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Role of pK_a of Nucleobases in the Origins of Chemical Evolution

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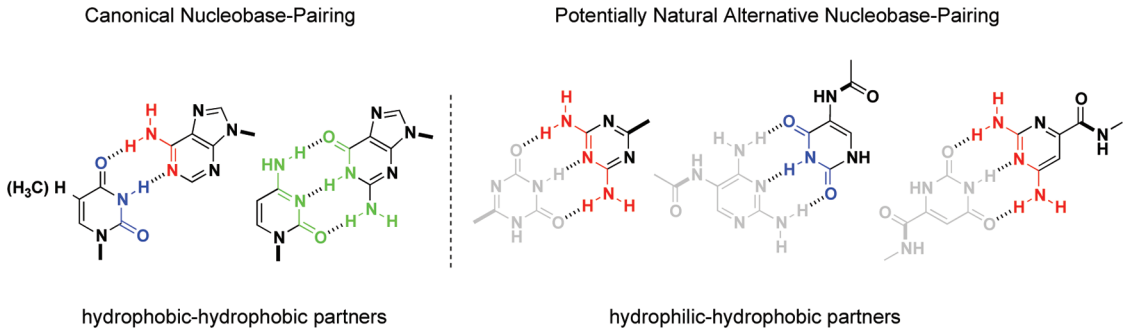

The formation of canonical base pairs through Watson–Crick hydrogen bonding sits at the heart of the genetic apparatus. The specificity of the base pairing of adenine with thymine/uracil and guanine with cytosine preserves accurate information for the biochemical blueprint and replicates the instructions necessary for carrying out biological function. The chemical evolution question of how these five canonical nucleobases were selected over various other possibilities remains intriguing. Since these and alternative nucleobases would have been available for chemical evolution, the reasons for the emergence of this system appear to be primarily functional.

While investigating the base-pairing properties of structural nucleic acid analogs, we encountered a relationship between the pK_a of a series of nonstandard (and canonical) nucleobases and the pH of the aqueous medium. This relationship appeared to correspond with the propensity of these molecules to self-assemble via Watson–Crick-type base-pairing interactions. A simple correlation of the "magnitude of the difference between the pK_a and pH" (pK_a–pH correlation) enables a general prediction of which types of heterocyclic recognition elements form hydrogen-bonded base pairs in aqueous media. Using the pK_a–pH relationship, we can rationalize why nature chose the canonical nucleobases in terms of hydrophobic and hydrophilic interactions, and further extrapolate its significance within the context of chemical evolution.

The connection between the physicochemical properties of bioorganic compounds and the interactions with their aqueous environment directly affects structure and function, at both a molecular and a supramolecular level. A general structure–function pattern emerges in biomolecules and biopolymers in aqueous media near neutral pH. A pK_a − pH < 2 generally prompts catalytic functions, central to metabolism, but a difference in pK_a − pH > 2 seems to result in the emergence of structure, central to replication. While this general trend is observed throughout extant biology, it could have also been an important factor in chemical evolution.

Introduction

The origins of chemical evolution are entangled with the events that led to the origins of life. Both physical and chemical processes must have played essential roles not only in prebiological and primitive biological processes but also during the emergence of the expanding and transforming sophisticated biochemical undertakings.[1] The physicochemical properties of the molecules, as determined by their interaction with the geophysical and geochemical surroundings, expressed both at the individual and at the supramolecular level (and the gradation of complexes in between), would have dictated and selected what sort of constituents and assemblies would have been able to form, transform, adapt, and provide for chemical evolution to supervene.

Of these various geophysical and geochemical components, water, by far, would have had the most influence in

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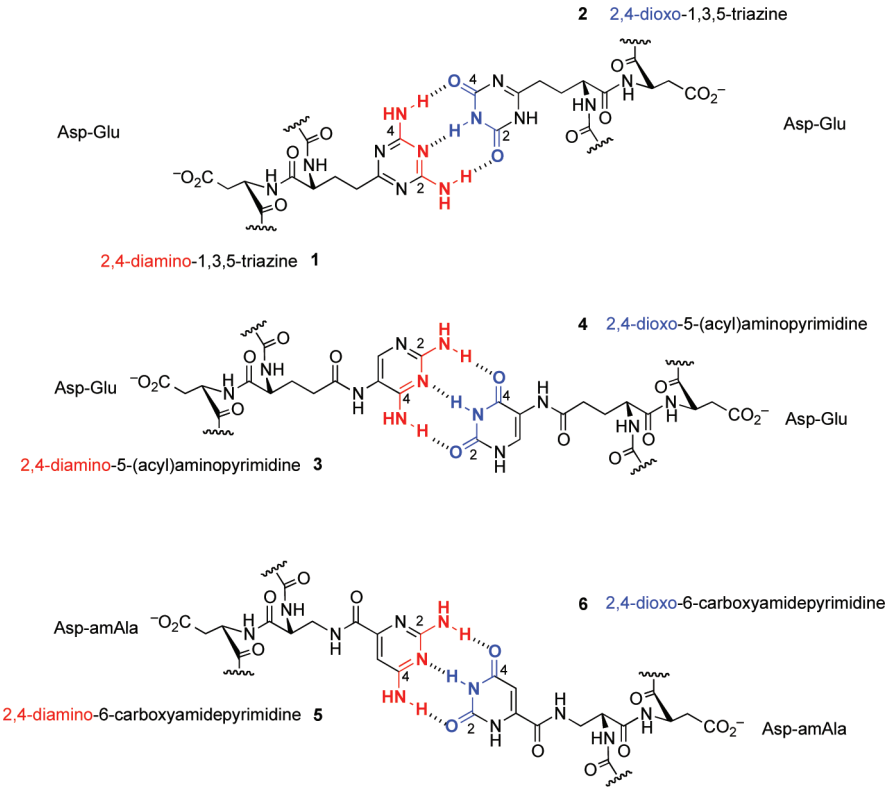


FIGURE 1. The alternative recognition elements and backbones investigated in mapping the landscape of potentially primordial informational oligomers.

