Nucleoside Analog Studies Indicate Mechanistic Differences Between RNA Editing Adenosine Deaminases

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Supplementary Information

Pages

Supplementary Methods

Gel Shifts

ADAR1 (130 nM final concentration) was mixed with ≤ 18 nM ^{32}P labeled RNA prepared by splint ligation (described in Experimental section) in assay buffer containing 15 mM Tris-HCl, pH 7.0, 1.5 mM EDTA, 60 mM KCl, 3% glycerol, 0.003% Nonidet P-40, 0.5 mM DTT, 160 U/mL RNasin. and 1.0 μg/mL yeast tRNA^{Phe}. Samples were incubated at 30 ºC for 30 min, then run on a 6% (80:1 acrylamide/bisacrylamide) native polyacrylamide gel and imaged using storage phosphor imaging plates from Molecular Dynamics on a Molecular Dynamics 9400 Typhoon phosphorimager. Data was analyzed using Molecular Dynamics ImageQuant 5.2 software. Gel images were combined to create one image as described in the figure legend (**Figure S1**).

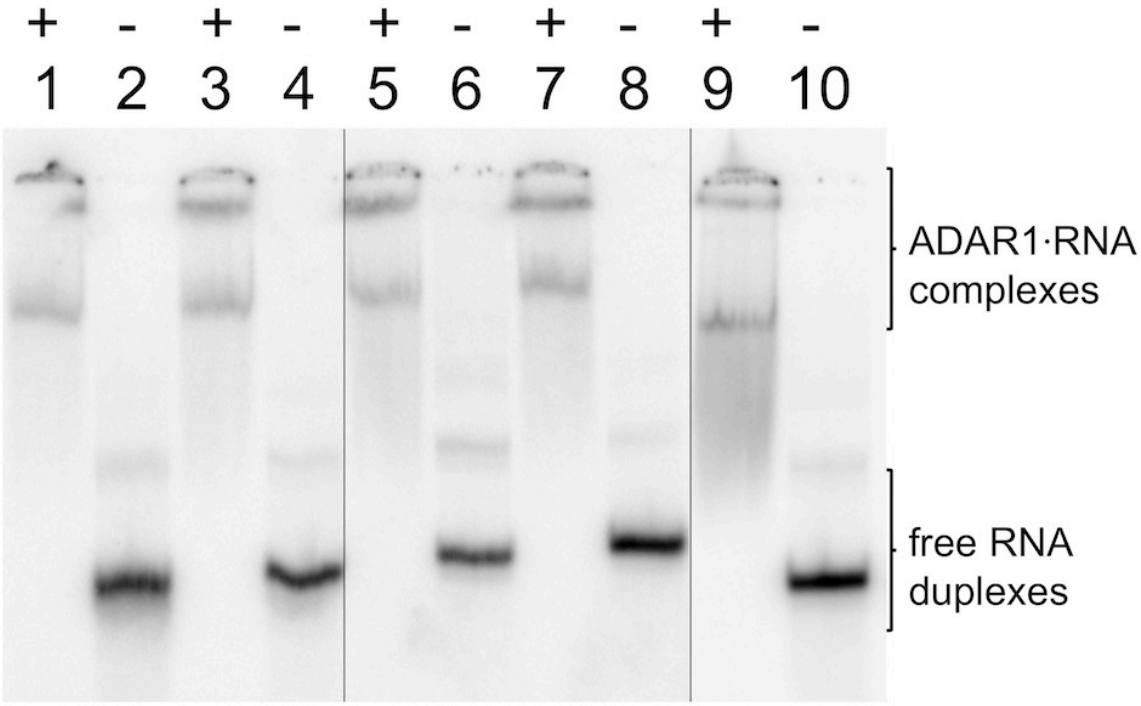

Figure S1. Gel mobility shift analysis indicating saturating concentration of ADAR1 used for kinetic experiments. Lines indicate where images of different gels were combined for this figure. Gel mobility shift analysis was carried out under the same conditions used for the deamination assay. Lanes 1, 3, 5, 7 and 9: duplex RNAs from Figure 3C in the presence of 130 nM ADAR1. Lanes 2, 4, 6, 8 and 10: free duplex RNAs from Figure 3C. RNAs with N equal to: Lanes 1 and 2: 7-deazaA, Lanes 3 and 4: : 8-aza-7-deazaA, Lanes 5 and 6: 2'-deoxyA, Lanes 7 and 8: 2'-*O*-methyl-A, and Lanes 9 and 10: adenosine.

