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REACTIONS OF REDUCING XENOBIOTICS WITH OXYMYOGLOBIN. FORMATION OF METMYOGLOBIN, FERRYL MYOGLOBIN AND FREE RADICALS: AN ELECTRON SPIN RESONANCE AND CHEMILUMINESCENCE STUDY

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Abstract—The oxygen–haem centre of oxymyoglobin reacts with reducing xenobiotics such as hydroxylamines and phenols with the concomitant formation of metmyoglobin and oxidation of the respective xenobiotic. Metmyoglobin formation rates were measured by visible spectroscopy with xenobiotic concentrations ranging from 100 μM to 30 mM. Analogous to previous results obtained with oxyhaemoglobin, the first step in the reaction of hydroxylamines with oxymyoglobin leads to the formation of the one-electron oxidation product of hydroxylamine, a nitroxyl radical detectable by electron spin resonance. A variety of paramagnetic secondary products were also found. The terminal oxidation product of hydroxylamine and hydroxyurea was the myoglobin–nitric oxide complex, one showing similar spectral characteristics to the analogous haemoglobin–nitric oxide adduct found in our previous experiments. On the other hand, the amount of low-spin ferric complexes obtained from metmyoglobin and an excess of the respective hydroxylamine was considerably lower than the corresponding results with methaemoglobin. A second important reaction intermediate was the compound I-type ferryl haem-species detected by a recently-published chemiluminescence assay. Partial spectral resolution of the emitted light using a set of cut-off filters indicated that maximum light emission occurred above 600 nm, most probably involving excited porphyrin states. The intensity of oxymyoglobin-related light emission was considerably higher than that reported earlier with oxyhaemoglobin. This indicates a difference in the excitation mechanism which leads to the formation of the compound I-type ferry haem species.

Key words: oxymyoglobin; ESR; chemiluminescence; ferryl myoglobin; xenobiotics

Reducing xenobiotics such as hydroxylamines [1–3] and phenols [4, 5] have previously been reported to convert oxyhaemoglobin to methaemoglobin. Reactive species such as ferryl haemoglobin and free radicals characterized by means of ESR† spectroscopy, chemiluminescence and spectrophotometry are formed as key intermediates [6]. Further oxidation of these intermediates was greatly dependent on the substituents bound to the respective xenobiotic. Unsubstituted hydroxylamine and hydroxyurea were oxidized to the haemoglobin–nitric oxide complex [1], whereas *N*-methylhydroxylamine and *N*,*N*-dimethylhydroxylamine did not form this species. A prerequisite for the observation of chemiluminescence was the binding of the haem centre to the globin moiety (Hb, Myo); haematin for instance did not emit light in the presence of H_2O_2 [6]. Furthermore, haem compounds whose sixth coordination site was occupied by internal ligands such as cytochrome *c* or cytochrome oxidase showed neither chemiluminescence nor free radical formation under the conditions studied [6]. The strength of external ligands such as cyanide or azide was inversely correlated to the light intensity [7]. The aim of this study was to investigate whether differences in the globin structure of myoglobin and haemoglobin affect the chemiluminescence and radical formation induced by reducing xenobiotics. Apart from the tetrameric structure of haemoglobin and the monomeric form of myoglobin, the main differences between the two compounds are related to oxidizable groups in the vicinity of the haem group which may influence redox reactions of the haem-iron. Adjacent to the proximal histidine ligand in position 92, the β-chain of haemoglobin contains a reactive cysteine group in position 93 which is easily oxidized to a thiyl radical [8]. In addition, haemoglobin has several tyrosine residues, one of which (α-42) has recently been found to be oxidized to its phenoxyl free radical [9] when hydrogen peroxide was added to MetHb. Equine myoglobin on the other hand does not have any thiol groups, but does have two tyrosine groups at positions 103 and 146. Tyr-103 has been reported to play a major

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† Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DETAPAC, diethylenetriaminepentaacetic acid; ESR, electron spin resonance, HbO_2, oxyhaemoglobin; MetHb, methaemoglobin; *p*-HyAn, *p*-hydroxyanisole; HyUr, hydroxyurea; $MyoO_2$, oxymyoglobin; MetMyo, metmyoglobin; UV–Vis, ultraviolet–visible.

role in the formation of the compound I type species [10–12].

