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Modulation of ADAR1 editing activity by Z-RNA *in vitro*

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ABSTRACT

RNA editing by A-to-I modification has been recognized as an important molecular mechanism for generating RNA and protein diversity. In mammals, it is mediated by a family of adenosine deaminases that act on RNAs (ADARs). The large version of the editing enzyme ADAR1 (ADAR1-L), expressed from an interferon-responsible promoter, has a Z-DNA/Z-RNA binding domain at its N-terminus. We have tested the *in vitro* ability of the enzyme to act on a 50 bp segment of dsRNA with or without a Z-RNA forming nucleotide sequence. A-to-I editing efficiency is markedly enhanced in presence of the sequence favoring Z-RNA. In addition, an alteration in the pattern of modification along the RNA duplex becomes evident as reaction times decrease. These results suggest that the local conformation of dsRNA molecules might be an important feature for target selectivity by ADAR1 and other proteins with Z-RNA binding domains.

INTRODUCTION

RNA editing enables eukaryotic organisms to efficiently increase the number of different functional proteins that can be derived from any given gene. Variety is generated as a result of nucleotide insertions, deletions or substitutions in pre-mRNAs (1). In one type of substitution editing adenosine is modified by hydrolytic deamination yielding inosine (A-to-I editing), which acts like a guanosine during translation. Currently, A-to-I editing is believed to be the most widespread editing phenomenon in higher eukaryotes (2,3).

In mammals, genes affected by RNA editing include the serotonin receptor 5-HT_{2C} mRNA (4), several glutamate receptor subunits (5) and a potassium channel (6), where single A-to-I editing events lead to amino acid recoding in mRNA transcripts with often substantial implication for protein function [reviewed in (3,7,8)]. Recently, intramolecular fold back structures in human mRNAs mediated by repetitive elements were shown to be frequent targets of editing that alter untranslated mRNA sequences with yet unknown functional consequences (9–12). Other dsRNA substrates of adenosine deaminases are measles virus genomes that become hypermutated (13) and the hepatitis delta virus antigenome in host cells where an amber stop codon is altered to yield a tryptophane codon (14). Adenosine deamination is catalyzed by members of an enzyme family known as ADARs (3,15). The two human ADAR enzymes that have been functionally characterized are ADAR1 and ADAR2. They are ubiquitously expressed in human tissues and their common features are three (ADAR1) or two (ADAR2) double-stranded RNA binding domains (dsRBDs) and a catalytic deaminase domain.

Human ADAR1 is expressed from three different promoters, one of which is interferon (IFN)-inducible (16,17). Stimulation of cells with IFN leads to the synthesis of the 150 kDa ADAR1 protein (ADAR1-L for long isoform, also termed hADAR1 p150) that contains a unique nucleic acid binding motif at its N-terminus. This domain consists of two subdomains, Zα and Zβ, and is able to bind to both Z-DNA and Z-RNA with high-affinity *in vitro* and *in vivo* (18–20). In the absence of interferon, a shorter 120 kDa form of ADAR1 is constitutively expressed with Zβ as its N-terminal domain. ADAR1-L is the only member of the ADAR family that is shuttled between the cytosol and the nucleus raising the possibility that it might play a role in interferon-induced antiviral defense pathways (21). Z-binding domains have also been

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identified in other proteins, such as DLM1 (22), viral protein E3L (23) and fish protein kinase PKR (24,25), but the functional roles and biological functions of the Z-binding domains in the context of these genes have to be uncovered.

It is not known how the sequence specificity of ADAR1-L is achieved in natural editing targets. On extended dsRNA molecules in the A-conformation, extensive editing occurs in a seemingly promiscuous way until ~60% of the adenosines have been modified (3). However, ADAR1-L displays a relative preference to deaminate certain adenosines depending on the sequence environment and a 5′-neighbor preference in the order A>>U>C>>G (26). It has been shown that, apart from the dsRBDs, the deaminase domain and the Z-binding domain directly or indirectly contribute to substrate binding but the mechanisms for interaction are unknown (27,28). The formation of Z-RNA is favored by alterations of purine and pyrimidine, especially alternating guanosine and cytosine repeats (29). The Z-binding domain of ADAR1-L binds to spontaneously forming Z-RNA sequences, which are in equilibrium with A-form RNA in solution (19), thereby shifting the equilibrium towards the Z-conformation (19), This suggests that the editing activity and site-selectivity of ADAR1-L might be influenced by local RNA conformation in addition to the known primary sequence preferences.

Here we demonstrate that the editing pattern *in vitro* on an extended dsRNA molecule is altered in the presence of a Z-RNA sequence motif. Furthermore we observed a preferential targeting of Z-motif containing dsRNA over pure A-form RNA. These results suggest that both the site-selectivity as well as enzymatic activity of ADAR1-L is influenced by its Z-binding domain through specific interactions with Z-RNA forming motifs.