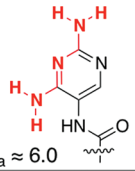
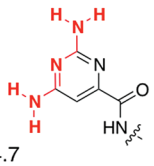
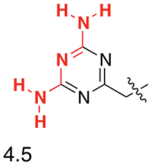
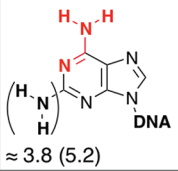
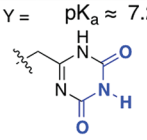
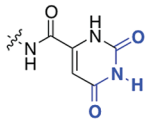
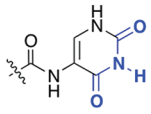
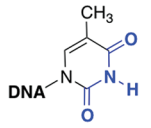
determining the physicochemical properties of the molecules and in beginnings of chemical self-organization toward life's origin. Not surprisingly, the presence of water is adjudged as one of the prerequisites for life to emerge. Two manifestations of the physicochemical properties of molecules, as dictated by their interaction with water, stand out: hydrophobicity and hydrophilicity. The interplay of these two characteristics plays a major role in determining the assemblage and, therefore, the function of (macro)molecules in extant biology. Such rudimentary interactions could have played a pivotal role also in the selection of molecules and in origins of chemical evolution.

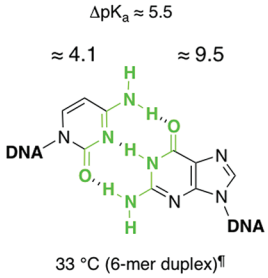
This Account outlines the work that led to the realization of one such physicochemical property, the pK_a (ionization or dissociation constant) of canonical and alternative nucleobases, which in turn dictates their hydrophobic and hydrophilic nature and thus their ability to function as base pairs in an aqueous medium. This work has its roots in (and is based on the fruits of) the seminal studies of Albert Eschenmoser and co-workers in the context of "chemical etiology of nucleic acid structure",[2] in which they examined the base-pairing properties of nucleic acids that were derived from various alternative sugar–phosphate backbones tagged with canonical nucleobases. The results of these extensive studies unambiguously demonstrated that Watson–Crick base pairing is not unique to the ribose–phosphate backbone of RNA/DNA but is also widespread among potentially natural alternative carbohydrate backbones. This conclusion, along with a host of other considerations, led Eschenmoser to expand this systematic investigation beyond the confines of the structural neighborhood of sugar backbone.[3] The strategy entailed experimentally mapping the landscape of potentially primordial informational oligomers, without any restrictions on the structure types of backbones and recognition elements, provided these candidates satisfied the requirements of emerging under likely prebiotic conditions and entertained the potential for function (information).

Mapping the Landscape of Potentially Primordial Informational Oligomers

In this frame of reference, the base-pairing properties of a family of oligomers, derived from various dipeptides (e.g., Asp-Asp, Asp-Glu), deoxodipeptides, and dipeptoids tagged with the noncanonical recognition elements 2,4-diamino- and 2,4-dioxo-substituted triazines (**1** and **2**) and 5-aminopyrimidines (**3** and **4**), were investigated in the

TABLE 1. Comparison of Intra- and Intersystem Base-Pairing Strengths of Dodecameric Duplexes Containing Alternative Recognition Elements Tagged to Oligodipeptides as Determined by UV−T_m Melting Curves[a]

UV-T$_m$ (°C)‡ 12-mer duplex xxxxxxxxxxxx yyyyyyyyyyyy	X =  pK$_a$ ≈ 6.0	 ≈ 4.7	 ≈ 4.5	 ≈ 3.8 (5.2)
Y =  pK$_a$ ≈ 7.2	ΔpK$_a$ ≈ 1.2 < 0 °C VERY WEAK	—	≈ 13	< 0 (19.4)*
 ≈ 6.6	—	< 0	< 0	< 0 (< 10)
 ≈ 8.9	15.5	19.2	16.0	**51.4** (41.9)
 ≈ 9.8	< 10	19.5	**53.8**	ΔpK$_a$ ≈ 6.0 (3.6) **41.6** (8-12 ° higher)++ STRONG



33 °C (6-mer duplex)¶
STRONG

[a]Taken from refs 4 and 5. ‡ indicates measured in 5 + 5 μM, 1 M NaCl, 10 mM Na_2HPO_4 buffer, pH 7.0, 0.1 mM Na_2EDTA; * indicates triplex. ++ indicates as determined for poly-d(D):poly-d(T) versus poly-d(A):poly-d(T);[13] ¶ indicates for a 6-mer homo-sequence duplex (25 + 25 μM) in 0.15 mM NaCl, 10 mM Na_2HPO_4 buffer, pH 7.0, 0.1 mM Na_2EDTA;[6] — indicates not measured.

research group of Eschenmoser and the present author at Scripps (Figure 1).[4]

