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New Findings Concerning the Mutual Action of Hormones and Receptors

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Abstract. *The actual mechanisms concerning the role of the hormone-receptor complex cannot satisfactorily explain the various hormone activities. Photobiological studies were performed in order to gain a deeper insight in this respect. 17β-estradiol (17βE_2) was used as representative hormone and methionine-enkephaline (ME) was used as an adequate model for a receptor. Their biological behaviors and mutual interactions were investigated in air-free media (pH~7.4; 37˚C) by excitation in singlet state, using monochromatic UV-light (λ=254 nm; E=4.85 eV/hν). It was found that tyrosine (Tyr) as a main component of ME, as well as ME itself, can eject solvated electrons (e_{aq}^-), when excited in singlet state. The observed quantum yields, Q (e_{aq}^-), in both cases decreased with an increase of the corresponding substrate concentration. The effect is explained by the formation of associates (unstable complexes of molecules prevailing in the ground state), which consume a proportion of the emitted e_{aq}^-. The ME transients, resulting from the electron emission, can partly regenerate by electron transfer from an efficient electron donor, e.g. ascorbate. 17βE_2, like other hormones, can also eject electrons under the same experimental conditions. In a mix of 17βE_2 and ME in air-free media (40/60 water/ethanol, pH~7.4; 37˚C), a mutual electron exchange takes place. Thereby 17βE_2 transients, being in status nascendy state, can partly regenerate by electron transfer from ME. Thus, the duration and action of 17βE_2 are prolonged. To our knowledge this fact is reported for the first time and it is a finding of basic biological and medical importance.*

There are various types of hormone receptors, which differ in their structure, location in the cell, as well as in their action. Generally it is assumed that receptors activate the action of the respective hormone in a particular cell. Being in the blood-stream, hormones can reach all tissue cells, but the hormone function is specific to a given kind of cell. According to current biological theories, the entire hormone action occurs only after complexing with the receptor. It was experimentally proven that: (i) Hormones can eject solvated electrons (e_{aq}^-) in polar media (1-3); (ii) the ejected e_{aq}^- have a frequency specific to the emitting hormone molecule and preserving the frequency by fast electron transfer *via* the brain can communicate with other biological systems in the organism. Thus the formation of a hormone receptor complex seems not to be necessary in all cases (4). (iii) The hormone transients resulting from the electron emission can, under given conditions, lead to the formation of metabolites initiating cancer (5, 6). (iv) The hormone transients during their life time (nascendent state) can, at least partly, be regenerated by electron transfer from a potent electron donor, *e.g.* vitamin C and enzymes (7, 8).

Considering recent experimental results, which are partly in contradiction with the actually accepted mechanisms of hormones as mentioned above, it appeared of interest to investigate the action mechanisms of the hormone-receptor complex. Thereby in this study 17β-estradiol (17βE_2) was applied as a typical representative hormone and methionine enkephaline (ME) as a representative of receptors.

Materials and Methods

All chemicals were of the highest purity available (Fluka-Aldrich; Merck) and were used as obtained. Depending on substrate solubility the samples were either dissolved in air-free triply-distilled water or in a mixture of water/ethanol (40/60 v/v) at pH~7.4; 37˚C. As a source for monochromatic light (λ=254 nm; E=4.85 eV/hν), a low pressure Hg-Lamp (HNS 12, OSRAM, 12 Watt) with incorporated VYCOR filter for elimination of the 185 nm line in a 4π-geometry irradiation vessel was used (9). For maintaining a constant temperature during the experiment, the apparatus was connected with a thermostat. The lamp intensity (I_0=1×10^{18} hν ml^{-1} min^{-1}) was determined by monochloric acetic acid as actinometer (10). The emitted e_{aq}^- from the actinometer were scavenged by 1×10^{-2} mol/l chlorethanol, where:

$$e^-_{aq} + ClC_2H_4OH \rightarrow Cl^- + {}^{\cdot}C_2H_4OH \quad (k=6.9\times10^9\ l\ mol^{-1}\ s^{-1})\ (11) \qquad \text{(Eq.1)}$$

