Tryptophan Synthase: Structure and Function of the Monovalent Cation Site

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SUPPORTING INFORMATION

RESULTS

Kinetic Simulation of the Transient Formation and Decay of the MVC-free $E(Q)_{indoline}$ in the Indoline Reaction. The kinetic and spectroscopic evidence presented in Figures 4 and 5 indicate formation of a closed, inactive E(A-A) consistent with Scheme 2. To test the validity of Scheme 2, a kinetic simulation was performed using the program Kinetic 3.11 (Figure S1B, Table S1). Reaction rates and equilibria calculated by Woehl and Dunn (9), for stage I of the β-reaction, together with relaxation rates for the reaction of E(A-A) with indoline, were used as rough estimates for the starting parameters in the simulation (9). The rate constants for formation of $E(Q)_{indoline}$ used in the simulation (Table S1) are incorporated into Scheme 2. The binding of L-Ser, and the conversion of E(Ain)(L-Ser) to $E(Aex_1)$, are much faster processes than is the conversion of $E(Aex_1)$ to E(A-A). These steps are essentially decoupled from the subsequent chemical steps and, because there is a strong forward commitment to give $E(Aex_1)$, do not influence the magnitude of the subsequent relaxation rates. Therefore for the sake of simplicity, the binding of L-Ser and the steps leading up to $E(Aex_1)$ formation were

omitted in the simulated time courses, and the steps included in the simulation begin with $E(Aex_1)$ (Scheme 3, Table S1). Simulations using the entire scheme (Scheme 2, data not shown) confirmed that the amplitudes and relaxation rates obtained gave essentially the same results as did the simulation where the fast steps are excluded (Scheme 3).

The rate constants used to simulate the times course under various MVC conditions are reported (Table S2). The simulated rate constants were constrained to prevent the yield of quinonoid species from exceeding the amount of $E(Q)_{indoline}$ observed in the SWSF experiments. Figure S1 and Table S2 compare the simulated time courses and the experimental SWSF data obtained at both 466 nm and 350 nm. The simulation of the 466 nm time course considers only the formation of $E(Q)_{indoline}$. The simulation of the 354 nm time course takes into consideration the formation of E(A-A), E(A-A)(indoline), and inactive E(A-A) by summing all of the values, and plotting these sums as a function of time. The extinction coefficients estimated for E(A-A), ε_{354} = 10,000 M^{-1} cm^{-1}, and for $E(Q)_{indoline}$, ε_{466} = 60,000 M^{-1} cm^{-1}, were used to estimate absorbance values at both 466 and 354 nm. Table S2 presents the relaxation rates and amplitudes for the formation and decay phases of the $E(Q)_{indoline}$ time course. This table compares the experimental data with parameters obtained from the simulation time courses for the reaction of L-Ser and indoline with the MVC-free form.

Table S1: Summary of Rate Constants Used in Scheme 3 to Generate the Kinetic Simulations Shown in Figure S1.								
MVC	k_1 (s^{-1})	k_{-1} (s^{-1})	k_2[a] (s^{-1})	k_{-2} (s^{-1})	k_3 (s^{-1})	k_{-3} (s^{-1})	k_4 (s^{-1})	k_{-4} (s^{-1})
MVC free	25	2.5	1000	1	1.4	1	88	200
CsCl, 0.5 mM	40	4	1000	1	0.14	1	88	100
CsCl, 10 mM	100	10	1000	1	0.14	5	166	100
NaCl, 0.5 mM	14	6	1000	1	1.4	2	166	100
NaCl, 10 mM	14	6	1000	1	1.4	10	166	20

[a]Pseudo first-order rate constant, $k_2 = k'_2$[indoline]

Table S2: Comparison of Relaxation Rates and Amplitudes From Analysis of Time Courses in Figure S1.								
	simulation (at 466 nm)				experiment (at 466 nm)			
MVC	A_1[b]	$1/\tau_1$ (s^{-1})	A_2[a]	$1/\tau_2$ (s^{-1})	A_1[b]	$1/\tau_1$ (s^{-1})	A_2[a]	$1/\tau_2$ (s^{-1})
MVC free	0.5	22.9	0.20	2.00	0.26	22.9	0.2	1.73
CsCl, 0.5 mM	0.86	33.4	0.04	1.26	0.66	40.3	0.13	1.20
CsCl, 10 mM	1.17	59.5	0.01	0.63	0.86	75.0	0.07	0.71
NaCl, 0.5 mM	0.92	12.7	0.02	2.61	1.38	10.7	0.50	2.00
NaCl, 10 mM	1.25	13.0	0.00	0.37	1.63	10.1	0.09	0.34

[a]Increasing absorbance.