Base-pair-mediated duplex formation between these potentially natural oligomers and complementary RNA and DNA sequences was prevalent, but with an unexpected result: while 2,4-diaminotriazine-tagged backbones in all these series were found to be uniformly stronger base-pairing partners, the corresponding complementary dioxotriazine-tagged series exhibited (very) weak or no base pairing. Furthermore, the exact opposite behavior was observed in the series tagged with 2,4-disubstituted 5-aminopyrimidines. There, the 2,4-dioxo-5-aminopyrimidine tagged backbones formed duplexes that were stronger (base pairing) compared with the duplexes from corresponding complementary 2,4-diamino-5-aminopyrimidine tagged series. Thus, the 2,4-diamino substituent on a triazine scaffold was functioning as a good base-pairing partner, but the same 2,4-diamino substituent on a 5-aminopyrimidine chassis was inferior; this trend is the exact opposite for the 2,4-dioxo moiety. Such contrasting base-pairing behavior could not be rationalized by invoking strengths of hydrogen bonding, keto−enol tautomerism, stacking effects or change in base pairing or backbone axes, or the nature of the backbone to which these recognition elements were attached.

While searching for clues for this incongruity of the base-pairing behavior, the existence of a correlation between acid ionization/dissociation constants (pK_a) of the complementary base-pairing partners and the base-pairing strength was noticed. The stronger base-pairing duplexes were formed whenever the difference between the pK_a of the "acceptor" and the pK_a of "donor" was at least 5 units or more; the smaller the ΔpK_a between the donor and the acceptor was, the weaker was the strength of the duplex (Table 1). For example, the 2,4-diaminotriazine unit (pK_a $\approx$ 4.5) paired strongly with deoxythymidine (pK_a = 9.8), corresponding to ΔpK_a of 5.3; however, the 2,4-diamino-5-aminopyrimidine unit (pK_a $\approx$ 6.0) paired weakly with thymidine, where ΔpK_a is 3.8. While the 2,4-dioxotriazine unit (pK_a $\approx$ 7.2) paired weakly with deoxyadenosine (pK_a 3.8), ΔpK_a of 3.4,

the 2,4-dioxo-5-aminopyrimidine unit ($pK_a \approx 8.9$) paired strongly with adenine; corresponding ΔpK_a is 5.1.

We further investigated the base-pairing properties of orotic acid, 2,4-dioxopyrimidine-6-carboxylic acid (**6**), and its complementary base-pairing partner, 2,4-diaminopyrimidine-6-carboxylic acid (**5**), tagged to an oligodipeptide backbone derived from alternating units of L-aspartic acid and L-3-aminoalanine (asp-amAla, Figure 1).[5] Oligodipeptide sequences tagged with 2,4-dioxo-derivative **6** were found to have extremely weak or no interactions with adenine-containing DNA/RNA sequences, while the corresponding complementary 2,4-diamino counterpart, **5**, exhibited stronger duplex formation with thymine-tagged DNA/RNA sequences. In contrast to the 5-amino-pyrimidine (**3** and **4**) tagged series, here, the 2,4-dioxo moiety (as opposed to a 2,4-diamino unit) in a pyrimidine nucleus was the inferior pairing partner. As was the case with previous studies of alternative heterocycles,[4] the property that is most consistent with the contradictory base-pairing behavior is the ionization constants; pK_a of 2,4-dioxo derivative **6** is 6.6, and that of the corresponding 2,4-diamino derivative **5** is 4.7. The weak base-pairing behavior of orotamide **6** with adenine ($\Delta pK_a \approx 3$) versus the stronger base pairing of **5** with thymine ($\Delta pK_a \approx 5$) is consonant with the ΔpK_a/base-pairing strength correlation (Table 1).

A comparison of the different pK_a values of the alternative heterocycles with those of the complementary canonical nucleobases, with a side-by-side qualitative relationship with base-pairing strengths (Table 1) accentuates this correlation between the magnitude of ΔpK_a of complementary partners with the base-pairing strength of the duplexes formed. While these correlations were largely consistent in their trends, they did not readily lend themselves to explanation.

While searching for explanations, the following sentence, "More precisely, the pK of an acid should be less than 4 and that of a base greater than 10 to ensure that only a small fraction of the compound remains in the un-ionized form at physiological pH. This general rule is not absolute", from Frank Westheimer's paper "Why Nature Chose Phosphates",[7] struck a chord. The pK values of 4 and 10 are exactly the same values around which the canonical nucleobases congregate; at physiological pH, the nucleobases are in the un-ionized form.[8] The alternative nucleobases, whose pK_a are more than 4 and less than 10, are probably in the "ionized form" under the measurement pH of 7.0.

Clues were obtained from the pH- and temperature-dependent UV-spectra behavior of orotamide derivatives. The pK_a value of 6.6 of the orotamide unit in **6** correlated with the deprotonation of the N(1)-H proton (and not the N(3)-H proton) based on the shift in the λ_{max} from 280 to 300 nm with increasing pH of the medium. Similar bathochromic shift was also observed in the temperature-dependent UV spectrum of the hexadecamer of **6** alone or in the presence of its pairing partner. All these facts indicated that the orotamide unit is deprotonated under the base-pairing measurement conditions and exists in its N(1)-anionic form. A similar λ_{max} shift, from 232 to 255 nm pointing to a N(1 or 5)H deprotonation, was also observed in the temperature-dependent UV spectra of 2,4-dioxo-triazine tagged oligodipeptides; such a behavior was absent in the 2,4-dioxo-5-aminopyrimidine series suggesting the absence of deprotonation of N(1)-H in **4**.

