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Electrochemical studies of the interaction of adriamycin to DNA

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Abstract

An electrochemical investigation of the interaction of adriamycin (DXH) with DNA on a Hg electrode is reported. In weakly acidic media of pH 4.0–7.0, the addition of DNA to DXH solution results in the decrease of redox peak currents. In the presence of DNA, no new peak appears and the standard rate constant k_s is not significantly changed. The binding of DXH to DNA by electrostatic attraction and intercalation forms a kind of supramolecular complex DXH–DNA, which is electrochemically non-active. The equilibrium constant for the complex is calculated. The decrease in peak current is proportional to DNA concentration and can be used to determine DNA concentration.

Keywords: Cyclic voltammetry; Hg electrode; Adriamycin; Interaction; DNA

1. Introduction

Adriamycin (DXH) (see Fig. 1) is an anthracycline antibiotic widely used as an antitumor agent [1]. It binds to DNA by intercalation between base pairs and inhibits RNA transcription [2]. Its activity against a broad spectrum of solid tumors has determined the importance of the studies on its interaction with DNA. The studies of DXH reacting with DNA by UV and fluorescence quenching spectroscopic techniques et al. have been reported [3–9]. By electrochemical method to study the difference of the electrochemical behavior of DXH with or without DNA, it can be found the influence of DNA on the electron transfer process of DXH. H. Berg et al. measured the complex formation of some anthracyclines with DNA with regard to the influence of the sugar residue by polarographic method [10]. From these results, conclusions can be drawn concerning biochemical reaction and some therapeutic effects of adriamycin. There are many factors affecting the reaction of DNA with DXH, such as temperature and ionic strength. Different results can be obtained in different conditions. In an attempt to address more fully the electrochemical interaction of DXH to DNA, we report here the studies on the binding of DXH to DNA by cyclic voltammetric technology.

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2. Experimental

2.1. Apparatus

Cyclic voltammetric experiments were performed using an EG&G PAR (Princeton Applied Research) Model 273 potentiostat/galvanostat controlled by an IBM microcomputer with EG&G PARC M270 software and with a PAR Model 303 static mercury drop electrode. The electrode surface area was 0.0162 cm^2, determined by weighing a large number of mercury drops. An Ag/AgCl electrode was used as the reference electrode with a platinum wire as the counter electrode. The UV-visible spectra were recorded by means of a CARY Model 1E spectrophotometer (Varian).

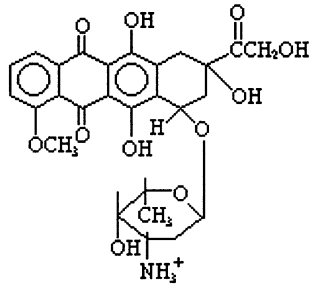

Fig. 1. Structure of adriamycin.

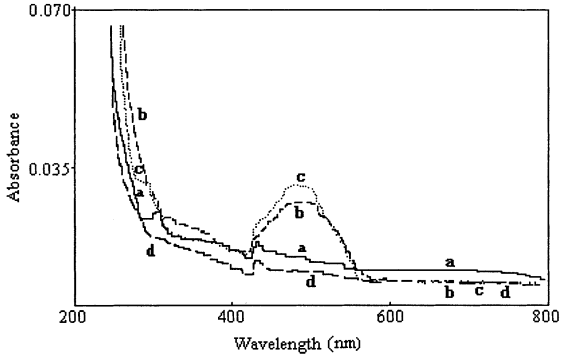

Fig. 2. Absorption spectra. (a) 0.12 M pH 6.0 McIlvaine buffer; (b) 2.0×10^{-6} M DNA mixing 5.0×10^{-6} M DXH in buffer; (c) 5.0×10^{-6} M DXH in buffer; (d) 2.0×10^{-6} M DNA in buffer.