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The produced Cl- ions were determined spectrophotometrically (12).

$$\text{Hence: } Q(e^-_{aq}) = Q(Cl\text{-}) \qquad \text{(Eq.2)}$$

HPLC analysis was performed before and after UV-irradiation of the systems [Hewlett-Packard model 1046/1050; programmable detectors for absorption (HP 1100), for fluorescence (HP1046) and for electrochemical measurements (HP1049) with computer on line]. A Zorbax Eclipse XDB-C18 column (150×4.6 mm ID, 5 μ particle size, Agilent) was used at 30˚C. The eluent was applied at a linear gradient between mobile phase A (2.5×10^{-4} mol/l ammonium acetate in water) and B (acetonitrile). The products resulting from 25-μl samples of ME were registered at 222 nm, whereas those of vitC at 250 nm and those of $17\beta E_2$ at 280 nm. The total run required 35 min, including a 5 min post-run, with a flow rate of 0.25 ml/min.

Results and Discussion

Experiments showed that ME as well as tyrosine (TYR) (as a main component of ME) emit electrons (e_{aq}^-) from their singlet excited state by UV-irradiation in aqueous solution (pH~7.4; 37˚C). The observed quantum yields, Q (e_{aq}^-), decrease with increasing substrate concentration (Table I). This unusual effect was attributed to the fact that both compounds form associates (unstable complexes of molecules). These complexes were spectrophotometrically established at concentrations $>10^{-7}$ mol/l. The associates consist of molecules prevailing in the ground state, which react with a proportion of the ejected e_{aq}^-. The reaction rate constants are: $k(e_{aq}^- + TYR)=3.4\times10^8$ l mol-1 s^{-1} (11) and k (e_{aq}^-+ME) ≈ 5.3×10^9 l mol^{-1} s^{-1} (calculated on the basis of the amino acids sequence); and for $17\beta E_2$ k ($e_{aq}^- + 17\beta E_2$) ≈ 2.7×10^{10} l mol^{-1} s^{-1} (11).

It has been recently established that hormone transients resulting by e_{aq}^- emission and being in a nascent state can be regenerated by electron transfer from an efficient electron donor, such as ascorbate (7, 8). Based on the outcome of these studies, the interaction of ME with vitamin C in air-free aqueous solution (pH~7.4; 37˚C) was investigated. The process was followed by HPLC analysis, where the remaining part (Rm, %) of the particular substrate, individually and in a mixture of both, ME and vitamin C, before and after each UV-treatment was registered as a function of absorbed UV dose (hv/l). The obtained results are presented in Figure 1.

From the course of the ME photolysis, registered by an HPLC peak at 10.3 min (retention) (curve A, Figure 1) and this of the mix of ME and vitamin C, registered also by the ME peak (curve A1, Figure 1), it is obvious, that ME is partly regenerated by electron transfer from vitamin C to ME transients. Curve B, Figure 1 (HPLC peak at 4.6 min) shows the very strong vitamin C degradation as a function of absorbed UV dose. However, curve B1, Figure 1, expressing the HPLC-peak of vitamin C in the presence of ME, shows at the same UV dose range, even a much higher maximum.

Table I. *[Q (e_{aq}^-)] obtained of TYR and ME in air-free, aqueous solution, containing 10^{-2} mol/l Chlorethanol as scavenger for e_{aq}^- by UV irradiation (λ=254 nm; pH~7.4; 37˚C).*

Series	Concentration (mol/l)	[Q (e_{aq}^-)]
A	5×10^{-5} TYR	0.096
B	1×10^{-4} TYR	0.020
C	1×10^{-5} ME	0.350
D	2×10^{-5} ME	0.070

Very likely the transients of ME and vitamin C have the inclination to form complexes, which facilitate the electron transfer process within both substrates. By extrapolation of curve A1 to nilUV dose and considering the calculated Qi (Rm) values, a ME regeneration of 14.6% was found (see insert I, Figure 1).

