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The 4-hydroxyestrone: Electron emission, formation of secondary metabolites and mechanisms of carcinogenesis

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Abstract

4-Hydroxyestrone (4-OHE₁), a typical cancer-inducing metabolite, originating from 17 β -estradiol (17 β -E2), was chosen as a model for the studies. The aim was to get a deeper insight in the mechanisms of its ability to initiate cancer. It was found, that 4-OHE₁ can eject electrons (e_{aq}^-), when excited in the singlet state by monochromatic UV-light ($\gamma = 254$ nm) in polar media (water:ethanol = 40:60 vol.%). The quantum yield $Q(e_{aq}^-)$, determined for various 4-OHE₁ concentrations, is found to be as high as that previously observed for 17 β -E2. It decreases with increasing substrate concentration, but it is enhanced at higher temperature. The ability of 4-OHE₁ to eject as well as to consume and to transfer electrons to other biological systems, classifies it as an electron mediator, similar to 17 β -E2.

The 4-OHE₁ transients resulting of the electron emission process are leading to the formation of secondary metabolites. Surprisingly, it was established that the secondary metabolites possess likewise the ability to eject as well as to consume electrons. Hence, they behave similar like 17 β -E2. However, the structure of the secondary formed metabolites, which determinates their biological properties and carcinogenity, depends on the nature of the available reaction partners involved in their formation. A probable reaction mechanism explaining the subject matter is discussed.

Keywords

4-Hydroxyestrone (4-OHE₁); Electron emission from 4-OHE₁; Secondary metabolites; Reaction mechanisms of carcinogenity

1. Introduction

Based on experimental evidence, 17 β -estradiol (17 β -E2) is blamed for the initiation of breast cancer [1-8]. Almost all of its A-ring metabolites exhibit two different biological actions on cell proliferation: at low concentrations (10^{-8} – 10^{-6} mol/L) 17 β -E2 stimulates, but at higher concentrations ($>10^{-5}$ mol/L) it inhibits the proliferation of cultured cells [3]. This effect was not observed for D-ring metabolites with exception for 16 α -hydroxyestrone. At higher 17 β -E2 concentrations, the metabolites: 2-hydroxyestrone, 2-hydroxyestradiol, 2-

hydroxyestriol, 4-hydroxyestrone and 4-hydroxyestradiol caused significant inhibition of the cell proliferation. Recent studies confirmed the previous findings that estrogens play a major role in promoting the proliferation of both, normal as well as the neoplastic breast epithelium cells [6,7]. It has been also found that patients with fibrocystic diseases are at elevated risk for developing breast cancer. Both, 16 α -hydroxyestrone and 4-hydroxyestrone are reported carcinogens [9,10]. Based on the above briefly reported results, it seems that estrogen metabolites obviously influence estrogen-target tissues and modulate estrogen-associated cancer disease by the mentioned metabolites.

In order to learn more about reaction mechanisms responsible for the specific behaviour of some estrogen metabolites initiating breast cancer, 4-hydroxyestrone (4-OHE₁) was chosen as a model for the present studies. In this respect it should be mentioned that 17 β -E2 as well as progesterone (PRG) [11] and testosterone (TES) [12] can emit as well as consume

“solvated electrons” (e_{aq}^-) in polar media and transfer them to other compounds. The same biological property has also been established for genistein [13] and adrenaline [14]. This capability of the hormones to act as electron mediator enables them to communicate with other biological systems [15]. The hormone transients resulting from the electron emission processes, e.g. the progesterone radical cation PRG^{•+}, can be regenerated in the presence of an appropriate electron donor [11]. The other types of hormone transients, such as phenoxyl type of ring A in the case of 17 β -E2, are leading to the formation of several metabolites. In the case of 17 β -E2, the electron ejection occurs from the π -electron structure of the A-ring resulting in the formation of a phenoxyl type radical [11]. It has several mesomeric structures, which enables the formation of various metabolites. As mentioned above, some of them can promote the proliferation of normal cells and other may initiate cancer, as it is the case of 4-OHE₁.

Therefore, the aim of the present investigations was besides studying the electron emission of 4-OHE₁, to investigate the fate of the resulting transients, their possible ability to form secondary metabolites and the behaviour of those, as well as the involved reaction mechanisms leading to carcinogenesis.

2. Materials and methods

A low pressure Hg-lamp (Osram HNS 12 W) with incorporated Vycor-filter for removal of the 185 nm line was used. The solutions were irradiated with a monochromatic UV(C)-light of 254 nm (4.85 eV/hm) in a special, double-wall 4 π -irradiation apparatus connected with a thermostat [16].