MATERIALS AND METHODS

Cell culture and protein extract preparation

Human embryonic kidney cells (HEK293) [American Type Culture Collection (ATCC) No.: CRL 1573] were grown in minimum essential medium alpha (MEM α) supplemented with 10% fetal calf serum (FCS) (Invitrogen). Stable cell lines ectopically expressing ADAR1-L (N6, N8), ADAR1-Lmut (NLM5) or ADAR2 (R10 and R13) were grown in DMEM α supplemented with 10% FCS and 278 ng/ml Geneticin (G418, Invitrogen). Based on prior characterization of the HEK293-derived stable cell lines (30), the N6 cell line was chosen as the source for ADAR1-L and the R13 cell line for ADAR2. Cytosolic extracts were prepared as previously described (31,32). The cytosolic extracts were eluted in 150 µl buffer A [10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, 8 µg/ml Leupeptin and 5 µg/ml Pepstatin). Extracts were assayed by western blot for ADAR1-L expression as described previously (30).

Preparation of the dsRNA substrates

Duplex RNA (Dharmacon) of 50 bp was prepared from a sense/antisense pair of RNA oligonucleotides. For the $[CG]_6$ substrate the sense strand sequence was: 5′-CGCGCGCGCGCGGGACAAAUUAACCAAGGAAAAUAACAAGGACAGGGACC-3′. The sense strand sequence for the $[CCGG]_3$-containing substrate was: 5′-CCGGCCGGCCGGGGACAAAUUAACCAAGGAAAAUAACAAGGACAGGGACC-3′). Thirty OD units of ssRNA from each strand in 50 mM NaCl and 10 mM Tris–HCl (pH 8.2) were heated for 2 min at 95°C and slowly cooled down to room temperature. The duplexes were purified using a FPLC MonoQ column with a gradient ranging from 0.25 to 2 M NaCl. Only the peak fraction (eluting at 0.658 M NaCl) was used for the described experiments.

In vitro editing assay

A 2 fmol/µl mix of both 50mer dsRNA substrates (1 fmol/µl each) was incubated at 37°C with either 2 µl of protein extract diluted into buffer D or 2 µl buffer D [20 mM HEPES (pH 7.9), 0.1 M KCl, 20% Glycerol and 0.2 mM EDTA] as negative control. The reaction was stopped by adding 0.5 µl of 0.5 M EDTA. Processed RNAs were purified by proteinase K digestion (1 h at 37°C), phenol/chloroform extraction and precipitation.

RT–PCR and editing analysis

The purified RNA substrates were reverse transcribed using SuperScript II reverse transcriptase (Invitrogen) and the oligonucleotide primer **R50RT** 5′-GACACGGTACCACACAACGGTCCCTGTCC-3′ (see Figure 1). A mock RT lacking reverse transcriptase was performed for all samples. Substrate-specific amplification of either the $(CG)_6$-containing or the $(CCGG)_3$-harboring sequences was done using primer pairs **R50CGD** (5′-GTGAATTCGCGCGCGCGG-3′) and **M13-4U** (5′-GACACGGTACCACACAAC-3′), or **R50CCGGD** (5′-GTGAATTCCGGCCGGC-3′ and **M13-4U**, respectively. PCR amplicons were subcloned into pBluescript II (Stratagene) and A-to-G changes in individually prepared plasmids detected by C-tracking using the Sequenase 2.0 cycle sequencing kit (USB) and sequencing primer-40 (5′-GTTTTCCCAGTCACGAC-3′). Sequencing products

Figure 1. dsRNA substrates for *in vitro* editing analysis. (**A**) The boxed sequence indicates the region of the substrate molecules in which editing is analyzed. Adenosines are numbered consecutively 1 through 16 from the 5′ end of the dsRNA substrate. The adenosines highlighted in red share the same 5′-neighbor. The two dsRNA substrates used differ in their 5′ sequence; the Z-forming substrate contains a 12 bp long repeat of alternating CG (underlined). (**B**) The non-Z-forming substrate contains a 12 bp segment with three CCGG-repeats (underlined), which has the same base composition as the $(CG)_6$-repeat, but does not convert into the Z-conformation (37).

were run out on 6% urea-PAGE and analyzed by autoradiography.

Statistics and error analysis

The error in all editing experiments was calculated using x = number of trials, n = number of successes and P = probability of achieving a success on any given trial. In our case, P is assigned as the observed editing percentage for the position of the adenosine and x is the total number of cDNA clones sequenced for the given experiment. On average the error ranges from 3% to 15% with the bigger error margins observed at positions which are edited at low levels. The error for positions that show high editing varies between 3% and 7%, translating to confidence values of 97% and 93%, respectively. Errors introduced by reverse transcriptase (10–13) and *Taq* DNA polymerase (10–14) are neglectable due to the shortness of the substrate. From the negative control incubations lacking editing enzyme the background editing rate, which reflects any sequence errors introduced during processing of the samples, was 8 A/G substitutions out of 2560 sequenced adenosines yielding an error rate of 0.04%.