2.2. Reagents

Fish sperm DNA (fsDNA) was purchased from ACROS (New Jersey, USA) and used as received. DNA concentrations per base pair were determined by the absorbance at 260 nm in 0.1 M NaCl. Stock solutions were stored at 4°C and are stable after 3 weeks. Adriamycin hydrochloride (DXH) was purchased from Mercian Corporation (Japan) and its chromatographic purity is 99.9%. Stock solution of DXH was prepared in water and stored in the dark at 4°C and can be dependable for 1 month. Dilution of DXH stock into the McIlvaine buffer was prepared immediately before use. Other reagents used were of analytical grade.

2.3. Procedure

0.12 M pH 6.0 McIlvaine buffer was used as supporting electrolyte. The measurements were taken after mixing DNA with DXH for at least 1 h under low light intensity to avoid photodegradation. Water was triply distilled from an all-quartz still. High purity nitrogen was used to deaerate for 12 min. All experiments were carried out at ambient temperature (ca. 16°C).

3. Results and discussion

3.1. UV-visible spectra

As shown in Fig. 2, DNA absorbs at 260 nm [11]. DXH shows the peaks at 252 nm, 292 nm and 480 nm [12]. To the mixture of DXH and DNA, the peak at 292 nm disappears and the peak at 480 nm decreases accompanied by the absorbance of 252 nm increasing. These are in accordance with Ref. [3], which means the formation of a new compound on this experimental condition.

3.2. Electrochemical behavior of DXH

As previously reported [10,13], the electroreduction of DXH is complicated, varying with the supporting electrolyte and pH value. In the pH

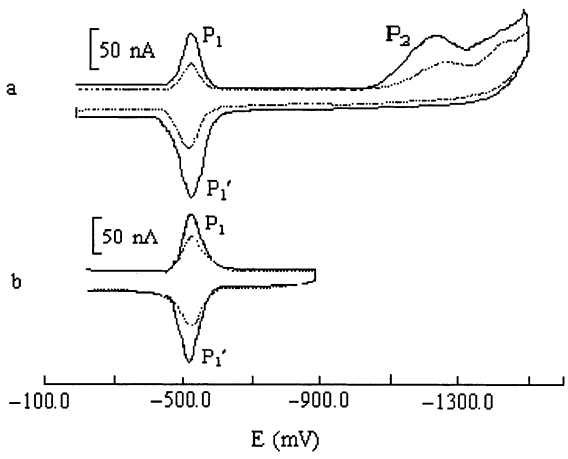

Fig. 3. Cyclic voltammograms for 5.0×10^{-6} M DXH in 0.12 M McIlvaine buffer of pH 6.0 in the absence (solid) and presence (dashed) of 2.0×10^{-6} M DNA. Rest time: 2 s. Scan rate: 100 mV s^{-1}.

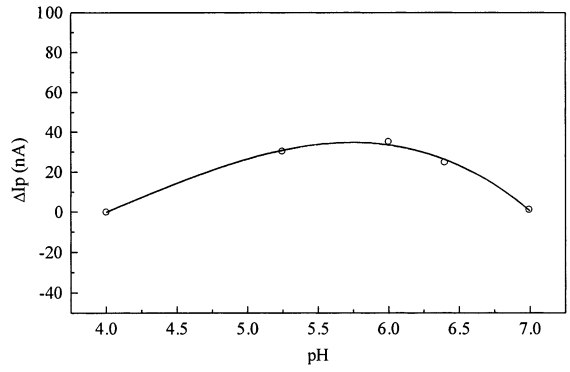

Fig. 4. The relationship of the decrease of the peak current (ΔIp) with pH values in 5.0×10^{-6} M DXH solution mixing with 2.0×10^{-6} M DNA.