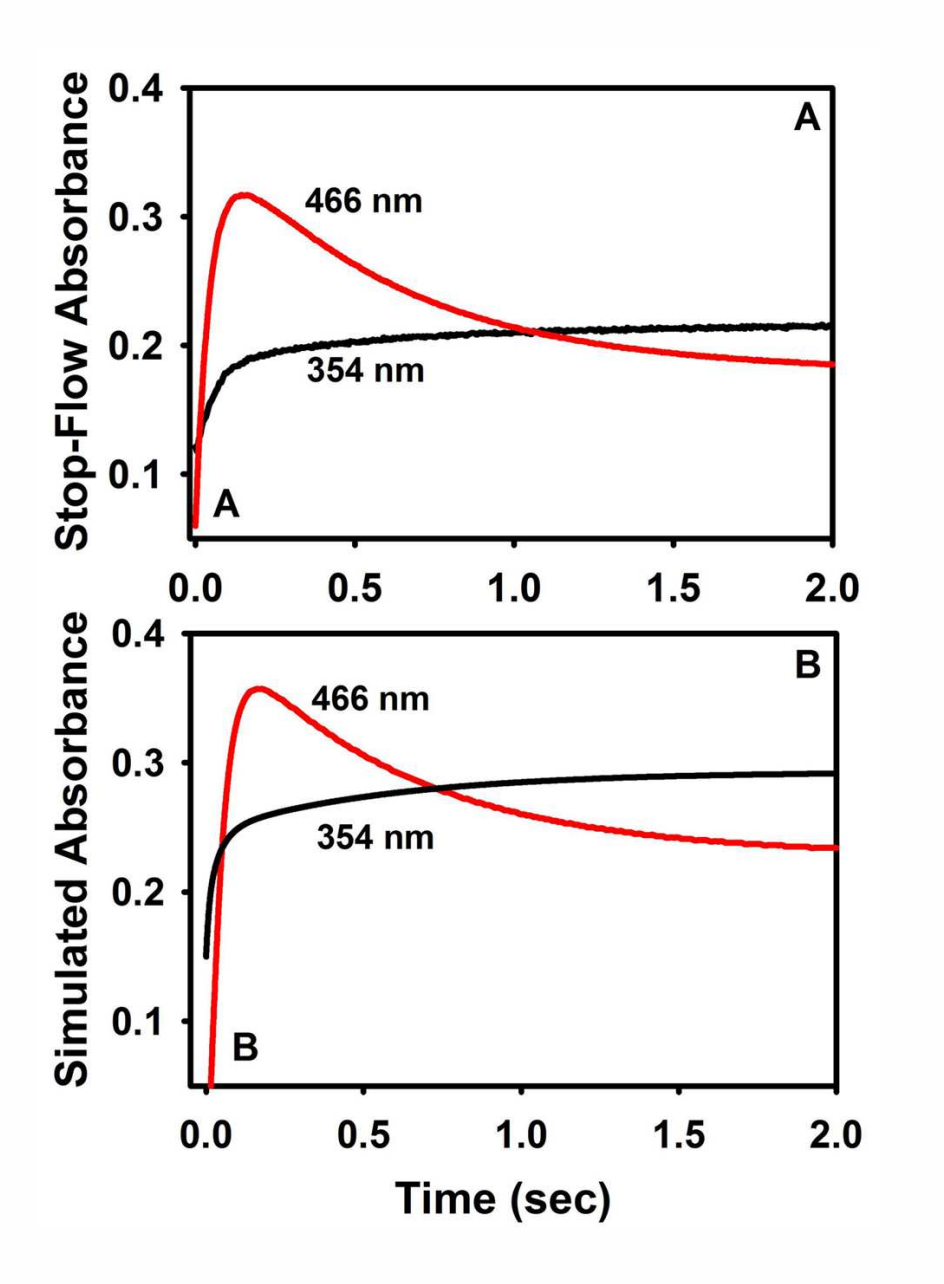

Figure S1. Comparison of observed (A) and simulated (B) time courses for reaction of indoline and L-Ser with the MVC-free enzyme at 354 and 466 nm, respectively. The time course simulations are based on Scheme 3 using rate constants given in Table S1. The observed Relaxation rates are given in Table S2.