MATERIALS AND METHODS

Hydroxylamine hydrochloride was purchased from Merck (Darmstadt, Germany). *N*-Methylhydroxylamine hydrochloride, *N*,*N*-dimethylhydroxylamine hydrochloride, hydroxyurea, BHA, BHT and *p*-HyAn were from Aldrich (Steinheim, Germany).

Preparation of oxymyoglobin. Horse heart MetMyo, obtained from Sigma (Deisenhofen, Germany), was dissolved in 50 mM phosphate buffer and reduced to its ferrous form using a 2-fold excess of sodium dithionite. Pure $MyoO_2$ was obtained by separation from dithionite and its oxidation products by separation on a Sephadex G-25 column (Pharmacia, Uppsala, Sweden) pre-equilibrated with 50 mM phosphate buffer, pH 7.4. The concentration of $MyoO_2$ was determined at its maximum absorption at 543 nm.

ESR experiments. The ESR experiments were carried out in a Bruker ER 200 D-SRC 9/2.7 spectrometer operating at 9.6 GHz with 100 kHz modulation frequency equipped with a rectangular TE_{102} microwave cavity and a rapid sampler. For measurements at 77 K, the samples were placed in a finger Dewar filled with liquid nitrogen.

Chemiluminescence measurements. For chemiluminescence measurements we used a red sensitive photomultiplier (EMI 9658 AM, Thorn EMI Electron Tubes Inc., Fairfield, NJ, U.S.A.) cooled to −25° and connected to an amplifier discriminator (model 1121; Princeton Applied Research, Princeton, NJ, U.S.A.) adapted for single photon counting (model 1109; Princeton Applied Research, Princeton, NJ, U.S.A.). Spectral analysis was performed with a set of nine cut-off filters (shortwave pass, L.O.T.-Oriel, Darmstadt, Germany) from 406 nm to 800 nm. In some cases it was necessary to use a combination of filters in order to obtain complete absorption beyond the indicated wavelength. All spectra were corrected for transmittance of the filters and for the photomultiplier response curve.

Spectrophotometric measurements. A Hitachi model 150-20 UV–Vis spectrometer was used for spectrophotometry. The MetMyo formation rate was calculated from the absorbance decrease at 543 nm relative to a calibration standard. All $MyoO_2$/xenobiotic reaction products were identified as MetMyo by comparing their spectra in the range of 450–650 nm with MetMyo obtained upon the addition of excess potassium hexacyanoferrate-(III) to a $MyoO_2$ solution. In all cases, no significant contribution of ferryl myoglobin to the absorption spectra was observed.

RESULTS

Metmyoglobin formation

Figure 1 gives an overview of the different MetMyo accumulation rates of $MyoO_2$ (0.83 mM) induced by various xenobiotics in the range of 0.1–30 mM. The inset shows the gradual change of the $MyoO_2$ spectrum to the MetMyo spectrum: 20 consecutive scans of the $MyoO_2/NH_2OH$ system were recorded at intervals of 3 min. Across the whole concentration range, unsubstituted hydroxylamine was found to have the highest reactivity. Experiments using BHT were not successful due to its poor solubility in