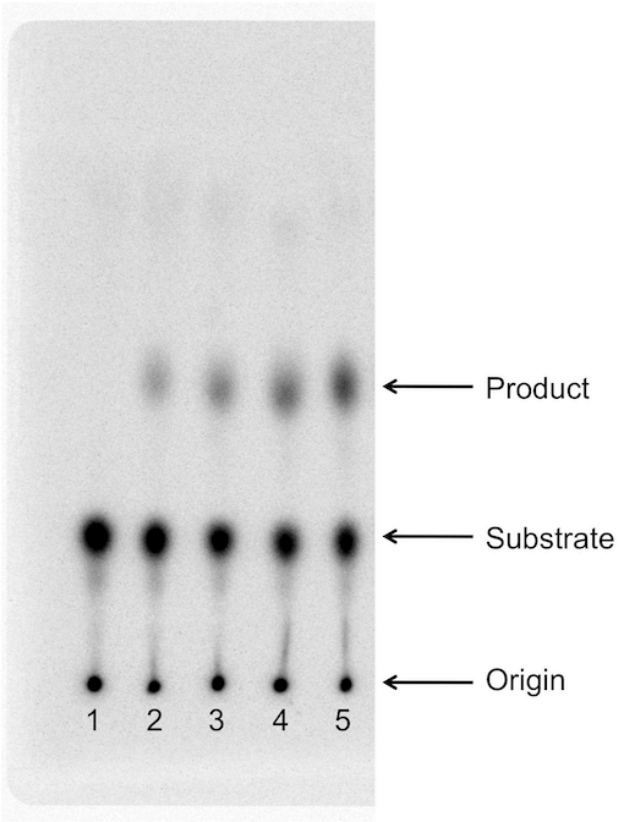

Figure S2. Single turnover kinetic analysis of the reaction of ADAR1 with A-containing RNA. Storage phosphor diagram of TLC plate used to separate deamination products arising from the reaction of 130 nM ADAR1 with ≤ 18 nM RNA. Lanes 1-5: reaction times of 0, 7, 15, 30, 45 min, respectively.

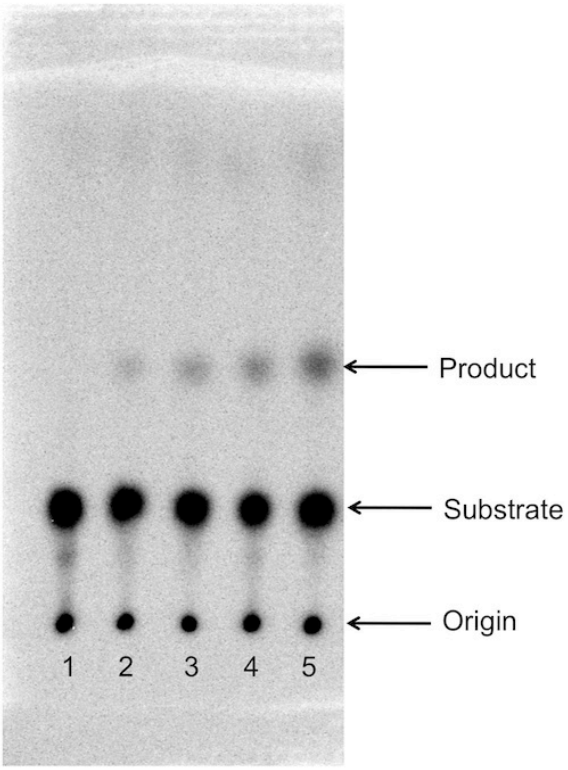

Figure S3. Single turnover kinetic analysis of the reaction of ADAR1 with 2'-deoxyA-containing RNA. Storage phosphor diagram of TLC plate used to separate deamination products arising from the reaction of 130 nM ADAR1 with ≤ 18 nM RNA. Lanes 1-5: reaction times of 0, 7, 15, 30, 45 min, respectively.

