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Similarity between enzymatic and electrochemical oxidation of 2-hydroxyacridinone, the reference compound of antitumor imidazoacridinones✪

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The present work is part of a wide research project aimed to elucidate the mechanism of the metabolic activation of the antitumor imidazoacridinone agent C-1311 selected for clinical trials. The objectives of the investigations presented here were: (i) to examine the enzymatic transformation of the reference compound 2-hydroxyacridinone and (ii) to test the similarity between enzymatic and electrochemical oxidation of acridinone compounds. This similarity was searched with respect to the usefulness of the electrochemical results for further studies on the metabolic oxidation of imidazoacridinone antitumor drugs. The enzymatic oxidation of 2-hydroxyacridinone was performed with a model system containing various amounts of horseradish peroxidase and hydrogen peroxide and was followed by UV-VIS spectroscopy and by HPLC. One product of the reaction was isolated and its chemical structure was identified. It was shown that 2-hydroxyacridinone was transformed by the studied system in a manner dependent on the amount of the enzyme and on the compound/H_2O_2 ratio. While under mild reaction conditions the transformation ran slowly to yield only one product, p1, independently of the reaction time, higher enzyme concentration resulted in several steps of transformation. Product p1 turned out to be a dimer: 1,1-bi(2-hydroxyacridinone). A comparison of the results of the enzymatic transformations of 2-hydroxyacridinone presented here with studies on the electrochemical oxidation reported earlier allowed us to show both transformations to be similar as far as the reaction pathway and two reaction products are concerned.

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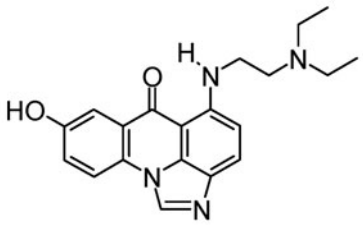
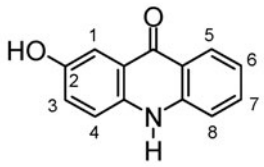
Abbreviations: HRP, horseradish peroxidase; Me_2SO, dimethylsulfoxide; ROESY, rotating frame enhanced spectroscopy; SPE, solid phase extraction.

2-Hydroxyacridinone is the reference compound of strongly active antitumor agents developed in our laboratory, imidazo- and triazoloacridinones (Cholody *et al.*, 1990a; 1990b; 1992; 1996). One imidazoacridinone derivative, compound C-1311, presented in Scheme 1 (Mazerska *et al.*, 1998a), was selected for phase I of clinical trials.



C-1311



2-hydroxyacridinone

Scheme 1.

We showed earlier that metabolic activation of imidazoacridinones is a prerequisite for their biological effects (Dziegielewski *et al.*, 1996; 1998). It was considered to be an important preliminary step in the biochemical mechanism of imidazoacridinone action. The metabolic transformation might facilitate or even enable direct reaction of imidazoacridinones with their targets such as DNA and proteins in the living cell (Mazerska *et al.*, 2001). Moreover, we have demonstrated that antitumor imidazoacridinones undergo oxidative activation with the model activation system of horseradish peroxidase and the ability to be transformed under such conditions is in reasonable correlation with the antitumor activity of these compounds (Mazerska *et al.*, 1998b).

At the same time, investigation into electrochemical oxidation of imidazoacridinones was undertaken (Mazerska *et al.*, 1997). We assumed that such studies can provide valuable insights into the oxidation reaction pathway of these agents, especially into the molecular mechanism of the oxidative metabolic activation of the imidazoacridinone drug C-1311 in the organism. However, we showed that electrooxidation of C-1311 was an irreversible process and that the reproducibility of the anodic region of the voltammogram was poor. Apparently, the anodic process was followed by a chemical reaction that led to filming and consecutive deactivation of the electrode surface (Cakala *et al.*, 1999). Therefore, electrooxidation of the reference compound 2-hydroxyacridinone presented in Scheme 1, which in contrast to C-1311 does not possess the imidazole ring and the aminoalkyl side chain, was carried out in organic aprotic solvents (Mazerska *et al.*, 1997) and in water (Mazerska *et al.*, 2002). We expected that, in the case of the reference compound, weaker deactivation of the electrode would occur because several amino groups were absent in this molecule in comparison with C-1311. In fact, the electrooxidation of the reference compound turned out to be an irreversible process like that of imidazoacridinones. Nevertheless, two main products of this reaction were isolated and their structures were identified. Furthermore, two pathways of 2-hydroxyacridinone electrooxidation were proposed and they were also discussed with respect to the metabolic activation of hydroxyacridinones in the organism. It was also suggested that electrooxidation might be a suitable method for the synthesis of adducts between antitumor hydroxyacridinones and DNA (Mazerska *et al.*, 2002).

In order to support the reliability of the above mentioned conclusions, which related to the transformation of 2-hydroxyacridinone in the organism but resulted directly from the studies on electrochemical oxidation of this compound, we undertook the studies presented in this paper. We aimed to verify that enzymatic and electrochemical processes of a wide group of antitumor acridinones are rele-

vant, as far as the reaction products and the molecular mechanism are concerned, considering that such a relevance has been reported in the case of other compounds (Chen & Dryhurst, 1982; Dryhurst, 1985; Hu & Dryhurst, 1997).