For editing correlation analysis we treated editing as a binary event, i.e. 0 for non-edited and 1 for an edited position. Our substrate with 16 possible editing positions on the sense strand thus has a total of 2^{16} possible pattern combinations. The null hypothesis (H_0) for our experiment states that there is no relationship between two positions (a and b) in the population, i.e.: $\rho = 0.0$ and the correlation is thus also 0 ($r = 0.0$). The significance was assessed with $x-2$ degrees of freedom and for the large sample sizes utilized, the values were found to be statistically significant at the $P \leqslant 0.01$ confidence interval.

RESULTS

Modulation of ADAR1-L site-selectivity by Z-RNA

To investigate the influence of different RNA conformations on human ADAR1-L enzymatic activity, we developed an *in vitro* assay with a set of dsRNA substrate molecules designed either with or without a Z-RNA forming sequence motif (Figure 1).

Each substrate is composed of a 50 nt synthetic RNA oligonucleotide annealed to its complement. The central sequence within the sense strand used for editing analysis (Figure 1 boxed insert) has been characterized previously using *in vitro* assays (26) and is known to be efficiently modified at several positions by human ADAR1-L. The two dsRNA substrate molecules constructed are identical in base composition. However, the Z-forming substrate begins at its 5′ end with a $(CG)_6$-repeat, whereas the non-Z-RNA forming substrate starts with a $(CCGG)_3$ repeat. In a prior *in vitro* study it was shown that a $(CG)_6$ RNA dimer will shift from the A to the Z-conformation after addition of human ADAR1-L Z-domain (19). Furthermore, it has been demonstrated that in DNA molecules a $(CG)_6$ motif surrounded by B-DNA can be shifted to Z-DNA by the ADAR1-L Zα domain, generating two flanking B-Z junctions (33).

Stably transfected cell lines have been generated that overexpress human ADAR1-L (30). Extracts from a high level ADAR1-L producing cell line (N6) were used for *in vitro* editing assays and extracts from the parent cell line (HEK293) served as controls.

Initially, two different hADAR1-L concentrations were tested with 2 or 20 fmoles of RNA substrate. Since Z-RNA formation has been shown to be most efficient at 37°C (19) the editing assay was performed at this temperature for 30 min. After incubation the RNAs were recovered, reverse transcribed and amplified by PCR. PCR amplicons were subcloned and ~100 individual clones derived from each incubation were sequenced.

Figure 2 shows the editing efficiencies at all 16 adenosines in both substrates when using 2 μg ADAR1-L containing cellular extract after normalization for the background editing activity of native HEK293 extract. The main observation from this initial experiment was that the overall Z-forming substrate was modified to a higher extent than the non-Z-forming substrate at almost every edited site. The overall percentage of cDNA clones that were edited at one or more positions were (80 ± 3)% for the $(CG)_6$-containing and (62 ± 2)% for the $(CCGG)_3$-containing substrate. In the non-Z-forming substrate with $(CCGG)_3$ the pattern and extent of modification at the different adenosines can be explained by the previously known properties and sequence preferences of ADARs (26). The most heavily edited nucleotides are in the center of the duplex and/or have an A or U as 5′-neighbor (adenosines 5, 6, 7, 8, 10).

Intriguingly, compared to the editing pattern on the non-Z-forming substrate, residues towards the 5′ end were edited to a higher propensity on the Z-forming substrate. Fourteen percent of all editing events occur at the four adenosines proximal to the Z-forming sequence in the $(CG)_6$ substrate (24 events compared to 171 total). In contrast, with the non-Z-forming substrate only 8% of the modifications map to the same four adenosines (8 events versus 102 total).

As an additional control, we tested extracts from another stable cell line (30) that overexpressed an ADAR1-L N-terminal point mutant (N173A), which has been shown to abolish Z-binding *in vitro* and *in vivo* (34). Although the expression level as judged by western blot analysis was equivalent to the wild-type ADAR1-L expression (data not shown), the editing activity of the point mutant after 30 min incubation with the dsRNA substrate was very low precluding a meaningful comparison to the wild-type protein with respect to the editing pattern. Out of 150 substrate molecules that were analyzed, only 9.3% contained one or more editing events. The resulting modification pattern was non-indicative of any preference. It is possible that the point mutation alters the enzymatic activity directly, or that a substantial fraction of the mutated protein is misfolded or unstable when overexpressed.

