Recognition of the spliceosomal branch site RNA helix on the basis of surface and electrostatic features

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ABSTRACT

We have investigated electrostatic and surface features of an essential region of the catalytic core of the spliceosome, the eukaryotic precursor messenger (pre-m)RNA splicing apparatus. The nucleophile for the first of two splicing reactions is the 2'-hydroxyl (OH) of the ribose of a specific adenineosine within the intron. During assembly of the spliceosome’s catalytic core, this adenine is positioned by pairing with a short region of the U2 small nuclear (sn)RNA to form the pre-mRNA branch site helix. The solution structure of the spliceosomal pre-mRNA branch site [Newby, M.I. and Greenbaum, N.L. (2002) Nature Struct. Biol., 9, 958–965] showed that a phylogenetically conserved pseudouridine (ψ) residue in the segment of U2 snRNA that pairs with the intron induces a markedly different structure compared with that of its unmodified counterpart. In order to achieve a more detailed understanding of the factors that contribute to recognition of the spliceosome’s branch site helix and activation of the nucleophile for the first step of pre-mRNA splicing, we have calculated surface areas and electrostatic potentials of ψ-modified and unmodified branch site duplexes. There was no significant difference between the total accessible area or ratio of total polar:nonpolar groups between modified and unmodified duplexes. However, there was substantially greater exposure of nonpolar area of the adenine base, and less exposure of the 2'-OH, in the ψ-modified structure. Electrostatic potentials computed using a hybrid boundary element and finite difference nonlinear Poisson–Boltzmann approach [Boschitsch, A.H. and Fenley, M.O. (2004) J. Comput. Chem., 25, 935–955] revealed a region of exceptionally negative potential in the major groove surrounding the 2'-OH of the branch site adenosine. These surface and electrostatic features may contribute to the overall recognition of the pre-mRNA branch site region by other components of the splicing reaction.

INTRODUCTION

Nucleic acids are highly charged, and their structure–function relationships and molecular recognition events are strongly influenced by electrostatic interactions (1,2). Therefore, the ability to model the electrostatic properties of RNA accurately is particularly critical. The irregular molecular surface resulting from unique structural motifs (3), noncanonical base pairing (4,5), bulged bases and base triples, all of which distort the backbone, creates unique electrostatic potential patterns. The different three-dimensional (3D) RNA structural motifs create more intricate backbone geometries than seen in the more regular RNA and DNA double helical stem regions. Some folds are comparable in complexity to protein structures, and generate spots with intense negativity due to altered backbone charge distribution. Regions of intense negative potential in some structures have been identified as recognition sites for cations (4,6).

Assembly of the precursor messenger (pre-m)RNA splicing machinery, the cellular (or organellar) ribonucleoprotein complex that catalyzes the removal of noncoding regions (introns) from RNA and ligates the flanking coding regions (exons), is an important problem in structural biology. In some cases, such as Group I and Group II introns, the catalytic power resides entirely within the RNA component. By comparison, the spliceosome, the supramolecular splicing apparatus found in the nuclei of eukaryotic cells, utilizes the same chemical mechanism as the Group II intron, yet requires both RNA and protein components for complete function. The five small nuclear (sn)RNA and numerous associated protein components comprising the spliceosome undergo a cycle of assembly and dissociation with the catalysis of each pre-mRNA substrate. The dynamic and highly specific nature of spliceosome activity makes its assembly a particularly fascinating and challenging target of study.

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Phylogenetic, mutational and biochemical studies have demonstrated the role of specific and highly conserved RNA interactions in the assembly of the catalytic core of the spliceosome (7). A critical RNA–RNA interaction is formed by pairing of a consensus sequence of the intron with a short region of the U2 snRNA forming a complementary helix of 7 bp with a single unpaired adenosine (called the branch site adenosine). The 2'-OH of this adenosine initiates a nucleophilic attack on the phosphodiester linkage at the 5' splice site during the first step of splicing.

