Supporting Information

F6 Binding to the Complexes Formed with L-Trp. The $(Na^+)E(Ain)$ complex gives a mixture of covalent intermediates when reacted with L-Trp. This mixture is dominated by the external aldimine and quinonoid species, $(Na^+)E(Aex_2)$ and $(Na^+)E(Q_3)$.[1-5] Tryptophan synthase cleavage of L-Trp to give indole and the $(Na^+)E(A\text{-}A)$ is an extremely slow process and is insignificant on the time scale of these experiments.[6] Titration of the F6 complex of $(Na^+)E(Ain)$ with L-Trp (Figure S1a) shows that at low L-Trp concentrations, the spectrum consists of peaks for free (-57.00 ± 0.01 ppm) and bound F6 (-56.52 ± 0.01 ppm). As the concentration of L-Trp increases, the -56.52 ± 0.01 ppm peak decreases in area and a new bound peak with chemical shift –55.45 ± 0.01 ppm appears. The area of this peak increases and then saturates at high L-Trp concentrations. The UV/Vis absorption spectrum of an NMR sample measured at the highest L-Trp concentration (Figure S1b), shows the sample is comprised of $(F6)(Na^+)E(Aex_2)$ with λ_{max} = 418 nm, together with a trace of $(F6)(Na^+)E(Q)_{L\text{-}Trp}$ (λ_{max} = 418 nm and 476 nm, respectively).[2,4,5,7-9]

Monovalent Cation Effects on the Reaction of L-His with the (F6)E(Ain) Complex. The substitution of Cs^+ for Na^+ in all of the systems under investigation herein, alters the redistribution of β-site chemical intermediates and the distribution of open and closed conformational states. Thus the distribution of chemical species (i.e., external aldimine vs. α-aminoacrylate vs. quinonoid species) is changed, but the chemical shifts observed for the bound forms of F6 and F9 are unchanged. For example, the observed chemical shift of $(F6)(Na^+)E(Ain)$ is identical to that of $(F6)(Cs^+)E(Ain)$, and the chemical shifts of F6 bound to E(A-A) species and to E(Q) species are unperturbed by substitution of Na^+ by Cs^+.

The L-Trp analogue, L-His, reacts with the Na^+ and Cs^+ forms of the (F6)E(Ain) complex to give equilibrating mixtures dominated by the external aldimine and quinonoid complexes.[5] The distribution of external aldimine and quinonoid species at equilibrium is dependent on the particular monovalent cation bound to the MVC site of the β-subunit.[3,10-15] The ^{19}F NMR spectra presented in Figure S2a establish that the distribution of bound intermediates is sensitive to the particular MVC, with Cs^+ favoring the complex with chemical shift -55.45 ± 0.01 ppm, whereas, Na^+ gives bound species with chemical shifts of -55.45 ± 0.01 ppm and -56.57 ± 0.01 ppm. The UV/Vis absorbance spectra presented in Figure S2b show that the Cs^+ complex (a) favors the $(F6)(Cs^+)E(Q)_{L\text{-}His}$ species (λ_{max} = 466 nm), while the Na^+ complex gives a mixture of species with λ_{max} = 466 nm, corresponding to the quinonoid species, and a broad envelope with λ_{max} ~ 423 nm corresponding to the $(F6)(Cs^+)E(Aex)_{L\text{-}His}$ species. The UV/Vis spectra in Figure S2c show the spectral changes resulting from the titration of the $(F6)(Na^+)E(Ain)$ complex with L-His. These spectra establish that the spectrum of the internal aldimine (λ_{max} = 412 nm) disappears as L-His reacts to form the $(F6)(Cs^+)E(Aex)_{L\text{-}His}$ and $(F6)(Cs^+)E(Q)_{L\text{-}His}$ species (Figure S2c). The plot of ΔAbs_{466nm} vs. [L-His] taken from the data in Figure S2c is shown in Figure S2d.

