POLAROGRAPHIC INVESTIGATION OF THE MOBILITY OF HALOGEN ATOMS IN HALOGEN-SUBSTITUTED COMPONENTS OF NUCLEIC ACIDS

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In order to establish quantitatively the reducibility of halogen-substituted components of nucleic acids, the half-wave potentials ($E_{1/2}$) of their electrochemical reduction have been determined. The electrochemical reduction of all compounds takes place in the interval from −0.5 to −1.8 V and consists in the splitting off of the halogen atom (iodine, bromine) from the given molecule. The iodine derivatives are reduced much more readily (500-800 mV) than the bromine derivatives.

In order to investigate the mobility of halogen in halogen-substituted components of nucleic acids, the halogen derivatives of bases, nucleosides, and nucleotides were studied polarographically. The electrochemical reduction of derivatives of purine and pyrimidine, nucleosides and nucleotides of the adenine series, and the adsorption of bromine derivatives of cytosine and uracil at the dropping mercury electrode have already been investigated; however, data on the electrochemical reduction of halogen-substituted components of nucleic acids are not available.

8-Bromoadenosine was selected as a model compound for the polarographic investigation of the mobility of halogen atoms. After the electrolysis in a phosphate buffer at pH 6.9 ($E_{1/2}$ = −1.15 V), the solutions were analyzed by UV spectroscopy. The obtained densities of the investigated solutions and the UV spectra of pure adenosine and bromoadenosine were analyzed graphically and by the method of main factors [5]. The results indicated that only two compounds were present in the mixture. Consequently, the electrochemical removal of bromine from 8-bromoadenosine is not complicated by side reactions. In order to determine quantitatively the reducibility of the halogen-substituted components of nucleic acids, the half-wave potentials ($E_{1/2}$) of the electrochemical reduction of compounds I-IX (Table 1) were determined at the dropping mercury electrode in the Britton−Ribinson aqueous universal buffer solution at pH 7.0.

Compounds Ia-VIIa, which do not contain halogen, are polarographically inactive; the bromo and iodo derivatives give one polarographic wave in the potential interval from −1.0 to −1.8 V and from −0.5 to −1.0 V respectively. For compounds having a half-wave potential of $E_{1/2} < -1.6$ V the waves are not well defined, because the region of the limiting-current plateau (i_{lim}) is close to the region of precipitation of the supporting electrolyte.

The amount of electrons (n) spent in the electrochemical reaction was calculated from the Il'kovich equation [6]. The diffusion coefficient (D) for the compounds IIb, Vb, VIIb, and VIIf was determined beforehand. The value of D was determined by two independent means: by using radioactive tracers with solutions of labeled compounds [7], and from pycnometric data according to the Stokes−Einstein equation [8]. From the results obtained it follows that the value of D, determined with the radioactive tracers, is higher by 10-15% than the value obtained by the pycnometric method.

This can probably be explained by an increase in the volume of the solvated molecules in dissolution. This tendency agrees with published data on the relationship of the diffusion coefficients of nucleosides and nucleotides of the adenine series, determined at the conditions of polarographic reduction and calculated from the Stokes−Einstein equation and pycnometric data [9]. The number of electrons, calculated with the use of the obtained values for D, was found to be equal to 2 (Table 2).

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TABLE 1. Half-Wave Potentials of the Investigated Components of Nucleic Acids in the Britton–Robinson Universal Buffer Solution (pH 7.0; depolarizer concentration 5×10^{-4} M)

No.	Compound	$-E_{1/2}$, V	No.	Compound	$-E_{1/2}$, V
Ia	Adenine	–	VIb	5-Bromouracil	1.76
Ib	8-Bromoadenine*	1.52	VIb	6-Bromouracil	1.65
Ic	8-Bromo-9-methyladenine	1.28	VId	5-Bromo-1-methyluracil	1.69
IIa	Adenosine	–	VIe	5-Bromo-3-methyluracil	1.74
IIb	8-Bromoadenosine	1.20	VIf	5-Iodouracil	1.11
IIc	8-Bromoadenosine monophosphate	1.10	VIg	6-Iodouracil	0.70
			VIIa	Uridine	–
IId	8-Bromoadenosine triphosphate	1.28	VIIb	5-Bromouridine	1.63
			VIIc	5-Bromouridine monophosphate	1.66
IId	8-Bromoadenosine-3',5'-cyclophosphate	1.15	VIId	5-Bromouridine diphosphate	1.67
IIf	8-Bromodesoxyadenosine	1.12			
IIg	8-Bromodesoxyadenosine monophosphate	1.16	VIIe	5-Bromouridine triphosphate	1.70
IIh	8-Bromodesoxyadenosine triphosphate	1.20	VIIf	5-Bromodesoxyuridine	1.64
			VIIg	2'-Bromodesoxyuridine	1.25
IIi	8-Iodoadenosine	0.52	VIIh	5-Bromodesoxyuridine monophosphate	1.66
IIIa	Inosin	–			
IIIb	8-Brominosin	1.17	VIIi	5-Iodouridine	0.98
IVa	Guanine	1.68	VIIk	5-Iododesoxyuridine	0.95
IVb	8-Bromoguanine*	1.40	VIIIa	Cytosine	1.77
IVc	8-Bromo-9-methylguanine	–	VIIIb	5-Bromocytosine	1.62; 1.78**
Va	Guanosine	1.13			
Vb	8-Bromoguanosine	1.58	VIIIc	5-Iodocytosine	0.83; 1.80**
Vc	8-Bromoguanosine monophosphate	1.46			
			IXa	Cytidine	1.68
Vd	8-Bromoguanosine triphosphate	1.16	IXb	5-Bromocytidine	1.49; 1.69**
Ve	8-Bromodesoxyguanosine	0.58	IXc	5-Bromocytidine monophosphate	1.62; 1.83**
VIa	Uracil	–			
			IXb	5-Iodocytidine	0.85; 1.67**