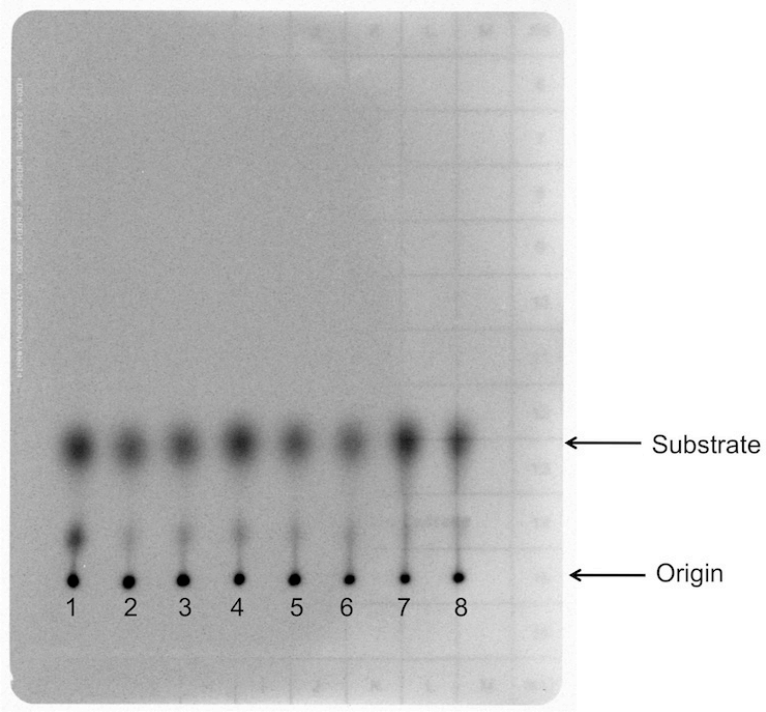

Figure S4. Single turnover kinetic analysis of the reaction of ADAR1 with 2'-*O*-methylA-containing RNA. Storage phosphor diagram of TLC plate used to separate deamination products arising from the reaction of 130 nM ADAR1 with ≤ 18 nM RNA. Lanes 1-8: reaction times of 0, 7, 15, 30, 45, 60, 90, 120 min, respectively.

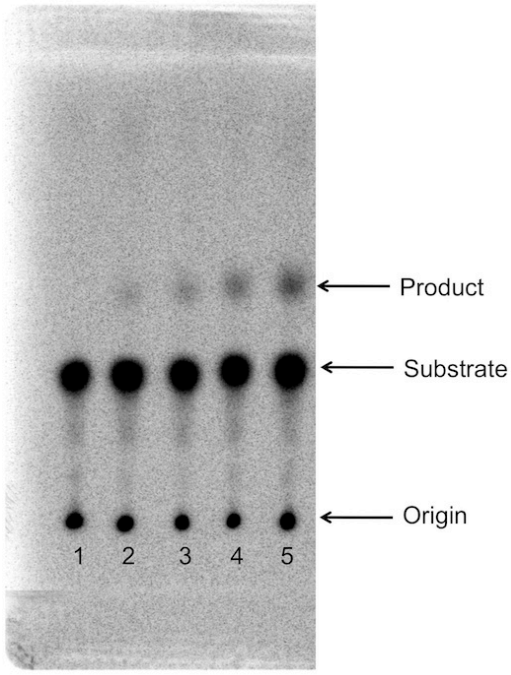

Figure S5. Single turnover kinetic analysis of the reaction of ADAR1 with 8-aza-7-deazaA-containing RNA. Storage phosphor diagram of TLC plate used to separate deamination products arising from the reaction of 130 nM ADAR1 with ≤ 18 nM RNA. Lanes 1-5: reaction times of 0, 7, 15, 30, 45 min, respectively.

