms, and 160 ms. [A minor contaminant protein (<3%) remaining after multiple purification steps is denoted by *]. **(B)** A time course of KDO8PS catalysis during single turnover reaction monitored by ESI-TOF MS in the positive ion mode. The E•PEP was mixed with the second substrate, A5P. Each trace represents the average of 15 mass spectra recorded in Trap Pulse Mode with 60000 pulses per mass spectrum. The peaks marked E, E•P_i , E•PEP, E•A5P, E•KDO8P and E•I correspond to KDO8P synthase and the enzyme complexes with phosphate (P_i), phosphoenolpyruvate (PEP), D-arabinose-5-phosphate (A5P), KDO8P and intermediate **I**, respectively, at 15+ and 16+ charge states at the reaction times: 7 ms, 10 ms, 16 ms, and 160 ms. [A minor contaminant protein (<3%) remaining after multiple purification steps is denoted by *].

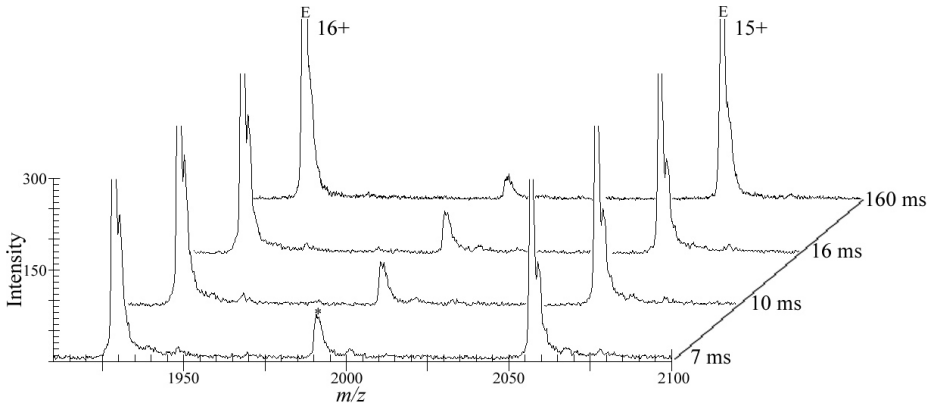

Figure 3. The positive-ion ESI-TOF mass spectra of PEP-free KDO8P synthase. KDO8P synthase was incubated with the same amount of the second A5P for 30 min at 4˚C, the substrate PEP binding originally on KDO8P synthase was removed away from KDO8P synthase. Each trace represents the average of 15 mass spectra recorded in Trap Pulse Mode with 60000 pulses per mass spectrum. The peak marked E correspond to KDO8P synthase at 15+ and 16+ charge states at the reaction times: 7 ms, 10 ms, 16 ms, and 160 ms. [A minor contaminant protein (<3%) remaining after multiple purification steps is denoted by *].

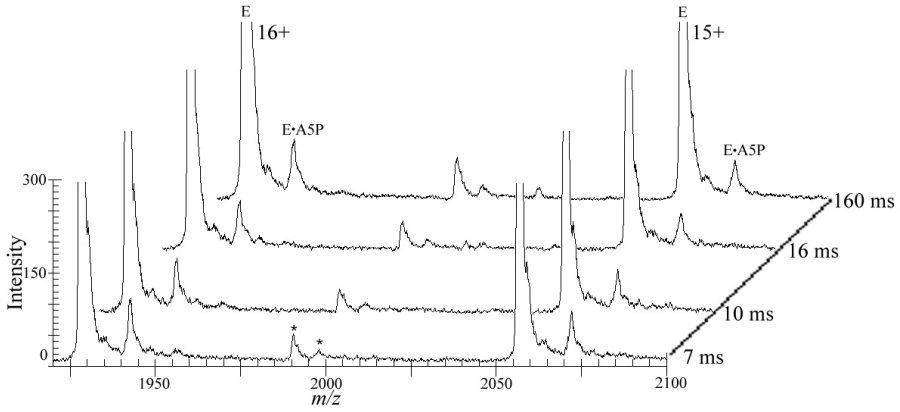

Figure 4. The positive-ion ESI-TOF mass spectra of PEP-free KDO8P synthase with A5P. KDO8P synthase containing the first substrate PEP was incubated with the same amount A5P for 30 min at 4˚C, and then put the second substrate, A5P back to PEP-free KDO8P synthase. This mixture was incubated for 30 min at 4˚C. Each trace represents the average of 15 mass spectra recorded in Trap Pulse Mode with 60000 pulses per mass spectrum. The peaks marked E and E•A5P correspond to the KDO8PS and KDO8PS with A5P bound, respectively, at 15+ and 16+ charge states at the reaction times: 7 ms, 10 ms, 16 ms, and 160 ms. [A minor contaminant protein (<3%) remaining after multiple purification steps is denoted by *].