Similar experiments were also performed using 5×10^{-5} mol/l vitamin C individually and in mix with 5×10^{-5} mol/l ME (insert II, Figure 1). It is interesting to note that the corresponding curve A1 did not show any maximum. The maximum of curve B1 (expressing vitamin C in mix with ME as a function of dose), was much smaller compared to that shown in Figure 1. This effect might be a sequence of the higher vitamin C concentration used and that of the reaction rate constants of the involved components mentioned above. The obtained initial quantum yields Q(Rm), and the regeneration of ME of 52.8% are given in insert II, Figure 1.

As well-known the steroid hormones are activated by complexing with the corresponding receptor. For the $17\beta E_2$ action the corresponding receptors, ERα and ERβ are responsible. Both receptors are complexing to $17\beta E_2$ with similar activity. It was now of interest to get a deeper insight into the very complicated mechanisms of the hormone receptor action and, in addition, to contribute, if possible, to a better understanding of the observed fast communication pathway of hormones on the basis of e_{aq}^- transfer process (4).

In order to get information about the interaction of ME with $17\beta E_2$, two series of experiments were performed. In I series 2.5×10^{-5} mol/l of each substrate were used, which were UV irradiated individually as well as in mixture of both. In II series the substrates concentration was two-fold each. Based on HPLC analysis Figure 2 shows the reminder (Rm, %) of the I series of UV irradiated ME and $17\beta E_2$ samples in air-free polar media (water/ethanol 40/60 v/v) at pH~7.4 and 37˚C, as a function of absorbed UV dose. The course of the curves representing the remainder (Rm, %) separately of each substrate (curves A and B) as well as of the given mixture (curves A1 and B1) are shown in Figure 2. It is obvious that the Rm-value of ME strongly decreases

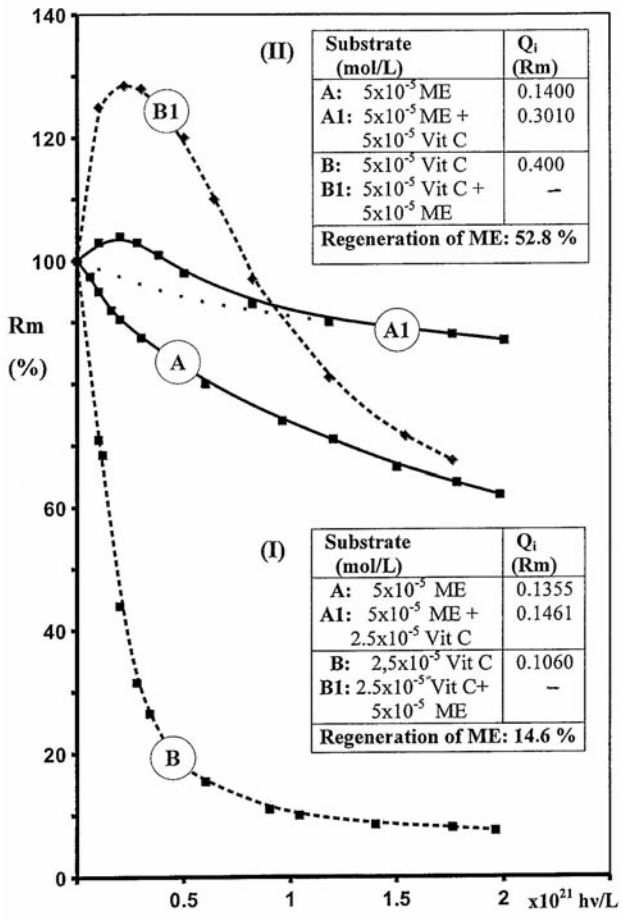

Figure 1. *HPLC analysis: Substrate remainder (Rm, %) as a function of absorbed UV-dose (hv/l) of (A) 5×10–5 mol/l ME; (A1) 5×10–5 mol/l ME in mix with 2.5×10–5 mol/l vitC; (B) 2.5×10–5 mol/l vitC; (B1) 2.5×10–5 mol/l vitC in mix with 5×10–5 mol/l ME in aqueous solution (pH~7.5) at 37°C. Insert (I). [Qi(Rm)] of the above systems. Insert (II). Similar data of studies using 5×10–5 mol/l ME and 5×10–5 mol/l vitC.*