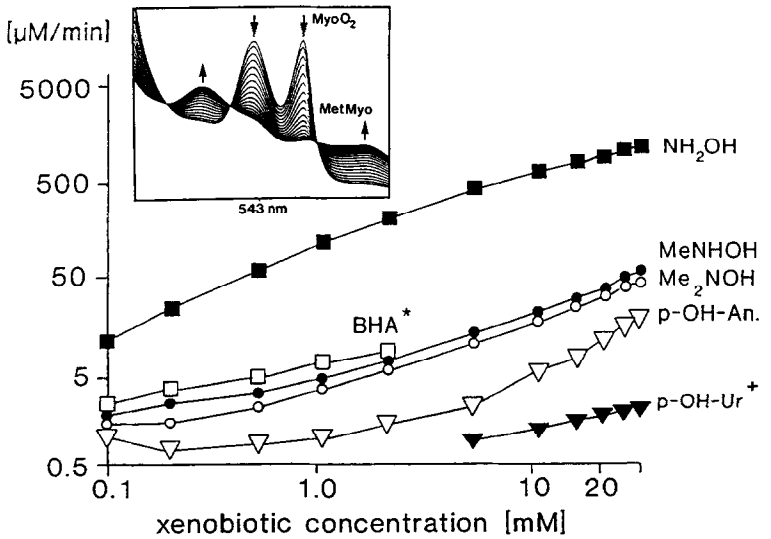

Fig. 1. Metmyoglobin formation rates (means of N = 6; SE < 4%) of several xenobiotics measured at 543 nm in 50 mM phosphate buffer, pH 7.4, 25°. The incubation mixture contained 0.83 mM oxymyoglobin and between 100 µM and 30 mM of the xenobiotic, except for butylated hydroxyanisole (BHA*), where the maximum solubility was 2 mM and *p*-hydroxyurea (*p*-OH-Ur^+) where the sensitivity limit of the spectrophotometer was reached at 5 mM. The inset shows 20 repetitive scans taken at 3 min intervals of an incubation containing 0.12 mM $MyoO_2$ and 0.15 mM NH_2OH.

phosphate buffer (< 100 μM). The sequence of descending reactivity was:

NH_2OH >> BHA > MeNHOH > Me_2NOH > *p*-HyAn >> HyUr

Formation of paramagnetic reaction intermediates

The formation of free nitroxide radicals from the same set of hydroxylamine compounds is shown in Fig. 2.

Using a flow system (total flow rate = 10 mL/min) of 1.36 mM $MyoO_2$ and 100 mM NH_2OH in the presence of 1 mM DETAPAC a transient ESR spectrum of a free radical was obtained (Fig. 2A) which immediately disappeared when the flow was stopped. According to its spectral characteristics (g = 2.0062, $a_N(1) = a_H(2)$ = 12.6 G) it was identified as the dihydronitroxide free radical ($NH_2O^{\cdot}$). The same species was obtained using oxyhaemoglobin instead of $MyoO_2$ under otherwise identical conditions [1] and in a chemical system in which NH_2OH was mixed with hexanitratocerate-(IV) in methanol [13]; spectral parameters in that case were slightly different due to solvent effects ($a_N(1) = a_H(2)$ = 11.9 G). A corresponding radical ($CH_3NHO^{\cdot}$) was also observed when the hydroxylamine derivative *N*-methylhydroxylamine (100 mM) was mixed with $MyoO_2$ (0.93 mM) at a total flow rate of 10 mL/min (Fig. 2 B1). Identification of this species was possible when our spectral parameters ($a_N(1) = a_H(4)$ = 11.9 G) were compared with previous results obtained in an HbO_2/CH_3NHOH system [2] or when $K_3Fe(CN)_6$ was used for the oxidation of CH_3NHOH [14]. The $CH_3NHO^{\cdot}$ radical is very unstable and rapidly dimerizes to the *N*-methyl-*N*-β-aminomethylnitroxide radical, which has previously been identified by computer simulation ($a_N(1)$ = 16.6 G, $a_H(3)$ = 14.4, $a_H(2)$ = 10.0 G, $a_N(1)$ = 1.7 G [2]. The corresponding ESR spectrum is shown in Fig. 2 B2 taken 5 min after the flow was stopped.

A stationary system could be used for the detection of the *N*,*N*-dimethylnitroxide radical ($(CH_3)_2NO^{\cdot}$), obtained from oxymyoglobin (2 mM) and *N*,*N*-dimethylhydroxylamine (20 mM) in 50 mM phosphate buffer, pH 7.4 containing 1 mM DETAPAC (Fig. 2C). Due to steric hindrance from the methyl groups this radical is persistent for a long period of time. It was also observed in previous experiments using HbO_2 [2]. The spectral parameters are: g = 2.0054; $a_N(1)$ = 17.0 G; $a_H(6)$ = 14.8 G.

Figure 2D shows the ESR spectrum obtained by repetitive scanning (50 times) of an incubation mixture consisting of *N*-hydroxyurea (200 mM) and oxymyoglobin (2 mM) in the presence of 1 mM DETAPAC and 50 mM phosphate buffer, pH 7.4. Due to its spectral parameters ($a_N(1)$ = 8.05 G; $a_H(1)$ = 11.7 G; g = 2.0063) it was identified as the aminocarbonylaminooxyl free radical described earlier by Lassmann *et al.* [15].