We investigated the reaction of enzymatic oxidation of 2-hydroxyacridinone in the model activation system, employed earlier (Mazerska *et al.*, 1998, 2001), of horseradish peroxidase/H_2O_2. The reactions were followed by UV-VIS spectroscopy and by HPLC. The structure of one main metabolite was determined by NMR spectroscopy. Finally, the spectra, chromatograms and the products of the studied horseradish peroxidase oxidation were compared with those observed earlier as a result of electrochemical oxidation (Mazerska *et al.*, 2002).

EXPERIMENTAL

Chemicals

2-Hydroxyacridinone was synthesised in our laboratory according to the method described earlier (Acheson, 1973). HRP (horseradish peroxidase, EC 1.11.1.7) and H_2O_2 were purchased from Sigma, and sodium mono- and diphosphate from Serva Feinobiochemica. Methanol (HPLC grade) was from Fluka (Buchs, Switzerland). All chemicals were used without prior purification. Ultra pure water, 18 MΩ, used in all experiments, was obtained with NANOpure water system, Barnstead (Dubuque, IA, U.S.A.).

HRP-mediated transformation of 2-hydroxyacridinone

Incubation mixtures containing 10^{-4} M 2-hydroxyacridinone and 0.5×10^{-4}–5×10^{-4} M H_2O_2 were prepared by dilution of stocks with 0.05 M phosphate buffer, pH 7.4, experiments were performed at room temperature. The reaction was initiated by the addition of an appropriate amount of the stock HRP solution to a final concentration from 0.0005 to 0.01 μg/ml.

Spectrophotometric analysis. Spectra of reaction mixtures were recorded in the range of wavelengths characteristic for acridinone chromophore (300–600 nm). Samples (2 ml) in 1 cm quartz cuvettes were placed in a LambdaBio UV-VIS spectrophotometer (Perkin-Elmer, Beaconsfield, U.K.) and the spectra were collected at 2, 4 and 5 min of the reaction.

HPLC monitoring of HRP-mediated transformations. The reaction mixture was prepared for HPLC analysis by solid phase extraction, SPE: 2–5 ml of the solution was passed through a Sep-Pak Classic C_{18} cartridge (Waters), 0.5 cm long. Subsequently, the cartridge was washed with 5 ml of water. After that the mixture of products was eluted with 0.5–1 ml of a 3:1 (v/v) methanol/water solution. The obtained product solution (5–20 μl) was analysed by a reversed phase analytical column, 0.4 × 25 cm (Spherisorb, ODS2, Jones, U.K.) with a Waters HPLC system equipped with a model 600K system controller, U6K pump system and 991 UV-VIS multidiode array detector (Waters-Millipore, U.S.A.). HPLC analyses were carried out at a flow rate of 1 ml/min with the following system: isocratic elution at 30% methanol in water for 5 min, followed by linear gradient from 30 to 45% methanol in water for 5 min, followed by isocratic elution at 45% methanol in water for 20 min, followed by linear gradient from 45 to 60% methanol in water for 10 min, followed by linear gradient from 60 to 100% methanol in water for 5 min.

Isolation and identification of the HRP-mediated oxidation product, p1

Seven-hundred and fifty milliliters of 10^{-4} M 2-hydroxyacridinone solution in 0.05 M phosphate buffer, pH 7, was incubated with 0.0005 μg/ml HRP and 10^{-4}M H_2O_2 at room temperature for 16 h. Then the incubation mixture

was concentrated by SPE: 50 ml of the solution was passed through a Sep-Pak Classic C_{18} cartridge (Waters). Subsequently, the cartridge was washed with 10 ml of water. After that the mixture of products was eluted with 2–5 ml of a methanol/water, 3:1 (v/v) solution. The eluate (0.7–1 ml) was injected onto an HPLC Waters instrument described above and was separated on a 1 × 25 cm Suplex pKb 100 reversed phase column (Supelco, Bellefonte, PA, U.S.A.) applying methanol/water, 65:35 (v/v), as the solvent under isocratic conditions, flow rate was 1 ml/min. The collected fractions of product p1 were lyophilized on a Freeze Dry System (Lymph, Lock 4.5, Labconco, U.S.A.) and the purity of the product was analyzed by HPLC under the conditions described above. The isolated product p1 is a bright yellow stable substance characterized by the retention time t_R=15.6 min and UV-VIS spectrum, λ_{max}: 262 nm, 430 nm. ^{1}H NMR (Me_2SO-d_6) exhibited signals at: δ 7.04 (dd, 1H, H-7, I_1=7.31 Hz, I_2=8.57 Hz); 7.28 (d, 1H, H-3, I=8.77 Hz); 7.36 (d, 1H, H-4, I=8.77 Hz); 7.45 (d, 1H, H-5, I=8.32 Hz); 7.53 (dd, 1H, H-6, I_1=7.31 Hz, I_2=8.32 Hz); 7.85 (d, 1H, H-8, I=8.57 Hz); 8.37 (s, 1H, OH); 11.20 (s, 1H, NH); after addition of D_2O the signals at 8.37 and 11.20 disappeared. ROESY (rotating frame enhanced spectroscopy) conjugated signals at 11.20 and 7.36 p.p.m. were observed. The NMR spectra were recorded on a VARIAN model 500 MHz Spectrometer.