6.0 McIlvaine buffer, the waves show two reduction peaks P_1, P_2 (see Fig. 3a). As shown in Fig. 3b, P_1 ,P_1' are a pair of reversible surface-confined peaks caused by two-electron transfer and two-proton uptake of the quinone group of DXH [10,13]. However, if we scan back after scanning the peak P_2, the peak P_1' increases significantly (see Fig. 3a), which is due to the adsorption of the reduction product of peak P_1. Peak P_2 is due to a kinetic or catalytic process [13]. Below pH 6.0, the behavior of the cyclic voltammograms of DXH fundamentally resembles that of the voltammograms obtained at pH 6.0, except for the peak potential. At elevated pH, it was found that the electrochemical behavior of DXH becomes complicated, showing different number of reduction waves and the first pair waves ascribed to the redox reaction of the quinone group.

3.3. The interaction of DXH with DNA

The interaction of DXH with DNA varied with supporting electrolyte, ionic strength, pH value and temperature [6,10]. By investigating the effect of pH value on the reaction of DXH with DNA, it was found that in weakly acidic media of pH 4.0–7.0, the addition of DNA to DXH solution and subsequent scanning produced no new waves. Both the reduction peak current and the oxidation peak current decrease accompanied by the peak potential of P_1, P_1' not changing. The peak potential of P_2 shifts negatively (Fig. 3a), which is similar to the decrease of the concentration of DXH. Because peak P_2 discharges near the base and the peak height is not easy to be exactly determined, only the changes of the redox peaks P_1 and P_1' in the absence and presence of DNA were studied (Fig. 3b). In the pH range from 4.0 to 7.0, the relation between peak potential and pH was directly investigated and a linear regression equation, $Ep = -176 - 56.3$ pH (Ep, mV; correlation coefficient, $r = 0.9952$) was obtained. This shows that the uptake of electrons is accompanied by an equal number of protons independent of the presence of DNA.

In neutral and alkaline media, the peak currents of DXH remain constant after the addition of DNA to the solution of DXH.

Because at pH 6.0 the peak current decrease is at a maximum (Fig. 4), pH 6.0 was selected as the optimum value.

Under the selected experimental condition, the peak currents of DXH solutions with DNA addition are linearly dependent on scan rate and increase with increasing preconcentration time, parallel to DXH in absence of DNA. These results indicate that the electrode reaction of DXH in presence of DNA is also a reversible surface electrochemical reaction where both the reactant and the product are strongly adsorbed on the

electrode surface [1,14]. For a reversible adsorption peak [15], the width of the peak at mid-height ($W_{1/2}$) is: $W_{1/2} = 90.6/n$ mV at 25°C. For the reduction peak P_1, $W_{1/2}$ is 54.8 mV. Thus, the estimated number of electrons transferred during the electrode reaction (n) is still 2. So we conclude that, no matter whether DNA is present or not, the electrode reaction processes are both two-electron transfer with two-proton uptake.

One probable explanation for the decrease of the peak current without change of peak potential is based on the competitive adsorption between DXH and DNA. By investigating the effect of accumulation time on peak area, we can calculate the surface coverage (θ) at different accumulation time (t_a). The amount of saturation adsorption is about 1.1×10^{-10} mol cm^{-2} [13]. To 5.0×10^{-6} M DXH solution, the reduction charges (Q), obtained by determining the reduction peak area of the voltammogram, are 0.036 and 0.062 μC, corresponding to $t_a = 2$ and 10 s, respectively. According to $\Gamma = Q/nFA$ and $\theta = \Gamma/\Gamma_s$, the coverage θ can be approximately calculated as 0.11 and 0.18, respectively. Thus at $t_a < 10$ s, the coverage of DXH is much smaller than 1 and the competitive adsorption between DXH and DNA hardly exists. As the peak currents are still decreasing at $t_a = 2$ s, this further confirms that the variation of peak currents is not due to competitive adsorption.