During the irradiation, the temperature was kept constant at 37 ± 1 °C or at 45 ± 1 °C. The intensity of the monochromatic lamp emission was determined at 254 nm ($I_0 = 1 \times 10^{18}$ h ν mL⁻¹ min⁻¹) by implementing chloroacetic acid actinometer [17].

The “solvated electrons” (e_{aq}^-), ejected from 4-OHE₁ were scavenged by chloroethanol (1×10^{-2} mol/L), where $k(e_{aq}^- + ClC_2H_4OH) = 2 \times 10^8$ L mol⁻¹ s⁻¹ [18]. The absorption of the UV-light by chloroethanol under the given conditions is negligible, however, it was taken into account. The resulting yield of Cl⁻ was determined by mercury (II) thiocyanate method [19], where $Q(Cl^-) = Q(e_{aq}^-)$.

The substrate, 4-OHE₁, as well as all chemicals used, were of highest purity available (Fluka–Aldrich). Since 4-OHE₁ is not soluble in water, a mixture of 40 vol.% water and of 60 vol.% ethanol was used as a solvent. It might be mentioned that the polarity of the

solvent plays an essential role in the electron ejection process [20]. Therefore, the water content in the solvent mixture is of crucial importance because its polarity is much stronger (dielectric constant, DC = 80) than that of ethanol (DC = 24.3).

3. Results and discussion

In the frame of the previous studies [11-15] it was stated that the above mentioned hormones (17 β -E2, PRG, TES, etc.) can eject electrons (e_{aq}^-) from their excited singlet states. On the other hand, their molecules in the ground state react with e_{aq}^- ($k = 10^9$ up to 10^{10} L mol $^{-1}$ s $^{-1}$) and consume a part of them. The probability of this reaction depends on the substrate concentration, on the reaction rate constant as well as on the nature of the solvent.

In order to enclose the multifunctional behaviour in the present studies, various substrate concentrations (1×10^{-5} up to 7.5×10^{-5} mol/L 4-OHE $_1$) were used.

As known, the electron emission process increases with rising the temperature at the expense of the fluorescence yield [20]. Hence, in order to verify this behaviour, the electron emission of 4-OHE $_1$ was investigated at two temperatures, 37 and 45 °C. The initial quantum yield of the ejected e_{aq}^- , $Q(e_{aq}^-)$, were calculated from the tangent of each curve (Fig. 1-4) in respect to the absorbed UV-quanta ($h\nu$ /mL) (formula (1)):

$$Q(e_{aq}^-) = [N(e_{aq}^-) \times N(A)] / [I_{ab} \times 10^3] \quad (1)$$

where $N(e_{aq}^-)$ = number of ejected electrons (mol/L); $N(A)$ = Avogadro constant (6.0221×10^{23} mol $^{-1}$); I_{ab} = absorbed UV-quanta/L ($I_{ab} \times 10^3$) and L = litre (1000 mL).

In Fig. 1, the yield of the ejected electrons (e_{aq}^- , mol/L) from 10^{-5} mol/L 4-OHE $_1$ at 37 °C is presented as a function of the absorbed monochromatic UV-quanta. The presented data are mean values of five individual series of experiments with a standard deviation of about 10%. Unexpectedly, the electron emission appeared as two sharp peaks. This indicates that two consecutive electron emitting processes were involved. The calculated initial quantum yields of both peaks are: (A) $Q(e_{aq}^-) = 2.1 \times 10^{-2}$ and (B) $Q(e_{aq}^-) = 0.3 \times 10^{-2}$ given as an insert in Fig. 1. The (e_{aq}^-) -yield of peak (A) is very similar to the previously reported of 17 β -E2, $Q(e_{aq}^-) = 1.76 \times 10^{-2}$ [11].

The electron emission expressed by two peaks in respect to the absorbed UV-quanta at 37 °C was also observed at higher substrate concentrations. Fig. 2 shows this effect for 5×10^{-5} mol/L 4-OHE $_1$. The corresponding electron yields are somewhat lower: for peak (A) $Q(e_{aq}^-) = 1.20 \times 10^{-2}$ and (B) $Q(e_{aq}^-) = 0.1 \times 10^{-2}$ as illustrated in Fig. 2 in insert.

Using 7.5×10^{-5} mol/L 4-OHE $_1$ in addition to peaks (A) and (B), a third peak appears at higher absorbed UV-doses ($\sim 12 \times 10^{18}$ $h\nu$ /mL).

