Alteration of the Diastereoselectivity of 3-Methylaspartate Ammonia Lyase by Using Structure-Based Mutagenesis

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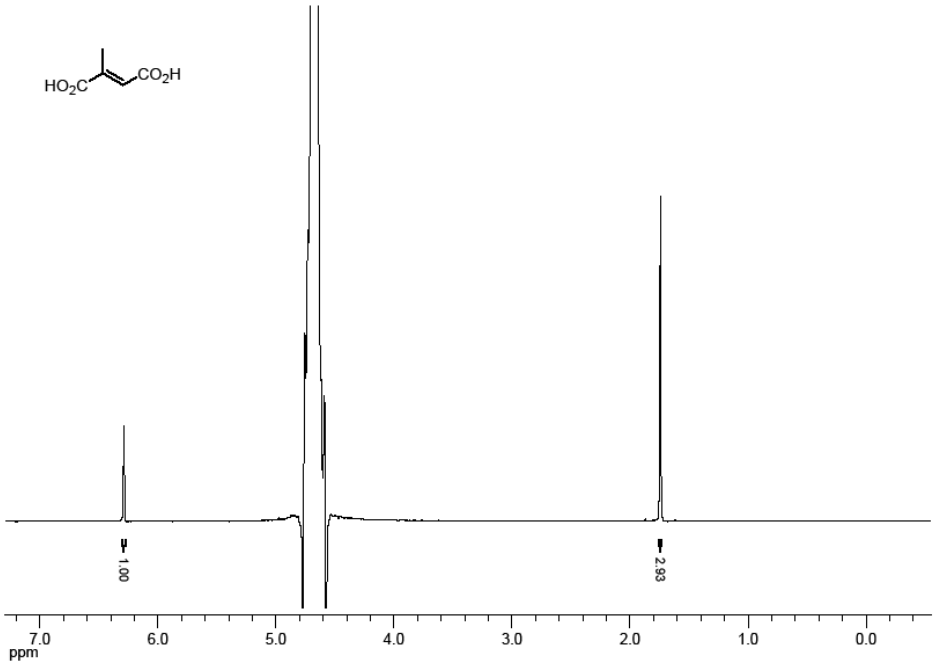

Figure S1. ^{1}H NMR spectrum of mesaconate (**1**). δ = 1.73 (s, 3H; CH_3), 6.28 (s, 1H; CH).

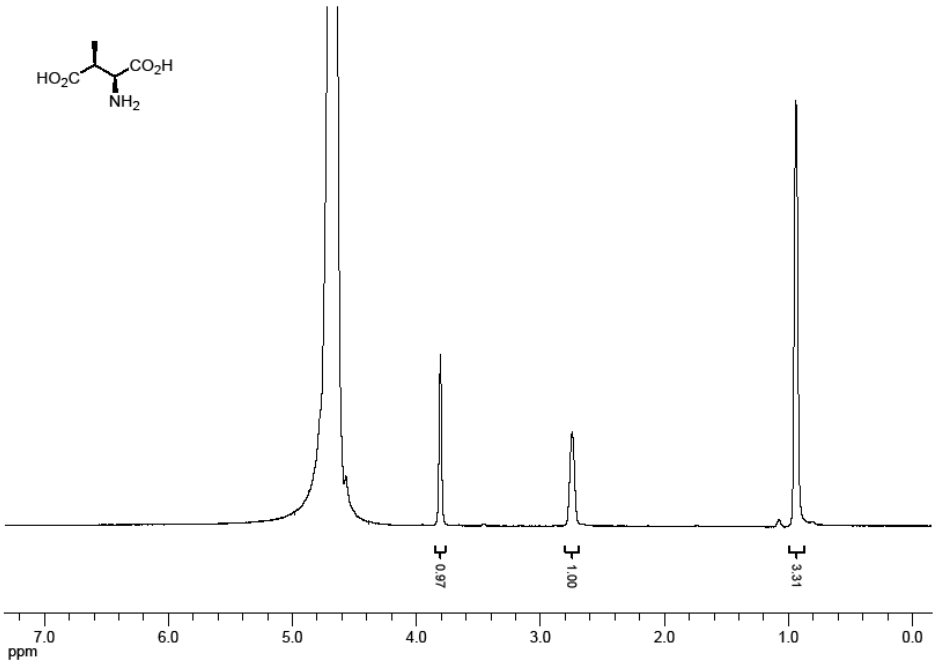

Figure S2. ^{1}H NMR spectrum of the material used as substrate **2**, which is a 1:1 mixture of the enantiomers (2*S*,3*S*)- and (2*R*,3*R*)-3-methylaspartic acid. The (2*R*,3*R*)-enantiomer is not a substrate nor an inhibitor of MAL. δ = 0.94 (s, 3H; CH_3), 2.74 (s, 1H; CH), 3.81 (s, 1H; CH).

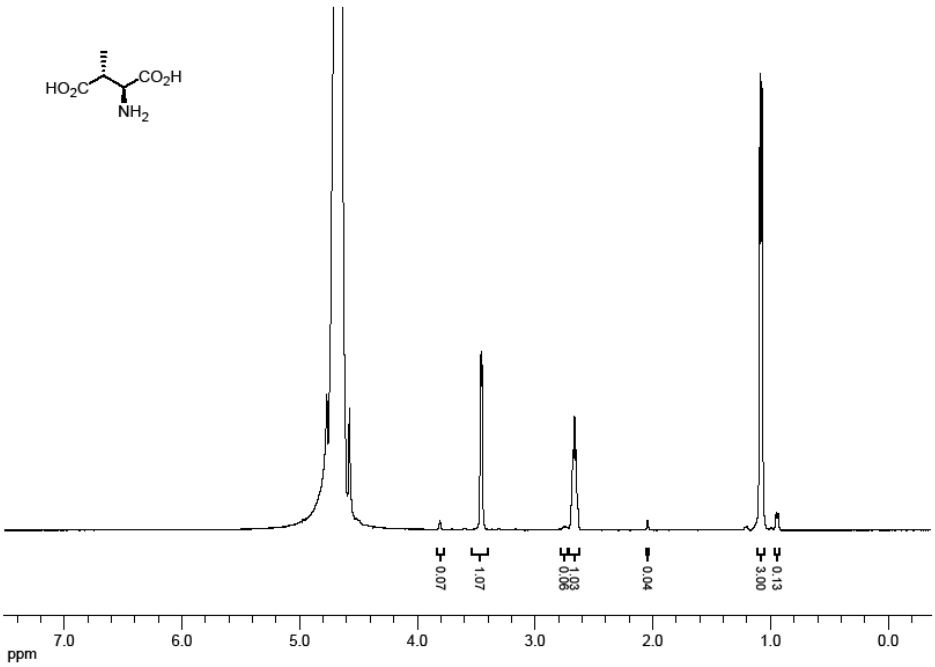

Figure S3. ^{1}H NMR spectrum of (2*S*,3*R*)-3-methylaspartic acid (**3**). δ = 1.08 (d, ^{3}J = 7.0 Hz, 3H; CH_3), 2.63-2.70 (m, 1H; CH), 3.46 (d, ^{3}J = 4.0 Hz, 1H; CH). Contaminant **2** (5-6%): δ = 0.94 (d), 2.04 (s), 2.74 (m), 3.81 (s).