If the formation of Z-RNA at the N-terminus of the $(CG)_6$-containing substrate is responsible for the change in editing pattern with ADAR1-L, then the editing enzyme ADAR2, which is also highly active on extended dsRNA molecules but lacks a Z-binding domain, should not display such a change in editing pattern. Thus the same experiment was carried out using extracts from either ADAR1-L or ADAR2 overexpressing cells (30). In all assays, both substrates were co-incubated and processed (± Z-forming sequence) for each reaction to reduce potential interassay variability. $(CG)_6$-containing and $(CCGG)_3$-containing molecules are

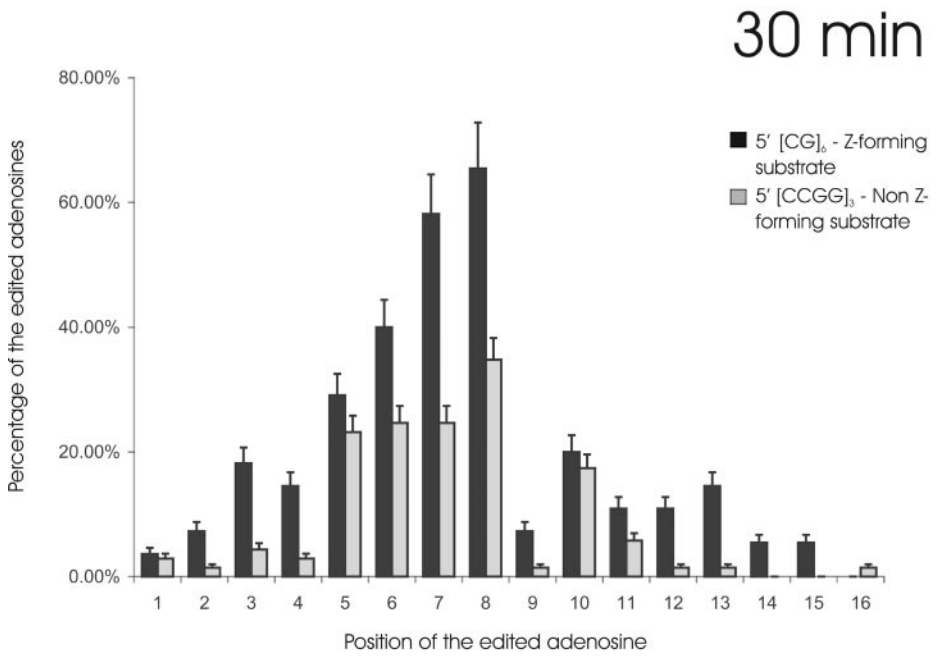

Figure 2. RNA editing by ADAR1-L. Editing activity was measured as the ratio of edited versus unedited adenosines at each position within the dsRNA molecules. Depicted are the averaged editing frequencies from three experiments for all 16 adenosines analyzed after 30 min incubation. For each edited position the background editing activity of native HEK293 cell extracts has been subtracted.

distinguished during sequencing thus allowing for the analysis of substrate-specific editing extents and patterns.

Figure 3A shows the results of RNA editing on Z-containing and Z-lacking substrates with either ADAR1-L or ADAR2 extracts. The patterns as well as the editing efficiencies of modification along the dsRNA substrate without the Z-forming motif are very similar after exposure to either ADAR1-L or ADAR2. However, with the Z-forming substrate the efficiency of ADAR1-L mediated editing is increased strongly in the 5′-half of the substrate molecule, confirming the initial results from individual substrate incubations. For ADAR2, the pattern and efficiency of modification on the Z-forming substrate is very similar to that on non-Z-forming dsRNA with maximum editing efficiencies at the central adenosines and decreasing editing extents towards the 5′ and 3′ ends.

The preference of ADAR1-L for the RNA with Z-forming motif when presented with both substrates in the same reaction is reflected by the fact that (78 ± 5)% of $(CG)_6$-containing substrates are modified at one or more positions after 30 min at 37°C compared to (42 ± 3)% for the $(CCGG)_3$-containing dsRNA (see Figure 3B). In contrast, ADAR2 does not display any detectable preference for either of the two RNA substrates (∼30% activity on both).

Preferential targeting of a Z-forming substrate by hADAR1-L *in vitro*

To further study the observed preference of ADAR1-L in modifying the Z-forming over the non-Z-forming RNA substrate, a time-course experiment was carried out. The coincubation of both substrates with each protein sample allowed us to compare directly the relative activity of ADAR1-L on both types of molecules as a function of time. Figure 4A and B show the results from 5 and 15 min incubations of ADAR1-L extracts with the $(CG)_6$-containing and $(CCGG)_3$-containing dsRNA substrates, respectively. Intriguingly, the difference in editing levels between the Z-forming and the non-Z-forming substrate after 30 min of incubation (Figure 3A) becomes more pronounced with decreasing incubation times, arguing for a preferential targeting of the Z-containing substrate by ADAR1-L. After 5 min (56 ± 3)% of the $(CG)_6$-containing substrate are modified at one or more positions [(59 ± 3)% after 15 min], whereas the non-Z-forming $(CCGG)_3$-containing dsRNA was modified (15 ± 2)% after 5 min [(19 ± 3)% after 15 min]. This means that at 5 min the Z-containing substrate is modified 3.7 times more efficiently than the dsRNA lacking a Z-motif (3.1 times after 15 min).

We also observe a more pronounced editing shift towards the 5′ end (adenosines 1–6) containing the Z-RNA forming sequence compared to the pattern of modification on the non-Z-forming substrate after 5 min exposure to ADAR1-L. This indicates that in the presence of the Z-forming motif the initial modifications of the substrate occur preferentially in proximity to the Z-forming RNA domain.