How exactly the deprotonation of the N(1 or 5)-H proton in **2** affects the base-pairing strength was not immediately clear, since it could be argued that it is the N(3)-H of 2,4-dioxo-triazine and -5-aminopyridmines that is involved in the hydrogen bonding. However, when the *deprotonation and protonation* (or the lack of it) of the recognition elements was linked via their corresponding "ionized" or "un-ionized" state in an aqueous environment with their *hydrophilicity and hydrophobicity* (and not solely with hydrogen-bonding capability), a qualitative understanding of the correlation between the base-pairing strength of the duplex, pK_a of the heterocycle, and the pH of the aqueous medium emerged.

In the specific examples considered above, when the pK_a of the heterocycles (**2**, **3**, and **6**) is close to the pH of the aqueous medium, they become deprotonated or protonated (ionized) and, therefore, hydrophilic (Figure 2). This, in turn, increases the solvation of the heterocycles by the aqueous medium (via polar interactions and hydrogen bonding), drastically hindering their ability to interact with their base-pairing partner (via stacking interactions and hydrogen bonding), weakening the duplex. With increasing number of such hydrophilic units, a "breaking point" is reached where no duplex formation is possible.[9] On the other hand, when the pK_a of the heterocycles (**1**, **4**, and **5**) is far removed from the pH of the aqueous medium, the heterocycles remain in their un-ionized form and, therefore, are hydrophobic (Figure 2). This coerces the heterocycles to minimize their interaction with the aqueous medium and congregate with their base-pairing partner (reinforced by stacking interactions[10] and specific hydrogen bonding), leading to stronger base pairing. In other words, here, when the difference between the pK_a of the heterocycle and the pH of the aqueous medium ($pK_a - pH < 2$) is smaller, the base-pairing strength is expected to be weaker; conversely,

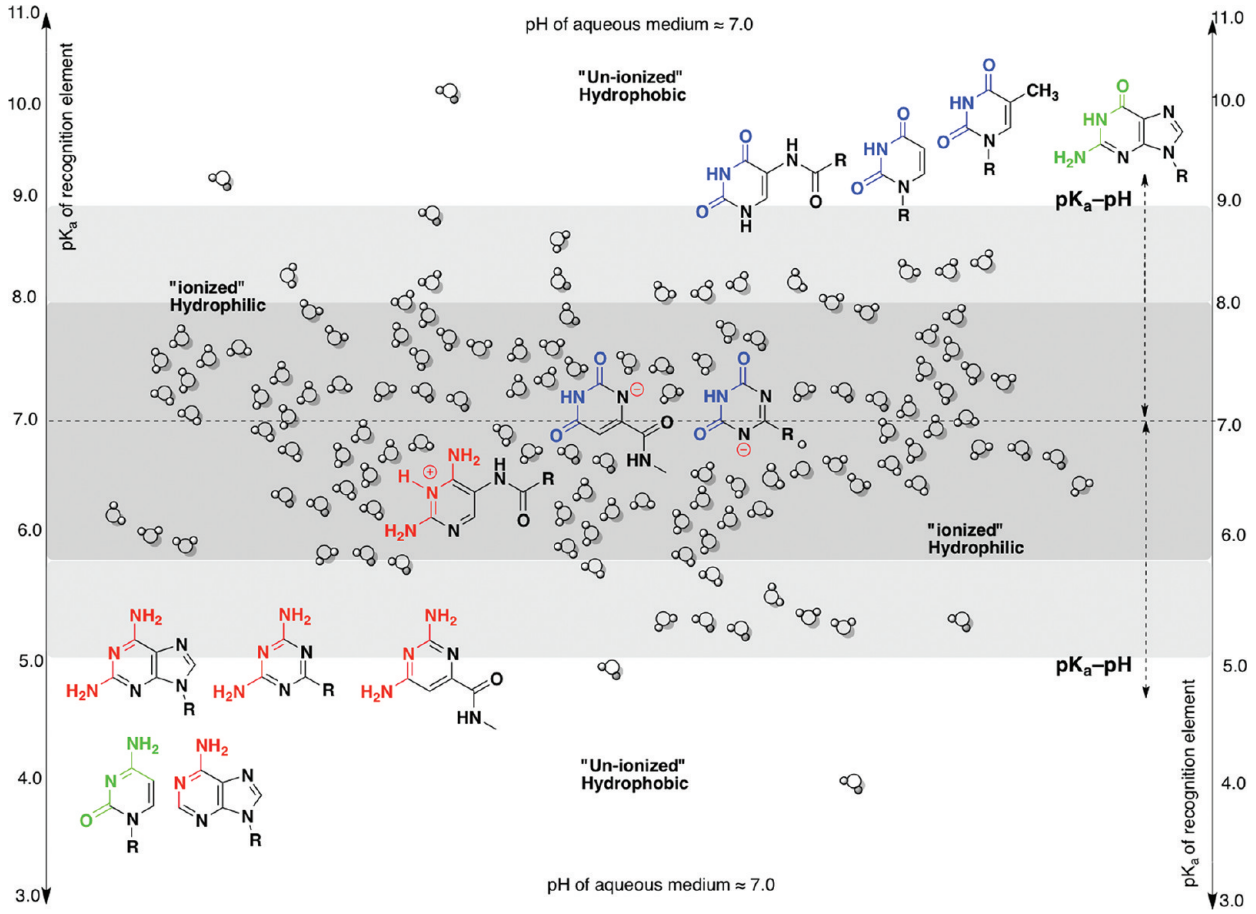


FIGURE 2. Juxtaposition of the pK_a values of the canonical nucleobases with those of the potentially natural alternative heterocycles, correlating with the degree of solvent interaction of the charged (hydrophilic) and uncharged (hydrophobic) nucleobases (in neutral aqueous conditions).

when the difference between the pK_a of the heterocycle and the pH of the aqueous medium is larger (pK_a − pH > 2), the base-pairing strength is anticipated to be stronger, all other things being equal.

