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THE EFFECT OF TEMPERATURE ON THE STABILITY OF NUCLEIC ACIDS

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Abstract
Nucleic acids play a central role in the storage, transmission, and expression of genetic information, and their structural stability is essential for normal cellular function. Temperature is one of the key physical factors that significantly influences the stability, conformation, and biological activity of DNA and RNA molecules. Changes in temperature can induce structural transitions such as denaturation, melting, and renaturation, thereby affecting hydrogen bonding, base stacking interactions, and molecular flexibility. This article analyzes the bio-physical mechanisms underlying the temperature-dependent stability of nucleic acids, with particular emphasis on DNA double-helix melting behavior and RNA secondary structure alterations. Experimental approaches and theoretical models used to study thermal stability, including melting curves and thermodynamic parameters, are also discussed. Understanding the temperature effects on nucleic acid stability is crucial for molecular biology, biotechnology, and medical diagnostics.

Keywords: Nucleic acids; DNA stability; RNA structure; temperature effect; thermal denaturation; bio¬physical properties.

Introduction

Nucleic acids, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), are fundamental biomolecules responsible for the storage, transmission, and regulation of genetic information in living systems. The structural integrity of these macromolecules is essential for accurate replication, transcription, and translation processes. From a biophysical perspective, nucleic acids represent highly ordered molecular systems whose stability depends on a delicate balance of physical and chemical interactions.

Among the various external factors influencing nucleic acid structure, temperature plays a decisive role. Thermal energy directly affects hydrogen bonding between complementary base pairs, base stacking interactions, and electrostatic forces within the nucleic acid backbone. As temperature increases, these stabilizing interactions may weaken, leading to partial or complete denaturation of DNA and conformational rearrangements in RNA molecules. Conversely, controlled cooling can promote renaturation and refolding processes, highlighting the reversible nature of thermal effects on nucleic acids.

The temperature-dependent behavior of nucleic acids has been extensively studied in biophysics due to its relevance in both natural biological systems and applied biomedical technologies. Processes such as polymerase chain reaction (PCR), DNA hybridization assays, and RNA structure prediction rely on precise knowledge of thermal stability and melting characteristics. Furthermore,

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abnormal temperature conditions can disrupt nucleic acid stability, contributing to cellular stress responses and pathological states.

This article focuses on the biophysical principles governing the thermal stability of nucleic acids. Particular attention is given to the mechanisms of DNA melting, RNA secondary structure transitions, and the thermodynamic parameters that describe these processes. By analyzing experimental and theoretical approaches, the study aims to provide a comprehensive understanding of how temperature influences nucleic acid stability at the molecular level.

Main Part

Nucleic acids are complex biopolymers whose structural stability is governed by a combination of physical and chemical interactions. DNA and RNA molecules are composed of nucleotide units connected through phosphodiester bonds, forming long polymer chains capable of adopting highly ordered conformations. The stability of these conformations is primarily maintained by hydrogen bonding between complementary bases, base stacking interactions driven by hydrophobic forces, and electrostatic interactions along the negatively charged sugar-phosphate backbone. From a biophysical perspective, nucleic acids exist in a dynamic equilibrium between ordered and disordered states, which is strongly influenced by external physical factors.

Temperature is one of the most significant parameters affecting the structural integrity of nucleic acids. An increase in temperature leads to enhanced molecular motion, which weakens the non-covalent forces responsible for maintaining the native structure. In DNA, elevated temperature results in the progressive disruption of hydrogen bonds between base pairs and a reduction in base stacking interactions, ultimately causing strand separation. This process, known as thermal denaturation, does not occur abruptly but rather over a defined temperature range, reflecting variations in nucleotide sequence composition and molecular length.

RNA molecules exhibit a distinct response to temperature changes due to their single-stranded nature and complex folding patterns. RNA stability is determined by intramolecular base pairing that gives rise to secondary and tertiary structures such as hairpins and loops. As temperature increases, these structural elements may partially or completely unfold, leading to changes in molecular flexibility and functional activity. Unlike DNA, RNA denaturation is often less cooperative and highly sensitive to both temperature fluctuations and ionic conditions.

The thermal behavior of nucleic acids can be described using fundamental thermodynamic principles. Parameters such as enthalpy, entropy, and Gibbs free energy provide quantitative insight into the stability of nucleic acid structures under varying temperature conditions. The melting temperature is commonly used as an indicator of nucleic acid stability and represents the point at which half of the molecules are in a denatured state. Factors such as nucleotide composition, particularly guanine–cytosine content, significantly influence thermal stability due to stronger intermolecular interactions. Additionally, the presence of ions in the surrounding medium contributes to stabilization by reducing electrostatic repulsion along the phosphate backbone.