The structure of an RNA duplex representing the U2 snRNA-intron pre-mRNA branch site helix from the yeast *Saccharomyces cerevisiae* was recently solved by Newby and Greenbaum using solution NMR techniques (8). In their study, the structural role of a phylogenetically conserved pseudouridine (ψ) residue within the segment of U2 snRNA directly opposite the AA dinucleotide at the intron’s branch site region was investigated by exploring the architectural features of a ψ-modified duplex (ψBP) and its unmodified counterpart (uBP). Duplex sequences were shown in Figure 1. Pseudouridine, a post-transcriptional modification of the canonical uridine (U) residue, is prevalent in structural RNAs such as tRNA, rRNA and snRNA of all eukaryotes. In particular, ψ residues in certain conserved locations in U2 snRNA, including the branch site pairing region, have been found to be important for spliceosome assembly and function (9–11). Structural models reveal that the conserved ψ induces a dramatically different structure from that seen in its unmodified counterpart (8). The structure of uBP adopts a continuous A-type helical RNA geometry, with the branch site adenosine stacked in the helix. ψBP, on the other hand, features a pronounced kink in the backbone of the intron part in the branch site region, resulting in the extrusion of the branch site adenosine from the helix (Figure 2). In contrast with the 2'-OH of the ribose in A-type RNA helical structures, the 2'-OH of this adenosine, the nucleophile in the first cleavage step of splicing, is oriented toward the major groove. As strong evidence that this structural change correlates with activity, Valadkhan and Manley (12) found that the presence of this pseudouridine greatly enhances the splicing reaction by protein-free U2/U6 complex.

In an attempt to achieve a detailed understanding of the interactions that stabilize the unusual structure of the branch site helix, and the features by which the 2'-OH of the branch site adenosine may be recognized by other RNA molecules, proteins or metal ions in the process of spliceosome assembly and activity, we calculated surface accessible areas and the surface and site electrostatic potentials of the ψ-modified branch helix and its unmodified counterpart. This information will help provide insight into the structure–function relationships in pre-mRNA splicing. Our calculations showed that in addition to the structural features associated with the ψBP, marked differences were found for the exposure of the polar and nonpolar surface areas around the branch site region and there is a region of significant negative electrostatic potential in the major groove, localized around the 2'-OH of the branch site adenosine. These features may contribute to the recognition of the branch site adenosine by other components and the activity in the first step of splicing.

METHODS

Structures

The branch site structural models used in this paper were the solution NMR structures solved by Newby and Greenbaum (8). Nine lowest energy structures for ψBP and ten lowest energy structures for uBP have been deposited in the Protein Data Bank (13) (PDB accession codes 1LMV and 1LPW for uBP and ψBP, respectively). The calculations in this paper were applied to all models of both structures. Here, we presented the results from model one of both structures. However, there were no significant differences among the results from other models. The NMR structures were not subjected to energy minimization prior to the calculations.

Calculation of solvent accessible surface areas

The overall polar and nonpolar solvent accessible surface area (SASA) of each RNA helix was modeled with NACCESS (14) and MSMS (15) programs. For the van der Waals radii of each atom, we used both the values of Alden and Kim (16) and the
default values of the NACCESS program. The solvent probe radius was set to 1.4 Å and the triangulation density was 1.4 vertex/Å² in the MSMS program. The slice size parameter that controls the level of accuracy in NACCESS was taken as 0.05 Å. Essentially, identical areas were obtained with parameters that provide a finer resolution surface mesh. The polar atoms were oxygen and nitrogen, and the nonpolar atoms were carbon and phosphorus. Hydrogen atoms were treated implicitly during the calculation by increasing the heteroatom van der Waals radius by a small amount. The solvent accessible surfaces provide information about the degree of solvent exposure of polar/nonpolar and charged atoms or residues of nucleic acids or other biomolecules.

**Solutions to the nonlinear Poisson–Boltzmann equation**

Because of the high charge density of nucleic acids generated by the sugar–phosphate backbone, and the irregular surface of RNA in particular, it is necessary to solve the complete nonlinear Poisson–Boltzmann (NLPB) equation (17). Numerical solutions to the NLPB equation used here were obtained with a hybrid boundary element (BE) and finite difference (FD) PBE algorithm (25). The NLPB equation for a biopolyelectrolyte immersed in a 1:1 salt solution, given below, was solved numerically subject to the appropriate boundary conditions (e.g. continuity of the electrostatic potential at the dielectric interface and zero potential when infinitely apart from the molecule):

\[
\nabla \cdot [\varepsilon(\vec{r}) \nabla \Phi(\vec{r})] - \kappa^2(\vec{r}) \sinh(\Phi(\vec{r})) + \frac{4\pi e}{k_B T} \rho(\vec{r}) = 0, \]

where \(\varepsilon\) is the dielectric constant, \(e\) represents the protonic charge, \(k_B\) denotes the Boltzmann constant and \(T\) is the absolute temperature of the solution, \(\nabla\) is the divergence operator, \(\Phi\) is the electrostatic potential, \(\rho\) is the solute charge density and \(\kappa\) is the Debye–Hückel screening parameter. The quantities \(\varepsilon, \Phi, \rho, \kappa\) and \(\kappa\) are all functions of the position vector \(\vec{r}\).