Another probable explanation of these experimental phenomena is that DXH binding to DNA forms an electroactive supramolecular complex with its diffusion coefficient decreasing responsible for the decrease of peak currents [16,17]. Investigation of the variation of the electrochemical parameters in absence and presence of DNA can judge whether the formed supramolecular complex is electrochemical active. Laviron [18] reported that the transfer coefficient α and the standard rate constant of the surface reaction k_s can be deduced from an experimental study of the variation of the peak potential as a function of the scan rate when $n\Delta Ep$ is smaller than 200 mV and α ranges from 0.3 to 0.7. Laviron provided several groups of data corresponding to m^{-1} and $n\Delta Ep$, respectively ($m = RTk_s/nFv$, where n, F, R and T have their usual significance). He also pointed out that the relative error on k_s is at the most about 6% if the relationship for $\alpha = 0.5$ was used.

Table 1 shows the relationship between $n\Delta Ep$ and v in absence and presence of DNA. As values of $n\Delta Ep$ smaller than 200 mV ($n = 2$) were obtained, this method could be used. Introducing the values of $n\Delta Ep$ into the regression equation, which was deduced from the data Laviron provided, the values of m^{-1} were obtained, and then k_s could be calculated. The average values of k_s are 2.2×10^3 s^{-1} and 2.4×10^3 s^{-1}, corresponding to the absence and presence of DNA, respectively (Table 1). The values of α and k_s did not change, independent of an addition of DNA. Obviously, the supramolecular complex formed has no electrochemical activity and the explanation that the formed complex is electroactive and the decrease of its diffusion coefficient leads to the peak currents decreasing is not suitable for this case.

It has been reported that 9,10-anthraquinone binds to DNA by intercalation owing to its planar hydrophobic structure [19,20]. There have been studies that some anthraquinone derivative anthracycline antibiotics such as adriamycin and

Table 1
The chosen values of ΔEp and the calculated k_s in the absence and presence of DNA

v (V/s)		10	15	20	30
Absenting DNA	$n\Delta Ep$ (mV)	14.4	24.0	29.8	39.6
	k_s ($\times 10^3$ s^{-1})	2.3	2.0	2.1	2.2
	Average k_s ($\times 10^3$ s^{-1})	2.2			
Presenting DNA	$n\Delta Ep$ (mV)	12.2	20.6	30.0	36.8
	k_s ($\times 10^3$ s^{-1})	2.8	2.3	2.1	2.4
	Average k_s ($\times 10^3$ s^{-1})	2.4			

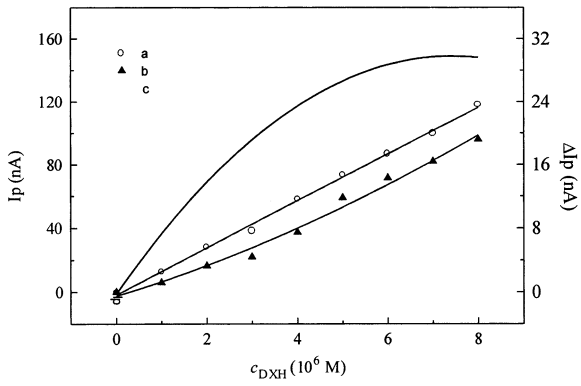

Fig. 5. Relationship between Ip and c_{DXH} (a, b), ΔIp and c_{DXH} (c). (a) $c_{DNA} = 0$; (b) $c_{DNA} = 6.0 \times 10^{-6}$ M; (c) $Ip_a - Ip_b$.

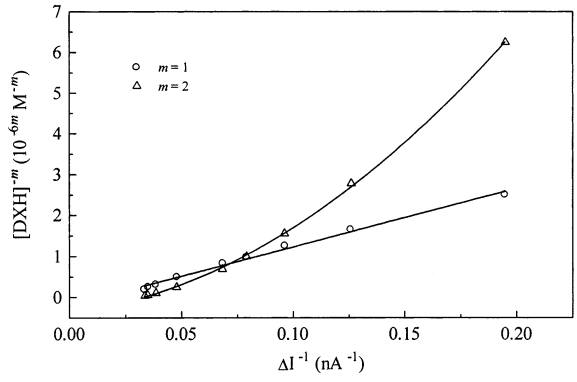

Fig. 6. Relationship between ΔI^{-1} and $[DXH]^{-m}$.