*Poor solubility ($C < 5 \cdot 10^{-4}$ M).

**$E_{1/2}$ value of second polarographic wave.

Based on the calculation of the number of electrons and the fact that only compounds incorporating bromine or iodine atoms are capable of undergoing electrochemical reduction, one can conclude, that dehalogenation of the compounds takes place at the dropping mercury electrode; the actual dehalogenation takes place in a two-electron stage according to the equation

$$RBr \xrightarrow[+2c]{+2H^+} RH + HBr$$

The participation of protons in the general electrode process is confirmed by the dependence of $E_{1/2}$ on the pH, as shown on the example of 8-bromoadenosine (Fig. 1). A similar correlation could not be obtained for 5-bromouridine, due to the coincidence of its reduction potentials with the potential at which precipitation of the supporting electrolyte occurs at low pH values.

Particular attention must be paid to the derivatives of cytosine and cytidine; their polarographic behavior differs significantly from that of the compounds discussed above. Thus, the unhalogenated compounds VIIIa and IXa give one polarographic wave, while VIIIb, c and IXb-d give two clearly defined polarographic waves. The second wave appears at potentials from -1.6 to -1.8 V and has a height approximately equal to that of the first wave. For instance, for IXb the values for the first and second wave are equal to 1.70 and 1.50 μA respectively; for VIIIc the values are 1.9 and 2.0 μA.

The coincidence of the $E_{1/2}$ values of compounds VIIIa and IXa with the $E_{1/2}$ values of the corresponding halogen derivatives (VIIIb, c and IXb-d) and the equal values of i_{lim} lead to the conclusion that the second wave of the electrochemical

TABLE 2. Parameters of Electrochemical Conversions and Diffusion Coefficient of Compounds IIb, Vb, VIIb, and VIIf

Compound	i_{lim}, μA	Δ $10^6 \cdot cm^2\ sec^{-1}$		n
		pycnometer method (in toluene)	radioactive-tracer method*	
IIb	1,34	6,6*4; 6,4	7,4*2	1,90
Vb	—	6,0		
VIIb	1,30	6,7		1,80
VIIf	1,32	6,6	7,9*3	1,84

*Solvent: Britton–Robinson buffer solution, pH 6.9.

*2By using 8-bromo(8-^{14}C) adenosine.

*3By using 5-bromo-2′-desoxy (2′-3H) uridine.

*4With water as the medium.

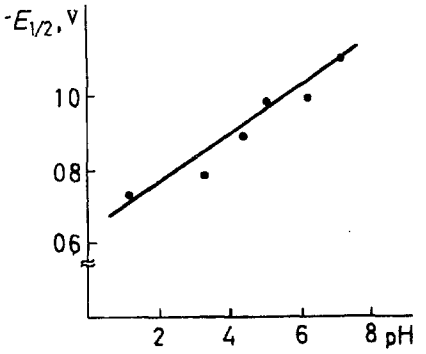

Fig. 1. Values for the electrochemical reduction wave $E_{1/2}$ of 8-bromoadenosine in Britton–Robinson buffer solutions as function of the pH. Depolarizer concentration $2.5 \cdot 10^3$ M.

reduction of the latter also represents a two-electron reaction and that it corresponds to the reduction of the cytosine ring. This agrees fully with the data for substituted 2-oxo-1,2-dihydropyrimidines [10].

The analysis of the obtained experimental data showed that the splitting off of bromine from purine bases is more difficult (by 500-600 mV) than from the corresponding nucleosides and nucleotides. Nucleotides are more difficult to reduce than nucleosides. This can be attributed in part to the fact that the ribose ring has an electron-acceptor effect and facilitates the reduction; the introduction of a negatively charged phosphate group enhances the repulsion of the molecule from the electrode surface and reduces its reactivity [11]. It is known that depolarizer molecules, diffusing to the electrode, assume a spatial orientation which corresponds to the minimum cross-sectional area; however, the nucleotides of the adenine series retain their spherical shape as a result of strong intramolecular hydrogen bonds. Consequently, the negative influence of the phosphate groups on the adsorption at the dropping mercury electrode is screened in the adenine nucleotides in comparison with the guanine nucleotides. This explains probably the easier polarographic reduction of IIc and IId as compared to Vc and Vd.