Statistical correlation observed between editing sites

In a recent study we analyzed the sequence environment and 5′- and 3- *cis*-preferences of more than 14 000 edited adenosine residues located in Alu repeat elements (11). One of the findings from this analysis was the preference of an editing site to be preceded or followed by another edited adenosine. This could be due to a coupling between editing reactions occurring at adjacent nucleotides within the ADAR-bound substrates.

To determine if the observed change in editing efficiency might be due to an alteration in the processivity of ADAR1-L on a Z-forming substrate, we performed a

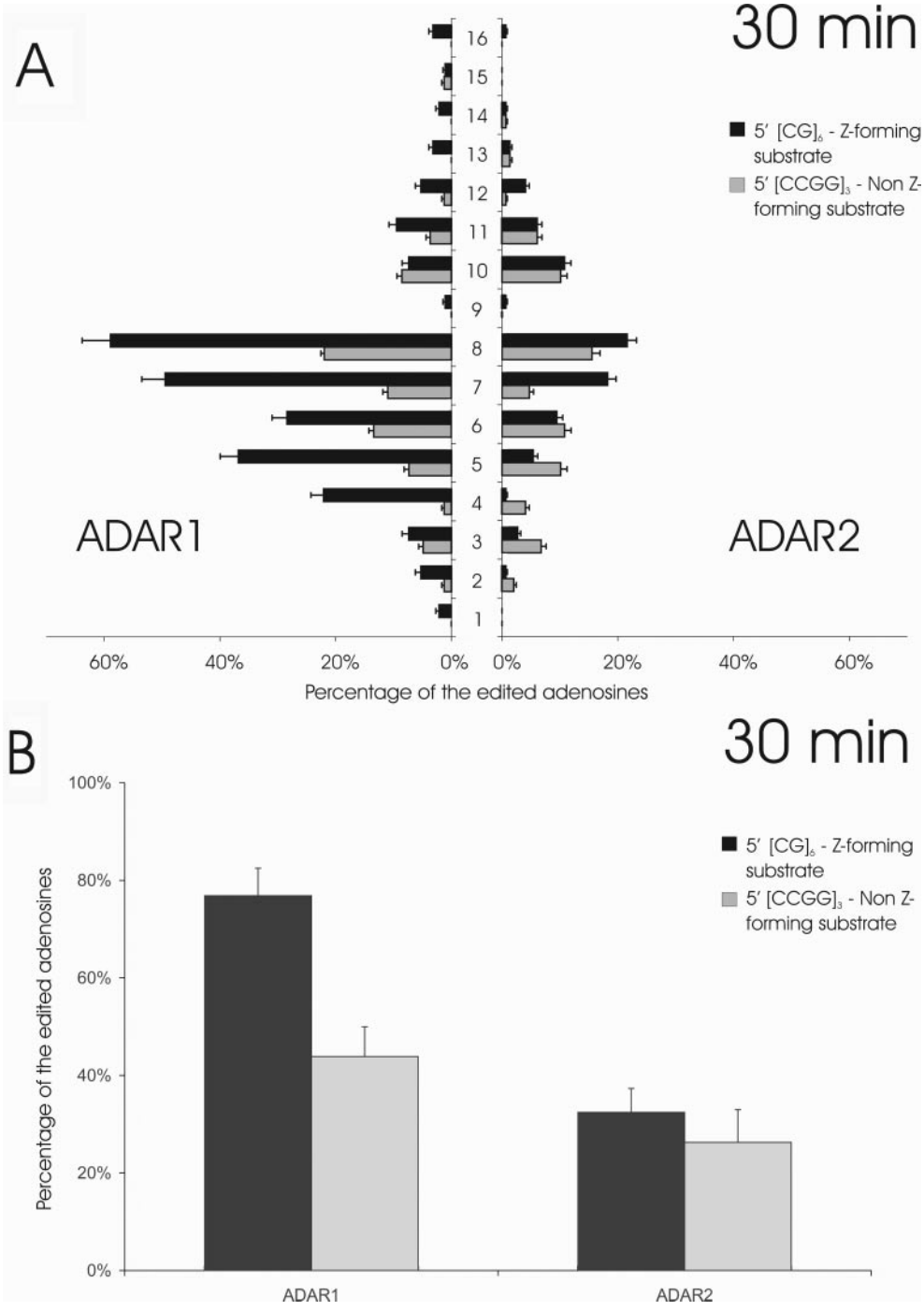

Figure 3. Comparison of the editing preferences of ADAR1-L and ADAR2. (**A**) The relative A-to-I editing values after 30 min of incubation is shown. Both substrates were co-incubated (each at a concentration of 0.16 fmol/l) with either ADAR1-L or ADAR2 containing cell extracts. (**B**) Preferential editing of Z-forming substrate by ADAR1-L. Overall editing efficiencies (fraction of substrate molecules that were modified at least once) by ADAR1-L or ADAR2 using the two dsRNA substrates with or without Z-forming motif.

