ECS Transactions, 53 (16) 17-25 (2013)
10.1149/05316.0017ecst ©The Electrochemical Society

The Electrochemical Approach towards Proton Coupled Electron Transfer Pathways for Oxidation of Thymine in Water

M. T. Soomro[a, b], G. Grampp[a], T. X. Nguyen[a], I. M. Ismail[b]

[a]Institute of Physical and Theoretical Chemistry Graz University of Technology,
Stremayrgasse 9, 8010-Graz Austria
[b]Center of Excellence in Environmental Studies, King Abdul Aziz University, Jeddah
Saudi Arabia

The work describes the use of cyclic voltammetry to investigate the proton coupled electron transfer (PCET) pathways for oxidation of thymine in water. Biologically important compounds like DNA bases, and amino acids showed irreversible behavior when oxidized electrochemically. The reversible redox potentials for such systems (say, irreversible electrochcemical systems) were estimated by analyzing the cyclic voltammograms recorded at different scan rates. The approach presents here is relatively simple and easy for obtaining reversible redox potential of electrochemically irreversible systems. Moreover, for the case of thymine, the obtained reversible oxidation potential also used to establish the PCET-pathways for oxidation of thymine in water.

Introduction

Cyclic Voltammetry (CV) is a multipurpose technique and finds application in many different areas of research due to flexibility it can provide (1, 2). It is also widely used for the study of electroactive organic compounds in solution. Quite a few numbers of papers have been already published describing the use of cyclic voltammetry in electro-organic analysis (3-16). DNA bases and amino acids are important organic biological compounds and played significant role in many biological and biochemical systems and, if investigate by cyclic voltammetry, cyclic voltammetry gives valuable insights into redox behavior (say, mechanism of reaction) of these compounds.

Photoinduced electrons are involved in many reactions even in biological systems for example in photosynthesis, in conversion of small molecules to other forms, and in semiconductors that can be used in solar cells. The fate of the electron transfer processes decides by the redox potentials (excited and ground state) of the donor and accepter molecules. Because DNA bases and amino acids can easily donate electron to an electron acceptor, these compounds more often use for the study of photoinduced electron transfer processes. The precise reversible oxidation potentials of DNA bases and amino acids are difficult to obtain as these compounds showed irreversible peak when electrochemically oxidized using glassy carbon, platinum, and/or other electrodes (8, 9, 17) and, in addition, most of the electrochemical data available in the literature can only provide information about the oxidation peak potentials (5, 11, 15, 16, 18).

Given that the importance of reversible redox potentials, in understanding the electron transfer process, these redox potentials can further render the calculation of thermodynamic parameters such as equilibrium constant, rate constant, kinetic parameters and etc (19-29). Fewer reports are found in the literature presenting methodologies for the determination of reversible redox potentials of electrochemically irreversible systems (30-32) and correlating these potentials with the possible electron transfer reaction mechanism.

One of the energy relationship derived by Rehm and Weller (33) for the excited state electron transfer processes can simply manipulate and use for the determination of reversible redox potentials of electrochemically irreversible systems using cyclic voltammetry. For this purpose cyclic voltammetric investigations at different scan rates were carried out to find a relationship between peak potentials and electron transfer coefficient for the oxidation of DNA bases and amino acids.

Compounds were investigated for their reversible redox potentials using cyclic voltammetry are; DNA bases (thymine, thymidine), and amino acids (alanine, histidine, methionine). No oxidation peak observed for adenine, thymidine and alanine at bare glassy carbon electrode. The reversible redox potentials were also studied at different pH of the solution for observing a variation in potentials with the change of the pH.

Experimental

Reagents

Adenine (Alfa Aesar), Adenosine (Alfa Aesar), Thymine (Alfa Aesar), Thymidine (Alfa Aesar), Alanine (Alfa Aesar), Histidine (Fluka), Methionine (Fluka) were used as received. HCl and NaOH were purchased from Carl Roth Gmbh. KH_2PO_4 and Na_2HPO_4 were purchased from Fluka. The supporting electrolyte potassium nitrate and recrystallized potassium ferricyanide were used in aqueous solution.

