Sequential Assignment of Nucleic Acids

Pascale Legault
Département de Biochimie
Université de Montréal

Outline

1) NMR Spectroscopy for Structural Studies of Nucleic Acids
2) Primary Structure of Nucleic Acids (RNA and DNA)
3) Sample Preparation
4) Resonance Assignment of RNA by Homonuclear NMR
5) Resonance Assignment of RNA by Heteronuclear NMR
1) NMR Spectroscopy for Structural Studies of Nucleic Acids

The Fundamental Significance of RNA Structure

- RNA plays an essential role in biochemistry
  - protein translation (tRNA, mRNA, rRNA)
  - rRNA modifications (snoRNA)
  - telomere maintenance (telomerase)
  - ribozyme (RNA enzymes)
  - viral infection (coronavirus (SARS), HIV (AIDS), etc.)
  - RNA interference (siRNA, miRNA, etc.)
  - etc.

- The complexity in RNA function correlates with a complexity in RNA structure.
  - Example: the ribosomal RNA (rRNA)
RNA Structural Motifs

- The secondary structure of RNA is composed of double-stranded regions, as well as single strands, bulges, internal loops, hairpins, junctions, etc.
- These non-paired regions contained structural motifs such as U-turns, GNRA tetraloops, cross-strand purine stack, etc., and are important for formation of the tertiary structure.
- A better structural understanding of these motifs is necessary for understanding basic biochemical processes, RNA structure prediction, and drug design.
NMR Spectroscopy is an Important Method for Structural Studies of Nucleic Acids

As of Tuesday Oct 14, 2008

<table>
<thead>
<tr>
<th>Molecule Type</th>
<th>Proteins</th>
<th>Protein-Nucleic Acid Complexes</th>
<th>Nucleic Acid</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray</td>
<td>42747</td>
<td>1975</td>
<td>1096</td>
<td>24</td>
<td>45842</td>
</tr>
<tr>
<td>NMR</td>
<td>6557</td>
<td>138</td>
<td>820</td>
<td>7</td>
<td>7522</td>
</tr>
<tr>
<td>Others</td>
<td>226</td>
<td>53</td>
<td>15</td>
<td>2</td>
<td>296</td>
</tr>
<tr>
<td>Total</td>
<td>49530</td>
<td>2166</td>
<td>1931</td>
<td>33</td>
<td>53660</td>
</tr>
<tr>
<td>% NMR</td>
<td>13.2</td>
<td>6.4</td>
<td>42.5</td>
<td>21.2</td>
<td>14.0</td>
</tr>
</tbody>
</table>

2) Primary Structure of Nucleic Acids (RNA and DNA)
Nucleic Acids are Polymers of Nucleotides

Phosphate

Pentose

Base

The Two Types of Bases

Pyrimidine Ring

Purine Ring
Common Pyrimidine Bases

Cytosine

Uracil (RNA)

Thymine (DNA)

Common Purine Bases

Adenine

Guanine
Pentose Sugars

Ribose (RNA)  2-Deoxyribose (DNA)

Example of a Nucleoside Triphosphate: GTP
3) Sample Preparation

- The typical sample is 1 mM in 500 µL: around 5 mg of a 30-nucleotide RNA.
- Samples are synthesized chemically or enzymatically (T7 RNA polymerase)
- Isotopically-labeled ($^{13}$C,$^{15}$N) RNA are produced by enzymatic synthesis using $^{13}$C,$^{15}$N-labeled NTPs.
Synthetic Method (3’ to 5’)


- Most efficient method: >99% coupling yields in < 90 sec.

- Not yet suitable for uniform $^{15}$N and $^{13}$C labeling

RNA Synthesis with Bacteriophage T7 RNA Polymerase

Preparation of $^{13}$C/$^{15}$N-Labeled RNA

\[ E.\ coli\ fermentation\ with\ ^{15}\text{N-NH}_4\text{Cl}\ and\ ^{13}\text{C-glucose} \]

\[ \text{Isolation of rRNA} \]

\[ \text{Nuclease P1 degradation of rRNA to 5' NMPs} \]

\[ \text{Enzymatic synthesis 5' NTPs} \]

\[ \text{RNA synthesis using T7 RNA polymerase} \]

4) Resonance Assignment of RNA by Homonuclear NMR

1. NMR Structure Determination
2. $^1\text{H}$ chemical shifts in RNA
3. Typical NOEs observed in A-form helices
4. General assignment strategy
5. Assignments of exchangeable protons
6. Assignments of non-exchangeable protons
7. Correlations between exchangeable and non-exchangeable protons
NMR Structure Determination

Choosing an Interesting Biological System
Sample Preparation
Assignment of $^1$H, $^{13}$C, $^{15}$N, $^{31}$P Resonances
Obtaining Structural Constraints
Structure Calculation
Structure-Activity Relationships

