J. Chem. Thermodynamics 83 (2015) 6–11

Contents lists available at ScienceDirect

J. Chem. Thermodynamics

journal homepage: www.elsevier.com/locate/jct

Thermodynamic studies on protonation constant of adenosine and guanosine at different temperatures and ionic strengths

Farahnaz Soleimani [a], Tooba Afshari [b], Fahimeh Mokhtari [b], Farrokh Gharib [b,*]

[a] Chemistry Department, Roudehen Branch, Islamic Azad University, Roudehen, Iran
[b] Chemistry Department, Shahid Beheshti University, G. C., Tehran, Evin, Iran

ARTICLE INFO

Article history:
Received 7 September 2014
Received in revised form 29 October 2014
Accepted 17 November 2014
Available online 27 November 2014

Keywords:
Adenosine
Guanosine
Protonation constant
Ionic strength
Temperature
Enthalpy and entropy changes

ABSTRACT

Stepwise protonation constants of two purine nucleosides (adenosine and guanosine) were determined at different temperatures (293.15 to 308.15) and various ionic strengths (0.101 to 3.503 mol · kg^{-1} $NaClO_4$) using a combination of potentiometric and spectrophotometric method. The thermodynamic parameters (*i.e.* enthalpy change, ΔH, and entropy change, ΔS) of the protonations were calculated at different temperatures using van't Hoff and virial equations. The dependence of the protonation constant on ionic strength is modeled by a Debye–Hückel type equation and discussed. Finally, the protonation constants of the nucleosides and the enthalpy change of protonations were determined at zero ionic strength.

1. Introduction

Adenosine and guanosine are purine nucleosides comprising a molecule of adenine or guanine attached to a ribose molecule moiety via N-9 site, scheme 1, and are the molecular building-blocks of DNA and RNA [1,2]. Nucleosides can be produced by synthesis pathways, in particular in the liver, but they are more abundantly supplied via ingestion and digestion of nucleic acids in the diet [3]. They are very important compounds due to their vital roles and various functions in biological systems [1,2]. They are involved in a wide variety of processes such as: cellular metabolism, cell bioenergetics, anticancer and immunodeficiency virus (HIV) markers, anti-inflammatory, antiviral, *etc.* [4–9]. They play also vital roles in regulation of blood flow to various organs through vasodilation and acting on presynaptic purinergic receptors located on sympathetic nerve terminals inhibits the release of norepinephrine [10]. In terms of its electrical effects in the heart, they decreases heart rate and reduces conduction velocity.

Acid-base behavior is a key parameter to predict the ionization state of a molecule with respect to pH. This information is essential in estimation of Absorption, Distribution, Metabolism, and Excretion of compounds (ADME properties) in biological systems. The ADME properties are highly affected by the ionization of the compound. Moreover, protonation constant values of ionizable drugs also affect their lipophilicity and permeability, which are important physicochemical considerations to predict its bioavailability. This parameter is also important in research areas such as pharmaceutical drug discovery and development, where it often has a vital role in understanding the pharmacodynamic properties of new drug substances [11–15].

In a previous work [13], the protonation equilibria of adenosine were studied in different aqueous solutions of methanol and ethanol but at constant temperature (298.15 K) and ionic strength (0.101 mol · kg^{-1} $NaClO_4$) and the procedure has been used in this work. The present work deals with the study of protonation constant of adenosine and guanosine at different ionic strengths (0.101 to 3.503 mol · kg^{-1} $NaClO_4$ as supporting electrolyte) and various temperatures from 293.15 K to 308.15 K in aqueous solution; using a combination of potentiometric and spectrophotometric techniques. The thermodynamic parameters for protonation of the nucleosides were obtained using the van't Hoff equation [15–17] and from a virial equation of log K measurements as a function of temperature [18]. Protonation constant dependence on ionic strength was studied using a Debye–Hückel type equation. The Debye–Hückel equation accounts only for electrostatic and long-range interactions. But, at higher concentrations, short-range and non-electrostatic interactions have to be taken into account. This is usually done by adding a term or terms to the Debye–Hückel expression [19–21]. The most popular examples are the Pitzer model [22–23] and Specific Ion Interaction Theory (SIT) [24–27].

* Corresponding author. Fax: +98 21 22431661.
E-mail address: f-gharib@sbu.ac.ir (F. Gharib).

http://dx.doi.org/10.1016/j.jct.2014.11.013
0021-9614/

2. Experimental

2.1. Chemicals

Adenosine and guanosine, scheme 1, as analytical reagent grade materials, were used without further purification. Sodium perchlorate was dried under vacuum at room temperature at least 72 h before use. Sodium hydroxide solution, prepared from concentrated ampoule, was standardized against potassium hydrogen phthalate. Perchloric acid, as analytical reagent grade material, was standardized against sodium carbonate solution. All dilute solutions were prepared from double-distilled water with conductance equal to (1.2 ± 0.1) μS. The details on chemicals used in this work are given in table 1.

