The Chemistry and Biology of RNA editing by Adenosine Deaminases

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ABSTRACT

Deamination of adenosines within mRNAs catalyzed by ADAR enzymes generates inosines at the corresponding nucleotide positions. Since inosine is decoded as guanosine, this reaction can lead to codon changes and the introduction of amino acids into a gene product not encoded in the gene. Translation of the different coding strands created by this process leads to protein structural diversity in the parent organism and is necessary for nervous system function in metazoa. The basis for selective editing of adenosines within certain codons is not well understood at the structural/biochemical level. Here we describe the use of synthetic nucleoside analogs incorporated into RNA editing substrates via the protected phosphorimidites to define aspects of the editing reaction mechanism and to carry out mechanism-based trapping of ADAR-RNA complexes. In addition, a high-throughput screen has been developed capable of rapidly identifying functional editing systems.

INTRODUCTION

The RNA editing ADAR enzymes convert adenosines to inosines in mRNAs, changing the coding properties of these messages (Fig. 1). Thus, ADARs play a pivotal role in the basic process of information transfer that takes place during protein expression. Moreover, proteins translated from edited messages have been implicated in a number of neurodegenerative, psychiatric and behavioral disorders such as stroke, epilepsy, Alzheimer’s disease, schizophrenia and episodic ataxia. Our laboratory has carried out studies directed at defining the molecular basis for the fundamentals steps in this editing reaction. Much of what we have learned about the ADAR reaction mechanism has come from studies with ADAR2 and RNA substrates bearing nucleoside analogs. Here, we describe the use of nucleoside analogs in the study of the ADAR-catalyzed editing reaction and a screening strategy for identifying functional editing enzymes or RNAs capable of undergoing the editing reaction.

RESULTS AND DISCUSSION

Advances in chemical synthesis and characterization of nucleic acids allows for "atom-specific mutagenesis" of complex RNAs, such as present in editing substrates. By preparing ADAR substrates by chemical synthesis, one can subtly alter the structure of the edited nucleotide. Evaluating the effect these changes have on the rate of enzyme-catalyzed deamination reveals features of the editing reaction and guides the design of inhibitors. This has been accomplished in our laboratory using 5'-dimethoxytrityl, 2'-tert-butyldimethylsilyl, 3'-N,N-diisopropylamino-β-cyanoethyl phosphorimidites and RNA editing substrates prepared from these compounds. With this approach we have shown that 2’-O-methylation at the editing site greatly reduces the deamination rate. This suggested a possible regulatory mechanism whereby editing could be inhibited by snoRNA-guided 2’-O-methylation. In addition, we demonstrated that 7-deazah modification of the reactive adenosine does not inhibit the ADAR2 reaction, whereas purine C2 amination does. This is in stark contrast to the known structure/activity relationships for the reaction catalyzed by the nucleoside-modifying enzyme adenosine deaminase (ADA), indicating these two classes of adenosine deaminase recognize substrates differently. These studies showed the ADA reaction to be an imperfect model for the ADAR reaction and suggested that inhibitors might be prepared that are selective for ADARs and do not bind ADA. Another significant observation coming from analysis of substrate analogs was that 8-aza modification of the purine

![Fig.1](https://example.com/fig1.png)

**Fig.1** The A to I RNA editing reaction catalyzed by ADARs.

![Fig.2](https://example.com/fig2.png)

**Fig.2** 8-Azapurine is hydrated by ADAR2 and binds tightly.
facilitates interaction with the ADAR2 active site. Since 8-aza modification was known to facilitate hydration of the purine ring, these results underscored the importance of a substrate's susceptibility to covalent hydration in the ADAR reaction and guided the design of high affinity ligands for ADAR2 capable of mechanism-based trapping of the protein-RNA complex (Fig. 2).

The studies with substrate analogs described above have shown that the 8-aza modification of purine facilitates interaction of the base with the ADAR active site. Furthermore, previous work indicated that the 7-deaza modification has a minimal effect. We have combined these two modifications and analyzed the ability of 7-deaza-8-azadenosine to function as an ADAR substrate. Importantly, this nucleoside analog is deaminated by ADAR2 12-times faster than adenosine. This suggests that 7-substituted-7-deaza-8-azapurines may function as inhibitors of ADARs (Fig. 3). This hypothesis is currently being explored in our laboratory.

![Fig. 3 Analogs of 7-deaza-8-azapurine may function as RNA editing inhibitors.](image)

Genetic strategies are effective for rapidly defining structure/activity relationships in enzyme reactions and for the discovery of mutant enzymes with new properties. Application of this approach to the study of ADARs requires coupling the generation of libraries of mutant ADARs or mutant substrates with simple screens for editing activity. ADARs are not naturally found in bacteria or yeast, so simple genetic manipulation of these organisms to study A to I RNA editing is not possible. However, active ADARs can be isolated from yeast overexpression systems. To allow for rapid identification of active ADAR mutants and new editing substrates capable of supporting the ADAR reaction, we developed a screening strategy in the yeast *Saccharomyces cerevisiae*. Our screen is based on the known ability of ADAR2 to deaminate within a stop codon converting the sequence to a tryptophan codon. Thus, we inserted RNA secondary structure known to support ADAR2 editing (human GluR-B R/G site hairpin stem) into an mRNA transcript and made the necessary sequence changes to maintain an open reading frame and create a stop codon at the editing site. We also reduced the length of the duplex and the size of the loop from that naturally found in the GluR-B pre-mRNA. Downstream of this ADAR substrate structure is sequence encoding α-galactosidase, which is a secreted enzyme readily assayed directly on agar plates containing the colorimetric substrate 5-bromo-4-chloro-3-indolyl-α-galactopyranoside (X-Gal). The key elements of the reporter system are shown in Fig. 4.

![Fig. 4 A reporter has been developed that identifies active combinations of RNA editing enzymes and substrate RNAs. Conversion of a stop codon to a Trp codon allows for translation of α-galactosidase in the yeast *S. cerevisiae*.](image)

Using this strategy, we screened libraries of ADAR enzymes varying at specific amino acid positions. These studies have illustrated the importance of different active site residues. In addition, we screened libraries of RNA substrates. These latter studies have shown how sequence changes in the RNA editing substrate distal to the editing site affect the editing reaction.

**CONCLUSION**

Studies with RNA editing substrates bearing nucleoside analog substrates have identified key features of the reactive nucleoside including the importance of the 2'-substituent and propensity to undergo covalent hydration. This has led to the development of small molecule inhibitors of the RNA editing reaction. Inhibitor libraries as well as libraries of RNA editing enzymes or substrates can be screened using a recently developed novel strategy involving editing within in mRNA encoding a reporter enzyme.

**REFERENCES**


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