The observed peaks indicate the formation of secondary metabolites. It is surprising that they are also able to emit electrons. From our previous studies [20] is known that in such case the π -electron structure of ring A of the molecule is the main source of electron ejection. Hence, ring A in the secondary metabolite obviously remains preserved. This effect is of biological importance, because in the formation of secondary metabolites exists the possibility of incorporation of carcinogenous components.

Preliminary HPLC-analyses of the UV-irradiated samples at various UV-doses and of various substrate concentrations showed that several products are formed, however, with very small yields. This indicates that the ring skeleton of the substrate-molecule remains more or less unchanged. The reaction occurs mainly on the periphery of the molecule. The transients resulting from 4-OHE₁ as a sequence of the electron emission may scavenge free radicals available in the present medium, leading to the formation of new types of secondary metabolites.

For illustration of the subject matter, the following reaction mechanism is suggested: reaction (2) demonstrates the electron ejection from the excited 4-OHE₁ by UV-light. In organism, this process is realized by energy transfer through biological processes. The scavenge of (e_{aq}^-) by chloroethanol under Cl^- formation is shown by reaction (3).

Under the given conditions, the 4-OHE₁ radical ($R_1^\bullet$) and the ethanol radical ($R_2^\bullet$) can react and result in a new metabolite (M1). Naturally, the biological properties of M1 will be entirely different from those of the starting one (4-OHE₁).

Reaction (5) demonstrates a pathway for the formation of secondary metabolites under the present experimental conditions. However, following this pathway, other free radicals ($R_x^\bullet$) available in the media can lead to the formation of metabolites with characteristic biological properties. If the radical $R_x^\bullet$ originates from a heterocyclic compound (e.g. pyrene, etc.) carcinogenic metabolites might be formed.

The biological attributes of the secondary metabolite, M1, are very likely contingent on environmental conditions, life habits of the person, such as nutrition and may have distinct structures favouring carcinogenesis. The M1-metabolite is on the strength of its molecular structure able to eject likewise electrons, similarly to other biological substances [12,21,22]. The observed electron emission characterized by peak (B) in Figs. 1 and 2 is attributed to a process originating very likely from metabolite M1 reaction (5), but also of radicals originating of 4-OHE₁ photolysis (formation of dimers). Obviously, this depends on the substrate concentration and on the specific reaction rate constant (k) of the involved reaction partners.

It was also stated that with increasing 4-OHE₁ concentration, the $Q(e_{aq}^-)$ -yield decreases, as shown in Figs. 1 and 2 for both peaks (A) and (B). This effect was also previously observed for other investigated hormones [11-15]. It was attributed to several factors: (i) formation of hormone associates (unstable complexes) in the solvent mixture, where the substrate molecules in ground state consume a part of the ejected e_{aq}^- . Hence, only a fraction can diffuse away and is scavenged by chloroethanol (reaction (3)) (ii) another part of the emitted e_{aq}^- reacts with the well dissolved substrate molecules. The reaction rate constant of the above mentioned hormones are in the range of $k = 10^9 - 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$ [11-14] (iii) the =CO group on the 17-position of the 4-OHE₁ molecule acts as an efficient electron scavenger $k \sim 1.6 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ [23]. Therefore, a further part of the emitted electrons can be consumed by reacting with this group. The importance of this process is proportional to the substrate concentration and can initiate the formation of further types of metabolites possibly by reactions (6a)–(6c):

As a sequence of the e_{aq}^- attack, the resulting transient with the very reactive =CO⁻ group can subsequently scavenge H⁺, another radical present in medium (R_x) or eventually undergo hydrolysis, as shown by reaction (6c).

The significance of reaction (6) is demonstrated by the appearance of the further peak (C) by using of somewhat higher substrate concentration and application of a higher UV-dose, as shown in Fig. 3.

Naturally, the electron ejection process is resulting into a transient (degradation species; see reaction (2), which subsequently is leading to a metabolite (final product; reactions (5) and (6)) of the process.

Preliminary HPLC-analysis of UV-irradiated 4-OHE₁ samples showed that at a low UV-dose ($\sim 0.3 \times 10^{18} \text{ h}\nu/\text{mL}$) the formation of just one metabolite is observed. At higher UV-doses, however, in the addition to the formation of the second peak, also the substrate and the metabolite are successively decomposed, resulting into simpler molecules.

Since the skeleton of the 4-OHE₁ molecule remains more or less unchanged under relative low UV-doses and the reaction takes place on the “periphery” of the molecule (reactions: (2)-(4) and (6)), several metabolites can be formed. The peak (C) in Fig. 3 represents the electron emission of metabolites resulting probably from the transients shown by reaction (5). The observed $Q(e_{aq}^-)$ -yield was lower as expected.