RESULTS AND DISCUSSION

Spectrophotometric monitoring of HRP-mediated oxidation

Influence of the enzyme amount

The studies on enzymatic transformations of 2-hydroxyacridinone were begun with the reaction at an equimolar drug : H_2O_2 ratio at various concentrations of horseradish peroxidase. The results of spectrophotometric monitoring of these reactions are presented in Fig. 1. There are three sets of spectra taken during the incubation with 0.0005, 0.002 and 0.01 μg/ml of HRP. The spectra observed at the low concentration of the enzyme (Fig. 1A)

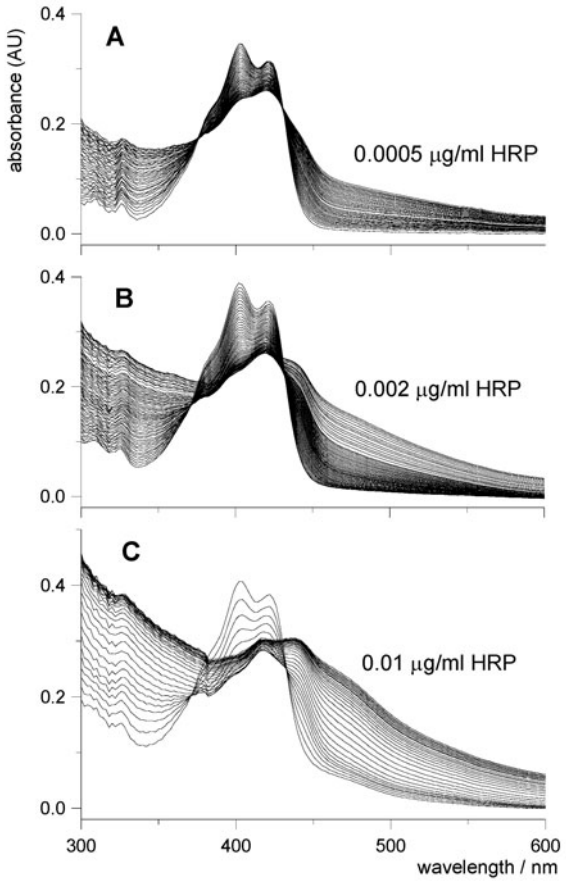


Figure 1. Absorbance spectra taken during the incubation of 10^{-4} M 2-hydroxyacridinone in 0.05 M phosphate buffer, pH 7.4, with equimolar amount of H_2O_2 and with HRP concentrations indicated in each panel.

The respective incubation times and the respective time intervals were the following: (A) 100/4 min; (B) 60/2 min and 40/4 min; (C) 80/2 min.

are characterised by two isosbestic points at 375 and 432 nm. At the higher concentrations of HRP (Fig. 1B and C) a new maximum at 436 nm and a wide band near 480 nm were observed, whereas those at 404 and 422 nm disappeared. Besides, no new isosbestic point was observed.

A direct comparison of the absorbance changes recorded during the reactions described above is presented in Fig. 2. There are two sets of curves, each of which relates to a

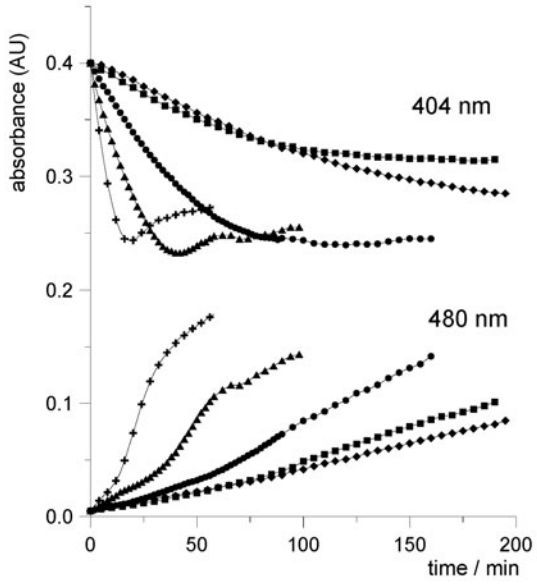


Figure 2. Time dependence of the absorbance at 404 and 480 nm during the incubation of 10^{-4} M 2-hydroxyacridinone with equimolar amount of H_2O_2 and various HRP concentrations: + 0.01 μg/ml; ▲ 0.005 μg/ml; ● 0.002 μg/ml; ■ 0.001 μg/ml and ◆ 0.0005 μg/ml.

different concentration of HRP and each set was collected at one wavelength value. One can notice that the slopes of the plots collected at two lower concentrations of HRP, 0.0005 and 0.001 μg/ml, are small and remain stable during the time studied. Higher enzyme concentrations, 0.005 and 0.01 μg/ml, resulted in higher rates of the absorbance changes in the first step of the reaction. These rates varied or even altered their signs in the next steps of this reaction as it was observed in the case of the plot at 404 nm.