In the presence of an excess of *N*-methylhydroxylamine (200 mM) relative to MetMyo (2 mM) a low-spin ferric complex was observed (Fig. 3). The formation of the respective ferric low-spin adducts from MetMyo (2 mM) and all other hydroxylamine compounds (200 mM) was much less than in our

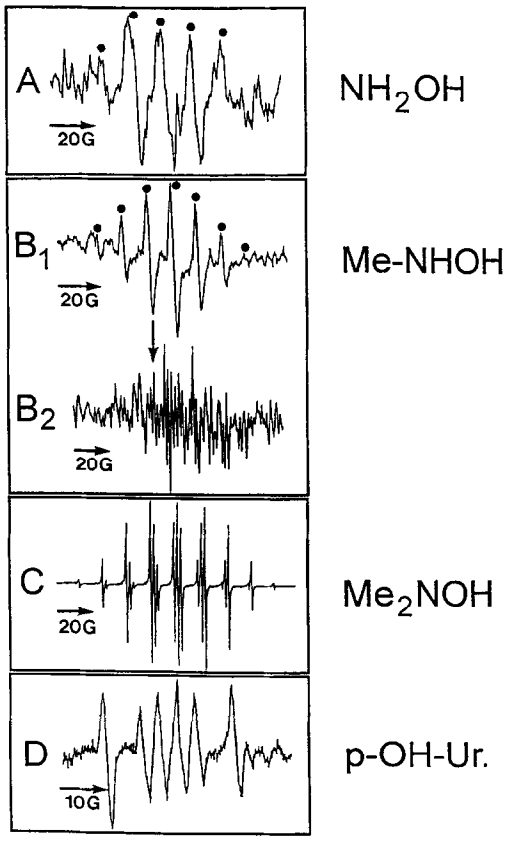

Fig. 2. ESR spectra of xenobiotic-derived free radicals. (A) $NH_2O^{\cdot}$ (lines marked '●'): The radical was detected using a flow system (10 mL/min) consisting of oxymyoglobin (1.36 mM, final concentration) and NH_2OH (100 mM, final concentration) in 50 mM phosphate buffer, pH 7.4 containing 1 mM DETAPAC. The ESR instrument settings were: scan range, 100 G; microwave power, 20 mW; receiver gain, 5×10^5; modulation amplitude, 5 G; time constant, 0.33 sec; scan rate, 71.5 G/min. (B1) $CH_3NHO^{\cdot}$ (lines marked '●'): The radical was detected using a flow system (10 mL/min) consisting of oxymyoglobin (0.93 mM, final concentration) and CH_3NHOH (100 mM, final concentration) in the presence of 1 mM DETAPAC at pH 7.4. The ESR instrument settings were: scan range, 200 G; microwave power, 20 mW; receiver gain, 5×10^5; modulation amplitude, 4 G; time constant, 0.16 sec; scan rate, 285 G/min. (B2) $CH_3NO^{\cdot}CH_2NH_2$: The same incubation system as in (B1) except that the flow was stopped. The ESR instrument settings were: scan range, 120 G; microwave power, 20 mW; receiver gain, 1×10^6; modulation amplitude, 0.4 G; time constant, 0.66 sec; scan rate, 21.5 G/min. (C) $(CH_3)_2NO^{\cdot}$: The radical was detected in a stationary system consisting of oxymyoglobin (2 mM) and $(CH_3)_2NOH$ (20 mM), pH 7.4 and 1 mM DETAPAC. The ESR instrument settings were: scan range, 150 G; microwave power, 20 mW; receiver gain, 1×10^5; modulation amplitude, 0.24 G; time constant, 0.33 sec; scan rate, 26.8 G/min. (D) $H_2N\text{-}CO\text{-}NHO^{\cdot}$: The radical was detected using repetitive scanning (50 scans) of a stationary system consisting of oxymyoglobin (2 mM) and hydroxyurea (200 mM) in 50 mM phosphate buffer, pH 7.4, containing 1 mM DETAPAC. The ESR instrument settings were: scan range, 50 G; microwave power, 20 mW; receiver gain, 1×10^6; modulation amplitude, 1 G; time constant, 0.33 sec; scan rate, 17.9 G/min.

