A C T A U N I V E R S I T A T I S L O D Z I E N S I S
FOLIA BIOCHIMICA ET BIOPHYSICA 2, 1983

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QUANTITATIVE ESTIMATION OF THE DAMAGE TO THE GAMMA IRRADIATED HAEMATIN IN AQUEOUS SOLUTIONS

The stationary radiolysis of 0.1 N KOH aqueous solutions of haematin was carried out in order to determine the effectiveness of the individual radicals. The samples were irradiated also in the presence of free radical scavengers. The radiosensitivity of haematin was determined under the investigated conditions and the radiation yield of haematin was also estimated. Results obtained for different radicals were compared. The effectiveness of hydrated electrons and hydroxyl radicals seems to be the highest, while the role of superoxide anion and the ethanol radical is rather small. In the presence of radicals of "opposite" nature, oxidizing and reducing, "reconstruction" reaction was observed.

Introduction

The influence of ionizing radiation upon haeme has usually been observed in order to determine the "so-called" process of "apoenzyme protective effect" for some of haemoproteins [10, 21]. Some works dealing with the kinetics of radiolysis of porphyrins had to be carried out under conditions protecting against aggregation, which was necessary for kinetic informations [20]. In these solvent mixtures the primary yield of radicals remained unknown. It was difficult, therefore, to determine the effectiveness of the individual radical. The present work is concerned mainly with the quantitative aspect of haematin radiolysis and the contribution of radicals to the destruction process. As the structure of porphyrins' solutions is still discussed we resigned to calculate the degree of aggregation, and expressed all data per gram atom of porphyrins' iron. The basic method applied in our experiments consisted of addition of free radical scavengers to the irradiated solutions, which caused limitation of chosen radicals down to values which could be neglected. Results of radiolysis performed in the presence of few radicals responsible for the final effect allowed to estimate the role of individual radicals concerning haematin destruction under given conditions.

Materials and methods

The reagents used were A.R.-grade, (POCh-Gliwice), without further purification. Solutions were prepared using bi-distilled water. Trade haematin (POCh-Gliwice) was purified according to the method of Shemin [25]. Haematin solutions were prepared by weight in 0.1 N KOH, and checked spectrofotometrically at 385 nm, after 15 minutes. The primary haematin concentration equalled $2 \cdot 10^{-3}$ M (E_{385} = 58 900), average standard deviation was about 5%. Samples were protected from light. The irradiation was carried out in an ionizing chamber by Co^{60} source at room temperature in an atmosphere of argon, air, oxygen, or nitrous oxide. Ethanol ($2 \cdot 10^{-2}$ M) and sodium formate (10^{-2} M) were applied as additional radical scavengers. The dose rate was 0.8 Mrad/h, the doses ranged from 0.1 to 4 Mrads. The absorbance was measured in 1 cm cuvettes after diluting samples with air-saturated 0.1 N KOH, using Specord UV-VIS and VSU-2p spectrophotometers (Carl-Zeiss, Jena).

Results and discussion

The irradiation caused an alteration of haematin spectrum, particularly significant in the Soret band. Spectra after, and before irradiation are shown in Fig. 1.

The percent of changed haematin was calculated from A_{385} value (absorbance at 385 nm). Because of aggregates which may be formed under studied conditions [6, 7, 8] the Lambert-Beer law do not apply in alkaline haematin solutions. However, deviations observed in the range studied did not exceed 20%, what would confirm hypothesis that an alteration of the molar extinction coefficient decreases for porphyrins' solutions for pH higher than 11 [7, 8]. Radiolysis of haematin solutions was performed in our experiments under conditions having well known primary radicals radiation yield. Results presented in Fig. 2 indicate that there is a significant dependence between conditions of radiolysis and degree of destruction.

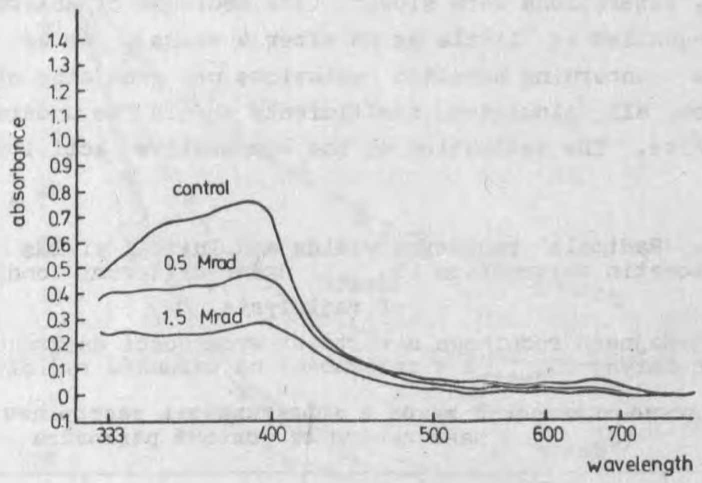

Fig. 1. Absorption spectra of haematin in the visible range for different doses of γ-radiation (air atmosphere)