In order to deepen the analysis of the absorbance changes observed during the incubation of 2-hydroxyacridinone with a high concentration of the enzyme, a set of plots collected at several wavelengths is presented in Fig. 3. There are, in addition, marked lines perpendicular to the time axis. They were drawn through the points at which the alter-

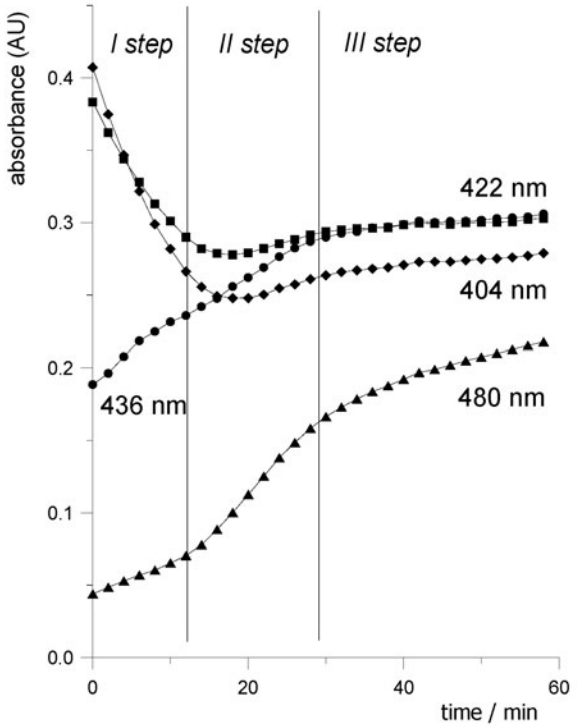


Figure 3. Time dependence of the absorbance at 404, 422, 436 and 480 nm during the incubation of 10^{-4} M 2-hydroxyacridinone with equimolar amount of H_2O_2 and 0.01 μg/ml of HRP.

ation of the plot rates occurred. In this way, the reaction time was divided into three steps. In the first step one product seemed to be formed in the reaction mixture, in the second at least one new appeared, whereas in the third step the reaction was running slowly to the plateau state.

Changes with different drug : H_2O_2 ratio

The influence of the drug : H_2O_2 ratio on the pathway of the enzymatic transformations of 2-hydroxyacridinone is illustrated in Fig. 4. There are sets of spectra recorded for the high amount of the enzyme, 0.005 μg/ml. The lowest drug : H_2O_2 ratio, 1 : 0.5, gave rise to two isosbestic points in the spectra set. Therefore, even at the high concentration of the enzyme, the low amount of the second substrate of this reaction, H_2O_2, resulted in only one product in the reaction mixture. At a 1:1 as well as 1 : 5 drug : H_2O_2 ratio more complicated sets of the spectra resulted. This led us to postulate that

more than one species was present in the reaction mixture at the higher drug : H_2O_2 ratios.

In order to support the latter conclusion, we analysed the course of the reactions by monitoring the absorbance changes with time. Figure 5 illustrates the dependence on H_2O_2

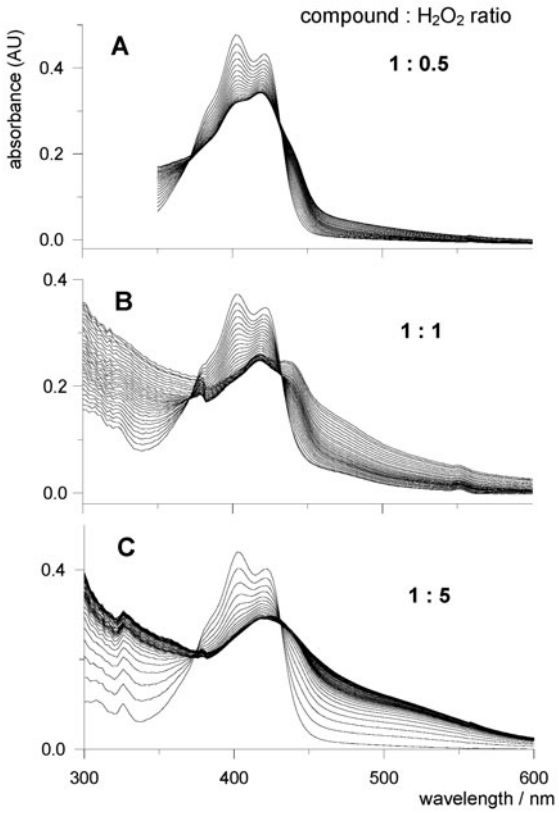


Figure 4. Absorbance spectra taken during the incubation of 10^{-4} M 2-hydroxyacridinone with 0.005 μg/ml HRP and the compound : H_2O_2 ratios indicated in each panel.

