Bioelectrochemistry and Bioenergetics, 27 (1991) 221–227 A section of J. Electroanal. Chem., and constituting Vol 342 (1991) Elsevier Sequoia S.A., Lausanne

JEC BB 01429

Short communication

The electrochemical redox behaviour of the antineoplastic 1,4-benzoquinone-guanylhydrazone-thiosemicarbazone (ambazone)

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INTRODUCTION

The antibacterial and antineoplastic properties of 1,4-benzoquinone-guanylhydrazone-thiosemicarbazone (ambazone: substance 1 in Table 1) encouraged the synthesis of derivatives. The ambazone structure was varied at the ring and/or the side chain to obtain compounds with better antibacterial and/or antineoplastic properties [1,2]. The oxidation and reduction behaviour of these substances was

TABLE 1

R_4 R_2 R_3			
R,	R₂	R,	R,
$1 = N - N - C (NH_2)_2$	= N - NH - C (S) NH ₂	н	Н
$2 = N - N = C (NH_2)_2$	= N - NH - C(0) NH ₂	н	н
$3 = N - N - C (NH_2)_2$	= N - NH - C(S) NH ₂	н	СН,
$4 = N - N - C (NH_2)_2^{-1}$	= N - NH - C(S)NH ₂	СН,	CH(CH,),

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studied by polarography and coulometric measurements in order to correlate the structure with the biological activity. The ambazone with the greatest biological effect [3] shows a particular redox behaviour, in contrast to the other not so active ambazone derivatives.

Changes in the structure of the molecule, produced by living cells, were studied by polarography.

MATERIALS AND METHODS

The compounds investigated were synthesized in this institute as reported elsewhere [1]. The experiments were carried out with four substances which are all poorly soluble in water [5,6]. Therefore stock solutions were prepared in dimethyl-sulphoxide (DMSO) with a concentration of 5×10^{-5} M (0.11 mg/ml of substance 1). The stability of the stock solution was checked by polarographic measurements. The maximum concentrations in 0.06 M phosphate buffer, pH 7, were in the range 3.5×10^{-5} to 2×10^{-6} M. For polarographic measurements, oxygen was removed from the stock solution as well as from the buffers. For electrochemical measurements, GWP 673 and ECM 700 polarographs from the Institut fuer Gerätebau der Akademie der Wissenschaften, Berlin, were used.

The coulometric reduction was studied on a mercury pool electrode [7] with an OH 404 potentiostat from Radelkis, Budapest. The spectrophotometric measurements were carried out with a Specord M 40 from Carl Zeiss, Jena.

RESULTS

Polarographic behaviour

The polarogram of all the substances in Table 1 consists of a cathodic wave of the quinone group, as shown in Fig. 1. In this polarographic reaction the quinone is reduced reversibly on the mercury dropping electrode (DME) to hydroquinone [8–10]. In addition, only substance 1 shows an oxidation wave on the positive part of the polarogram, which cannot be separated from the cathodic wave as shown in Fig. 2. The difference in the structures of substances 1 and 2 is the substitution of oxygen by sulphur in the side chain (R₂ in Table 1). Therefore, the anodic oxidation current of substance 1 is due to the >C=S bound in the side chain of the molecule. This anodic current is diffusion-controlled and proportional to the concentration. The ratio of the height of the anodic and cathodic waves is 1:2. The reduction current corresponds to the two reversible electrons was found by the dependence of log $i/(i_d - i)$ on the potential of the polarographic wave and also controlled by coulometric measurements. The anodic–cathodic wave of substance 1 can be recorded over the whole pH range without separation.

In neutral or acid buffer solution, the wave heights did not change. Under strong alkaline conditions, however, the height of the anodic and cathodic wave



Fig. 1. Substance 2. Dependence of the polarographic wave height on the concentration. 0.06 M phosphate buffer, pH 7; initial potential -0.2 V; 25°C; SCE. $10^5 c/M$: (1) 0.9; (2) 1.4; (3) 1.9; (4) 2.3; (5) 2.8; (6) 3.2; (7) 3.7.

decreases. The polarographic behaviour of substances 3 and 4 is more or less the same as that for substance 1, but at higher concentrations $(3 \times 10^{-5} \text{ M})$ the anodic part seems to be present.

Influence of oxygen

Under oxygen-free conditions, the wave height of substance 1 is constant. In the presence of oxygen, the anodic part of the wave disappears (Fig. 3) and the cathodic part is lowered. During this oxidation process, the half-wave potential of this wave shifted about 50 mV to more negative values. Under alkaline conditions, the oxidation reaction takes place faster. In acid buffer solution, no oxidation takes place [5,6].