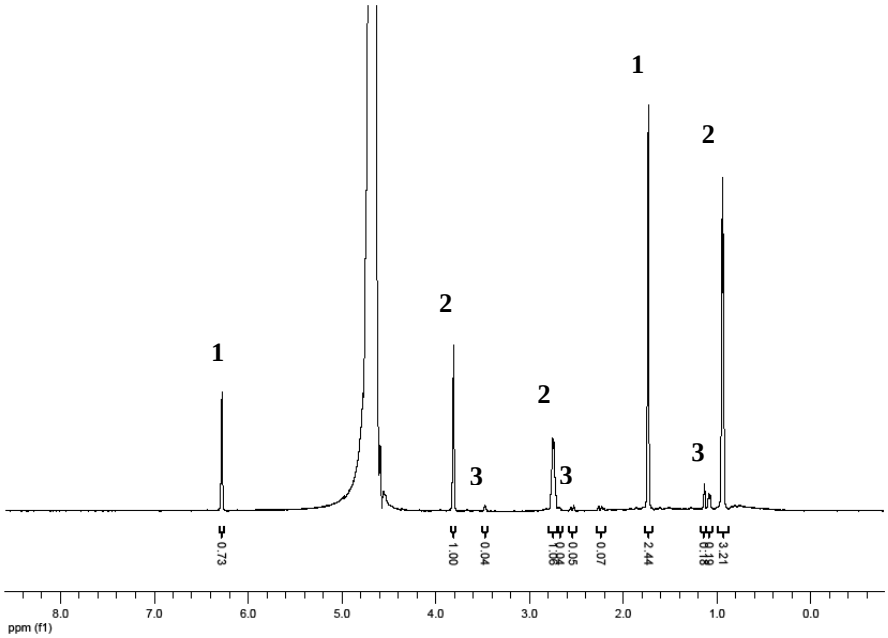

Figure S4. ^{1}H NMR spectrum identifying the products of the MAL-catalyzed deamination of **2**. The spectrum was taken after a 13 day-incubation period. Ratio **1** : **2** : **3** = 41 : 57 : 2. δ = 0.93 (d, ^{3}J = 6.5 Hz, 3H; CH_3), 1.08 (d, ^{3}J = 7.5 Hz, 3H; CH_3), 1.74 (s, 3H; CH_3), 2.71-2.78 (m, 2H; CH), 3.46-3.48 (m, 1H; CH), 3.81 (s, 1H; CH), 6.28 (s, 1H; CH_3). Impurity δ = 1.13 (s), 2.24 (d), 2.54 (d).