The respective incubation times and the respective time intervals were the following: (A) 100/4 min; (B) 60/2 min and 40/4 min; (C) 80/2 min.

amount of such plots collected at 404 and 480 nm. Single rate value of the absorbance changes was observed for the 1 : 0.5 drug : H_2O_2 ratio in the time range studied, whereas the 1 : 1 and 1 : 2 ratios resulted in at least three rate values in the same time range. However, it is surprising that at the 1 : 5 drug : H_2O_2 ratio only a single rate of the absorbance changes was noticed at both 404 and 480 nm. Moreover, it was the highest rate. Combining these results one can conclude that the low amount of H_2O_2 allowed the formation of only one product. In the case of the equimolar drug : H_2O_2 ratio and of the low excess of H_2O_2, the reactions ran in three steps. Finally, the presence of high excess of H_2O_2 with the high concentration of the enzyme probably induced such an intensive processes

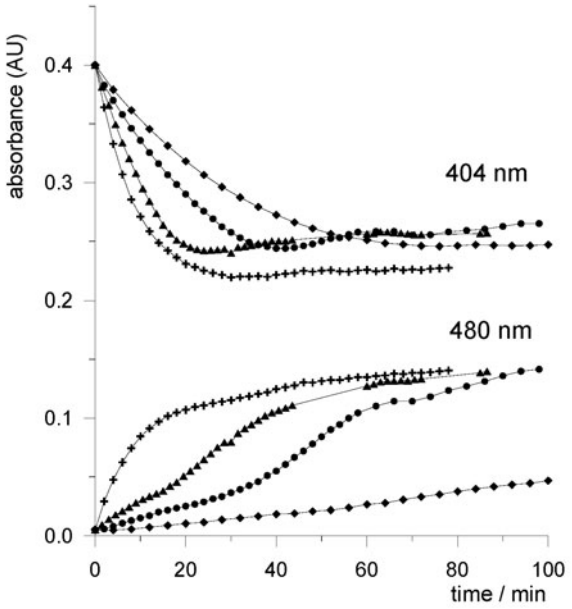


Figure 5. Time dependence of the absorbance at 404 and 480 nm during the incubation of 10^{-4} M 2-hydroxyacridinone with 0.005 μg/ml HRP and with compound: H_2O_2 ratios: + 1 : 5; ▲ 1 : 2; ● 1 : 1 and ◆ 1 : 0.5.

that we were not able to observe the separated steps of this reaction.

HPLC analysis of the reaction mixture

The results mentioned above gave us some general and preliminary knowledge about the products and the pathway of 2-hydroxyacridinone enzymatic oxidation. In order to identify the products and to be able to draw some conclusions about the mechanism of this transformation, we analysed the reaction mixture by means of HPLC. Such a chromatogram taken after an incubation of 2-hydroxyacridinone with the equimolar amount of H_2O_2 and with low concentration of HRP is presented in Fig. 6A. Two main

chromatographic peaks were found. They represent the substrate and one main product, named p1. It was observed that, in spite of the long incubation time, a considerable amount of the substrate remained in the reaction mixture and the amount of p1 was not high. Despite this finding, such a low concentration of HRP would be suitable for the synthesis of p1, because only this product was found under the conditions applied. Figure 6 contains also UV-VIS spectra of the substrate and of p1, which were drawn out directly from the recorded chromatogram. One can observe the spectrum of p1 being shifted towards longer wavelengths in comparison with that of the substrate. This indicates that a more extensive delocalised electron system exists in product p1 than in the substrate.

An HPLC analysis of the reaction mixture obtained with a high amount of the enzyme was also performed and is presented in Fig. 7A. Examination of the chromatogram let us notice, firstly, that the reaction mixture was much more complex than in the case of the low HRP concentration (Fig. 6A). Several new peaks representing new products of this reaction were observed (Fig. 7A), whereas the peaks of the substrate and of p1 disappeared. Selected spectra of the peaks named p2, p3 and p4 are shown in Fig. 7B. As can be seen, the spectrum of peak p3 differed significantly from that of the substrate, thus p3 is a result of a degradation of the acridinone heterocyclic moiety. On the other hand, the spectrum of p4 retained the absorbance in the visible region, however, the