An electrochemical reduction wave of 2-bromoadenine could not be obtained: It is probably reduced in a more negative region than 8-bromoadenine Ib. The higher mobility of bromine in 8-bromoadenine than in 2-bromoadenine is in agreement with the available data, indicating that bromine is removed more easily from position 8 in the catalytic liquid-phase hydrogenolysis with gaseous hydrogen [12].

No significant difference exists in the pyrimidine series in the ease of the polarographic reduction of the bromine derivatives of the corresponding bases, nucleosides, and nucleotides.

The reduction of 5-bromouracil is more difficult by 100 mV than the reduction of 6-bromouracil; even more noticeable is the difference between the 5- and 6-iodo derivatives of uracil, where it reaches 400 mV. This conforms with the published data on the mobility of the 5-C−Br and 6-C−Br bonds in the reducing dehalogenation of 5,6-dibromouracil with gaseous tritium in neutral and weakly alkaline media [13]. In the catalytic dehalogenation 5-10% 5-bromo(6-^{3}H)uracil is formed besides (5,6-^{3}H)uracil, i.e. uracil is removed from the position 5 of uracil somewhat more difficultly than from the position 6.

The influence of the methyl group on the ease of the polarographic reduction of the bromo derivatives of nucleic acid components depends on its position. Thus, the introduction of the methyl group into position 1 or 3 of 5-bromouracil has no noticeable effect. On the other hand, the value of $E_{1/2}$ for 5-bromo-6-methyluracil could not be determined because the introduction of the methyl group made the reduction difficult and shifted the half-wave potential into the region of precipitation of the supporting electrolyte. This must be attributed to the electron-donor properties of the methyl group and to the steric hindrances caused by it.

In the purine series the introduction of the methyl group in position 9 of adenine and guanine eased the polarographic removal of bromine from position 8 by 270-280 mV. This is probably related to the fact that methylation of the imino group of the imidazole ring eliminated its acid dissociation. However, a more complex picture is also possible, related to the fact that intermolecular hydrogen bonds cannot be formed in N-alkylated derivatives.

As a whole, the bromine derivatives of the purine series are reduced more easily than the derivatives of the pyrimidine series. No significant differences were found in the ease of electrochemical reduction of analogous bromine derivatives of the ribo and the desoxyribo series.

The polarographic removal of iodine proceeds much more easily (minimum at 550 mV) than the removal of bromine. The chlorine derivatives of thymine and uracil were found to be polarographically inactive.

The present study represents the first attempt of a systematization of the quantitative data for the polarographic dehalogenization of derivatives of components of nucleic acids. For a deeper understanding of all factors affecting the electrochemical reduction, it is essential to investigate a wide circle of problems, including the dependence of the $E_{1/2}$ values on the pH, on the quantum-chemical characteristics of the initial molecules, on their adsorption at the dropping mercury electrode, etc.

EXPERIMENTAL

The polarographic investigations were carried out on a PAR-170 electrochemical system by using a three-electrode scheme. The cathode was a dropping mercury electrode with enforced droplet detachment t = 0.44 sec, m = 1.09 mg·sec^{-1}); the anode was a platinum wire and the auxiliary electrode an aqueous saturated calomel electrode.

REFERENCES

1. J. Stradins and V. Kadysh, Bioelectrochem. Bioenerg., **4**, 508 (1977).
2. H. Klukanova, M. Studnickova, J. Kovac, J. Turanek, and V. Kahle, Bioelectrochem. Bioenerg., **215**, 317 (1986).
3. B. Janik and P. J. Elving, Chem. Rev., **68**, 75 (1968).
4. J. Jursa and V. Vetterl, Studia Biophys., No. 13, 75 (1986).
5. I. Ya. Bershtein and Yu. L. Kaminskii, Spectrophotometric Analysis in Organic Chemistry [in Russian], Khimiya, Leningrad (1986), pp. 36, 132.
6. S. G. Mairanovskii, Ya. P. Stradyn', and V. D. Bezuglyi, Polarography in Organic Chemistry [in Russian], Khimiya, Leningrad (1975), p. 83.
7. V. B. Luk'yanov and S. S. Berdonosov, Radioactive Tracers in Chemistry [in Russian], Vyssh. Shkola, Moscow (1975), p. 260.
8. A. Weissberger, Physical Methods of Organic Chemistry [Russian translation], Moscow (1950), p. 79.
9. B. Janik and P. J. Elving, J. Am. Chem. Soc., **92**, 235 (1970).
10. V. P. Kadysh, Ya. P. Stradyn', E. L. Khanina, G. Ya. Dubur, and D. Kh. Mutsenietse, Khim. Geterotsikl. Soedin., No. 1, 117 (1985).

11. B. Janik and P. J. Elving, Chem. Rev., **68**, 295 (1968).
12. J. Davoll and B. A. Lowy, J. Am. Chem. Soc., **74**, 1563 (1952).
13. I. Filip and L. Bogachek, Materials of the 2nd Symp. of Member Countries of SEV on Organic Compounds Labeled with Radioactive Isotopes, Leningrad, December 8-11, 1981 [in Russian], Moscow (1982), p. 102.