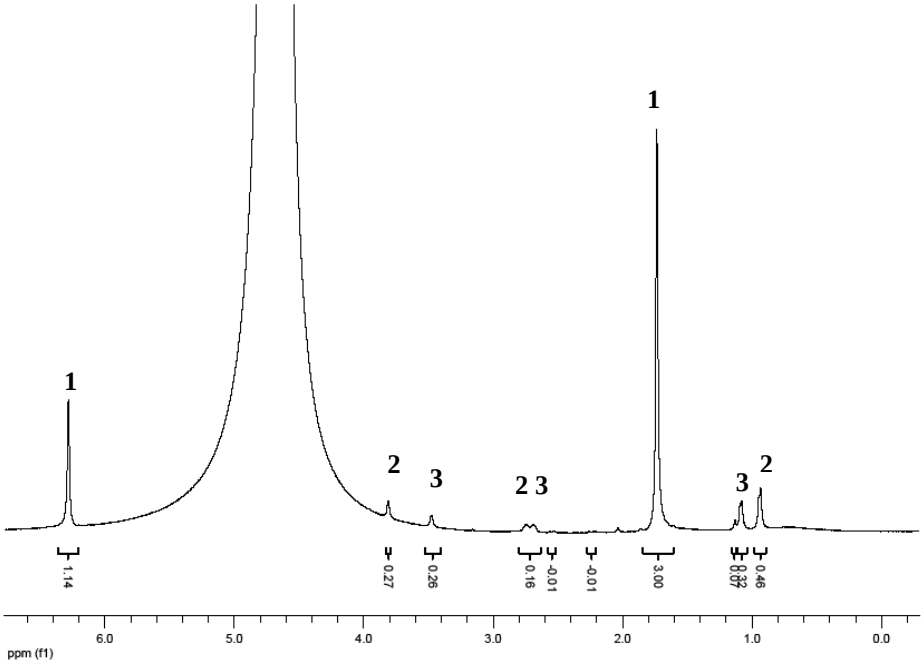

Figure S5. ^{1}H NMR spectrum identifying the products of the MAL-catalyzed deamination of **3**. The spectrum was taken after a 13 day-incubation period. Ratio **1** : **2** : **3** = 79 : 12 : 9. δ = 0.93 (d, ^{3}J = 6.5 Hz, 3H; CH_3), 1.08 (d, ^{3}J = 7.5 Hz, 3H; CH_3), 1.74 (s, 3H; CH_3), 2.65-2.78 (m, 2H, CH), 3.47 (s, 1H; CH), 3.81 (s, 1H; CH), 6.28 (s, 1H; CH_3). Impurity δ =1.13 (s), 2.24 (d), 2.54 (d).

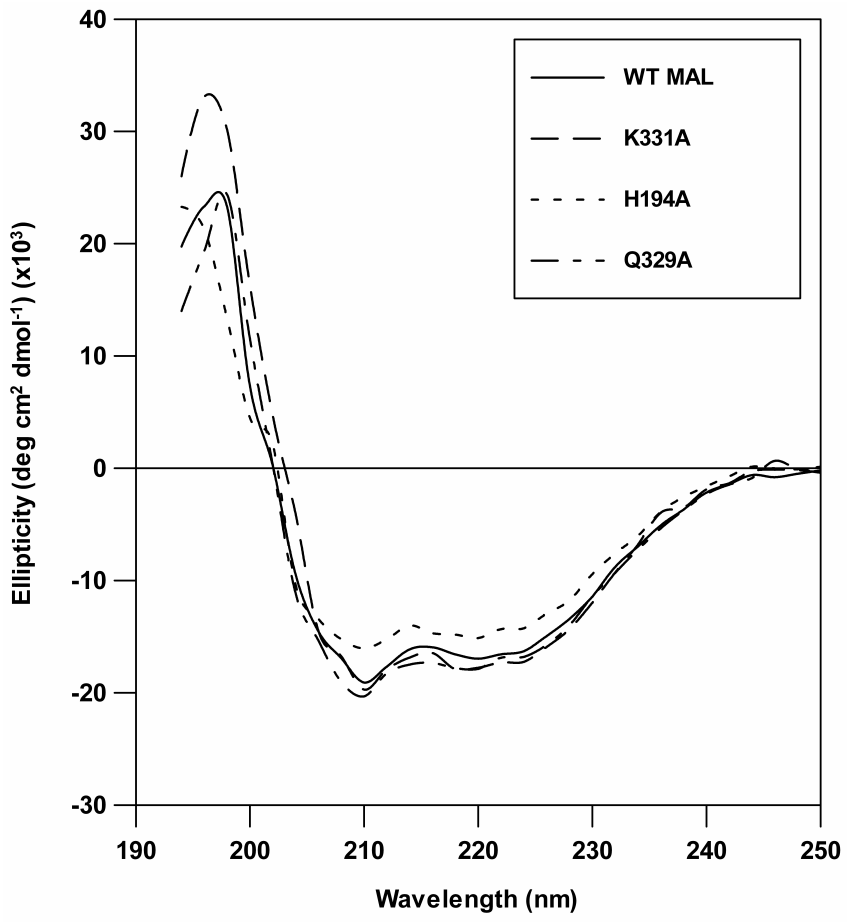

Figure S6. Superimposed far-UV CD spectra of wild-type and mutant enzymes. The CD spectra of all the mutants were comparable to that of wild-type MAL, but for clarity only the CD spectra of the K331A, H194A, and Q329A mutants are shown. Spectra were measured in 10 mM Tris buffer (pH 8.0), containing 2 mM $MgCl_2$ and 0.1 mM KCl, at a protein concentration of approximately 3.2 µM.