It is through the Debye–Hückel parameter that the effects of mobile ions on the electrostatic potential are captured. The second term in Equation (1) represents the charge density of the mobile ions in solution, which are treated in a 'mean field' way as a continuous Boltzmann distribution. The dielectric interface that separates the regions of different polarizability was here represented by the solvent excluded molecular surface. The ionic solution, which contains 0.1 M added NaCl [close to the NMR experimental condition (19)], was treated as the region of high dielectric, here taken as 80 (25°C), and the solute interior had a low dielectric constant of 2 in order to facilitate visual inspection, color mapping of the electrostatic potential was finely scaled as follows: green (most positive), followed by blue, white (neutral), red and yellow (most negative).

**Calculations of site electrostatic potentials**

The electrostatic potentials surrounding specific atom sites were calculated by taking the average electrostatic potential of sampled grid points outside the molecule around a particular atom. The grid points were sampled by generating two layers of spheres 0.5 and 1.0 Å away from the van der Waals surface of the atom. The electrostatic potentials of the grid points were obtained by NLPB calculations using the hybrid PBE approach with Amber94 atomic radii and charge parameters.

**RESULTS**

**Surface areas of the duplexes**

We calculated the surface accessible area of the two branch site RNA structural models. The SASA determined by NACCESS, which is based on the method of Lee and Richards (24) with the atomic radii published by Alden and Kim (16), is shown in Table 1. Repetition of the calculations with the MSMS algorithm and with default NACCESS atomic radii indicated that the results did not vary significantly with the choice of surface method or atomic radii.

| Branch site RNA helices | Total SASA (Å²) | Polya/n polar SASA | Nonpolar SASA of branch site adenosine (Å²) | SASA of 2‘-OH branch site adenosine (Å²) |
|------------------------|----------------|--------------------|-------------------------------------------|------------------------------------------|
| BBP                    | 3770 ± 168     | 1.95               | 110 ± 30                                  | 0.4 ± 1.8                                |
| uBP                    | 3670 ± 39      | 1.9                | 56 ± 6                                    | 18 ± 3.9                                  |

The calculations are averaged over the ensembles of all NMR models for both modified and unmodified RNA structures. Values were calculated by NACCESS using the radii published by Alden and Kim (16).
The total SASA for uBP is 3670 ± 39 Å² and that for ψBP is 3770 ± 168 Å². The two duplexes exhibit, at most, a 2% difference in the total solvent accessible area (well within SD). The ratio of polar/nonpolar exposure level is also similar for the two duplexes. Because the stable form of any polyanionic system such as RNA in solution may be the one with maximal polar exposure and minimal nonpolar part exposure, this result is in accord with the thermal denaturation experiment results (19), which showed that ψBP has only a slightly smaller free energy than uBP (−0.7 kcal/mol).

Our data revealed significant differences in the vicinity of the branch site base, however. The nonpolar SASA of the branch site adenosine in ψBP is 110 ± 30 Å², a much greater value than that for the same base in the unmodified helix (56 ± 6 Å²). Since adenine has the most nonpolar atoms among all bases, this is consistent with the structural model of the branch site adenine of ψBP extruding into the solvent instead of stacking in the helix, as is the analogous adenine of uBP. We speculate that the cost of nonpolar exposure is compensated by the formation of the base triple and an additional water-mediated hydrogen bond formed by ψBP in the major groove.

The most surprising result was the finding that the SASA of the 2'-OH of ψBP's branch site adenosine is much smaller than that of its counterpart in uBP or any other 2'-OH in either duplex (0.4 ± 1.8 Å² versus 18 ± 3.9 Å²). These data imply that the 2'-OH of ψBP branch site adenosine does not protrude into solvent in the major groove, but is level with the lower dielectric environment of the groove itself.

Surface electrostatic potentials of the RNA branch site

The surface electrostatic potentials of uBP and ψBP were calculated using the hybrid PBE approach with the atomic partial charges and radii taken from the AMBER94 force field, except for the atomic partial charges for ψ, which were obtained by quantum chemical calculations using the Gaussian98 program (http://www.gaussian.com) (Prof. Maria Nagan, Truman State University, unpublished results). The electrostatic potential mapped on the surface of the unmodified branch site has similar features to that of a typical A-form RNA helix. As expected, the backbones have mostly negative electrostatic potential as a result of the negatively charged phosphate groups, although the grooves display some patches of positive potential, reflecting the presence of electropositive base atoms (Figure 3a–c). The overall surface potential of the major groove is considerably more negative than that of the minor groove, typical of the A-form RNA helix. As shown in Figure 3a and b, and reported in prior studies (6,25), this feature of A-form helices is independent of base sequence (26). Presence of the additional adenosine stacked in the helix does not impact the electrostatic surface.