General Applicability of the pK_a–pH Relationship and Implications

The relationship between pH of the medium, pK_a of the heterocycle, and its base-pairing capacity is widely documented and has been exploited by various research groups largely in the context of mismatch discrimination and triplex formation, pertaining to diagnostic and antisense applications.[11] The "pK_a–pH rule" can be useful in understanding the base-pairing behavior of many of these nucleobase variations (Figure 3).[12]

A classic example is the 2,6-diaminopurine–thymine base pair, which is weaker compared with guanine–cytosine (though both base pairs have three hydrogen bonds) and in some instances not as stable as A–T base pair.[13] The higher basicity of 2,6-diaminopurine, pK_a 5.2, leads to more protonation at neutral pH, increasing its hydrophilicity and interaction with water, thereby impeding its interaction with its complementary partner.[14]

Another instructive example is the strong base pairing between two purines, guanine (pK_a 9.5) and isoguanine (pK_a 9.2) in aqueous medium.[15] Here $\Delta pK_a < 1$, which according to the original ΔpK_a-correlation[4] predicts weak or no base pairing;[16] however, the experimental observation is in concordance with what would be expected from pK_a–pH criterion (here >2). This case illustrates clearly that the ΔpK_a criterion that was used before[4] is actually a special manifestation of the pK_a–pH correlation.[17]

This analysis is also valid for other noncanonical nucleobases and is exemplified here by one of the size-expanded guanine (dxG)–cytosine base pair, which is destabilizing compared with the inverse expanded cytosine (dxC)–guanine couplet (even though they both have three-hydrogen bonds and similar dimensions). When the pK_a of dxG (7.2) is taken into consideration, this contrasting behavior falls in line with the pK_a–pH/base pairing correlation.[18]

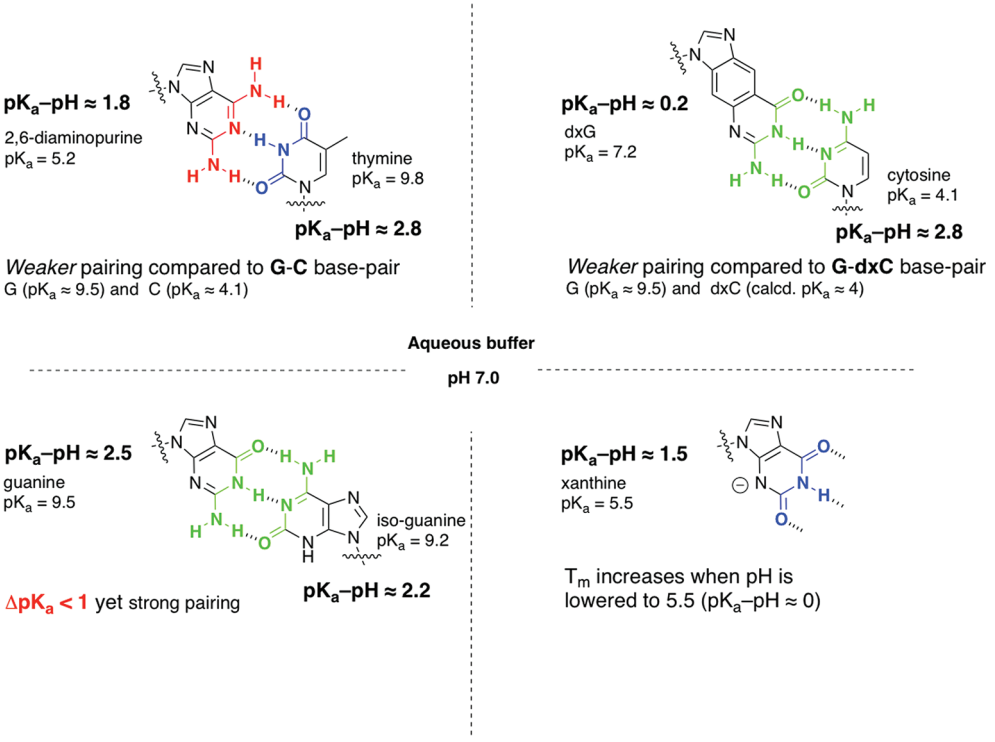


FIGURE 3. Selected nucleobase pairings illustrating the general applicability of the magnitude of the difference between pK_a of the heterocycle and the pH of the medium ("pK_a–pH rule") in explaining the effect on thermal stability of the duplexes.

There are informative exceptions to the pK_a–pH/base pairing correlation in aqueous medium, such as the nucleobases that do form self- and cross-base pairs upon protonation, for example, cytosine, adenine, and 8-aminoguanosine.[19] Herein, protonation is needed for complementarity to be fulfilled, and therefore, the pK_a of the nucleobase must be close to the pH of the medium. There are other cases of halogenated nucleobases[20] that have pK_a–pH < 2 but still base pair strongly, perhaps by maintaining their hydrophobicity (due to the nature of the substituents).