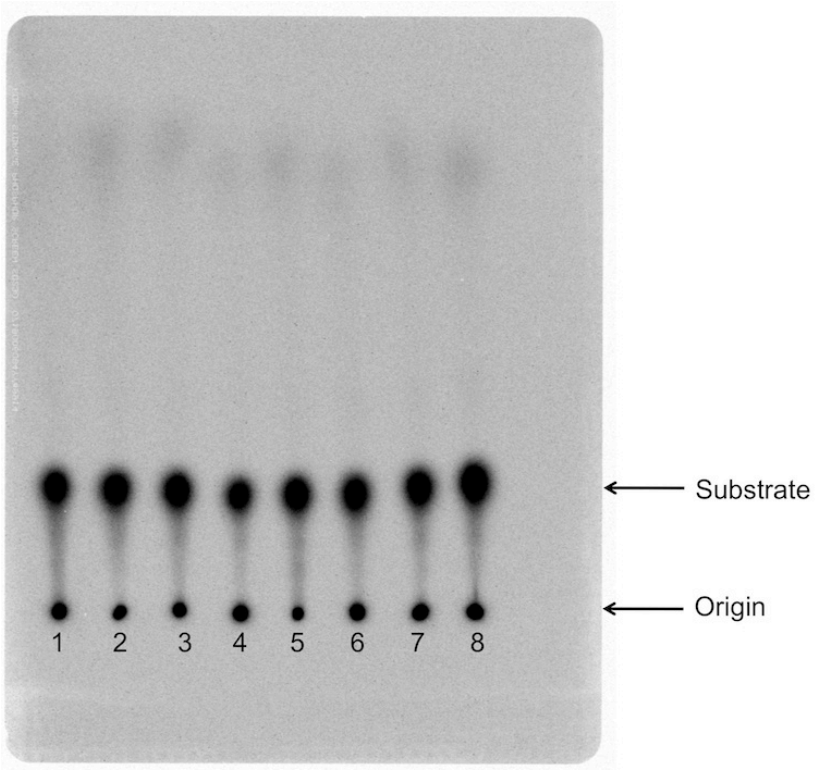

Figure S6. Single turnover kinetic analysis of the reaction of ADAR1 with 7-deazaA-containing RNA. Storage phosphor diagram of TLC plate used to separate deamination products arising from the reaction of 130 nM ADAR1 with ≤ 18 nM RNA. Lanes 1-8: reaction times of 0, 7, 15, 30, 45, 60, 90, 120 min, respectively.

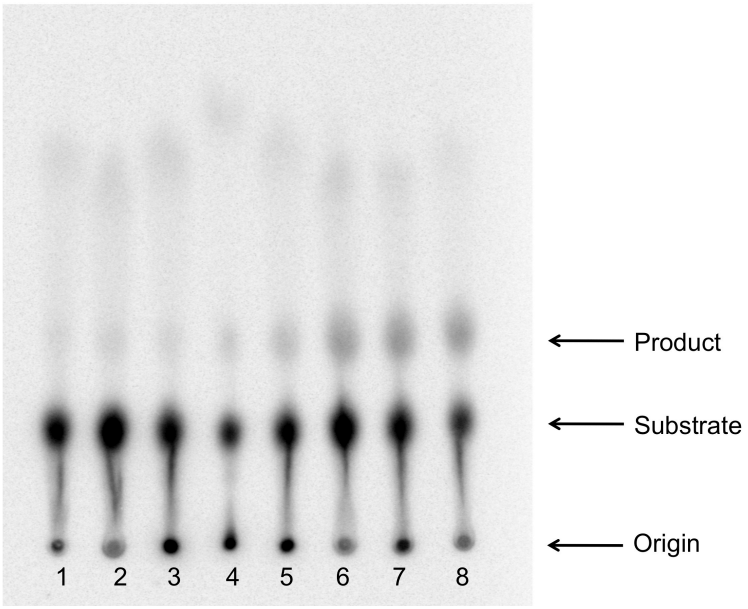

Figure S7. Single turnover kinetic analysis of the reaction of ADAR2 R455A with 7-deazaA-containing RNA. Storage phosphor diagram of TLC plate used to separate deamination products arising from the reaction of 250 nM ADAR2 R455A with 25 nM RNA. Lanes 1-8: reaction times of 0, 1, 3, 10, 20, 40, 60, 90 min, respectively.

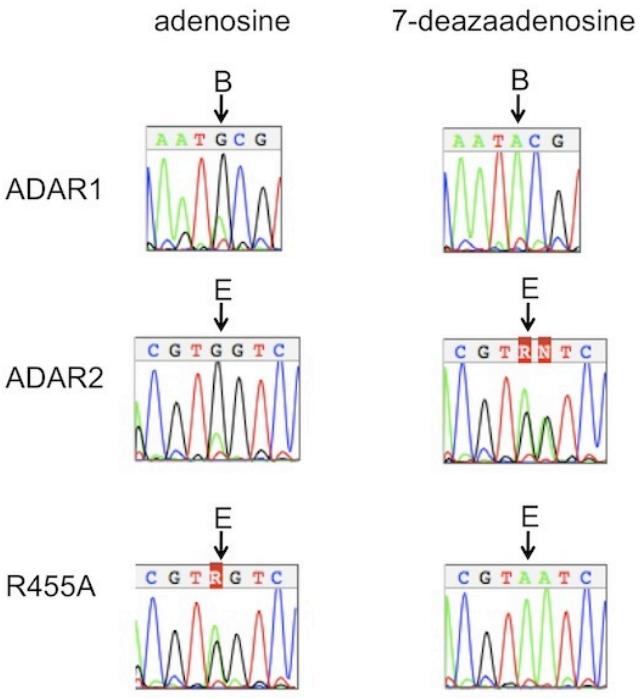

Figure S8. Representative sequencing traces showing editing of adenosine and 7-deazaadenosine by ADAR1, ADAR2 and ADAR2 R455A. The B and E sites are indicated.