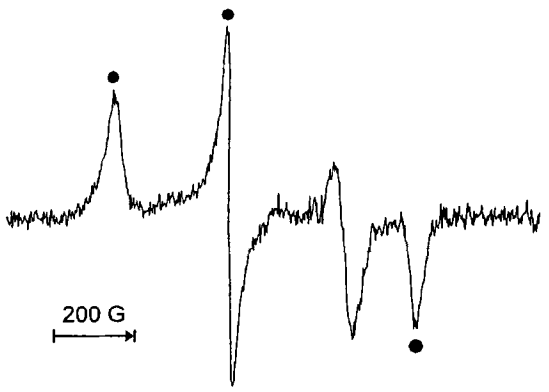

Fig. 3. The low-spin ferric myoglobin (2 mM) complex (marked '●') with *N*-methylhydroxylamine (200 mM) measured at 77 K. The unmarked line is the spectrum of the $CH_3NHO^{\cdot}$ radical which is not resolved at 77 K. The ESR instrument settings were: scan range, 2000 G; microwave power, 20 mW; receiver gain, 6.3×10^4; modulation amplitude, 5 G; time constant, 0.66 sec; scan rate, 357 G/min.

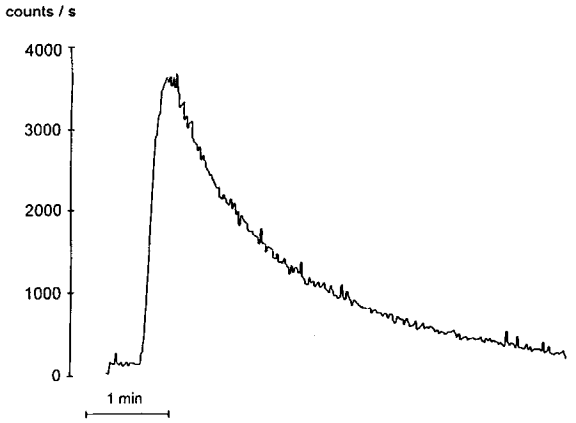

Fig. 4. Chemiluminescence time scan of an incubation containing oxymyoglobin (3 mM) and NH_2OH (4 mM) measured at 25° in 200 mM phosphate buffer, pH 7.4. The peak intensity was reached 15 sec after the addition of NH_2OH.

Table 1. EPR parameters of low-spin ferric complexes of various hydroxylamines with methaemoglobin and metmyoglobin

	$MetHb^{3+}$	$MetMyo^{3+}$
NH_2OH	$g = 2.46; 2.20; 1.91$	$g = 2.50; 2.22; 1.89$
	secondary species: (2.80; 2.32; 1.72)	
MeNHOH	$g = 2.38; 2.17; 1.93$	$g = 2.56; 2.30; 1.97$
Me_2NOH	$g = 2.49; 2.22; 1.89$	$g = 2.68^*; 2.20; 1.65^*$
N-HyUr	$g = 2.52; 2.24; 1.86$	$g = 2.56; 2.23; 1.85$

* Due to low peak intensity values are only approximations.

previous experiments with HbO_2 [1–3]. Table 1 summarizes the *g*-values of the different low-spin complexes formed from haemoglobin or myoglobin and the various hydroxylamines. 2,2-Diphenyl-1-picrylhydrazyl was used as internal standard ($g = 2.0036$). The observed *g*-values were clearly distinct from those of previously reported low-spin complexes such as sulphmyoglobin or hemichrome [16, 17].

Experiments using the phenolic xenobiotics *p*-HyAn, BHA and BHT failed to produce detectable concentrations of the expected phenoxyl radicals. The major obstacle seems to have been the limited solubility of these compounds and the slightly lower solubility of $MyoO_2$ compared to HbO_2, which produced the phenoxyl radicals of *p*-HyAn and BHA in concentrations just above the detection limit [4, 5]. When haemoglobin was allowed to react with BHA the phenoxyl radical formed in this reaction was added to a reactive thiol group on the globin moiety of haemoglobin. The resulting paramagnetic reaction product exhibited characteristics of an immobilized radical (single ESR line [5]). No such species was observed when $MyoO_2$ was incubated with BHA. This is in line with the fact that myoglobin does not have any thiol groups on the globin moiety.