daunomycin interact with DNA by intercalation and electrostatic attraction by their basic sugar residue with the sugar-phosphate backbone of DNA [10,21,22]. The investigation of Zhao [9] and Frederick [23] showed that the chromophore of DXH is intercalated at the CG–GC steps at the DNA helix with the amino sugar extended into the minor groove. In our experiment, in the pH 4.0–7.0 region, there is an electrostatic interaction between the protonated amine group of the sugar residue of DXH ($pK_a = 8.2$ [24]) and the negatively charged phosphate group of DNA. Above pH 7.0, DXH forms a neutral species or a charged anion and below pH 4.0, DNA has no net charge, so the electrostatic attraction disappears. If we used the single-strand DNA instead of the native double-strand DNA, it was found that there is no interaction between DXH and DNA. So we conclude that the interaction of DXH with DNA is that the outside electrostatic attachment of the basic sugar residue is followed by the intercalation and the main effect on the interaction is the intercalation of DXH to DNA. DXH binding to DNA by intercalation in weakly acidic media forms a kind of electrochemically non-active supramolecular complex, which cannot be reduced on the electrode. In presence of DNA, the equilibrium concentration of DXH decreases, which results in a decrease of redox peak currents.

3.4. Determination of the stoichiometry of DNA–mDXH

After a little modification to the method of Li and Qu [25], we can conclude the equation

$$\frac{1}{\Delta I} = \frac{1}{\Delta I_{max}} + \frac{1}{\beta \cdot \Delta I_{max}} \times \frac{1}{[DXH]^m}$$

According to this equation, the composition of this supramolecular complex and the equilibrium constant can be calculated.

In Fig. 5, curve b typically represents the current change at 6.0×10^{-6} M DNA with different concentration of DXH. Curve a shows the relationship of the peak current and c_{DXH} in absence of DNA. A linear regression equation for curve a and a polynomial regression for curve b were obtained by programming. Then curve c was plotted using the difference between the above two, which represents the relationship between ΔI ($Ip_a - Ip_b$) and the concentration of DXH.

It is assumed that DNA and DXH forms a single complex and that m is 1 and 2. If the plot $1/\Delta I$ versus $1/[DXH]^m$ is linear, the assumed value of m is reasonable. The results of $m = 1$ and $\log \beta = 5.3$ were obtained from the experimental data (Fig. 6), which means that DXH binding to DNA forms a 1:1 complex of DNA–DXH.

3.5. Analytical application

In 0.12 M pH 6.0 McIlvaine buffer solution, the

Table 2
Results of sample determination

Number	SM[a] ($\times 10^{-6}$ M)	EM[a] ($\times 10^{-6}$ M)	RSD (%)	Added ($\times 10^{-6}$ M)	Found ($\times 10^{-6}$ M)	Recovery (%)
1	1.20	1.15		2.0	3.30	108
2	1.20	1.13	8.1	3.0	4.3	106
3	1.20	1.31		4.0	5.20	97
Average	–	1.20	–	–	–	104

[a] SM, spectrometric method; EM, electrochemical method.

addition of DNA to DXH solution results in a decrease of the reduction peak current. This can be applied to the determination of DNA concen-tration. The decrease of the reduction peak height is proportional to the DNA concentration in the range from 5.0×10^{-7} to 8.0×10^{-6} M (correlation coefficient $r = 0.9948$) using 8.0×10^{-6} M DXH solution.

Genomic DNA sample was obtained from human peripheral leukocyte by the method of Sambrook et al [26] and was determined after suitable dilution. Table 2 shows the result of the sample determination. The result was consistent with the value by UV spectroscopy, which means the method is suitable for the determination of DNA.