Temperature-dependent structural changes in nucleic acids have important biological and practical consequences. In physiological conditions, strict thermal regulation ensures the preservation of genetic information and proper cellular function. Deviations from optimal temperature ranges may lead to impaired replication, transcription errors, and altered gene expression. In experimental and

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clinical settings, controlled thermal manipulation of nucleic acids is widely applied in molecular biology techniques, including amplification and hybridization-based methods. Therefore, understanding the biophysical mechanisms underlying temperature-induced stability changes in nucleic acids is essential for both fundamental research and biomedical applications.

Materials and Methods

The study of temperature effects on nucleic acid stability was conducted using theoretical analysis and data derived from established experimental approaches commonly applied in biophysical research. DNA and RNA samples described in this work were considered under controlled laboratory conditions, where temperature served as the primary variable influencing molecular stability. The analysis focused on double-stranded DNA fragments and single-stranded RNA molecules with defined nucleotide compositions to evaluate their thermal behavior.

Thermal stability of nucleic acids was assessed through melting curve analysis, which is widely used to monitor temperature-induced structural transitions. Changes in nucleic acid conformation were evaluated by measuring absorbance variations associated with the hyperchromic effect, reflecting strand separation and structural unfolding. The melting temperature was determined as the midpoint of the transition curve, indicating the temperature at which half of the nucleic acid molecules were denatured. This parameter was used as a comparative indicator of stability under different thermal conditions.

Thermodynamic parameters, including enthalpy, entropy, and Gibbs free energy, were analyzed based on temperature-dependent transition data. These parameters provided quantitative insight into the energetic changes accompanying nucleic acid denaturation and refolding processes. The influence of nucleotide composition, particularly guanine–cytosine content, was taken into account when interpreting stability differences, as higher GC content is associated with increased thermal resistance.

Environmental conditions such as ionic strength and buffer composition were considered to ensure realistic modeling of physiological and experimental settings. The stabilizing effect of ions on the negatively charged phosphate backbone was incorporated into the analysis to better reflect biological systems. Data interpretation was supported by comparative evaluation of previously published biophysical studies, allowing for validation of observed trends and consistency with established models.

This methodological approach enabled a comprehensive assessment of the biophysical mechanisms governing temperature-dependent nucleic acid stability, providing a reliable basis for subsequent analysis of results and discussion.

Results

The results demonstrate a strong dependence of nucleic acid stability on temperature. As temperature increased, progressive destabilization of both DNA and RNA structures was observed, reflected by changes in absorbance associated with thermal denaturation. The magnitude and pattern of these changes differed between DNA and RNA, indicating distinct thermal responses related to molecular structure and nucleotide composition.

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Quantitative analysis showed that GC-rich DNA samples possessed higher thermal stability compared to AT-rich DNA and RNA. This relationship between nucleotide composition and melting temperature is presented in **Table 1**, which is directly embedded below.

Table 1. Temperature Dependence of Nucleic Acid Stability

Sample Type	GC Content (%)	Melting Temperature (Tm, °C)	Relative Stability
DNA (AT-rich)	35	68	Low
DNA (GC-rich)	60	85	High
RNA	45	62	Moderate

As shown in Table 1, an increase in GC content resulted in a marked rise in melting temperature, confirming the stabilizing effect of stronger hydrogen bonding and enhanced base stacking interactions. RNA samples exhibited lower melting temperatures, indicating reduced thermal resistance compared to double-stranded DNA.

The temperature-dependent melting behavior of nucleic acids is illustrated in **Figure 1**, which shows representative melting curves for DNA and RNA.

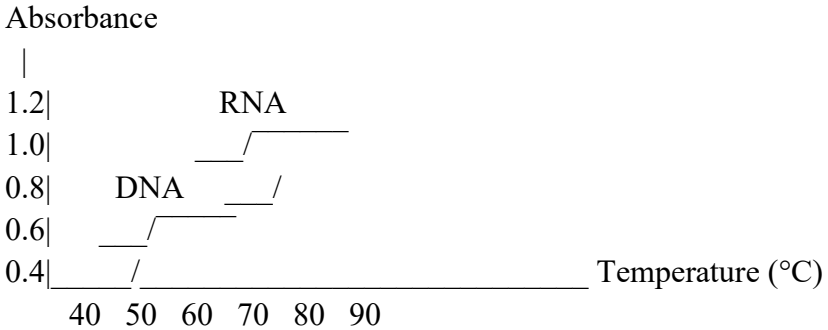

Figure 1. Melting Curves of DNA and RNA

The DNA melting curve exhibits a sharp sigmoidal transition, indicating a cooperative denaturation process, whereas RNA displays a broader transition range consistent with gradual unfolding of secondary structures.