Light-emitting reaction intermediates

In order to detect reaction intermediates exhibiting excitation states such as was recently demonstrated in the HbO_2/NH_2OH system [6] we incubated $MyoO_2$ with various xenobiotics in a photon counter.

Figure 4 shows the kinetics of low-level chemiluminescence developed in an incubation system consisting of 3 mM of $MyoO_2$ and 4 mM of NH_2OH. The maximum of approx. 3400 counts/sec is reached after approx. 15 sec and quickly decreases to less than 10% of the maximum intensity within approx. 5 min. The general feature of light emission is similar to previous results obtained with HbO_2 [6], except that chemiluminescence intensity in the $MyoO_2$ system was more pronounced (approx. 3400 counts/sec compared to 540 counts/sec), indicating the existence of a different reaction mechanism. In Table 2 light intensity of the $MyoO_2$ system is compared with the respective values for HbO_2 [6]. With the exception of $(CH_3)_2NOH$ and *p*-hydroxyurea, light emission of the $MyoO_2$ system was considerably higher. A comparison of the chemiluminescence data with MetMyo formation rates is given in Fig. 5. It can clearly be seen that a rough correlation exists between light emission and the reaction rate $d[MetMyo]/dt$. Spectral resolution of the light emission is shown in Fig. 6, where a set of cut-off filters were used in the range between 406 and 800 nm (corrected for the transmittance of the filter and the response of the photomultiplier). In Figs 6(a) and (b) a wavelength analysis of the chemiluminescence of the $MyoO_2/NH_2OH$ and HbO_2/NH_2OH systems is presented. Most of the emitted light is in the red or near-infrared area. This finding excludes the participation of excited carbonyls whose emission maxima are approx. 450 nm. In contrast, $MetMyo/H_2O_2$ (Fig. 6(c)) and $MetHb/H_2O_2$ (Fig.

Table 2. Comparison of the chemiluminescence peak intensities obtained with incubations containing HbO_2 (3 mM) or $MyoO_2$ (3 mM) and various xenobiotics (4 mM) in phosphate buffer (200 mM, pH 7.4) at 25°

$MyoO_2$ values (counts/sec)							
NH_2OH >>	BHA >	MrNHOH >	*p*-HyAn >	BHT >>	Me_2NOH >	HyUr	
[3367]	[800]	[660]	[175]	[142]	[10–20]	[5–10]	
HbO_2 values (counts/sec)							
NH_2OH >>	MeNHOH >	BHA >	Me_2NOH >	*p*-HyAn >>	BHT >	HyUr	
[540]	[350]	[100]	[90]	[80]	[55]	[20]	

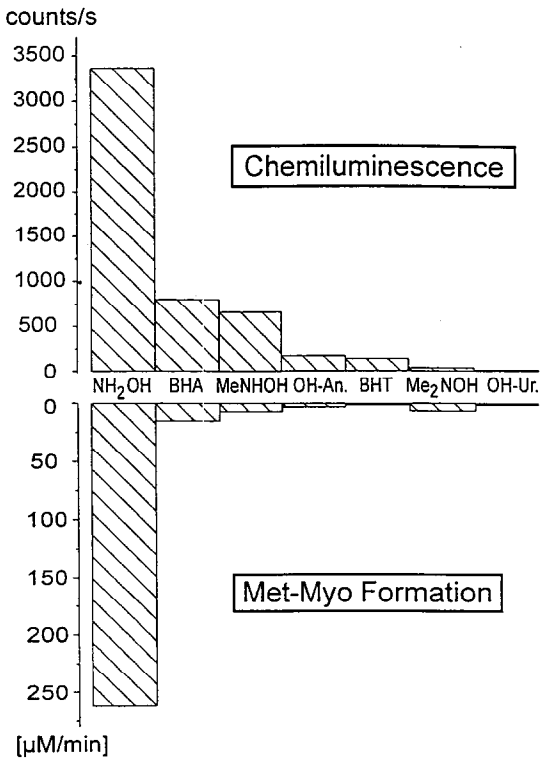

Fig. 5. Comparison of chemiluminescence peak intensities obtained from oxymyoglobin (3 mM) in the presence of various xenobiotics (4 mM) and measured at 25° in 200 mM phosphate buffer, pH 7.4, with the MetMyo formation rates, measured spectrophotometrically at 543 nm (0.83 mM $MyoO_2$, 2 mM xenobiotic).