$^1$H Chemical Shifts in RNA
Typical NOEs in A-Form Helices

General Assignment Strategy

A) Exchangeable protons: 1D \(^1\)H, 2D NOESY

B) Non-exchangeable protons

- Aromatic Spin Systems: 2D DQF-COSY (H5-H6), 2D NOESY
- Sugar Spin Systems: 2D DQF-COSY, 2D TOCSY
- Sequential Assignment: 2D NOESY, 2D \((^{31}\)P, \(^1\)H) HETCOR

C) Correlation of exchangeable and non-exchangeable protons: 2D NOESY
Assignments of Exchangeable Protons:
1D Imino Proton Spectrum

Assignments of Exchangeable Protons:
2D NOESY
Assignments of Non-Exchangeable Protons:
H5-H6 Region of DQF-COSY

Assignments of Non-Exchangeable Protons:
H1'-H2' Region of DQF-COSY
Assignments of Non-Exchangeable Protons:
Sequential Assignments
Correlations Between Exchangeable and Non-Exchangeable Protons

![Diagram showing correlations between exchangeable and non-exchangeable protons.](Image)

Limitations of Homonuclear NMR:

1. Inefficient through-bond transfer of magnetization
2. Spectral overlap particularly in the ribose region
3. Homonuclear techniques limited to small RNAs (<15 nt)

Advantages of Heteronuclear NMR:

1. Allow unambiguous through-bond transfer of magnetization
2. Increase resolution provided by heteronuclear correlations
3. Larger RNAs can be studied (at least up to 45 nt)
5) Resonance Assignment of RNA by Heteronuclear NMR

1. Assignment strategy
2. $^{13}$C and $^{15}$N chemical shifts in RNA
3. Assignments of exchangeable protons
4. Assignments of non-exchangeable protons
5. Correlations between exchangeable and non-exchangeable protons

Assignment Strategy

A) Exchangeable protons:
- 1D $^1$H, 2D NOESY
- 2D ($^{15}$N, $^1$H) HSQC
- 3D $^{15}$N-edited NOESY-HSQC

B) Non-exchangeable protons
- Aromatic/Sugar Spin Systems:
  - 2D ($^{13}$C, $^1$H) HSQC
  - 3D HCCH-COSY
  - 3D HCCH-TOCSY
  - 2D ($^{15}$N, $^1$H) HCN

- Sequential Assignment:
  - 3D/4D $^{13}$C-edited NOESY-HSQC
  - HCP-type only for small RNA

C) Correlation of exchangeable and non-exchangeable protons:
- 2D G-specific H(NC)-TOCSY-(C)-H
- 2D A-specific (H)N(C)-TOCSY-(C)-H
- 2D C-specific H(NCCC)H
- 2D U-specific H(NCCC)H
- 3D/4D $^{13}$C-edited NOESY-HSQC
$^{15}$N Chemical Shifts in RNA: 
($^1$H, $^{15}$N) HSQC Spectrum

$^{13}$C Chemical Shifts in RNA: 
($^1$H, $^{13}$C) HSQC Spectrum Sugar Region


13C Chemical Shifts in RNA: 
(1H, 13C) HSQC Spectrum Aromatic Region

Assignments of Non-Exchangeable Protons: 
HCCH-Type Experiments

1H → 13C → 13C → 1H 

One-bond INEPT COSY, RELAY, or TOCSY One-bond Reverse INEPT

\[ J_{1H, 1C} = 145-165 \text{ Hz} \quad J_{1C, 1C} = 38-42 \text{ Hz} \]

Allows for unambiguous assignments of 1H and 13C ribose as well as H6C2H6 in pyrimidines

A) DQF-COSY

B) HCCH-COSY

1H Chemical Shift (ppm)

13C Chemical Shift (ppm)
Assignments of Non-Exchangeable Protons: HCCH-Type Spectra

All 2D planes at C1' frequency of G2 and C12

3D HCCH-COSY
3D HCCH-RELAY
3D HCCH-TOCSY
Methylene-filtered
3D HCCH-TOCSY
Methylene-edited

Assignments of Non-Exchangeable Protons:
2D (1H, 15N) HCN

Allows for unambiguous correlations between 1H of ribose and H6/H8 of base

[([GGUGUGAACC])2]
Correlation of Exchangeable and Non-Exchangeable Protons: G-specific H(NC)-TOCSY(C)H

Correlation of Exchangeable and Non-Exchangeable Protons: A-specific (H)N(C)-TOCSY(C)H
Correlation of Exchangeable and Non-Exchangeable Protons:
U-specific H(NCCC)H

Correlation of Exchangeable and Non-Exchangeable Protons:
C-specific H(NCCC)H