Supplemental References

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Figure Captions: Supporting Information

Figure S1a: Titration of the (F6)(Na^+)E(Ain) complex with L-Trp. Concentrations: αβ, 0.800 mM; L-Trp (increasing from bottom to top): 0.00, 53 μM, 122 μM, 254 μM, 382 μM, 793 μM 1.25 mM, 2.03 mM. **Figure S1b**: UV/Vis absorption spectra of the NMR samples in Figure S1a corresponding to the (F6)(Na^+)E(Ain) complex (black) and of the species formed when a saturating concentration of L-Trp is reacted with the (Na^+)E(Ain) complex (red).

Figure S2a: Comparison of the effects of 20 mM Na^+ (red) and 200 mM Cs^+ (black) on the ^{19}F NMR spectra resulting from reaction of 100 mM L-His with (F6)E(Ain). [αβ] = 1.40 mM; [F6] = 2.8 mM. **Figure S2b**: UV/Vis absorbance spectra for NMR samples presented in 7a. Na^+ complex, red, Cs^+ complex, black. **Figure S2c**: The UV/Vis spectra for the titration of the (F6)(Na^+)E(Ain) complex with L-His. [αβ] = 10mM; [F6] = 2.8 mM; [αβ] = XX; [Na^+] = 100 mM; L-His concentrations: 1.59 mM, 3.92 mM, 11.32 mM, 21.42 mM, 43.75 mM, 100.00 mM. **Figure S2d**: Plot of ΔAbs_{466nm} vs. [L-His]. Data taken from Figure S2c.

Figure S1

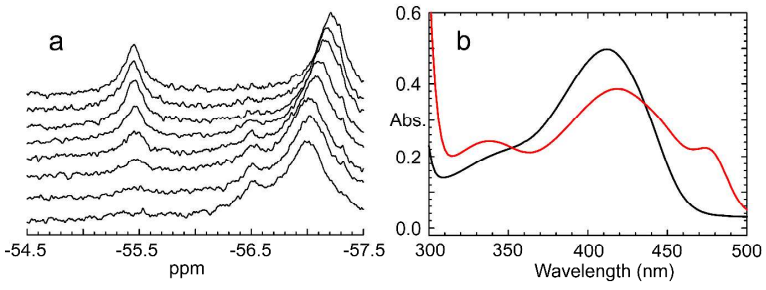
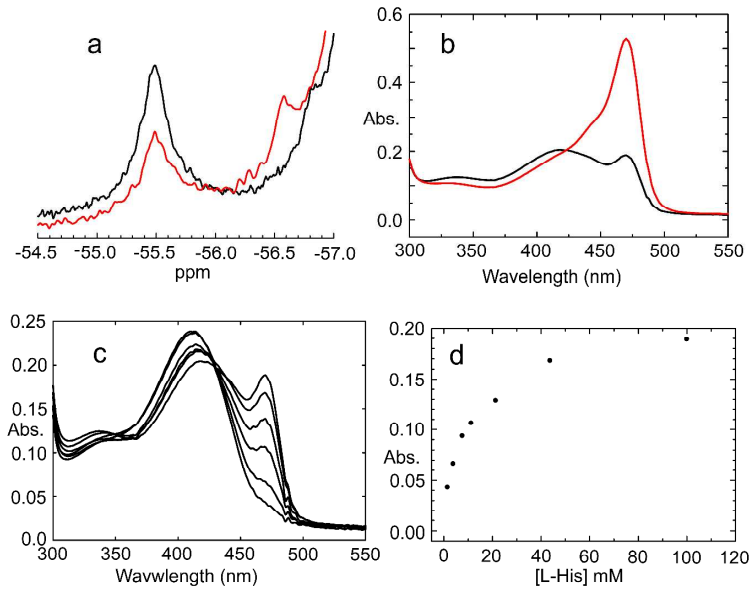


Figure S2


a
-54.5 -55.0 -55.5 -56.0 -56.5 -57.0
ppm
b
0.6
0.4
Abs.
0.2
0.0
300 350 400 450 500 550
Wavelength (nm)
c
0.25
0.20
0.15
Abs.
0.10
0.05
0.0
300 350 400 450 500 550
Wavwlength (nm)
d
0.20
0.15
0.10
Abs.
0.05
0.00
0 20 40 60 80 100 120
[L-His] mM