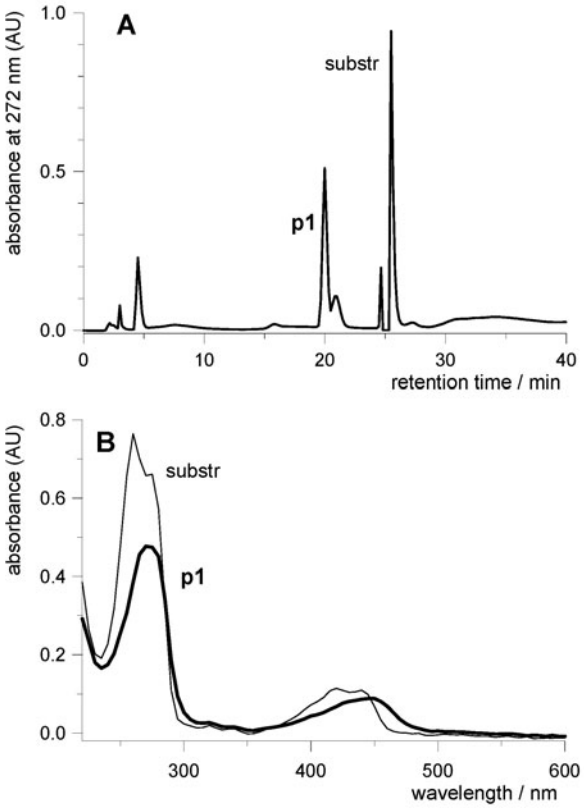


Figure 6. HPLC chromatogram (A) (reversed phase, methanol + water gradient system, 272 nm) taken after 16-h incubation of 10^{-4} M 2-hydroxyacridinone with 0.0005 μg/ml HRP and 10^{-4} M H_2O_2; and UV-VIS spectra (B) collected for HPLC peaks of substrate and p1.

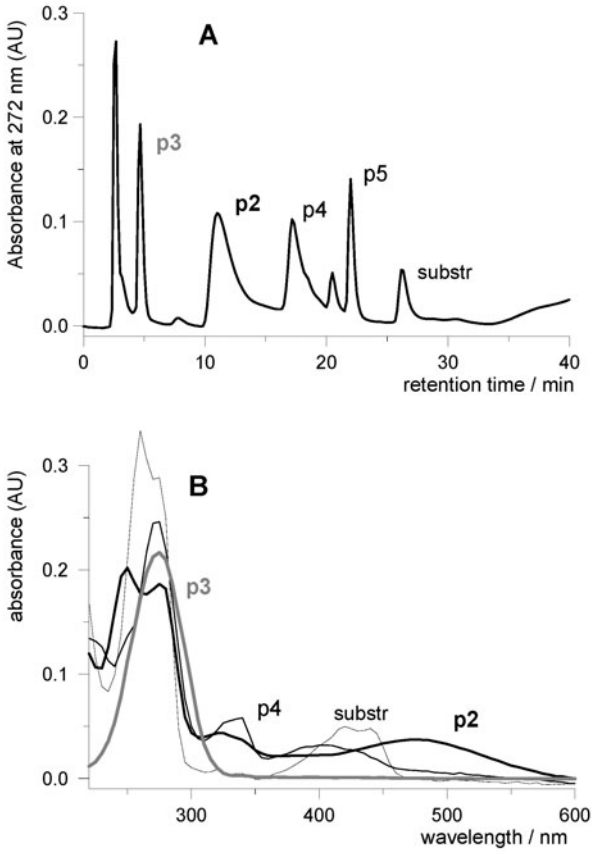
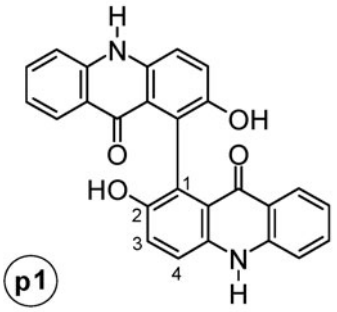


Figure 7. HPLC chromatogram (A) (reversed phase, methanol + water gradient system, 272 nm) taken after 16-h incubation of 10^{-4} M 2-hydroxyacridinone with 0.005 μg/ml HRP and 10^{-4} M H_2O_2; and UV-VIS spectra (B) collected for HPLC peaks of substrate and products p2, p3 and p4.

shape of the spectrum did not resemble that of the substrate. Probably the structure of this product is similar to that of 2-hydroxyacridinone. The spectrum of the product p2 was also far from that of the substrate displaying the specific broad band near 480 nm. This band indicates a notable alteration in the chromophore structure of p2.

Preparative synthesis and the structure of the p1 product

In order to propose the mechanism of the enzymatic oxidation of 2-hydroxyacridinone with respect to the metabolic transformation of antitumor imidazoacridinones, the synthesis of p1 was carried out. Searching for optimal conditions of the synthesis we took into consideration the fact that the amount of the product should be sufficient for NMR experiments. Considering the results presented above we performed incubation of 2-hydroxyacridinone with a low amount of HRP, 0.0005 μg/ml, and the equimolar drug : H_2O_2 ratio for 16 h. The reaction mixture was extracted by SPE and then the p1 fraction was separated by HPLC under preparative mode. A lot of HPLC fractions of the pure product were lyophilised. NMR studies on the p1 structure (described in the experimental part) identified it to be a dimer 1,1′-bi(2-hydroxyacridinone), the structure of which is presented in Scheme 2. Despite repeated attempts we failed to isolate any pure fractions of products p2 and p4. They were extremely unstable.



Scheme 2.

It should be noticed that a dimer structure, like that of p1, is quite common among the products of peroxidase mediated oxidation. It was obtained earlier as a result of, for instance, tyrosine (Michon *et al.*, 1997) or hydroxytryptamine (Favretto *et al.*, 1998) oxidation. Moreover, the dimer structure was also found in naturally occurring anthraquinones (Fujitake *et al.*, 1998).