4. Conclusion

It was observed by cyclic voltammogram that DXH interacting with DNA by electrostatic attraction and intercalation in weakly acidic media forms an electrochemically non–active supramolecular complex DXH–DNA, which causes the equilibrium concentration of DXH reducing and results in the decrease of the peak current. The interaction of DNA with DXH can be applied to determine DNA.

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References

[1] D.W. Henry, Cancer Treat. Rep. 63 (1979) 845–850.
[2] M.J. Waring, Annu. Rev. Biochem. 50 (1981) 159–165.
[3] J.B. Chairs, N. Dattagupta, D.M. Crothers, Biochemistry 21 (1982) 3933–3940.
[4] E.J. Gabbay, D. Grier, R.E. Fingerle, R. Reyner, R. Levy, S.W. Pearce, W.D. Wilson, Biochemistry 15 (1976) 2062–2070.
[5] D.E. Graves, T.R. Krugh, Biochemistry 22 (1983) 3941–3947.
[6] F. Barcelo, J. Martorell, F. Gavilanes, J.M. Gonzalez-Ros, Biochem. Pharmacol. 37 (1988) 2133–2138.
[7] C.A. Frederick, L.D. Williams, G. Ughetto, G.A. Marel, J.H. Boom, A. Rich, H.J. Wang, Biochemistry 29 (1990) 2538–2549.
[8] F.A. Fornari, D.A. Gewirtz, G.B. Baner, D.J. Abraham, G.E. Kellogg, Biophys. J. 64 (1993) A284.
[9] X.J. Zhao, S. Jiang, D.S. Lu, Y.C. Fan, Z.G. Li, Chin. Biochem. J. 10 (1994) 325–328.
[10] H. Berg, G. Horn, U. Luthardt, Bioelectrochem. Bioenerg. 8 (1981) 537–553.
[11] J. Marmur, J. Mol. Biol. 3 (1961) 208–218.
[12] J.B. Chaires, N. Dattagupta, D.M. Crothers, Biochemistry 21 (1982) 3933–3937.
[13] K. Kano, T. Konse, N. Nishimura, T. Kubota, Bull. Chem. Soc. Jpn. 57 (1984) 2383–2390.
[14] E. Laviron, J. Electroanal. Chem. 52 (1974) 355–393.
[15] A.J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals and Applications, Wiley, New York, 1980.
[16] M.T. Carter, M. Rodriguez, A.J. Bard, J. Am. Chem. Soc. 111 (1989) 8901–8911.
[17] Z. Zhu, N.Q. Li, Mikrochim. Acta 130 (1999) 301–308.
[18] E. Laviron, J. Electroanal. Chem. 101 (1979) 19–28.
[19] W.Y. Li, J.G. Xu, X.Q. Guo, Q.Z. Zhu, Y.B. Zhao, Chem. J. Chin. Univ. 17 (1996) 1706–1707.
[20] Z. Zhu, N.Q. Li, Microchem. J. 59 (1998) 294–306.
[21] E. Calendi, A.D. Marco, M. Reggiani, B. Scarpinato, L. Valenti, Biochim. Biophys. Acta 103 (1965) 25–49.
[22] C. Molinier-Jumel, B. Malfoy, J.A. Reynaud, G.A. Sadron, Biochem. Biophys. Res. Commun. 84 (1978) 441–449.
[23] C.A. Frederick, L.D. Williams, G. Ughetto, G.A. Marel, J.H. Boom, A. Rich, A.H.J. Wang, Biochemistry 29 (1990) 2538–2549.

[24] R.J. Sturgeon, S.G. Schulman, J. Pharm. Sci. 66 (1977) 958–961.
[25] F. Qu, N.Q. Li, Y.Y. Jiang, Microchem. J. 58 (1998) 39–51.
[26] J. Sambrook, E.F. Fritsch, T. Manicatis, in: Cold Spring Harbor Laboratory (Ed.), Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, New York, 1989.