with absorbed UV-dose, comparing curve A and A1. This illustrates that ME is acting as an e_{aq}^- donor for 17βE$_2$. Simultaneously, the Rm-value of 17βE$_2$ is increasing in the presence of ME, comparing curve B with B1, which demonstrates, that 17βE$_2$ is partly regenerated by e_{aq}^- transfer from ME. Thereby, 7.5% ME is consumed for the regeneration of 2% 17βE$_2$.

From the course of the respective curves resulting of II series (not graphically presented) it could be determined that 1% ME was consumed as electron donor for regeneration of 2.3% 17βE$_2$ (see insert II, Figure 2) The obtained results demonstrate that several reaction mechanisms are involved in the interaction of ME (used as a receptor model) and 17βE$_2$ (hormone representative). Thereby their reactivity with e_{aq}^- was already mentioned.

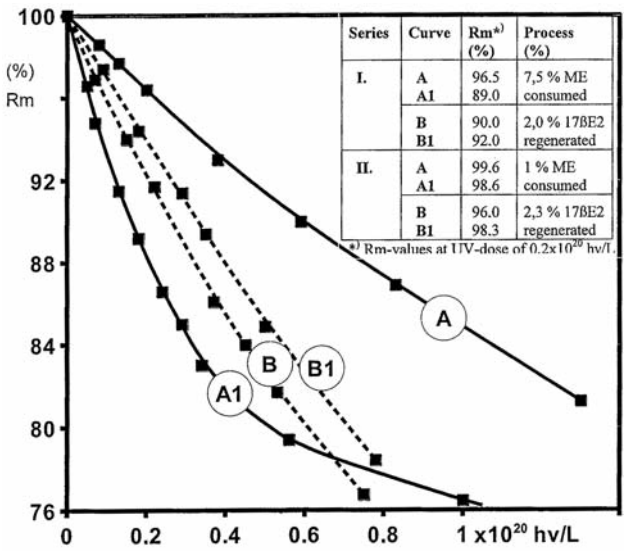

Figure 2. *HPLC analysis. Substrate remainder (Rm, %) of substrates dissolved in water/ethanol (40/60 v/v), airfree; pH~7.5. Series I. (A) 2.5×10–5mol/l ME, (A1) 2.5×10–5 mol/l ME + 2.5×10–5 mol/l 17βE$_2$; (B) 2.5×10–5 mol/l 17βE$_2$ (B1) 2.5×10–5 mol/l 17βE$_2$ + 2.5×10–5 mol/l ME. Series II. The same kind of studies, but 5×10–5 mol/l of each substance was applied. The obtained data of both series are given as insert.*

Conclusion

By summarizing the results, it can be stated that both substrates, ME and 17βE$_2$, are able to eject electrons as well as to react with e_{aq}^- and to undergo mutual electron interaction. TYR, as an important component in receptors, emits e_{aq}^- in aqueous media depending on its concentration and on the pK-values of the functional groups, which was previously reported for various compounds (13). The same is also valid for phenylalanine and all other amino acids incorporated in ME. It is also interesting that the studied compounds, as well as practically all hormones (7, 8), tend to form associates at higher concentrations. This ability favors a mutual electron transfer, even in the presence of air.

It is finally emphasized that up untill now, to our knowledge, an unknown ability was experimentally observed for the first time, namely: a prevailing electron transfer from receptor (ME) to the actual hormone (17βE$_2$) transients, causing a partial hormone regeneration. Consequently, the hormone life time and thus its action is prolonged. This ability of the receptor is of particular importance for the biological sciences and medicine.

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