statistical correlation analysis for all possible combination of editing events in the test substrates. A MatLab script was designed that tests for all possible combinations and computes the relevant statistics. Due to the large sample sizes used, the values were found to be statistically significant at the $P \leqslant 0.01$ confidence interval. As shown in Figure 5, we detect positive correlations (i.e. pairs of adenosines that are modified within the same substrate molecule at a higher rate than theoretically expected) as well as negative correlations (i.e. lower than expected co-modification). Most strikingly, adenosine pairs #5/6 and #7/8 are edited together to a much larger extent than statistically expected. However, we see no significant difference in the number and strength of correlations found within the two substrates for assays with ADAR1-L or ADAR2. Therefore, it is likely that intrinsic properties of the editing enzymes or other substrate features not affected by the

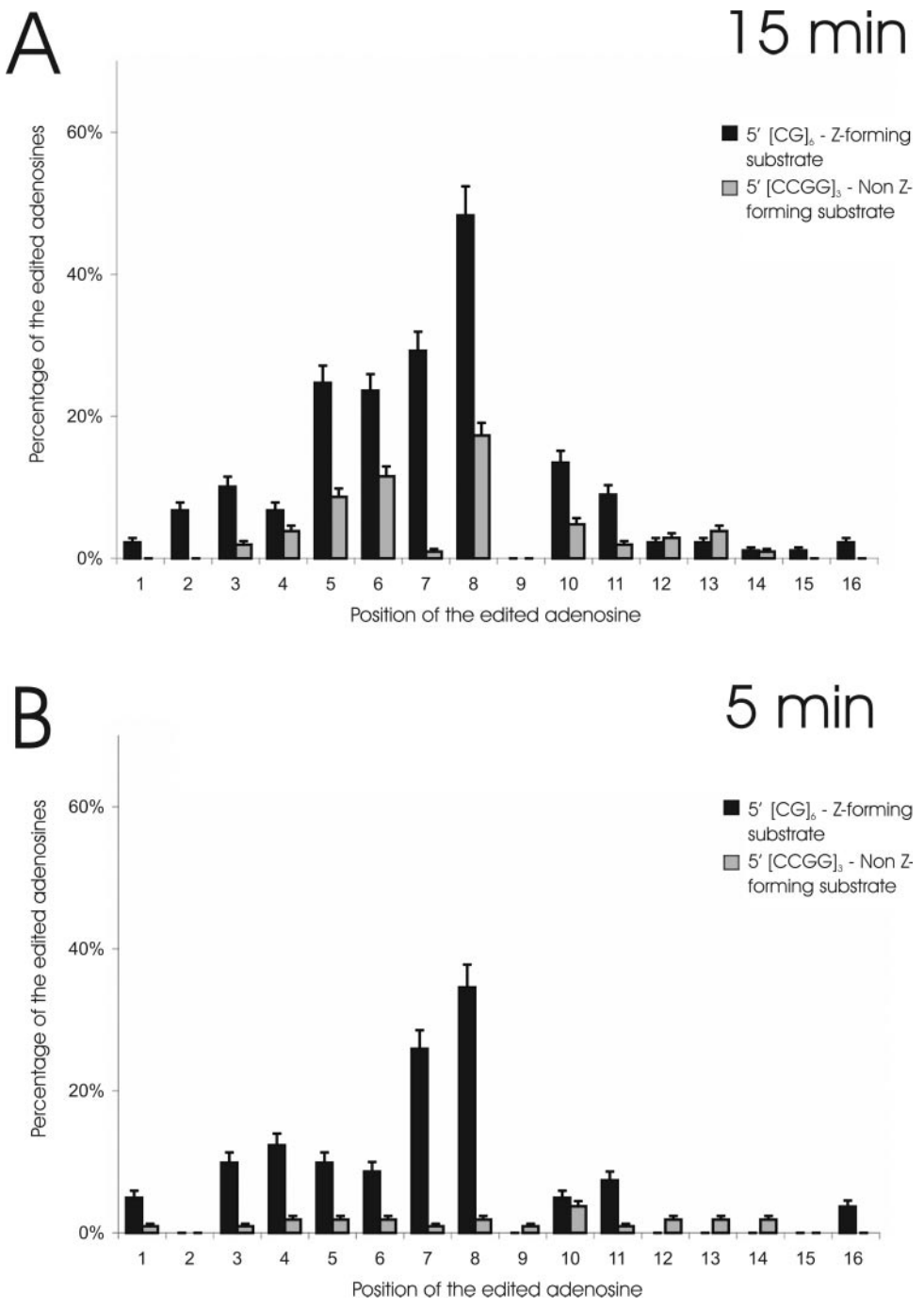

Figure 4. Editing time-course experiment. Editing patterns from 15 min (**A**) and 5 minute (**B**) incubations of ADAR1-L extract with dsRNA substrates.

Z-forming motif are responsible for the observed correlations of editing.

