# IONIZATION POTENTIALS AND DONOR PROPERTIES OF NUCLEIC ACID BASES AND RELATED COMPOUNDS

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The first vertical ionization potentials of guanine, adenine, hypoxanthine, xanthine, cytosine, thymine, uracil and purine have been determined by HeI photoelectron spectroscopy. The potentials increase in the above order and are assigned to ionization from the highest $\pi$ level. The experimental results are compared with valence shell SCF calculations, and the correlation between the association constants of these molecules with riboflavin and their donor properties is discussed. Detailed spectra will be presented and discussed in a forthcoming paper.

## 1. Introduction

The bases of the nucleic acids are of fundamental importance to molecular biology. Molecular orbital calculations on their electronic structure of varying degrees of sophistication have previously been carried out by Pullman [1–3], Clementi [4] and their co-workers. These have successfully predicted the relative magnitude and direction of dipole moments of nucleo bases. Theoretical ionization potentials may be obtained from these calculations by applying Koopmans' theorem*. Experimentally, Lifschitz et al. [5] have obtained estimates of the first ionization potential of some purines and pyrimidines from mass spectrometric appearance potentials by way of semi-logarithmic extrapolation. The values so obtained are in qualitative agreement with previous theoretical predictions [1–4].

We have now obtained the UV photoelectron spectra of these compounds. The oxygen "lone pair" peaks are quite distinct in some of these due to the planarity of the molecules concerned. The full spectra and assignments will be presented later. The first ionization in these molecules is assigned to removal of an electron from the highest filled $\pi$ molecular orbital. Our values are lower than those estimated by mass spectrometry by up to 0.6 eV. The precision in our experiments is estimated to be ±0.03 eV, while in the appearance potential method is generally believed to be around ±0.3 eV [6].

* At present, ground-state wavefunctions available are of insufficient quality to permit reliable calculations of Koopmans' defect corrections due to electron relaxation and changes in correlation energy accompanying ionization to be made.

## 2. Experimental results

Puriss grade samples of the bases from the Fluka and Koch-Light laboratories were used generally without further purification apart from pre-pumping below the sublimation temperature in the spectrometer.

Purity was checked before measurement by mass spectrometry. A Perkin–Elmer PS 18 HeI photoelectron spectrometer with heated probe was used to record the spectra. Where thermal or photodecomposition was suspected, the material remaining in the sample tubes after measurement was also examined by mass spectrometry.

The temperature was maintained to within ±1°C and the resolution at the argon doublet was less than 30 meV. The experimental precision was estimated to be ±0.03 eV. The $^2P_{3/2}$ and $^2P_{1/2}$ peaks of xenon and argon were used for calibration. The first vertical

Table 1
Observed and calculated ionization potentials (eV)

| Molecule | $I_1$ observed | $I_1$(calc) scaled INDO (this work) | $I_1$(calc) PPP [1] |
|---|---|---|---|
| guanine (G) | 8.24 | 8.38 | 7.59 |
| adenine (A) | 8.44 | 8.42 | 7.92 |
| hypoxanthine (HX) | (8.55) (adiabatic) [a] 8.89 (vertical) | 8.64 | 8.00 |
| xanthine (X) | 8.89 | 8.93 | 8.82 |
| cytosine (C) | 8.94 | 8.81 | 8.16 |
| thymine (T) | 9.14 | 9.37 | 8.80 |
| uracil (U) | 9.50 | 9.58 | 9.15 |
| purine (P) | 9.52 | 9.11 | 8.87 |
| pyrimidine | 9.73 [7] [b] | | |

a) The lower value is estimated by analysis of the vibrational structure.
b) The first ionization potential of pyrimidine is attributed [7] to removal of an electron from a nitrogen "lone pair".