Widma absorpcji hematyny w zakresie widzialnym dla różnych dawek promieniowania γ (w atmosferze powietrza)

Спектры поглощения видимого света гематином для разных доз γ-облучения (в атмосфере воздуха)

According to B u t l e r, E v e r s et al. [9, 17, 20] some radicals may react with haematin leading to phlorin derivatives, e.g.

$$Fe^{III}_{haem} \longrightarrow Fe^{II}_{haem} \longrightarrow \text{phlorin} + Fe^{II} \qquad (1)$$

As all these intermediates might have an appreciable absorption in the Soret band, we found experimentally, comparing spectra obtained for different doses of irradiation, that just 385 nm is the best wavelength for characterizing post-irradiated changes. The applied method was also quick enough to avoid the problem of slow spectral changes described as "ageing" of haematin solutions. The influence of fast processes as green complex forming [5] eliminated by waiting until was absorbance of samples was stable. Since the rate constant of autooxidation was equal to 5.6×10^{-4} min^{-1} [7] the influence of this reaction could be neglected. Our observations of haematin solutions stored for several weeks confirmed the results obtained by Brown et al., however, alterations were slower (the decrease of absorbance at 460 nm equalled as little as 6% after 4 weeks). As we expressed all data concerning haematin solutions per gram atom of porphyrins iron, all calculated coefficients should be understood to be relative. The estimation of the comparative activity of in-

Table 1

Radicals' radiation yields and initial yields of haematin destruction (G_{destr}) under different conditions of radiolysis

Wydajność rodnikowa a wartości wydajności destrukcji hematyny (G_{destr}) w zależności od warunków radiolizy

Радиационно-химический выход а эффективность деструкции гематина (G_{destr}) в зависимости от условий радиолиза

Conditions	Oxygen[1]	Air[1]	Air ethanol[2]	Air HCOONa[3]	N_2O[1]	Argon[1]	Argon HCOONa
G_{OH}	3.17	3.17	0	0	4.60	3.17	0
G_{O_2}	2.73	2.73	2.73	5.90	0.40	0	0
G_{Et}	0	0	3.17	0	0	0	0
$G_{e_{aq}}$	0	0	0	0	0	2.73	2.73
G^o_{destr}[4]	0.100	0.100	0.045	0.045	0.120	0.020	0.072

[1] Without additional scavengers added; [2] EtOH concentration equaled to 2×10^{-2} M; [3] HCOONa concentration equal to 0.01 M; [4] average standard deviation equaled to 10%

dividual radicals upon the presence of others is independent of the structure of solutions until structure differences caused by different conditions are negligible. The ratio of absorbances at 580 nm to 540 nm taken as a measure of complex formation [4, 5] did not depend significantly on the conditions applied and was 0.8 ±0.10. Radical radiation yield values summarised in Tab. 1 were taken from literature according to authors investigating the radiolysis of systems similar to ours [11, 12, 13, 14, 15, 16, 18, 19, 22].

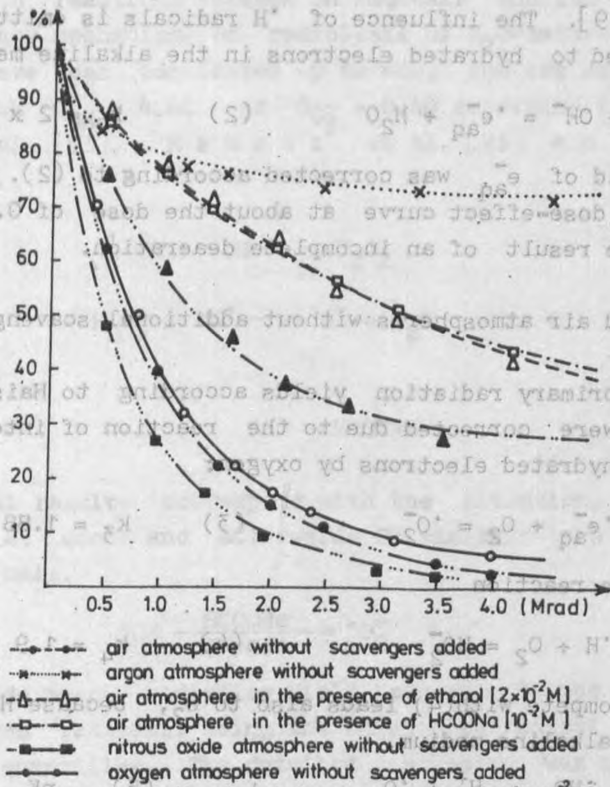

Fig. 2. The percent decrease of concentration of irradiated haematin solution as a function of the dose

Procentowy spadek stężenia napromieniowanych roztworów hematyny w funkcji dawki

Процентное понижение концентрации облученных растворов гематина в функции дозы

The values of haematin initial destruction yield presented in Tab. 1 were calculated from experimental data - slope of the doseeffect curves for a dose range of 0.2-0.4 Mrad, according to Fig. 2.