Coulometric reduction

For the study of the redox properties of the ambazone derivatives the substances were reduced at a mercury pool electrode. Under these conditions, the substances are completely reduced; the quinone changes to hydroquinone. The reaction can be followed polarographically as shown for substance 1 in Fig. 4. After total reduction, the hydroquinone part plus the anodic wave can be observed (curve 5 in Fig. 4). Substance 2 exhibits the same behaviour, however without an anodic wave part. Hydroquinone can be reversibly oxidized by oxygen, and sub-



Fig. 2. Substance 1. Dependence of the polarographic wave height on the concentration. 0.06 M phosphate buffer, pH 7; initial potential -0.2 V; 25°C; SCE. $10^5 c/M$: (1) 0.9 (2) 1.4; (3) 1.9; (4) 2.3; (5) 2.8; (6) 3.2.



Fig. 3. Substance 1. Oxidation by dissolved molecular oxygen with time. Concentration 0.4×10^{-5} M; 0.06 M Britton-Robinson buffer, pH 9.0; 25°C; SCE. Duration of aeration/min: (1) 0.0; (2) 3; (3) 5; (4) 45; (5) 60; (6) 90.



Fig. 4. Substance 1. Coulometric reduction at the mercury pool electrode. 0.06 M phosphate buffer, pH 7; 25°C; SCE; concentration 0.4×10^{-5} M. (1) Polarogram at the beginning of the reduction (quinone); (2-4) polarogram during coulometric reduction; (5) complete reduction (hydroquinone); (6) after reoxidation with dissolved molecular oxygen (quinone — whole polarogram, hydrogen peroxide is formed); (7) after addition of catalase (hydrogen peroxide is destroyed).

stance 2 then shows the same cathodic wave as before its reduction. In contrast, the anodic part of the wave of substance 1 disappears and the cathodic part is lowered somewhat. During this reoxidation, hydrogen peroxide is generated, which can be destroyed by adding the enzyme catalase (compare Fig. 4, curves 6 and 7). During this redox reaction cycle, the yellow colour disappears and then reappears, as measured spectroscopically.



DISCUSSION

The electrochemical behaviour of a quinone group is very well known from the literature [8-10] and holds true for the quinone group of all the ambazone derivatives of this study. Regarding the polarographic behaviour, only substance 1 differs from all the other derivatives by an additional anodic oxidation wave,



created by the C=S group in the side chain of the molecule. The anodic part disappears after oxidation with oxygen (Fig. 4) and a new substance is formed. Therefore the oxidation reaction takes place in the thiosemicarbazone part of the molecule, as can be shown by oxidation of the isolated thiourea side-chain. This polarographic behaviour of substance 1 during oxidation is probably connected with its biological activity.

According to the oxidation of thiourea [11], the following pathway can be postulated for the oxidation reaction of the ambazone. In the first step, one electron is removed, which creates a positive charge in the side chain at the nitrogen atom (A) [12]. After deprotonization, a radical is formed at the sulphur atom (B), which is capable of forming dimers.

This scheme is in agreement with the polarographic findings. First, during reoxidation the C=S bond is reduced slightly and then the anodic wave disappears, which may lead to dimerization resulting in a decrease of the diffusion coefficient. The reduction of the -S-S- bond of the dimers is indicated by a 50 mV more negative polarographic wave. Dimers are formed only in neutral, alkaline solutions — not in acid solutions.

It was shown by RP-HPLC that the retention time of the electrochemically produced dimer is smaller than that for ambazone itself (substance 1) [13] and that the dimer is also less polar than ambazone.

Owing to the solubility in water, higher concentrations of the dimer cannot be formed. Substances 3 and 4 have a >C=S bond in the side chain like substance 1, but the ratio of the anodic-cathodic wave is not 1:2. The formation of dimers may be influenced by the substituents of the ring. It is possible that steric effects and/or the electron distribution in the molecule suppress the formation of the anodic wave. Further experiments are necessary.

The actions of the four substances in biological experiments are different. Only substance 1 shows a strong anticancer activity. This may be the result of one or more of the following items:

(1) the formation of radicals during redox processes;

(2) splitting of dimers by reduction connected with the reformation of the initial substance;

(3) the formation of hydrogen peroxide during the redox reaction of the quinone group; and

(4) the formation of complexes with metal ions [4].

These properties might be involved in the biological efficacy of these substances.

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