Apparatus

For cyclic voltammetric measurements, Autolab PGSTAT 302N (Metrohm) fully computer controlled and with installed software GPES (version 4.9.007) was used. A conventional three electrode system with 20-25 mL electrochemical cell equipped with gas bubbler and gas outlet (nitrogen gas purging system) was used. Bare glassy carbon electrode and platinum electrode imbedded in Teflon with diameter of 2 mm were used as working electrodes. The Ag/AgCl reference electrode was made by simply inserting silver wire (coated with AgCl) in a glass tube containing 0.1 M KNO3 solution and/or phosphate buffer. To separate a reference solution and sample solution vycor frit was used. Pt wire was used as counter electrode.

Procedure

Solutions were made in double distilled water. For supporting electrolyte buffer systems were used with and without potassium nitrate salt. 0.01 M phosphate buffer solutions of different pH were prepared. 1-5 mM concentrations of DNA bases, and amino acids were used for investigation in aqueous solution. Before taking each measurement using potentiostat all solution were deaerated with argon at least for 25-30 minutes. All experiments were performed at 298 K. Potassium ferricyanide was used as external standard for checking the potential of reference electrode. All reported potential are in volts and will be referenced to the Ag/AgCl reference electrode.

Glassware washed carefully in three steps (i) left for overnight in dilute HNO_3 acid solution (ii) thoroughly washed with detergent and rinsed at least 3 times (iii) oven dried at 110 ^oC. For cleaning the working electrode manual procedure was followed using diamond paste and alumina slurry on the polishing pad. The electrodes were then rinsed with deionized water, immersed in concentrated nitric acid for 5 second, rinsed second time and then dried in an air. During the analysis no deterioration of the working electrode was observed. The potential range of solvent depends on certain factors such as electrode material, electrolyte systems and solvent itself and for water we have found that compared to Pt electrode, glassy carbon electrode has by far the widest oxidative window from 0 to + 1.5 V vs. Ag/AgCl but reductive window limited to + 1 V vs. Ag/AgCl. The background CV's also recorded in buffer solutions with different pH in the absence of compounds.

Results and Discussion

Using glassy carbon electrode the potential was scanned between 0 V to + 1.5 V vs. Ag/AgCl reference electrode. Thymine, thymidine, alanine, adenosine, histidine, and methionine were investigated for their oxidation peak using pH 2 to 12 buffer solutions. Three of the compounds thymidine, adenine, and alanine didn't show any peak when electroxidize whereas other four compounds thymine, adenosine, histidine, and methionine gave well defined oxidation peak at around + 1.25 V vs. Ag/AgCl but no reduction peak on the reverse scan. Figure 1(a) shows the oxidation of thymine at pH 4. The behavior shows that upon oxidation the products formed were highly reactive and consumed or dimerized before reduction was observed. Therefore the reaction can be referred to the EC mechanism that is electron transfer reaction coupled with the homogeneous chemical reaction.

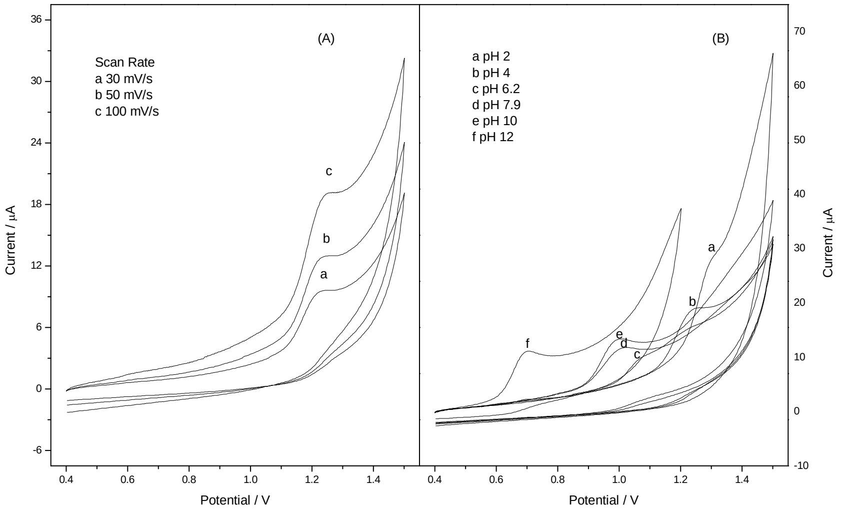

Figure 1: Cyclic voltammograms of 1mM of thymine (A) at different scan rates in pH 4 buffer and (B) in different pH buffer solutions at 100 mV/s scan rate.

Electrochemical reversibility was checked by the analysis of thymine cyclic voltammogram (Figure 1(a)) recorded at various scan rate (34). The result shows that the current in the foot of the oxidation peak does not change while recording the cyclic voltammogram at different scan rates and the electron transfer from thymine is electrochemically irreversible. This was first reported by Reinmuth in 1960 (35). The absence of reverse electron transfer step provides enough evidence to the highly unstable oxidized cation. The other compounds showed similar behavior. There are other criteria for checking the electrochemical reversibility for example peak potential dependence on scan rate. With increasing scan rate, anodic peak potentials shifted to more positive values.

To obtain the reversible oxidation potential for an irreversible system a very well established energy equation is used for the present work originally derived by Rehm and Weller (33). The equation describes dependence of photoinduced electron transfer reactions on free energy. In the equation, ΔG is the free energy which is zero for a self exchange electron transfer reaction i.e., $\Delta G^{\ddagger} = \Delta G^{\ddagger}(0)$ at ΔG = 0. This free energy relationship, if manipulate mathematically, offers us possibility of determining the reversible oxidation potential of irreversible systems from the cyclic voltammetric measurements (34, 36-40). The transformation of Rehm-Weller equation is shown below, see equation 1-3. Equation 2 was obtained by taking the first derivative of equation 1 with respect to ΔG and shows the dependence of β on the free energy. While equation 3 was obtained by substituting ΔG in equation 2 with $\Delta G = E_{ox}^{0} - E_{ox}^{p}$. The reversible oxidation potential now can be obtained from equation 3 by plotting oxidation peak potential vs. β.

$$\Delta G^{\ddagger} = [(\frac{\Delta G}{2})^{2} + \left(\Delta G^{\ddagger}(0)\right)^{2}]^{\frac{1}{2}} + \frac{\Delta G}{2} \qquad (1)$$

$$\beta = \frac{\Delta G}{4}[(\frac{\Delta G}{2}) + \left(\Delta G^{\ddagger}(0)\right)^{2}]^{-\frac{1}{2}} + \frac{1}{2} \qquad (2)$$

$$E_{ox}^{p} = E_{ox}^{0} + \frac{(1-2\beta)}{[\beta(1-\beta)]^{\frac{1}{2}}}\Delta G^{\ddagger}(0) \qquad (3)$$

The peak potentials were measured from cyclic voltammetric analysis and are given in Table 1. Whereas β values were calculated from the equation 4. Equation 4 was taken from the pioneering work by Nicholson and Shane on the theory of stationary electrode polarography (41, 42). In the equation E_{ox}^{p} and $E_{ox}^{\frac{p}{2}}$ are the oxidation peak potential and oxidation half peak potential where other notations are representing convention constants.

$$\beta = \frac{1.857\,RT}{nF}\,[E_{ox}^{p} - E_{ox}^{\frac{p}{2}}]^{-1} \qquad (4)$$

The peak width and β, both are scan rate dependent and vary with changing the scan rate while doing the cyclic voltammetry. The calculated peak width and β values are given in Table 1 and were taken at scan rate of 100 mV/s. The calculated values of β at different scan rate further use to draw a linear plot between oxidation peak potential vs β. From the plots the reversible oxidation potential can readily be obtained. Figure 2(a) shows that data were plotted as E_{ox}^{p} vs. β for thymine using equation 3. From the graphs the reversible oxidation potentials were calculated and are represented in Table 2. The reversible oxidation for other compounds are also shown in Table 2.

Table 1: Cyclic voltammetric data acquired for donar molecules.

Compd	pH	E_{ox}^{p} / V	$E_{ox}^{p} - E_{ox}^{\frac{p}{2}}$ / mV	β
Thymine	4	1.26	99	0.48
Adenosine	4	1.33	102	0.48
Histidine	6	1.08	127	0.38
Methionine	6	1.04	186	0.27

All CV measurements presenting in Table 1 were recorded at 100 mV/s vs. Ag/AgCl reference electrode. The widths of the oxidation and reduction peak and electron transfer coefficient were calculated according to equation 4.

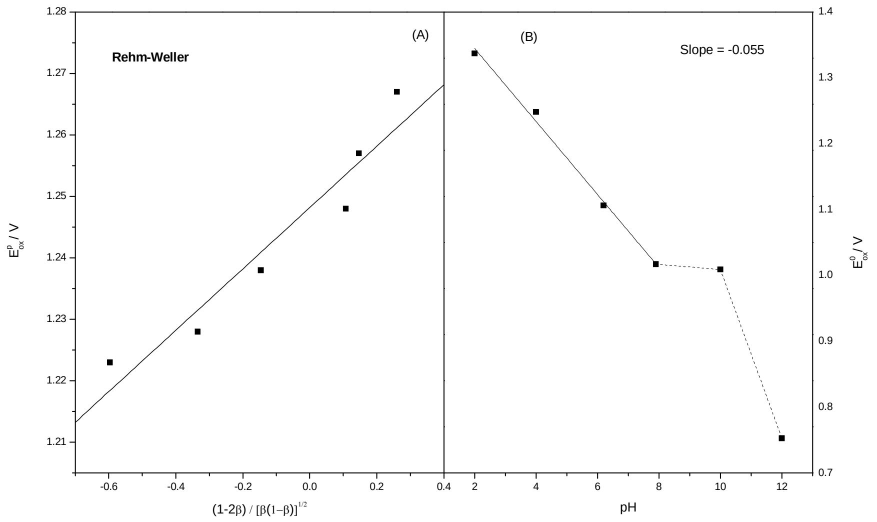

Figure 2: (A) The E^p_{ox} of thymine plotted as a function of β using equation 3 at pH 4. (B) The plot of reversible oxidation potential E^0_{ox} vs. pH for oxidation of thymine.

Table 2: Oxidation peak potential and reversible (apparent standard) potential obtained from Rehm-Weller plot E^p_{ox} vs. β.

Compd.	pH										
	2	3	4	5	6	7	8	9	10	11	12
E^p_{ox} / V											
Thymine	1.31	-	1.26	-	1.11	-	1.02	-	1.03	-	0.71
Adenosine	1.37	-	1.33	-	1.30	1.29	1.28	-	1.20	-	-
Histidine	-	-	-	-	1.08	-	0.84	-	0.80	0.72	0.62
Methionine	1.07	-	1.03	-	1.04	-	-	-	0.95	0.91	-
E^0_{ox} / V											
Thymine	1.34	-	1.25	-	1.11	-	1.02	-	1.01	-	0.75
Adenosine	1.42	-	1.31	-	1.30	1.29	1.14	-	1.00	-	-
Histidne	-	-	-	-	0.98	-	0.95	-	0.92	0.69	0.66
Methionine	0.90	-	0.90	-	0.65	-	-	-	0.88	0.69	-

The presented cyclic voltammetric data was recorded at 100 mV/s vs. Ag/AgCl reference electrode.