Conditions of radiolysis

Argon atmosphere without additional free radical scavengers

All data are based on results obtained by H a i s s i n s k y [18, 19]. The influence of ˙H radicals is omitted as they are converted to hydrated electrons in the alkaline medium.

$$\dot{H} + OH^- = \dot{e}_{aq}^- + H_2O \qquad (2) \qquad k_2 = 2 \times 10^7\ M^{-1}\ s^{-1}$$

The yield of e_{aq}^- was corrected according to (2). The shoulder on the dose-effect curve at about the dose of 0.1 Mrad is probably the result of an incomplete deaeration.

Oxygen and air atmospheres without additional scavengers added

Taking primary radiation yields according to Haissinsky, final yields were corrected due to the reaction of intensive scavenging of hydrated electrons by oxygen:

$$\dot{e}_{aq}^- + O_2 = \dot{O}_2^- \qquad (3) \qquad k_3 = 1.88 \times 10^{10} \ [19]$$

Possible reaction

$$\dot{H} + O_2 = HO_2^- \qquad (4) \qquad k_4 = 1.9 \times 10^{10} \quad [2]$$

which may compete with(4) leads also to O_2, because HO_2 dissociates in the alkaline medium.

$$\dot{H}O_2, \quad H^+ + \dot{O}_2^- \qquad (5) \qquad pK_5 = 4.88 \ [23]$$

and does not influence the final result either from the qualitative or quantitative point of view.

The irradiation in the atmospheres of oxygen and air was carried out in parallel in order to exclude the influence of nitrogen oxides which might be generated in air during irradiation. But such an influence was not observed.

N_2O atmosphere

The main reaction which occurred was the conversion of hydrated electrons to hydroxyl radicals.

$$\cdot e^-_{aq} + N_2O + H_2O = \cdot OH + OH^- + N_2 \qquad (6) \qquad [13]$$

According to many authors the concentration of OH radicals is high enough to form O_2 - either in the OH-OH recombination process or in reactions between OH radicals and its deprotonated form O^-. Many mechanisms of radiolysis of N_2O-saturated alkaline solutions have been considered up to now, and are still discussed. We took $G_{OH} = 4.60$ and $G_{O_2^-} = 0.40$ according to D a i n t o n et al. [13], R a b a n i et al. [23] and Z e h a v i et al. [26].

$$\cdot OH + \cdot OH \longrightarrow H_2O_2 \qquad (7)$$

$$H_2O_2 + \cdot O^- \longrightarrow \cdot O^- + H_2O \qquad (8)$$

Air + HCOONa

The final results correspond with the situation, where reactions (3), (4) occur and afterwards OH radicals are transformed to $\cdot O_2^-$ radicals.

$$\cdot OH \xrightarrow[\text{oxygen}]{\text{HCOONa}} \cdot O_2^- \qquad (9)$$

and superoxide anion radiation yield is equal to the sum of all primary formed radicals, being the only free radical present in significant quantities. The detailed discussion was presented by D r a g a n i ć et al. [16].

Air + ethanol

S e d d o n and A l l e n [24] found that final results of oxygen-saturated ethanol solutions to forming $\cdot O_2^-$ and ethanol radicals in reactions

$$e^-_{aq} \xrightarrow{O_2} \cdot O_2^- \qquad (10)\ [24]$$

$$\cdot OH \xrightarrow{EtOH} \cdot R_{ethanol.} \qquad (11)\ [24]$$

Argon + HCOONa

Hydroxyl radicals react very efficiently with formate ions

$$HCOO^- + \cdot OH = OH^- + H_2O + COO^- \qquad (12)$$

and $\cdot e^-_{aq}$ remains main active specie.

Calculations

The estimation of G_{destr} of haematin based on the definition of the destruction yield (the amount of molecules damaged by 100 eV of ionizing radiation energy) [1].

$$G_{destr} = \frac{10^9 \cdot \Delta c}{\Delta x}, \qquad (13)$$

where: c - the change of haematin concentration (M); Δx - the dose in rads which caused the change of concentration Δc.

In order to evaluate quantitatively the role of individual radicals in the radiolysis, the ratio of the yield of destruction caused by any radical to the yield of this radical was calculated

$$f = \frac{G_{destr}}{G^o}, \qquad (14)$$

where: G_{destr} - the radiation yield of destruction caused by the radical caused by the radical formed with the yield equaled G^o.