Comparison of enzymatic and electrochemical oxidation of 2-hydroxyacridinone

The results described above draw our attention to a possible relevance of HRP-mediated oxidation presented here to the electrochemical oxidation reported for 2-hydroxyacridinone earlier (Mazerska *et al.*, 2002). A comparison of the two obtained results is shown in Figs. 8–10. The figures were prepared by com-

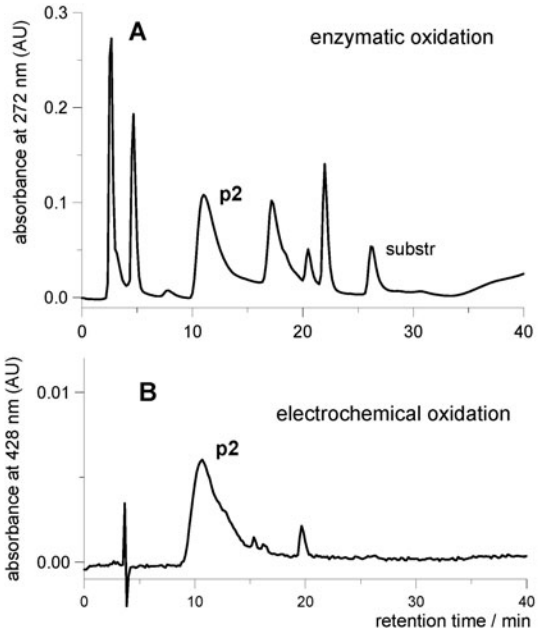


Figure 8. A comparison of the absorbance spectra taken during enzymatic (A) and electrochemical (B) transformation of 2-hydroxyacridinone.

Panel (A): The incubation mixture contained 10^{-4} M 2-hydroxyacridinone, 10^{-4} M H_2O_2 and 0.0005 μg/ml HRP in 0.05 M phosphate buffer, pH 7.4. Panel (B): spectrum set from Fig. 5 of (Mazerska *et al.*, 2002). The spectra were taken during 1 mV s^{-1} potential scan and were obtained for 0.55×10^{-3} M 2-hydroxyacridinone in 0.05 M, phosphate buffer pH 7.4,+ ethanol, 1 : 1 with 0.3 M $NaClO_4$.

bining the results presented in this paper with those presented earlier. Firstly, in Figs. 8A and 8B there are corresponding sets of UV-VIS spectra recorded during the two steps of the enzymatic and electrochemical oxidation, respectively. They are close to each other in their shape as well as in the presence of isosbestic points. Secondly, a comparison of the HPLC analyses of the enzymatic and electrochemical reaction mixtures is presented in Fig. 9. Although the chromatograms taken after incubation with the enzyme and after the electrolysis were not similar, the broad chromatographic band named p2 observed in both chromatograms was identical in character

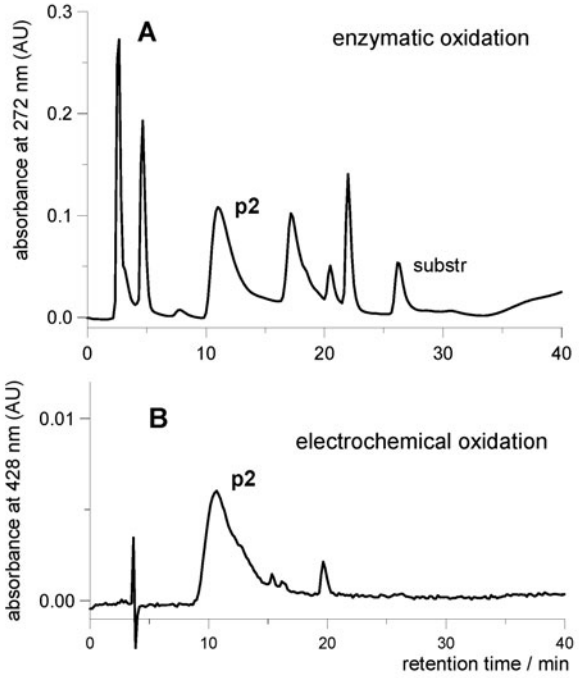


Figure 9. A comparison of HPLC analyses of the product mixtures obtained after enzymatic (A) and electrochemical (B) transformation of 2-hydroxyacridinone.

Panel (A): the chromatogram (reversed phase, methanol + water gradient system, 272 nm) obtained after 16-h incubation of 10^{-4} M 2-hydroxyacridinone with 0.005 μg/ml HRP and 10^{-4} M H_2O_2 in 0.05 M phosphate buffer, pH 7.4. Panel (B): the chromatogram from Fig. 8 of (Mazerska *et al.*, 2002) (reversed phase, methanol + water gradient system, 428 nm) obtained after 60 min of controlled potential electrolysis at 620 mV of 10^{-3}M 2-hydroxyacridinone solution in 0.05 M phosphate buffer, pH 7.4, + ethanol, 1 : 1 and 0.2 M $NaClO_4$.

and retention time. Likewise, the HPLC analyses of the enzymatic and electrochemical oxidation indicated the identity of products p1 obtained in both reactions (not shown).