6(d)) couples caused significant light emission in the 450–600 nm range. A possible explanation is the involvement of excited carbonyls as a consequence of energy transfer in oxidation reactions. The predominant light intensity in Figs 6(c) and (d) is, however, between 600 and 750 nm. In a set of experiments the participation of $^1O_2$ dimol emission could be excluded [18] since no endoperoxide formation was detected in incubation of the $^1O_2$-trap 9,10-anthracenedipropionic acid in the presence of the MetMyo/H_2O_2 system, followed by HPLC analysis of the products formed.

Therefore, the most likely candidate for light emission is an excited porphyrin state. Recent reports of excited haematoporphyrin systems in dimethylsulphoxide solution have also described maximum light emission in the red above 650 nm [19]. Formation of the excited state is probably linked to the compound I type ferryl species since previous investigations using the MetHb/H_2O_2 model system revealed a good kinetical correlation between light emission and the compound I related ESR spectrum [6].

DISCUSSION

Our results show a remarkable similarity in the reactivity of HbO_2 and $MyoO_2$ with a series of hydroxylamines and phenol compounds. In all cases, the haem-bound dioxygen cooxidizes the haem-iron (Fe(II) → Fe(III)) and the xenobiotic (to the respective free radical). This process is accompanied by low level chemiluminescence, which we have shown to be indicative of the intermediate existence of a compound I-type ferryl haem intermediate [6]. In the case of the hydroxylamines we have also been able to demonstrate the transient existence of a free radical intermediate, the one-electron oxidation product of the respective xenobiotic (reaction 1):

$$\text{Myo(II)}O_2 + \text{Xen-OH} \rightarrow [\text{Myo(III)-OOH}] + \text{Xen-O}^{\cdot} \quad (1)$$

$$[\text{Myo(III)-OOH}] \rightarrow \text{compound I} + H_2O \quad (2)$$

$$[\text{Myo(III)-OOH}] + 2\ \text{Xen-OH} \rightarrow \text{Myo(III)-OH} + 2\ \text{Xen-O}^{\cdot} + H_2O \quad (3)$$

The reaction stoichiometry of xenobiotic oxidation (1 e^-) requires the intermediate existence of [Met(III)-OOH], where the haem oxygen has the oxidation state of hydrogen peroxide. Spectrophotometric investigations of the analogous Hb(III)-OOH revealed that this species exists but can be detected only at very low temperatures [20]. In a similar system the release of H_2O_2 into the solution has been excluded, since the addition of catalase showed no effect on the reaction rate [21]. As has been shown in model experiments using MetMyo plus H_2O_2 [6, 18] a compound I-type ferryl species is formed (reaction 2). With excess xenobiotic present, a direct reaction with concomitant xenobiotic free radical formation might also be possible (reaction 3). This would be in line with the absence of spectrophotometrically detectable concentrations (accumulation) of the ferryl species.