The results of the electrostatic surface potential calculation of the ψ-modified helix highlight an intriguing difference between the two duplexes that is likely to play an important role in recognition. The backbone region and the grooves of the regions several base pairs away from the branch site have similar features to uBP, but there is a distinct and exceptionally negative region in the major groove (Figure 4b and c) corresponding to the region of the branch site adenosine, the 2'-OH of which is the nucleophile in the cleavage reaction at the pre-mRNA 5' splice site. Since the electrostatic properties calculated with continuum PBE models are sensitive to the atomic radii and partial charges used, we repeated the electrostatic potential calculations with the atomic charge and radii for every atom including the pseudouridine taken from the Discover force field parameter set. A comparison of the electrostatic potentials generated using each force field revealed no visible difference between the two sets of data (data not shown). The electrostatic potential in the minor groove was not significantly different from that of the minor groove of uBP (Figures 3a and 4a).

In order to determine if the negative potential region in ψBP is sequence dependent, we performed calculations in which a −0.5e charge placed on each nonbridging phosphate oxygen atom was considered (i.e. no partial charges on the nucleotides). As shown in Figure 5, the backbone charges are responsible for the overall enhanced negativity of the electrostatic potential in the major groove as opposed to the minor groove in both uBP and ψBP. Thus, this electrostatic trademark, which is characteristic of both A-DNA and A-RNA, is largely due to the sugar–phosphate backbone charges. Furthermore, the negative potential region in the major groove is still present in the ψBP, albeit less pronounced than when all the partial charges of the RNA were included. This finding supports a model in which both the kinked backbone and the bases are each partially responsible for creating the exceptionally negative electrostatic potential region. However, according to
more quantitative results discussed below, the backbone still makes the largest contribution to the total potential in this pocket of enhanced negative electrostatic potential.

Electrostatic potentials at 2'-OH of nonterminal residues

Although the images of the surface potential of the ψBP RNA duplex indicate some interesting electrostatic features associated with its major groove, such results are at most qualitative. Thus, to quantify the electrostatic potential around the branch site adenosine, we calculated the electrostatic potentials at the 2'-OH of each nonterminal residue. The results for uBP are shown in Table 2 and those for ψBP are shown in Table 3. For uBP, the electrostatic potential at the 2'-OH of each nonterminal residue is approximately −1.3 kcal/mol/ε. The electrostatic potential at the 2'-OH of the branch site adenosine is similar to that of the other 2'-OH. This is consistent with the surface electrostatic potential depiction.

The results for the ψ-modified helix show markedly different values for the branch site adenosine and neighboring residues than for the rest of the duplex. The 2'-OH of the branch site adenosine has a very strong negative electrostatic potential (−3.7 kcal/mol/ε) compared with that of the unmodified branch site adenosine, which may steer cationic species into this potential binding pocket. Because the branch site...
The distance is from the specific atom to the 2'-OH of the branch site adenosine (denoted by *). The surface is the solvent accessible surface of the specific atom calculated by the NACCESS program.

The electrostatic potential at specific atom sites became less negative as distance from the branch site adenosine increased. The electrostatic potential at this local site and surroundings.

Thus, the localized kink and overall distortion of the backbone of uBP (5) revealed some insightful and unique sequence- and structure-dependent electrostatic patterns that have helped understand electrostatic-mediated recognition principles employed during binding processes of cationic drugs and proteins to a variety of nucleic acid structures (33,34). The classical work of Pullman and co-workers (32,35) was the first to show the concerted role of electrostatics and solvent accessibilities in governing the recognition and binding events in nucleic acids.

The regions or pockets of intense negative potential in RNA structures, such as found in loops or major grooves, are potential binding or recognition sites for cationic species such as metal ions (e.g. Mg$^{2+}$), cationic drugs, and amino acid side chains. Such regions have been identified using surface electrostatic potential calculations or Brownian dynamics simulations, both of which are based on the nonlinear PBE (3,36–38). In fact, many crystallographically observed metal ions are found to be located in regions of high negative potential in a variety of RNA structures such as the P4–P6 domain from the *Tetrahymena* Group I intron, the loop E fragment of 5s rRNA, the hammerhead ribozyme, tRNA, *Escherichia coli* 58 nt 23S rRNA and the P5b stem loop of the Group I intron (3,6,37,38).