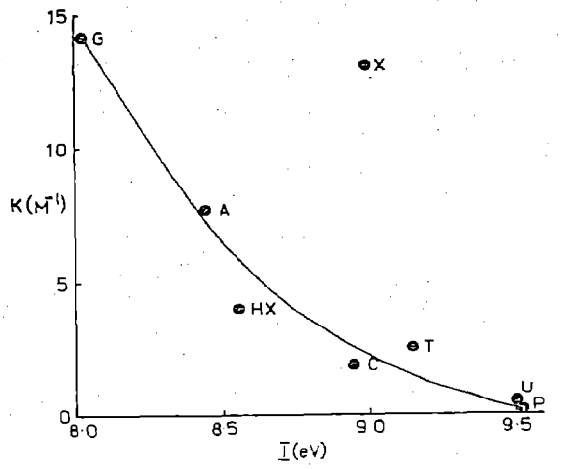


Fig. 1. Association constant $K$ ($M^{-1}$) for riboflavin with purines and pyrimidines in aqueous solution [10] as a function of first ionization potential $I_1$ of base (nomenclature as in table 1).

ionization potentials ($I_1$) are listed in table 1.

It is interesting to note that for the DNA bases, the ionization potential difference between adenine and guanine is the same as the difference between thymine and cytosine (0.2 eV). It follows that the difference of ionization potential between the purine and pyrimidine bases in the T–A and C–G pairs will also be equal (0.7 eV).

Ab initio SCF calculations have so far been carried out only for adenine, guanine, cytosine and thymine [3,4]. The calculated highest occupied molecular orbital energies are in general higher than our observed first ionization potential by about 1 eV.

We have carried out INDO calculations [8] for all the purines and pyrimidines studied here using experimentally determined molecular geometry from X-ray crystallographic works [12]. From these we obtain the energy $\epsilon$ of the highest filled $\pi$-level, and comparison with $I_1$ is made with scaled binding energies related to $\epsilon$ (both in eV) by

$$I_1 \text{ (calc) (scaled INDO)} = 3.29 + 0.55\,\epsilon.$$

These are given in the third column of table 1 and are seen to reproduce the experimental trend of $I_1$ quite well. In the last column, Pullman's [1] PPP values are listed for comparison. These do not correlate as well with the experimental results.

## 3. Molecular complexes with riboflavin

Purines and pyrimidines are known to form 1:1 complexes with riboflavin. McCormick and co-workers [10] have measured association constants $K$ of these molecules with riboflavin in aqueous solution at pH 7 by fluorescence quenching. Slifkin [11] has established a near-linear relationship between the highest occupied Hückel $\pi$ orbital energies of these molecules with the association constants for complex formation with riboflavin. However, the correlation is less satisfactory when PPP orbital energies are used. A more reliable test for such a correlation is made by using the experimental ionization potentials. The relationship between $I_1$ determined by photoelectron spectroscopy and $K$ is shown in fig. 1.

All molecules except xanthine conform to a reasonable trend of higher $K$ with lower $I_1$. In terms of the free energy $\Delta G_A$ of association with riboflavin under these conditions, we have (except for xanthine)

$$\delta\Delta G_A \approx -0.09\,\delta\Delta I_1 \text{ (eV)}.$$

In a more detailed comparison, separate correlations would be sought for purine and pyrimidine associations. This overall correlation would be consistent with the description of the complexes as of "charge-transfer" type, with the nucleobases acting as donors and riboflavin as the constant acceptor. On the naive

charge-transfer valence bond interpretation of the above relationship [9], the average amplitude $|\lambda|$ of ionized wavefunction $\Psi_{D^+A^-}$ in the ground state wavefunctions of the donor–acceptor complexes in 0.3.

Although the geometry of complexes of this kind has not been determined unequivocally, it appears to be accepted that the stacking form is generally preferred in aqueous solution [13], while the coplanar hydrogen-bonded form is preferred in nonpolar media [14]. The correlation with the $\pi$-donor properties for complex formation in water would also be most easily interpretable in terms of the stacking model.

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