2.2. Apparatus

The emf (electromotive force) was measured using a Jenway research pH-meter model 3520 (resolution ± 0.1 mV), using a combined glass-pH electrode (Jenway). All titrations were carried out in a 80 mL thermostatted double-wall glass vessel. Spectrophotometric measurements were performed on a UV–Vis Shimadzu 2100 spectrophotometer (220 nm to 300 nm and in the interval of 0.5 nm) with a Pentium 4 computer and using thermostatted matched 10 mm quartz cells. The measurement cell was of the flow type. A peristaltic pump allowed circulation of the solution under study from the potentiometric cell to the spectrophotometric cell, so the absorbance and the emf of the solution could be measured simultaneously. To exclude carbon dioxide from the system, a stream of purified nitrogen was passed through a sodium hydroxide solution and then bubbled slowly through the reaction solution.

2.3. Procedure

All measurements were performed at temperatures and ionic strength ranging from 293.15 K to 308.15 K and from (0.101 to 3.503) mol · kg^{-1} sodium perchlorate, respectively, at atmospheric pressure (83.993 kPa). The protonation constants were evaluated from the measurement of absorbance versus emf by titration of 25 mL of the nucleosides ($5.0 \cdot 10^{-4}$ to $2.0 \cdot 10^{-5}$ mol · kg^{-1}) with 0.1 mol · kg^{-1} sodium hydroxide solution both with the same ionic strength at the desired temperature. Potentiometer calibration was performed by the Gran's method [28] in terms of hydrogen ion concentration, by titrating a strong acid (0.01 mol · kg^{-1} HCl) with a strong base (0.1 mol · kg^{-1} NaOH) at the same condition of temperature and ionic strength to be used in the later experiments. The potential, E, was allowed to stabilize after each addition of the titrant and is defined as:

$$E_{cell} = k_1 + k \log[H^+] + k \log \gamma_H^+, \quad (1)$$

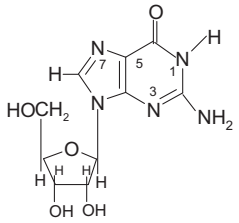
where k_1 comprises $E°C_{ell}$ and the junction potential, and $k = 2.303RT/F$ in which R, T, and F, the gas constant, temperature and the Faraday constant, respectively. The calculated Nernst parameter, k, was very close to its theoretical value and always within the range of 59.1 to 59.2. As the ionic strength is kept constant in each run, so the activity coefficient of hydrogen ion, γ, is constant too, and so:

$$E_{cell} = E'_a + k \log[H^+], \quad (2)$$

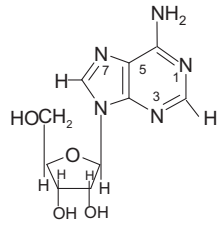
where E'_a (the specific constant of the potentiometric cell) is $k_1 + k \log \gamma_H^+$. The non-ideality of solutions is then included in E'_a.

TABLE 1
Purities and sources of materials used in this work.

Materials	Purification method	Mass fraction	Sources
Adenosine	Used as received	0.99	Fluka
Guanosine	Used as received	0.99	Fluka
Sodium perchlorate	Vacuumed 72 h at room temperature	0.99	Merck
Sodium hydroxide		Solution	Merck
Perchloric acid		Solution	Merck



Guanosine



Adenosine

SCHEME 1. The chemical structures of the nucleosides.

3. Results and discussion

Adenosine and guanosine shown in scheme 1 are di- and tribasic species, respectively. Adenosine and guanosine may accept the first proton at N1 and N7 sites in the purine moiety, respectively, the second proton at N1 site in guanosine and finally the last one at the ribose groups in a very alkaline pH range, pK > 12 (not considered in this work). The results obtained for various acidity constants of the proton donors of adenosine, equation (3), and guanosine, equation (4), in different ionic strengths and various temperatures, using the computer program Squad [29], are listed in table 2 together with some values reported in the literature for comparison [13,30–33].

$$H_nL^{n-1} + H^+ \leftrightarrows H_{n+1}L^n \quad K = [H_{n+1}L^n]/[H_nL^{n-1}][H^+], \quad (3)$$

$$H_nL^{n-2} + H^+ \leftrightarrows H_{n+1}L^{n-1} \quad K_n = [H_{n+1}L^{n-1}]/[H_nL^{n-2}][H^+], \quad (4)$$

where L represents adenosine or guanosine and n may be 1 or 2 for the different protonation equilibria of the nucleosides. In figure 1, the species mole fractions of both systems are shown for 0.101 mol · kg^{-1} $NaClO_4$ and T = 298.15 K.

The protonation constant values obtained in this work are in agreement with those reported before. The small differences are possibly due to the different experimental method and the different background electrolyte and temperature used.

3.1. Dependence of protonation constant on ionic strength and temperature

The thermodynamic protonation constant of the two bases is expressed, for example for guanosine, as: