Qatar Univ. Sci. J. (1994), 14 (C) : 199-202

CHARGE TRANSFER COMPLEX FORMATION CONSTANTS OF EDTA WITH METHYLVIOLOGEN AND ITS ZWITTERIONIC DERIVATIVES

By

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ثوابت التكوين لمتراكب انتقال الشحنة الـ EDTA مع المثيل فيلوجن ومشتقات أيونه المزدوج

كمال زكي إسماعيل

في الدائرة الفوتوكيميائية لثلاثي (٢,٢' - بيردين) روثنيوم (٢) يحدث أكسدة للروثنيوم إلى الروثنيوم (٣) عن طريق المثيل فيلوجن وينتج شق ذو الكترون منفرد للفيلوجن مع المعدن الذي يقوم بدوره في التفاعل مع الماء لإنتاج فوق أكسيد الهيدروجين الذي يتم التعرف عليه كهروكيميائياً حيث يعطي اشارة مميزة . وفي وجود مركب الـ EDTA فإن الروثنيوم (٣) يختزل مرة أخرى إلى الروثنيوم (٢) وتكمل الدائرة . في الحالة العادية يحدث تفاعل بين الفيلوجن والـ EDTA ويتكون متراكب إنتقال الشحنة، وهذا بدورة ينتج تيار أصلي كبير . استبدال كاتيون المثيل فيلوجن بالشق المزدوج الأيوني المتعادل يعطي خط القياس الأصلي بصوره جيدة . وقياس ثوابت التكوين لمتراكبات انتقال الشحنة المتكونه بين الـ EDTA والمثبطات مثل داى بروبان سلفونات - ٤,٤' - بيبردنييم يعتبر ذو أهمية . وقد طبق كل من طريقة تحليل بنس - هلبراند وناشن لحساب ثابت التكوين وكانت هناك قيم منخفضة لمشتقات المزدوج الأيوني .

Key Words: Charge transfer, Methylviologen, Zwitterion

ABSTRACT

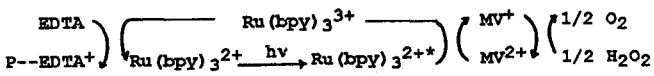
In the photochemical cycle of the labelled, tris (2,2'bipyridine) ruthenium(II), $Ru(bpy)_3^{2+}$, the photo excited label is oxidized through electron transfer quenching by methylviologen (1,1'-dimethyl-4,4'-bipyridinium dication, MV^{2+}) producing the Ru(III) complex and $MV^{+\bullet}$. The resulting radical cation, $MV^{+\bullet}$, reacts with water to produce hydrogen peroxide which is detected electrochemically to produce the analytical signal. In the presence of EDTA, as a sacrificial donor, Ru(III) is reduced back to Ru(II) and the cycle is completed. The strong ground state interaction between viologen and EDTA results in a charge-transfer complex formation. This interaction competes with the excited-label quenching step and produces quite large background current. Replacing the dipositive viologen cation with the corresponding, net zero-charged zwitterionic derivatives is expected to reduce such background problem. Two derivatives were chosen, 1,1'-dipropanesulfonate-4,4'-bipyridinium, PVS, and 1,1'-dibutanesulfonate-4,4'bipyridinium, BVS to replace methyl viologen MV^{2+}. Better base line was obtained using both derivatives. Determination of the formation constants of the charge transfer complexes formed between EDTA and the three quenchers is very important. Such values are crucial for any theoretical or experimental study of the already complicated complex equilibrium involved in such biosencing scheme. Both Benesi-Hilebrand analysis and Nash treatment were applied to determine the formation constants spectrophotometrically and lower values were obtained for the zwitterionic derivatives.

INTRODUCTION

Since radioimmunoassay was introduced by Yallow and Berson in 1950's and its application in the detection of very low concentrations of antigens in the early sixties[1] a great deal of research is going on in this field. Although radioimmunoassay proved to be very effective, there still some disadvantages such as health hazards, disposal difficulties and short lifetimes of active isotopes. One of the solutions to this problem was the introduction of enzymes and fluorescent labels to replace isotopes and thus enzymeimmunoassays and fluoroimmunoassays were developed. Although the catalytic turnover of enzymes is

very high (high efficiency), they are expensive and require long procedures for purification and immobilization.

The photochemistry of the inorganic complex tris-(2,2'-bipyridine)ruthenium(II) and its derivatives in relation to their electrochemical detection and the possibility of using them as chemical labels to replace hydrogen peroxide producing enzymes in biosensors was investigated[2,,3]. The chemistry follows the following scheme:



Scheme 1

In the absence of oxygen, methylviologen, MV^{2+}, is used to oxidize (oxidative quenching) the excited complex, $Ru(bpy)_3{}^{2+*}$, to produce $Ru(bpy)_3{}^{3+}$ and the radical cation, $MV^{+\bullet}$, which is dark blue and absorbs at about 605 nm with molar extinction coefficient of $1.37 \times 10^4\ M^{-1}cm^{-1}$ [14]. The concentration of such species is proportional to the concentration of the label. In the presence of oxygen, however, the intense colour is bleached instantaneously by the fast reaction ($k > 5 \times 10^6\ M^{-1}S^{-1}$, in aqueous solution at pH = 7.4[5], between the radical cation, $MV^{+\bullet}$ and the dissolved oxygen in solution and hydrogen peroxide is produced according to the following reactions:

$$2MV^{+\bullet} + 2H^+ + O_2 \xrightarrow{k_1} 2MV^{2+} + H_2O_2 \quad .....(1)$$

$$2MV^{+\bullet} + 2H^+ + H_2O_2 \xrightarrow{k_2} 2MV^{2} + 2H_2O \quad .....(2)$$

Thus, in the presence of oxygen, the concentration of H_2O_2, measured amperometrically, is proportional to the concentration of the inorganic label. Competition of back reaction of hydrogen peroxide with the radical cation, reaction (2), is expected to decrease or even destroy the signal. Fortunately, the effect of such reaction on the concentration of $MV^{+\bullet}$ was proven to be minimal. Calculations showed that in an air saturated solution at ambient temperatures, where $[O_2] = 2.8 \times 10^{-4}$ M, the fraction of $MV^{+\bullet}$ disappearing by back reaction is only about 10% (of the total radical cation concentration) when the concentration of H_2O_2 is about 0.01 M. Such concentration is extremely large compared with trace analysis concentrations. Accordingly, reaction (2) represents no danger within the concentration range of the application.

Scheme 1, shows that EDTA is used as a sacrificial electron donor. It reduces Ru(III) back to Ru(II) and thus regenerate the photochemically active label. The pK_a values of EDTA are 0.0, 1.5, 2.0, 2.8, 6.1 and 10.2[6]. It is reported by Hofffman et. al.[7] that in neutral or alkaline mixtures of EDTA and methylviologen, photoactive complexes between EDTA anions and methylviologen dication are formed. Photolysis of such complex induces a charge transfer from EDTA to MV^{2+} producing the radical cation $MV^{+\bullet}$. The later will react with oxygen and produces hydrogen peroxide. formation of H_2O_2 form this route (not through the photochemistry of the label) will increase the background (current measured in the absence of label) of the measurements. To solve this problem, one should find a way to prevent or reduce the formation of the photoactive complex. One method is to replace the relatively highly charged dipositive MV^{2+} methylviologen cation with less positive, net zero-charged, zwitterionic viologen derivatives.

EXPERIMENTAL

Reagents

Water was deionized and passed through activated carbon bed before distillation. Methylviologen and EDTA were analytical grade (Aldrich, Milwaukee, . WIS., USA) and used as received. Both propylviologensulfonate and butylviologensulfonate (Molecular Probes, USA) were used as received. Tris (2,2'bipyridine)ruthenium(II) chloride (Stern Chemicals, Newbury Port, MA., USA) was used as received without further purification.

Measurements

IBM model 9420 and Hewlett-Packard UV-visible (diode array) spectrophotometers were used to record the absorption spectra in 1 cm quartz cuvetts.

RESULTS AND DISCUSSION

In an actual photolysis experiment, 0.5 ml sample solution was photolysed for 0-10 minutes. The sample contains 0-100 nmoles of $Ru(bpy)_3{}^{2+}$ (label), 10 mmoles viologen and 20 mmoles of EDTA in a pH 7.4 phosphate buffer. Air bubbles were used to stir the solution. An Argon-ion laser, 30-160 mW power at 457.9 nm, was used as an excitation light source. The measured current, resulting from the oxidation of hydrogen peroxide at a platinum electrode, is then correlated to the label concentration[8].

It has been reported[7] that methylviologen forms a 1:1 charge-transfer complex with EDTA, triethanolamine and cysteine. These complexes are photoactive and their formation constants are pH dependent. Photolysis of such complex produces, through electron transfer from EDTA to MV^{2+}, the radical cation $MV^{+\bullet}$ which reacts with the dissolved oxygen to produce H_2O_2. This produces considerable current in the absence of label. It is important to reduce background and thus improve signal-to-background ratio of the measurement. Reduction of background can be achieved by minimizing the possibility of complex formation between viologen and EDTA. This is done by replacing the dipositive methylviologen, cation with less positive derivatives. We choose two derivatives of 1.1'-dimethyl-4,4'-bipyridinium dication, MV^{2+}, namely, 1,1'-dipropane sulfonate-4,4'-bipyridinium (PVS) and 1,1'-dibutane sulfonate-4,4'-bipyridinium (BVS). Both compounds were successful in reducing the background appreciably but did not eliminate it completely. Solution evaporation and interference of air bubbles with the light beam still cause some noise and drift in the signal.

The reaction scheme for the protolysis and detection of the label concentration involves very complicated multiequilibria[7]. For good understanding and/or trying to

build a mathematical model, the formation constants of EDTA/viologens complexes should be estimated. The equilibrium constant for the 1:1 complex formation between EDTA and methylviologen derivatives as well as the molar absorptivities of the formed complexes at selected wavelengths can easily be calculated using several techniques. In Benesi-Hilderbrand treatment[9] both the equilibrium constant (K_{eq}) for the complex formation and the molar absorptivity of the complex at a given wavelength (ε_l) are obtained from the relation

$$[A]_o / \Delta A_\lambda = 1 / (\varepsilon_\lambda l) + 1 / (K_{eq}\, \varepsilon_\lambda l\, [D]_o)$$

Where ΔA_λ is the change in solution absorbance resulting from complex formation at the selected wavelength l, i. e. the difference between the absorbance of both donor and acceptor before mixing and the absorbance resulting after their mixing. The formed complexes between the viologen derivatives and EDTA showed a new absorption at λ 350 nm with a tail extending to the region of 500 nm. Such absorbance does not exist for either viologens or EDTA in their separate solutions. $[A]_o$ is the molar concentration of the acceptor: MV^{2+}, PVS or BVS which showed absorption maxima at 257 nm ($\varepsilon = 2.09 \times 10^4\ M^{-1}cm^{-1}$), 261 nm ($\varepsilon = 2.42 \times 10^4\ M^{-1}cm^{-1}$), 261 nm ($\varepsilon = 2.15 \times 10^4\ M^{-1}cm^{-1}$), respectively. $[D]_o$ is the molar concentration of the donor, EDTA. A plot of ($[A]_o/\Delta A_l$) *Vs.* $1/[D]_o$ is linear for l = 1 cm, where ε_l = 1/intercept and K_{eq} = intercept/slope. Other methods of analysis of absorbance data based on algebraic rearrangement of the above equation in order to minimize the extrapolation errors and identify multiple equilibria are available in literature including:

Foster[10]

$$\Delta A_l/[D]_o = K_{eq}\, \varepsilon_\lambda l\, [A]_o - K_{eq}\, \Delta A_\lambda$$

Scott[11]

$$[A]_o\, [D]_o / \Delta A_\lambda = [D]_o / (\varepsilon_\lambda l) + 1/(K_{eq}\varepsilon_\lambda l)$$

Scatchard[12]

$$\Delta A_\lambda / ([A]_o\, [D]_o) = K_{eq}\, \varepsilon_\lambda l - K_{eq}\, \Delta A_\lambda / [A]_o$$

When the absorbance of one of the components, such as the acceptor (viologen), is not negligible, the recorded absorbance change upon complex formation is due to the increased absorbance of the complex and the diminished absorbance of the free component. Under such condition, Nash treatment[13] is appropriate. Here, a plot of ($1/[D]_o$ vs $1/(1-(A_t/A_A))$, where A_t is the total absorbance of solution and A_A is the absorbance of acceptor, viologen, is linear with an intercept equal to ($-K_{eq}$) and a slope equal to [K_{eq}-($K_{eq}\, \varepsilon_l/\varepsilon_A$)] where ε_l and ε_A are the molar absorptivities of the complex and the acceptor respectively at the wavelength of interest. Both Benesi-Hildebrand plots and Nash treatment were applied to calculate the formation constants of EDTA/viologen complexes. Figure 1, displays Benesi-Hildebrand plots for the three viologenss. Table 1, lists both K_{eq} and molar absorptivities of the complexes measured in phosphate buffer at pH 7.4 at three selected wavelengths. No measurements were made at the wavelength of maximum absorptivities of the complexes. Such absorbance is obscured by the absorption of viologen

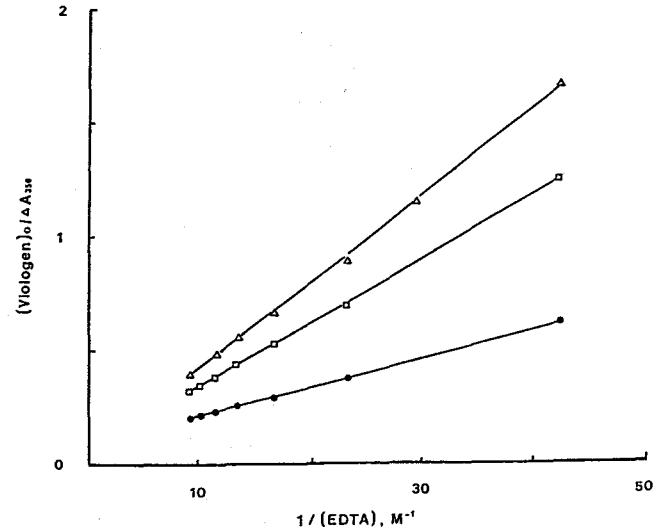


Fig. 1. Benisi - Hildebrand plots for solutions containing 0.025m viologen ($MV^{+\bullet}$, PVS <> and BVS Δ) and EDTA in pH=7.4 phosphate buffer at 350 nm.

Table 1

Equilibrium constants (K_{eq}) for the formation of 1:1 complex between the donor EDTA and the acceptor viologen derivatives in phosphate buffer (pH = 7.4) at 22° C.

λ (nm)	Benisi-Hildebrand Treatment		Nash Treatment	
	K_{eq}, M^{-1}	ε_λ, $M^{-1}cm^{-1}$	K_{eq}, M^{-1}	ε_λ, $M^{-1}cm^{-1}$
MV^{2+}				
350	7.17	11.25	7.11	11.6
370	7.16	7.55	7.10	7.9
400	6.34	3.91	7.13	3.9
PVS				
350	2.20	16.47	2.18	18.7
370	2.34	10.34	2.27	12.1
400	2.69	6.65	2.56	5.7
BVS				
350	0.84	16.33	0.67	18.0
370	1.43	11.55	0.18	12.1
400	1.29	4.22		

derivatives in the UV region. Based on 1:1 complex formation, the values of K_{eq} are in the order MV^{+2} > PVS > BVS. Charge transfer complexes rather than contact ion pairs are formed, K_{eq} for the latter less than unity[14]. For some charge transfer complexes, such as I_2/amine, the formation constants are in the order of several thousands, but for most organic 1:1 charge transfer complexes the value is usually less than 200[15]. Charge transfer complexes are formed as a result of electron transfer from the highest occupied molecular orbital, HOMO, of a donor to the lowest unoccupied molecular orbital, LUMO, of the acceptor in the ground state. The band maximum of absorption of the complex will depend on the extent of interaction between the donor and the acceptor and the red shift of the complex absorption relative to the absorption of the uncomplexed pair reflects the lowering in the energy of system as a result of complexation. Charge transfer complex formation constants of EDTA/viologen are pH dependent[7,14], K_{eq}

increases with pH. The formation constant for EDTA/MV^{+2} complex is reported to increase from 1.3 at pH 4.6 (EDTA is dinegative) to 18 at pH 11.2 (EDTA is tetranegative)[14]. More interaction (stronger complexation) is expected between more negative donors and highly positive acceptors. On the other hand, neutral zwitterionic cysteine, at pH 3.5, forms weaker complexes, $K_{eq} = 0.29$ with methylviolo-gen[14]. Thus, for a given donor/acceptor pair the values of equilibrium constant will depend on the electron transfer ability between them. Weaker complexation will result from dipole or dispersion force type of interaction between pairs[14]. Accordingly, the expected lower values for K_{eq} obtained for PVS and BVS, compared with those of methylviologen are justified since both have zero net charge and weaker interaction with EDTA is expected for both. The obtained K_{eq} values will be used as inputs in the multiequilibrium model designed to study and optimize the $Ru(bby)_3^{2+}$/EDTA/viologen sensor system. Substituting methylviologen with both PVS and BVS is proven to be effective in reducing background and thus improving signal to background ratio.

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