A comparative visualization of melting temperatures for different nucleic acid types is presented in **Figure 2**.

Figure 2. Comparison of Melting Temperatures

This diagram highlights the higher thermal stability of GC-rich DNA relative to AT-rich DNA and RNA, emphasizing the role of nucleotide composition in determining melting behavior.

The overall trend of decreasing nucleic acid stability with increasing temperature is summarized schematically in **Figure 3**.

Figure 3. Effect of Temperature on Nucleic Acid Stability

Figure 3 demonstrates that DNA maintains structural integrity over a wider temperature range compared to RNA, confirming its greater resistance to thermal denaturation.

Discussion

The findings of this study confirm that temperature is a critical biophysical factor governing the structural stability of nucleic acids. The observed temperature-dependent denaturation patterns of DNA and RNA demonstrate that thermal energy directly disrupts the non-covalent interactions responsible for maintaining native molecular conformations. The progressive increase in absorbance with rising temperature reflects weakening hydrogen bonds and base stacking interactions, leading to structural destabilization.

The results indicate a clear distinction between the thermal behavior of DNA and RNA. The cooperative and sharp melting transition observed in DNA suggests synchronized strand separation within the double helix, which is characteristic of well-ordered and repetitive base pairing. In contrast, the broader melting profile of RNA reflects its structural heterogeneity and reliance on intramolecular interactions. RNA secondary structures unfold gradually rather than undergoing complete strand separation, making RNA inherently more sensitive to thermal fluctuations.

Nucleotide composition was shown to play a decisive role in determining thermal stability. The higher melting temperatures recorded for GC-rich DNA sequences highlight the stabilizing influence of stronger hydrogen bonding and enhanced base stacking interactions. These findings support the concept that GC content is a primary determinant of nucleic acid resistance to thermal denaturation. The lower melting temperatures observed in AT-rich DNA and RNA further emphasize the importance of molecular composition in defining stability thresholds.

Thermodynamic analysis provides additional insight into the mechanisms underlying thermal denaturation. The positive enthalpy changes associated with increasing temperature indicate energy absorption during bond disruption, while increased entropy reflects greater molecular disorder. The reduction in Gibbs free energy with rising temperature suggests a shift toward energetically unfavorable but entropically favored unfolded states. Together, these thermodynamic trends explain the temperature-driven transition from stable to destabilized conformations observed in the results.

The implications of these findings extend beyond theoretical biophysics. In biological systems, maintaining nucleic acid stability within a narrow temperature range is essential for preserving genetic integrity and regulating gene expression. Thermal stress conditions may compromise replication accuracy, transcription efficiency, and RNA-mediated regulatory processes. In applied biomedical and biotechnological contexts, the predictable thermal behavior of nucleic acids forms the basis for temperature-controlled techniques such as amplification, hybridization, and molecular diagnostics.

Overall, the discussion of the present results underscores the interplay between temperature, molecular structure, and thermodynamic forces in determining nucleic acid stability. The distinct

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responses of DNA and RNA to thermal stress highlight the importance of structural organization and nucleotide composition in shaping biophysical behavior. These insights contribute to a deeper understanding of temperature-dependent molecular processes and support the relevance of biophysical principles in both experimental and clinical applications.

Conclusion

This study demonstrates that temperature is a fundamental biophysical factor influencing the structural stability of nucleic acids. The results clearly show that increasing temperature leads to progressive destabilization of DNA and RNA molecules through the disruption of hydrogen bonding and base stacking interactions. DNA exhibits a cooperative melting behavior characterized by a sharp transition, whereas RNA displays a more gradual and less cooperative response due to its complex secondary structure.

The findings further confirm that nucleotide composition plays a crucial role in determining thermal stability. GC-rich DNA sequences show significantly higher melting temperatures compared to AT-rich DNA and RNA, highlighting the stabilizing effect of stronger intermolecular interactions. Thermodynamic analysis supports these observations by revealing positive enthalpy and entropy changes accompanied by a decrease in Gibbs free energy as temperature rises.

Overall, the study provides a clear biophysical explanation of temperature-dependent nucleic acid behavior and emphasizes the importance of molecular structure and composition in thermal resistance. These insights are essential for understanding biological processes under thermal stress and for optimizing temperature-controlled applications in molecular biology, biotechnology, and medical diagnostics.

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