This magnitude was called in our work "the effectiveness coefficient" of an individual radical, because it indicate radiosensitivity of haematin to this radical. Under conditions where we can assume that the effect is caused by one radical only, equation

$$G_{destr} = f \cdot G^{o} \tag{15}$$

is true.

When results could be atributed to several species the total effect is equaled to the sum of destructions caused by all radicals, as far as inter-radicals interaction is negligible.

$$G_{destr} = \sum_{n} f_n \cdot G_n^{o}. \tag{16}$$

Based on eq. (16) approximate values of "f" may be determined if G_{destr} is known (in our experiments G_{destr} was using to relative calculations only, because we did not estimate percent of dimers in solution, and we did not know exactly therefore mollar concentration) - c (see eq. (13)). However results of calculations are independent on radiolysis conditions since present radicals are all oxidizing, (or all reducing, e.g. results obtained in systems: Argon + HCOONa and Argon + EtOH are the same).

Only experiments with argon-saturated solutions lead to different data (see Tab. 2).

Table 2

Values of the effectiveness coefficients of haematin destruction calculated from the values of G_{destr} obtained in the presence of radicals having similar nature

Wydajność aktywności destrukcyjnej wobec hematyny wykazywana przez rodniki w warunkach obecności rodników innych, ale o tej samej naturze

Эффективность деструкции гематина вычисленные на основе значений G_{destr} полученных в присутствии радикалов сходной природы

Radical	$^{\cdot}OH$	$^{\cdot}\bar{e}_{aq}$	$R_{ethanol}$	$^{\cdot}O_2$
Effectiveness coefficient	0.0255	0.0264	0.0076	0.0076

Conclusions

1. Application of mathematician methods may allow to determine some parameters, like radicals' activity to many compounds, or radiation destruction yield - in easy way. In that work we estimated mainly relative values, however it should be possible to found also absolute data, by comparing our results with the data concerning structure of investigated solutions.

2. Radiosensitivity is different often to various rádicals. For haematine alkaline solutions sensitivity to radicals formed during water radiolysis is the highest in the case of hydrated electrons and hydroxyl radicals, while the role of superoxide anion seems to be rather slight ($^{\cdot}\bar{e}_{aq}$: $^{\cdot}OH$: $^{\cdot}O_2^-$ = 1.00 : 1.00 : : 0.29).

3. Similar results obtained for systems: HCOONa-water, and EtOH-water in the presence of oxygen may indicate that ethanol radicals, usually reducing species are converted in oxygen-saturated solutions into oxidizing radicals.

4. Very slight destruction effect observed in system argon--water, without additional scavengers added suggests fast interaction between present species formed in the course of radiolysis, because radicals like hydrated electrons or hydroxyl radicals usually react with haematin very efficiently. The effect described above may be due to fast "reconstruction" processes of haematin, reducing in one step and then being reoxidizing.

5. Generation of nitrogen oxides, under given condition (air atmosphere, dose rate of 0.8 Mrad/h) was not observed.

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OCENA ILOŚCIOWA DESTRUKCJI NAPROMIENIONYCH PROMIENIAMI γ WODNYCH ROZTWORÓW HEMATYNY

W pracy prowadzono radiolizę roztworu hematyny w 0,1 N wodnym KOH. W celu oszacowania promienioczułości hematyny wobec poszczególnych rodników zastosowano dodawanie do napromienianego roztworu zmiataczy wodnych rodników. Analizując otrzymane wyniki określono wielkość wydajności radiacyjnej destrukcji hematyny, liczonej na gramoatom żelaza porfirynowego, a także określono promienioczułość hematyny w badanych warunkach. Porównanie wpływu różnych rodników, powstających w procesie radiolizy wody wskazuje, że w alkalicznych warunkach największe niszczące działanie na hematynę wykazują: rodniki hydroksylowe i elektrony uwodnione, podczas gdy wpływ anionorodników ponadtlenkowych i rodników etanolowych jest nieznaczny. Wydajność radiolizy szczególnie malała, gdy w środowisku znajdowały się jednocześnie rodniki o utleniającym i redukującym charakterze, co może być spowodowane zachodzeniem szybkich procesów "rekonstrukcji".

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КОЛИЧЕСТВЕННАЯ ОЦЕНКА ДЕСТРУКЦИИ
γ-ОБЛУЧЕННЫХ ВОДНЫХ РАСТВОРОВ ГЕМАТИНА

В работе определяли эффективность отдельных радикалов в процессе радиолиза раствора гематина 0,1 н КОН, применяя метод "сметателей" свободных радикалов.

Установлено радиочувствительность гематина в исследуемых условиях и радиационно-химический выход деструкции гематина. Сравнивались результаты, полученные для отдельных радикалов. Эффективность гидратированного электрона и гидроксильного радикала является самой высокой, в то время как влияние супероксидного и этанолового радикалов является относительно небольшим.