Peak potential and peak current also vary with the pH of the solution indicating that that the electrode process is controlled simultaneously by diffusion and adsorption. The cyclic voltammograms of thymine obtained at different pH values, while keeping the concentration constant, have suggested that the oxidation of thymine is more easier at higher pH values (acidic to alkaline), see figure 1(b). This was expected because at more alkaline medium the quenching rate constant for the photoinduced electron transfer reaction between thymine and acceptor molecule increases. Both the peak current and peak potential vary with the pH of the solution and provide a basis to explore the nature of electron (proton) transfer reaction either it is one electron transfer or two electron transfer reaction on the time scale of cyclic voltammetry. To see the variation of reversible oxidation potential with pH, the E_{ox}^{p} was plotted vs. pH, figure 2(b). pH dependence of the reversible oxidation potential (apparent standard oxidation potential) can be used as an electrochemical too to determine the reaction mechanisms which are involved in proton coupled electron transfer (PCET) pathways (43-45). The 55 mV value of slope obtained from figure 4 suggested that the oxidation of thymine is one electron and one proton transfer reaction (43, 44). Based on these observations the three possible PCET-pathways were suggested (scheme not shown here). The dissociation constant for the neutral and radical cation thymine have been known, thus, the standard oxidation potentials by proposed PCET-pathways are also calculated. These obtained values are shown in Table 3.

Table 3: pK_a and potential values of Thymine

pK_a	$pK_{QH^{\cdot +}} = 3.2^{a}$
	$pK_{QH} = 9.9^{b}$
	$pK_{H_2PO_4^- / HPO_4^{2-}} = 7.2$
Potential (in Volts vs. Ag/AgCl reference electrode)	$E_{ETPT}^{0} = 1.27$
	$E_{PTET}^{0} = 1.01$
	$E_{CPET}^{0}\ (HPO_4^{2-}) = 1.05$

[a]Ref (46), [b]Ref(47)

Conclusion

The preliminary results are presented here and it is concluded from that the Rehm-Weller energy equation was successfully manipulated for determining the reversible oxidation potentials for electrochemically irreversible systems which is on the other hand difficult to get from simple cyclic voltammetric analysis. The reversible oxidation potential for thymine was further used to elucidate the oxidation mechanism of thymine in water and based on the observations PECT pathways for oxidation of thymine in water were proposed. The next goal is to use the Marcus free energy equation for determining the reversible redox potentials for electrochemically irreversible systems and to compare it with the above results.

Acknowledgements

Financial support provided by Higher Education Commission of Pakistan (HEC) is thankfully acknowledges. Acknowledgement is also made to Institute of Physical and Theoretical Chemistry, Graz University of Technology.