Among the majority of the alternative heterocycles and canonical nucleobases (investigated at neutral pH), in order to become ionized, the ones that have pK_a values lower than pH of the medium are to be protonated, while the ones with pK_a values higher are to be deprotonated; here, the smaller the difference between the pK_a of the molecule and the pH of the medium, the greater would be the degree of ionization, and a weaker duplex formation is expected (and observed). However, there are instances where it is the other way around, with the exact opposite expectations and results. An example is xanthosine (p$K_a \approx 5.5$), which is present as a monoanion at neutral pH and has been shown to pair more strongly with adenine when the pH is lowered from 7.5 to 5.5;[21] this is due to the increasing hydrophobicity of xanthosine when the pK_a of nucleobase and the pH of medium become equal.[16]

There are examples that suggest there may be boundaries to the pK_a–pH correlation (Figure 4): at one end of the spectrum there is 7-deazaguanine (p$K_a \approx 10.3$) and 3-deazaguanine (12.3), which exhibit decreased duplex stability although they have a pK_a – pH > 2.[22] At the other end, 5-aza- and 6-azacytosine (pK_a of 2.6 and 2.8) are also known to decrease duplex stability.[23] Thus, when the pK_a of the nucleobase is below 3 or higher than 10, the base-pairing capability with complementary partner is compromised, perhaps, also due to an increase in hydrophilic character of the heterocycles. Such a suggestion finds support in the decreased lipophilic behavior of 3-deazaguanine and 5-aza- and 6-azacytosine nucleosides (compared with the parent canonical nucleosides).[24] These limited examples indicate that there could be upper and lower limits to the pK_a–pH correlation in this specific scenario, namely, 3.5 > pK_a – pH > 2. Therefore, in an aqueous medium at near neutral pH, there appears to be a narrow window of pK_a values of heterocycles, 3.5–4.5 and 9–10, wherein duplex formation mediated by base pairing seems to be optimal (Figure 4).

In arguing as to "Why Nature Chose Phosphates", Westheimer has emphasized the "*importance of being ionized*",

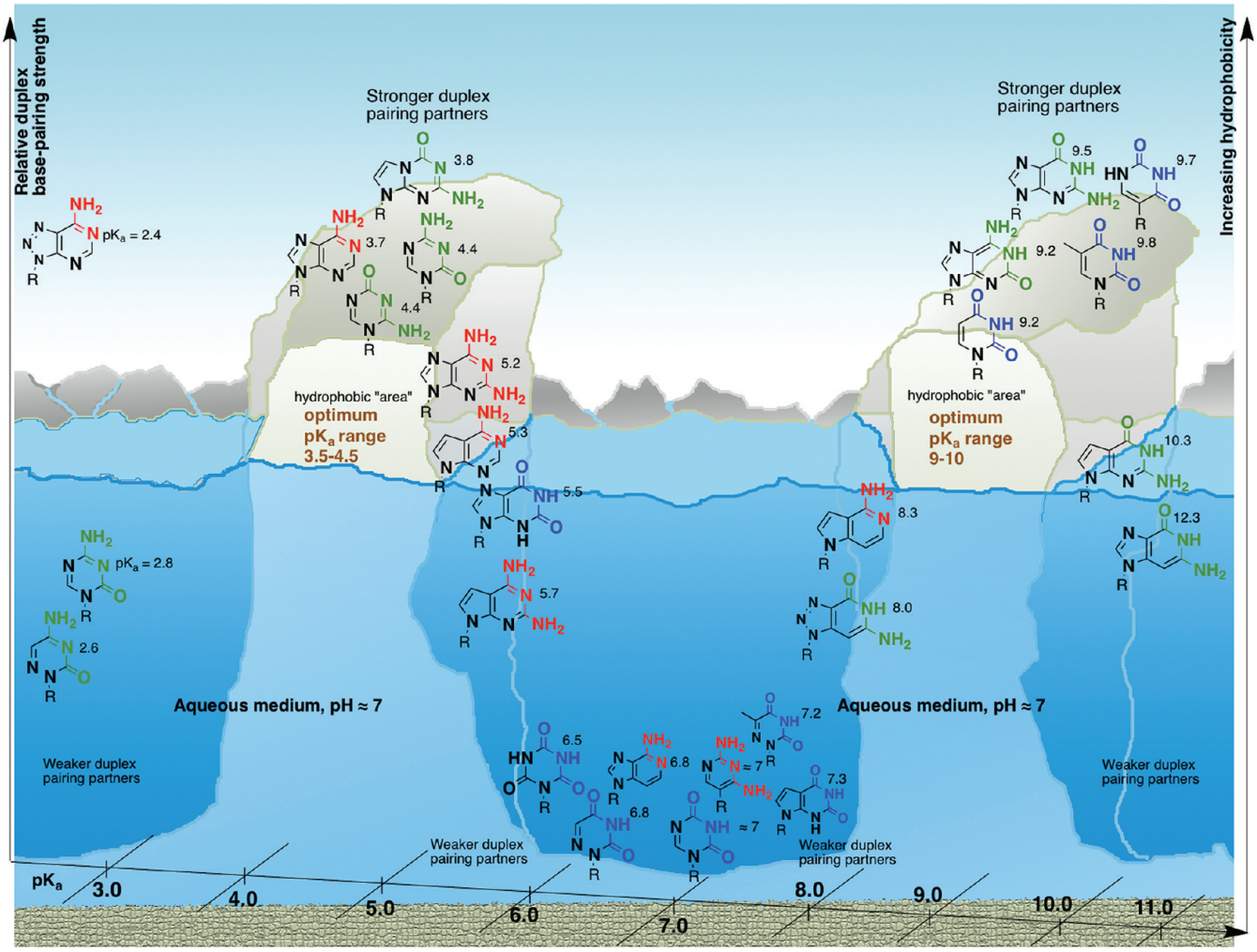


FIGURE 4. A qualitative landscape associating the pK_a of canonical nucleobases and their close structural analogs (in neutral aqueous medium) with their relative hydrophobicity and relative base-pairing strength (in oligomeric duplexes, as judged by thermal stability). In this context, the canonical nucleobases straddle a narrow pK_a range and represent an "optimum" in terms of base-pairing capabilities.