In the next step of the presented comparison UV-VIS spectra of p1 and p2 are presented in Fig. 10. Two spectra are identical in both sets. The p1 spectrum is similar in shape to that of

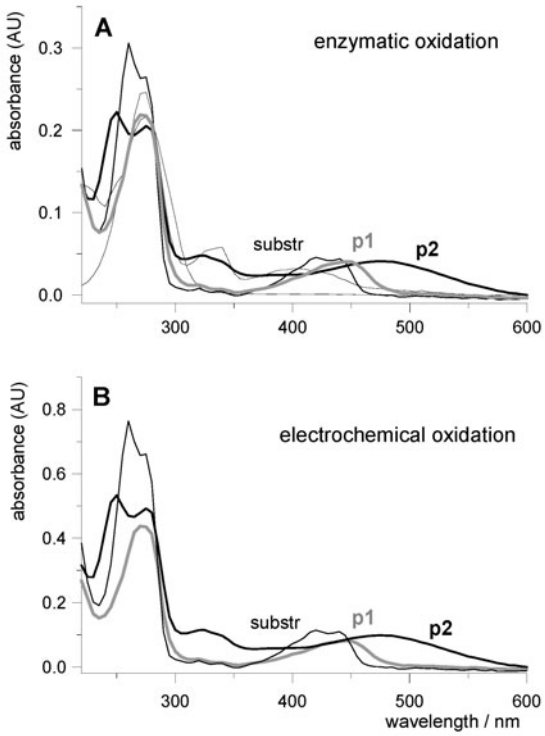


Figure 10. A comparison of UV-VIS spectra of the main products of enzymatic (A) and electrochemical (B) transformation of 2-hydroxyacridinone.

Panel (A) the spectra collected for the HPLC peaks presented in Fig. 9A. Panel (B) the spectra from Fig. 9 collected for HPLC peaks (Mazerska *et al.*, 2002).

the substrate but it is shifted to longer wavelength, whereas p2 displayed a spectrum with a broad band near 500 nm. Finally, a comparison of the NMR results obtained here for the p1 product of the HRP mediated oxidation with similar studies reported recently (Mazerska *et al.*, 2002) for p1 obtained electrochemically showed the p1 structure to be identical in both cases.

Combining the comparisons presented above we show that two products of the enzymatic and the electrochemical oxidation of 2-hydroxyacridinone are identical. The p1

product was identified to be a dimer (Scheme 1), whereas p2 was an orthoquinone derivative the structure of which was determined earlier (Mazerska *et al.*, 2002). One can add that several dimer-like products as well as orthoquinone-like products of enzymatic (Michon *et al.*, 1997; Favretto *et al.*, 1998) and electrochemical oxidation (Goyal, 1994, Massini *et al.*, 1997; Hapiot *et al.*, 1996) have also been found earlier for various biologically active compounds with aromatic hydroxyl groups.

CONCLUSIONS

The presented studies were aimed at the HRP-mediated oxidation of 2-hydroxyacridinone. We investigated: firstly, a model system of enzymatic activation in the living cell and secondly, the reference compound of antitumor acridinone agents. We also intended to find out whether exists any similarity between the enzymatic and electrochemical oxidation of 2-hydroxyacridinone, which would allow us to apply the electrochemical results to our further studies on the metabolic oxidation of the imidazoacridinone antitumor compound.

Summing up the results of HRP mediated oxidation, it was shown that this transformation ran slowly to yield one p1 product under mild conditions, 0.0005 μg/ml of HRP and equimolar drug: H_2O_2 ratio. This was observed spectrophotometrically and with the aid of HPLC and was confirmed by identifying the p1 structure. On the other hand, a higher enzyme concentration in the incubation mixture, 0.005 μg/ml resulted in several steps of the oxidation. The conclusion about the reaction stages was confirmed by HPLC analysis which showed several new products, whereas the amount of the first one, p1, was diminished in the reaction mixture.

The comparison of the enzymatic and electrochemical reactions showed that they are comparable with regard to the reaction pathway, as well as to the reaction products. The resemblance of the enzymatic to the electrochemical reaction pathway was shown by the spectrophotometric and HPLC analysis of the reaction mixtures and by the identification of the reaction products. Therefore, the mechanism of the electrochemical oxidation presented earlier (Mazerska *et al.*, 2002) may be transferred to the mechanism of the enzymatic oxidation of 2-hydroxyacridinone where it relates to the formation of product p1 and p2 at pH = 7.4. The product p1 was postulated there to be formed by one-electron oxidation of the substrate to the cation-radical. Then, recombination of two radicals should take place to yield the p1 dimer. The formation of p2, which was found to be an orthoquinone derivative, ran probably through dissociation of the substrate and two consecutive steps of one-electron oxidation followed by one step of two-electron oxidation.

In conclusion, the similarity demonstrated above of the enzymatic to the electrochemical oxidation of 2-hydroxyacridinone allows us to apply the electrochemical investigations to further studies on the mechanism of metabolic activation of the antitumor acridinone derivatives developed in our laboratory.

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