References

1. A. Bard and L. Faulkner, *Electrochemical methods: fundamentals and applications*, Wiley (2001).
2. J. Heinze, *Angewandte Chemie International Edition in English*, **23**, 831 (1984).
3. J. Xu, Y. Wang, Y. Xian, L. Jin and K. Tanaka, *Talanta*, **60**, 1123 (2003).
4. M. A. T. Gilmartin and J. P. Hart, *Analyst*, **117**, 1613 (1992).
5. S. C. Brooks and M. M. Richter, *Chem. Ed.*, **7**, 9 (2002).
6. R. P. Deo, N. S. Lawrence and J. Wang, *Analyst*, **129**, 1076 (2004).
7. H.-S. Wang, H.-X. Ju and H.-Y. Chen, *Analytica Chimica Acta*, **461**, 243 (2002).
8. A. M. Oliveira Brett and F.-M. Matysik, *Journal of Electroanalytical Chemistry*, **429**, 95 (1997).
9. A. M. Oliveira Brett and F.-M. Matysik, *Bioelectrochemistry and Bioenergetics*, **42**, 111 (1997).
10. Y. Xu and B. J. Venton, *Physical Chemistry Chemical Physics*, **12**, 10027 (2010).
11. G. Dryhurst, *Talanta*, **19**, 769 (1972).
12. G. Dryhurst, *Analytica Chimica Acta*, **57**, 137 (1971).
13. V. Vetterl, *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, **19**, 169 (1968).
14. V. Brabec and V. Mornstein, *Biophysical Chemistry*, **12**, 159 (1980).
15. J. A. Reynaud, B. Malfoy and P. Canesson, *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, **114**, 195 (1980).
16. B. Malfoy and J. A. Reynaud, *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, **114**, 213 (1980).
17. R. A. Kenley, S. E. Jackson, J. C. Martin and G. C. Visor, *Journal of Pharmaceutical Sciences*, **74**, 1082 (1985).
18. A. P. Darmanyan, W. S. Jenks and P. Jardon, *The Journal of Physical Chemistry A*, **102**, 7420 (1998).
19. C. R. Bock, J. A. Connor, A. R. Gutierrez, T. J. Meyer, D. G. Whitten, B. P. Sullivan and J. K. Nagle, *J Am Chem Soc*, **101**, 4815 (1979).
20. J. K. Nagle, W. J. Dressick and T. J. Meyer, *J Am Chem Soc*, **101**, 3993 (1979).
21. N. Sutin and C. Creutz, *J Chem Educ*, **60**, 809 (1983).
22. R. Breslow and R. Goodin, *J Am Chem Soc*, **98**, 6076 (1976).
23. M. Patz, H. Mayr, J. Maruta and S. Fukuzumi, *Angewandte Chemie International Edition in English*, **34**, 1225 (1995).
24. B. Jaun, J. Schwarz and R. Breslow, *J Am Chem Soc*, **102**, 5741 (1980).
25. R. Breslow and J. L. Grant, *J Am Chem Soc*, **99**, 7745 (1977).
26. E. M. Arnett, K. Amarnath, N. G. Harvey and J. Cheng, *J Am Chem Soc*, **112**, 344 (1990).
27. D. D. M. Wayner and D. Griller, *J Am Chem Soc*, **107**, 7764 (1985).
28. G. Grampp, S. Landgraf and C. Muresanu, *Electrochimica Acta*, **49**, 537 (2004).
29. G. Grampp, C. Muresanu and S. Landgraf, *Electrochimica Acta*, **53**, 3149 (2008).
30. D. R. Arnold, X. Y. Du and K. M. Henseleit, *Can J Chem*, **69**, 839 (1991).
31. J. González-Velasco, *Electroanalysis*, **6**, 711 (1994).
32. J. G. Velasco, *Electroanalysis*, **9**, 880 (1997).
33. D. Rehm and A. Weller, *Israel J Chem*, **8**, 259 (1970).
34. R. J. Klinger and J. K. Kochi, *J Am Chem Soc*, **102**, 4790 (1980).
35. W. H. Reinmuth, *Analytical Chemistry*, **32**, 1891 (1960).

36. S. Fukuzumi, K. Hironaka, N. Nishizawa and T. Tanaka, *B Chem Soc Jpn*, **56**, 2220 (1983).
37. S. Fukuzumi, S. Koumitsu, K. Hironaka and T. Tanaka, *J Am Chem Soc*, **109**, 305 (1987).
38. S. Fukuzumi, N. Nishizawa and T. Tanaka, *Journal of the Chemical Society, Perkin Transactions 2*, 371 (1985).
39. T. Yago, Y. Kobori, K. Akiyama and S. Tero-Kubota, *The Journal of Physical Chemistry B*, **106**, 10074 (2002).
40. Y. Kobori, T. Yago, K. Akiyama and S. Tero-Kubota, *J Am Chem Soc*, **123**, 9722 (2001).
41. R. S. Nicholson, *Analytical Chemistry*, **37**, 1351 (1965).
42. R. S. Nicholson and I. Shain, *Analytical Chemistry*, **36**, 706 (1964).
43. C. Costentin, C. Louault, M. Robert and J.-M. Savéant, *Proceedings of the National Academy of Sciences*, **106**, 18143 (2009).
44. C. Costentin, M. Robert, J.-M. Savéant and A.-L. Teillout, *Proceedings of the National Academy of Sciences*, **106**, 11829 (2009).
45. M. Sjödin, S. Styring, H. Wolpher, Y. Xu, L. Sun and L. Hammarström, *J Am Chem Soc*, **127**, 3855 (2005).
46. J. J. Christensen, J. H. Rytting and R. M. Izatt, *The Journal of Physical Chemistry*, **71**, 2700 (1967).
47. C. Sonntag, *Free-Radical-Induced DNA Damage And Its Repair: A Chemical Perspective*, Springer (2006).