concluding that the ionization of phosphates has important consequences in an informational polymer as a linker under physiological conditions by (a) solubilizing the polymer in an aqueous medium, (b) stabilizing the backbone against hydrolysis, and (c) preventing the leakage of the charged polymer from the confines of the bilayer membrane.[7] If one could reason, "why nature chose the canonical nucleobases", then a significant part of the answer would be "*the importance of being not ionized*" under physiological conditions, the exact opposite of phosphate. The significance of the pK_a values of the canonical nucleobases being less than 4 and greater than 9, correlating to their neutral forms and their ability to form Watson–Crick base pairing, has been pointed to by others in the context of structural studies.[25]

Among the whole range of canonical and noncanonical nucleobases, the complementary base-pairing sets as exemplified by A–T/U and G–C seem to be the most advantageous set of informational–recognition elements, based on their physicochemical properties as expressed and manifested in an aqueous environment at near neutral pH,[26] satisfying the narrow window of pK_a (3.5–4.5 and 9–10) where they are able to function as base pairs (Figure 4). Considering the potentially natural alternatives where one of the heterocycles is able to pair, the corresponding complementary partner seems to be inefficient. These two observations, more importantly, seem to be (largely) independent of the nature of the (plethora of) backbones to which the canonical and alternative nucleobases are attached, strengthening the implication that, in chemical evolution, the composition and structure of the recognition elements could have played a more influential role in Nature's choice of an informational system than the backbone.[4b]

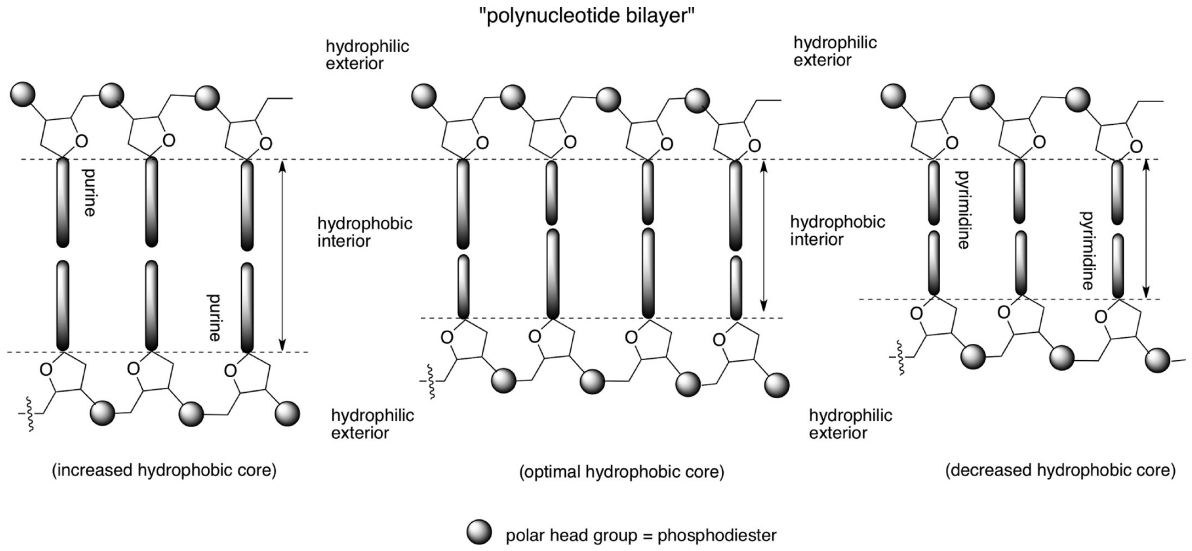


FIGURE 5. A balance between the magnitudes of hydrophobic interior versus hydrophilic exterior, regulating optimal base-pairing strength, may be a reason for the selection of a purine–pyrimidine (over a purine–purine or a pyrimidine–pyrimidine) base pair.

When pK_a − pH < 2, It Leads to Catalytic Function Coupled with Structural Diversity

Among the major canonical RNA (DNA) nucleobases, only A and C are known to shift their pK_a's toward near neutrality by virtue of the unique environments created by RNA folding and strategic positioning of the nucleobases and the sugar–phosphate backbone, in conjunction with cations.[27,28] This results in the protonation of A (shifted pK_a 5.5–7.5) and C (shifted pK_a 5.9–7.2) at near neutral pH;[29] shifts in pK_a of G and U (or T) do not seem to occur.[28] For example, it is known that cytosine acquires a histidine-like pK_a, which is optimal for general acid–base chemistry, implying that the catalytic sites in ribozymes may have a greater chance of possessing nucleobases that have shifted their pK_a values; and not surprisingly most of the catalytic sites in smaller ribosomes seem to involve charged bases.[30]

Numerous examples of charged nucleobases and their contribution to (a) catalysis in ribozymes and (b) structural diversity stemming from non-Watson–Crick base-pairing modes are available from contemporary biology. Protonation is also known to increase the number of possible pairing modes leading to structural diversity.[31] In Nature, the same nucleobases (A and C) that are used for both structural and informational purposes (Watson–Crick mode) in DNA and RNA are also used, in addition, for catalytic and functional purposes (non Watson–Crick mode) but in RNA.[32]