DISCUSSION

Within a double-stranded RNA substrate molecule site-selectivity and efficiency of modification by ADAR1 and ADAR2 proteins is largely determined by *cis*-sequence preferences that have been characterized for both enzymes *in vitro* and *in vivo* (10,11,26,35). Furthermore, the efficiency of modification at a given adenosine decreases the closer it is to the end of the dsRNA molecule (26). When analyzing the modification pattern and rates on a dsRNA molecule [$(CCGG)_3$ds50] that is likely to assume a complete A-conformation, the results for both ADAR1-L and ADAR2 are similar and in agreement with previous data on other completely base-paired dsRNA substrates and follow known *cis*-sequence preferences. However, when using a dsRNA identical in sequence composition but harboring a Z-RNA forming motif at its 5′ end, the pattern of modification produced by ADAR1-L is significantly altered. The observed

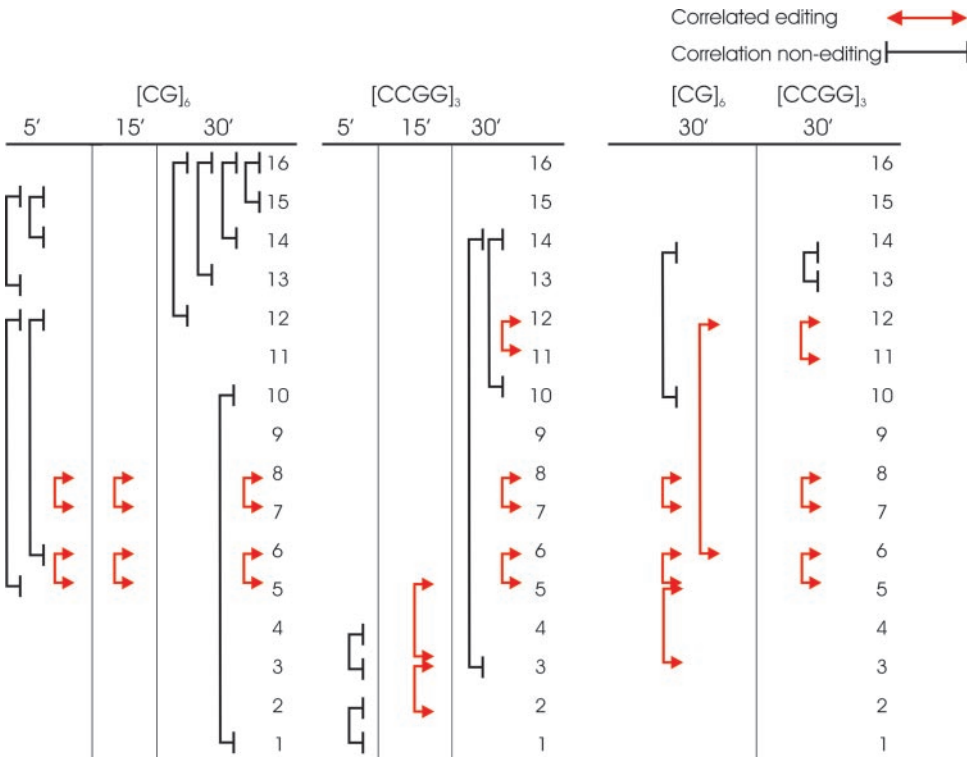

Figure 5. Editing site correlations. Pairs of adenosines that are modified in a correlated fashion (with correlation value $\geqslant 0.7$; $P < 0.01$) are indicated. Red arrows indicates positive correlations and black lines indicates negative correlations. Results calculated from three experiments (5, 15 and 30 min incubations) with each of the dsRNA substrates are shown for ADAR1-L (left and center panel). The right panel shows the results derived from 30 min incubation with ADAR2 extracts. See Supplementary Table S1 for numerical values.

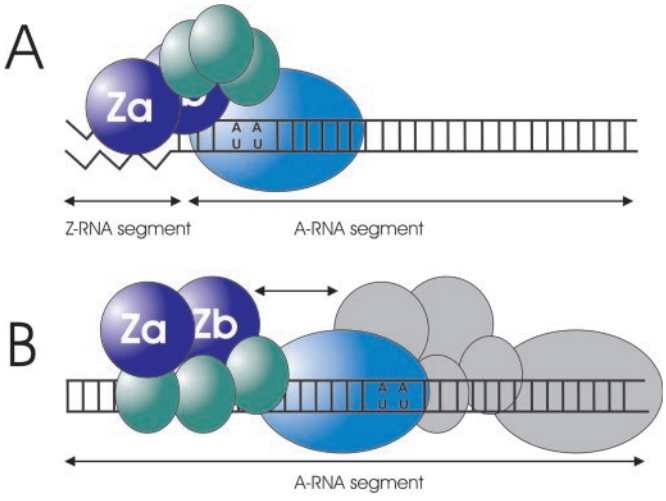

Figure 6. Model for the interaction of ADAR1-L with Z-motif containing or lacking substrate (**A**) The Zα motif binds with high-affinity to the Z-motif within the $(CG)_6$-containing dsRNA substrate, thereby restricting the movements of the catalytic domain (sky blue) and causing a modification pattern that is shifted towards the 5′ end of the substrate molecule. (**B**) A double-stranded A-RNA substrate is bound by the dsRBDs (teal) with high-affinity, but without sequence specificity, allowing ADAR1 to move along the length of the substrate.

shift of the editing pattern towards the end of the molecule with the Z-forming sequence is probably due to the Z-binding domain present in the ADAR1-L protein. This is supported by the fact that the ADAR2 enzyme, which lacks a Z-binding domain but otherwise largely shares ADAR1 *cis*-sequence preferences and dsRNA binding properties, shows the same modification pattern irrespective of the presence of a Z-forming sequence in the substrate.