The very high negative charge density of nucleic acids requires the complete nonlinear form of the PBE for a rigorous calculation of electrostatic potentials. However, the full PBE does not possess an analytical solution for anything beyond very simple symmetrical geometries such as infinite planar and cylinder cases in certain limits. In the 1980s, several numerical approaches were developed in order to solve the PBE for any arbitrary 3D shape and charge distribution (39–41). The algorithms most widely used (DelPhi, UHBD) fall in the category of finite difference solutions to the PBE (29,42). However, these and newer nonlinear PBE implementations (43) still suffer two main deficiencies: convergence issues and inadequate treatment of outer boundary conditions, especially in the limits of low salt and high charge. This is a big limitation since many macromolecular assemblies such as nucleosomes and ribosomes are highly charged.

Boschitsch and Fenley (25) recently developed a novel hybrid PBE approach that employs both the boundary element and finite difference methods. It combines the intrinsic advantages of a BE and finite difference scheme to solve the nonlinear PBE for complicated molecular geometries by decomposing the electrostatic potential into a linear component satisfying the linear PBE solved by a fast multipole-accelerated BEM (18) and a correction term to account for the nonlinear effects. One of the main advantages of the hybrid PBE approach lies in its robust and superior treatment of outer boundary conditions and solution convergence, with the latter being accelerated by multigrid techniques [for a more detailed discussion of this novel PBE approach, see (25)]. The hybrid BE/FD PBE approach predicts surface and site (at specified locations) electrostatic potentials and electrostatic solvation free energies for realistic nucleic acid structures with high accuracy and efficiency.

The calculation of solvent accessible surface area, first introduced by Lee and Richards (24) to quantify the molecular surface, provides information about the interaction of a biomolecule’s functional groups with other solute and solvent molecules. Measurement of the curvature, surface area and precise geometrical shape of a molecular surface are routinely used by the structural biology and biophysics communities when predicting intra- and intermolecular interaction and associated conformational change.

Here, we compared results of solvent accessible surface areas and nonlinear PBE calculations of the ψ-modified and unmodified U2 snRNA-intron branch site sequence in order to evaluate the molecular basis of stability and recognition.
Although the ψ-modified RNA branch helix and its unmodified counterpart have similar global values for total surface area and polar/nonpolar exposure, the two helices have marked differences in the polar/nonpolar exposure in the region of the branch site. We also found that ψBP exhibits a region of profound electrostatic negativity in the more accessible widened major groove in the vicinity of the 2'-OH of the branch site adenosine. This finding indicates that the presence of ψ not only affects the structure of this biochemically important region of the spliceosome, but also impacts on other properties of the molecule not readily seen from the structure.

We propose that this heterogeneity contributes to molecular recognition of the branch site adenosine by other components of the spliceosome in order to facilitate splicing activity. Biochemical studies in which individual functional groups of the branch site adenosine were modified imply an extrahelical orientation of the adenine base and recognition of its topology by other components of splicing (44); this conclusion was supported by NMR structural studies (8,19). Apparently, the energetic cost of exposure of the nonpolar groups is balanced by formation of a base triple including the branch site adenine base (8) and by a water-mediated hydrogen bond involving ψNH1 (45).

Unusual electronegative pockets sometimes correspond to metal ion binding sites (6). Unusual electrostatic negativity surrounding the branch site adenosine in the ψ-modified duplex, largely due to the distortions created in sugar-phosphate backbone analogous to those observed in RNA bulges and K-turn motifs, suggests it might represent a cation binding site. NMR studies by Newby and Greenbaum found no evidence of a site-bound magnesium ion in the U2 snRNA–intron duplex (19). However, because the duplex they studied is only one component of the catalytic core, it is possible that this region may attract a hydrated metal ion or other cation in the context of other components. It may also help to minimize electrostatic repulsion between the components of the spliceosome during the first step splicing interactions. In addition to topological features associated with ψ-dependent structure, there are also marked electrostatic features that may contribute to recognition and activity.

In summary, we have calculated surface areas and electrostatic potentials of the ψ-modified and unmodified branch site RNA helices. We have found unusually small 2'-OH exposure and have identified a region of exceptionally negative electrostatic potential surrounding the 2'-OH of the branch site adenosine in the major groove of the ψ-modified duplex. In addition to structural features associated with the presence of this highly conserved ψ residue, surface area and electrostatic features may contribute prominently to the recognition, and perhaps indirectly to the activation, of the branch site nucleophile of the spliceosome’s catalytic core.

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