Thus, employing this phenomenon of perturbation/non-perturbation of pK_a of nucleobases, Nature seems to have transformed a chameleonic set of "phenetic and genetic" alphabets (in RNA) to a pedantic and restricted set of "genetic only" alphabets (in DNA/RNA).[33]

pH of Early Oceans and Implications in the Context of pK_a–pH Correlations

The pH of the early hydrosphere is thought to lie more on the acidic side (ranging from pH 4.5 to 6.0).[34] If self-replicating oligomeric systems could have existed in their current canonical forms under acidic pH conditions, then the base-pairing rules would be expected to be different from what they are today, with the caveat that this acidic aqueous environment would have also been capable of harboring chemical evolution. Then, protonated base pairs might have been the rule, rather than the exception. It is widely accepted that the free energy minimum for folding in most RNAs occurs around pH 5.5. There are some ribozymes and aptamers that function at a pH as low as 4.0.[29c,35] Considering these in the context of the RNA world, it is beguiling to consider whether RNA acted in these early stages as a better catalyst than replicator or followed a different set of base-pairing rules (e.g., Hoogsteen mode) for replication.

pK_a (of Nucleobases) As a Driver of Congregation and As a Modulator of Emergent Properties

The magnitude of the pK–pH difference does not only influence the way nucleobases interact with themselves in aqueous surroundings;[8] when amplified at a supramolecular level, it also modulates the expression of the emerging (chemical and physical) properties and the functioning of a bioassemblage.

In this context, the chemical constituents of RNA/DNA present an interesting contrast: while pK_a − pH is greater than 2 for the canonical nucleobases rendering them hydrophobic at neutral pH, the phosphate diester backbone is ionized and exists as a polyanion. This creates a diverging hydrophobic and hydrophilic zone within the same polymer (akin to the phospholipids). In an aqueous environment, the hydrophobic zones tend to aggregate, sequestering themselves from interactions with water molecules, while the hydrophilic side is exposed to water (akin to lipid bilayer). Therefore, RNA/DNA duplexes could be viewed, superficially, as a "polynucleotide bilayer" (Figure 5) with a higher level of sophistication in terms of structure, information storage, and function (compared with the lipid bilayer).

This hydrophobic interior, along with the well-known role played by water[36] and a charged hydrophilic (versus uncharged hydrophobic[37]) backbone, enables the two polynucleotide strands to hybridize in a robust and dynamic manner. Such an assemblage is fine-tuned for optimal base pairing between complementary nucleobases and mismatch discrimination, sensed by weakening of the duplex (more so in DNA than in RNA[38]).

Changing the "internal hydrophobicity" of RNA/DNA (e.g., by varying the length of the bases) would affect not only the base-pairing strength but the also the sensitivity to base-pairing mismatch. This is exemplified by the studies of Kool and co-workers in their extended nucleobases, wherein a single mismatch in x-DNA leads to overall higher strength of the mismatched duplex of x-DNA compared with that of a mismatch in DNA; in the former case there is about a 25−44% drop in thermal stability compared with almost a 32−58% in the latter.[39] A similar trend is also observed for the y-DNA series.[40] Conversely, shortening of the bases (e.g., pyrimidine−pyrimidine base pairs) would be expected to weaken duplex strength. However, there may not be meaningful discrimination of base-pairing partners if the duplex strengths are compromised beyond a limit. Thus, the selection of a purine−pyrimidine base pair (over a purine−purine or pyrimidine−pyrimidine) may be a reflection of the *optimization (and not maximization) of base-pairing strength*,[2] a result of the balancing act between strength of the assemblage and its sensitivity to mismatch or defects by control of the internal hydrophobicity versus external hydrophilicity (Figure 5). Such a cooperative effect and the tendency to minimize exposure of hydrophobic surfaces may also be at play in the selection of the Watson−Crick (over Hoogsteen and other) mode of pairing within the confines of a ribofuranosyl-phosphodiester backbone.[41]

The hydrophobic−hydrophilic interactions, arising from the fundamental physicochemical properties of molecules in an aqueous environment, are known to have important consequences in extant biology. The hydrophobic effect is a well-recognized driving force for assemblage[42] and concentration[43] in an aqueous medium. That these interactions would have also been consequential in the origins of chemical evolution has been illustrated, here, by considering the pK_a of the canonical nucleobases, in the context of chemical etiology of nucleic acid structure.[2]

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Ramanarayanan Krishnamurthy received his B.Sc. in chemistry from Vivekananda College (University of Madras) in 1984 and M.Sc. in Chemistry (1986) from the Indian Institute of Technology, Bombay. He obtained his Ph.D. from The Ohio State University, Columbus, OH, in 1992 under the guidance of Professor David Hart. Captivated by a lecture given by Professor Albert Eschenmoser (OSU, 1990), he did his postdoctoral work at Swiss Federal Institute (ETH), Zürich, with Professor Eschenmoser. Following a NASA-NSCORT fellowship (1994−1996) with Professor Gustaf Arrhenius at Scripps Institution of Oceanography, UCSD, La Jolla, CA, he rejoined Professor Eschenmoser at the Skaggs Institute of Chemical Biology at The Scripps Research Institute, La Jolla, in 1996, spawning a nearly 13-year research collaboration. He is currently an Associate Professor of Chemistry at TSRI applying synthetic organic chemistry to understand the chemistry behind the origins of life and, in the process, developing molecular tools to probe biology and novel molecular leads for chemical therapeutics.

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