In addition to the preferential modification of proximal adenosines to the location of the Z-forming motif, the dsRNA substrate with a Z-forming sequence is targeted with a much higher frequency than the substrate that lacks the Z-forming motif.

The central region of the substrate that harbors the adenosines targeted for modification by the enzyme is likely to be in the A-conformation at all times. This is supported by the fact that ADAR1-L as well as ADAR2 are both active on the substrate with the Z-forming motif on its 5′ end. Since ADAR2 lacks Z-binding domains it can only bind and modify the dsRNA if at least part of it is in A-conformation.

Taken together, these results argue that in the *in vitro* system, site-selectivity and editing efficiency of ADAR1-L are modulated by its Z-binding domain. The implications of this finding suggest that it would be useful to search for specific ADAR1-L target sequences in viral RNA genomes or within cellular dsRNAs. It could also shed light on the different target specificities and functional roles of the short and long ADAR1 isoforms, which include (p150) or lack (p110) the Z-binding motif. A great deal is known about DNA sequences that readily form Z-DNA (36–38). It is generally assumed that similar sequence preferences apply for Z-RNA, but only limited experimental data is available. Certain repeat sequences, such as $(CG)_n$ with $n > 2$ are known for their ability to easily switch into the Z-DNA conformation (33).

The processivity of ADAR1-L seems to be the same on each tested substrate with a strong correlation of co-editing in positions 7 and 8 of the duplex. It can be speculated that the initial A-to-I modification of either one of the two adenosines would weaken the local base-pairing character of the RNA duplex in such a way that the neighboring adenosine becomes more accessible for modification by the enzyme either without or after dissociation of the initial enzyme–substrate complex. Experiments that employ RNA substrate variants that mimic such intermediate products could shed light on this mechanistic question.

Since editing levels are in part influenced by the nature of the nucleotide preceding the edited adenosine the comparison of editing efficiencies between individual adenosines must take this into account. Comparing the editing of adenosines with the same 5′-neighbor eliminates the *cis*-preference parameter from the analysis. Interestingly, when looking at all the adenosines of the used substrates that are preceded by another A (positions 3, 4, 6, 8, 10, 11, 12, 14 and 16), the difference in editing pattern by ADAR1-L between the Z-containing and the Z-lacking substrate becomes even more apparent.

In vitro the ADAR1 Zα domain alone is able to stabilize a dsRNA $(CG)_6$-repeat in the Z-conformation (19) leading to a transition from the right-handed A-conformation to the Z-form in solution. Two models that could explain this observation have been discussed previously with respect to Z-DNA binding and B-to Z-DNA conversion by Zα (33). One suggests that Zα binds the non-Z-conformation and actively flips it into Z, whereas according to the other model Zα binds to small amounts of transiently forming Z-form that are present in solution due to Brownian motion (33).

The currently available data is still compatible with both models. The observed change in editing pattern during short incubation times suggests that high-affinity binding to the Z-form RNA motif is favored over binding to other regions of the dsRNA. The preference is lost with extended incubation times, since additional editing events accumulate that are due to ADAR1 binding to A-form sequences. Since the overall catalytic activity of ADAR1-L is also altered in presence of the Z-forming substrate, the observed changes in editing pattern could also be the result of the enhancement of catalytic activity after A-to-Z-conversion while ADAR1 is bound to the substrate. In this scenario ADAR1-L may bind anywhere along the dsRNA duplex (through its dsRBDs) and facilitate the A-to-Z-conformational switch, which then anchors ADAR1 Zα at the Z-motif and stimulates adenosine deaminase activity.

According, to either model, the Z-forming motif acts as a cofactor that increases the site-selectivity of the ADAR1-L editing enzyme. Figure 6 shows a diagram for how a Z-RNA motif in dsRNA might be bound by ADAR1-L directing the enzymatic activity to a subregion of the whole substrate molecule, whereas dsRNA completely in the A-conformation is bound and modified as expected from non site-specific binding by the dsRBDs. In the presence of the Z-forming motif the Z-binding domains might dominate the initial enzyme–substrate interactions. In contrast, the dsRBDs direct the interactions in perfect A-form targets.

It would be interesting to determine how a 5′ Z-RNA stretch acts on a longer dsRNA substrate and how Z-RNA sequences within the context of a natural substrate affect the observed pattern of editing.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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