Contrary to the results obtained with HbO_2, the

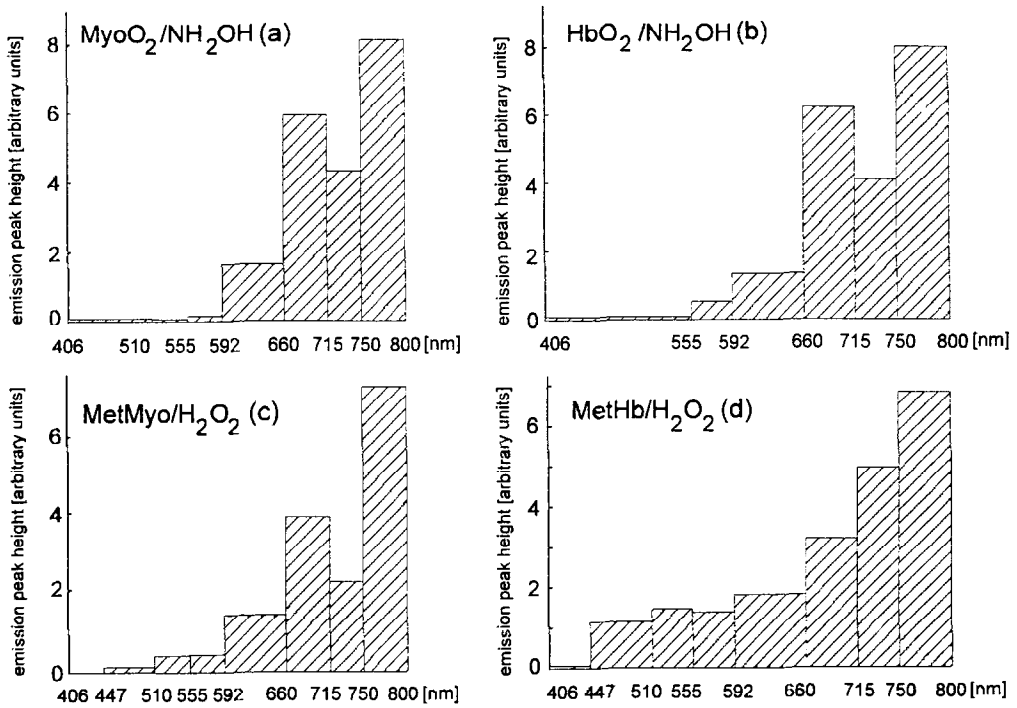

Fig. 6. Spectral resolution of the chemiluminescence taken at approximate intervals of 50 nm between 406 and 800 nm. (a) $MyoO_2$ (3 mM), NH_2OH (4 mM; (b) HbO_2 (3 mM), NH_2OH (4 mM); (c) MetMyo (0.28 mM)/H_2O_2 (0.56 mM); (d) MetHb (0.28 mM)/H_2O_2 (0.56 mM).

investigated phenols (BHA, BHT and *p*-HyAn) did not produce detectable amounts of the respective phenoxyl radicals. This might be due to the slightly lower solubility of myoglobin in the presence of higher concentrations of xenobiotics required to produce steady-state radical concentrations above the detection limit of the ESR spectrometer (approx. 10^{-9} M). A major difference was observed, however, in the reactivity towards BHA. Whereas in the HbO_2/BHA system the predominant species in the ESR spectrum was a paramagnetic BHA-globin adduct, this species could not be detected in the $MyoO_2$/BHA system. A likely explanation is that the addition reaction takes place at a reactive SH-group of haemoglobin, most probably at position β-93 which is easily accessible. Myoglobin, on the other hand, does not possess any thiol groups.

Furthermore, differences in the structure of the globin moiety are also reflected in the reactivity of the compound I-type ferryl intermediate. Light intensity of the chemiluminescence observed in the oxymyoglobin/xenobiotic systems is considerably higher than in the respective haemoglobin systems. This might be due to the existence of a higher ratio of the excited state myoglobin species which decays via the light emitting pathway, whereas in the haemoglobin species more xenobiotic free radicals are produced which decay via a non-chemiluminescent pathway:

Compound I + Xen-OH → compound II + Xen-O· (4)

Compound II + Xen-OH → ferric haem + Xen-O· (5)

This reaction sequence provides an additional explanation for the higher free radical steady-state concentration detected in the reactions of *p*-HyAn or BHA with HbO_2. The observed difference in the intensity of light emission may also be due to the availability of various oxidizable amino acid residues on the globin side chain of haemoglobin and myoglobin possibly involved in the one-electron shuttle process to haem-iron, assumed to establish an excited state and subsequent light emission. Chemiluminescence is also dependent on the reaction pathway by which the ferryl species is formed. This can be seen in the spectral resolution of the emitted light (Fig. 6). If MetHb is incubated with an excess of H_2O_2 the pattern of the emitted light changes, especially in the range between 450 and 550 nm. A possible explanation is secondary reactions involving excited carbonyls (emission at approx. 450 nm) and also a shift of peak emission towards higher wavelengths (approx. 800 nm or above). This might be caused by heat evolution from secondary reactions such as dismutation of H_2O_2.

Despite these gradual differences in reaction kinetics the similarities between these haem compounds predominate. The general reaction scheme is the same. This is of toxicological interest since both haem compounds become powerful oxidants if activated to the ferryl state by reducing xenobiotics such as hydroxylamines or phenolic compounds.

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