As previously stated, the electron emission process, fluorescence, etc., depends on the temperature [20]. This effect was also observed in the case of other hormones [13,14]. Hence, for the sake of completeness, the electron ejection of 4-OHE₁ was verified at 45 °C. The results are presented in Fig. 4. The obtained quantum yields of e_{aq}^- for peak A and B are higher compared to those in Fig. 1 for the same substrate concentration, but at 37 °C.

It is worth mentioning that the pH of the media decreases (formation of H⁺) simultaneously with the increase of the e_{aq}^- -yield (reaction (1)). This gives a hint that the OH-groups are involved in the electron ejection process, as stated previously [20]. The effect is shown for 37 and 45 °C in Fig. 5. The pH-change as a consequence of the absorbed quanta (hν/mL) does not show any peaks, but is linear, which indicates that the electron ejection proceeds continuously during the UV-irradiation.

4. Conclusion

4-Hydroxyestrone (4-OHE₁) is a carcinogenic metabolite originating from 17β-E2 hormone. In order to get a deeper insight in the reaction mechanisms of its biological properties, especially in respect to cancer, adequate investigations were performed. The obtained results are summarized as follows:

- 4-OHE₁, similar to 17β-E2, is able to emit electrons from its excited singlet state. The quantum yield $Q(e_{aq}^-)$ is nearly the same like this of 17β-E2 and decreases with increasing substrate concentration, but enhances with temperature.
- The 4-OHE₁ transient derived from the electron ejection process is leading to the formation of a secondary metabolite by reaction with the present $\cdot C_2H_4OH$ radical. This fact indicates, that depending on the life habits of a person, a variety of metabolites can be formed, also such initiating cancer.
- This is likewise able to emit electrons, as expressed by peak B (see Figs. 1-4).
- The appearance of a free radical on 17-position of 4-OHE₁-molecule, resulting by a specific reaction of the =CO group with a part of the emitted electrons, leads to the formation of a further type of metabolite, which also emits electrons and which is expressed by peak (C), Fig. 3.
- The pH-decrease as a consequence of the absorbed UV-dose elapses linearly. This indicates a permanent e^- -emission from the excited 4-OHE₁ and subsequently from the secondary metabolites, naturally in competition with the electron consuming processes, mentioned in the text.
- The accomplishment of the observed peaks is a result of opposite acting processes: electron emission on the account of substrate degradation, self-consumption of e^- within the substrate, competing with the formation of the secondary metabolite, which likewise is able to eject electrons. All these processes interact with each other with different reaction rate constants and in dependence of the respective concentration of the reaction partners at given temperature.

- Summing up it can be stated that the involved reaction mechanisms of the sexual hormone 17 β -E2 and its various metabolites are rather complicated. The carcinogenicity of the in each case formed metabolite depends on the available reaction partners in the organism. This means that environmental conditions, nutrition, living habits, etc. can provide reaction partners favouring the generation of secondary, cancer initiating metabolites.

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Fig. 1.

Yield of ejected electrons (e^-_{aq} , mol/L) at 37 °C from 1×10^{-5} mol/L 4-OHE₁ in airfree solution (40 vol.% water and 60 vol.% alcohol) in neutral media as a function of absorbed UV-quanta (hv/mL).

Fig. 2.

Yield of ejected electrons (e_{aq}^- , mol/L) at 37 °C from 5×10^{-5} mol/L 4-OHE₁ in airfree solution (40 vol.% water and 60 vol.% alcohol) in neutral media as a function of absorbed UV-quanta (hv/mL).

Fig. 3.

Yield of ejected electrons (e^-_{aq} , mol/L) at 37 °C from 7.5×10^{-5} mol/L 4-OHE₁ in airfree solution (40 vol.% water and 60 vol.% alcohol) in neutral media as a function of absorbed UV-quanta (hv/mL).

Fig. 4.

Yield of ejected electrons at (e^-_{aq} , mol/L) 45 °C from 1×10^{-5} mol/L 4-OHE_I in airfree solution (40 vol.% water and 60 vol.% alcohol) in neutral media as a function of absorbed UV-quanta ($h\nu/mL$).

Fig. 5. pH-change as a function of the absorbed UV-dose ($\text{h}\nu\text{/mL}$) using $1 \times 10^{-5} \text{ mol/L}$ 4-OHE₁ in 40 vol.% water and 60 vol.% ethanol, airfree: (A) at 37 °C and (B) at 45 °C.