THE STEADY STATE LEVEL OF CATALASE COMPOUND I IN ISOLATED HEMOGLOBIN-FREE PERFUSED RAT LIVER *

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1. Introduction

The long study of the catalytic and peroxidatic activities of catalase has led to little information on its function *in vivo*, particularly in mammalian systems [1, 2]. In bacteria, e.g. *Micrococcus lysodeikticus*, spectroscopic evidence indicated that catalase is largely saturated with H_2O_2 in the form of the primary intermediate, compound I [3]. In mammalian tissue, the correspondence of the order of reaction and the overall flux rates for methanol metabolism in the rabbit were suggestive of catalase's role in methanol oxidation [1]. Similar conclusions were reached by Tephly, Parks and Mannering [4] for methanol metabolism in the rat. Heppel and Porterfield [5] found coupled nitrite oxidation in rat liver homogenates, and Portwich and Aebi [6] demonstrated the coupled oxidation of formate in rat liver slices.

In the present investigation, spectrophotometric measurements of absorbance in the 660 nm band region of catalase compound I from isolated perfused rat liver have been performed. Experimental evidence which supports an interpretation of changes of the observed signal to be due to the catalase system will be presented; it includes the effects of the above-mentioned hydrogen donors, of hydrogen peroxide generating substance, of oxygen tension, and of inhibitors of the catalase system.

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2. Experimental

Spectrophotometry of light transmitted through a lobe of perfused liver was performed with the Rapidspektroskop of Howaldtswerke Deutsche Werf, Kiel, as adapted by Brauser [7]. Dual wavelength absorbance photometry by sinusoidal wavelength modulation [7] was used for more sensitive study of absorbance changes as a function of time (see also [8]). Bandwidth of monochromatic light was 4 nm. Compensation for spectral characteristics of the apparatus was afforded by a reference beam which was reflected from a magnesia surface.

In preliminary experiments, the rotating filter apparatus of Chance [9] as adapted for absorbancy measurements [10] was used with a 660 nm measuring filter and a 720 nm reference filter, having bandwidths of ± 30 and ± 7 nm, respectively. Light transmitted through a liver lobe was collected by a light pipe and was detected by an infrared-guarded silicone diode.

Oxygen uptake was followed with Ag-Pt-microelectrodes inserted into the perfusion circuit before

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and after the liver. Reduced pyridine nucleotide surface fluorescence was measured with the Ultropak technique [11], using light of 366 nm for Ultropak and wavelengths > 420 nm for emission. Electronics were from Netheler and Hinz, Hamburg.

Livers from male Wistar rats, 120–150 g weight, fed on stock diet, were perfused with bicarbonate-buffered salt solution, pH 7.4, 37°, containing 7 g of dextran (M.W. 40,000; kindly supplied by Knoll Co., Ludwigshafen per 100 ml, as described [8]). Ethyl hydrogen peroxide was obtained from Ferrosan, Malmö, 3-amino-1,2,4-triazole from Schuchardt, München, the other chemicals from Merck, Darmstadt.

3. Results

3.1. *Cycle of anoxia and effect of hydrogen donors*

The difference spectrum of catalase compound I with respect to catalase has a peak at around 660 nm and an isosbestic point at around 640 nm, as shown in [12] for the enzyme from horse blood. In this spectral region there is also absorbance by other pigments, e.g. cytochromes. However, these pigments contribute with broad bands. In fig. 1, the absorbance difference between 640 and 660 nm, Δ A (640–660), as recorded from perfused liver is shown. The signal responds to a cycle of anoxia, indicating that the steady state level of part or all of the compounds contributing to the measured signal is a function of oxygen supply. Furthermore, fig. 1 shows that methanol, a hydrogen donor for peroxidatic decomposition of compound

$$\underset{\text{free catalase}}{Cat(OH)_4} + H_2O_2 \rightarrow \underset{\text{compound I}}{Cat(OH)_3OOH} + H_2O \quad (1)$$

$$Cat(OH)_3OOH + AH_2 \rightarrow Cat(OH)_4 + A + H_2O \quad (2)$$

I (reaction 2) (see [13]), causes a deflection of the trace in direction of the anoxia response. Other hydrogen donors such as ethanol, formate, and nitrite give similar responses. A subsequent cycle of anoxia results in a negligible further increase, indicating that the hydrogen donor response and the anoxia response are not additive. When the hydrogen donor is added during anoxia, then no absorbance change occurs at that time or upon later reoxygenation.

For one of the hydrogen donors, ethanol, the effect on the absorption spectrum is shown (fig. 2). While an absorbance decrease with respect to the control spectrum occurs in the compound I region when ethanol is added, there is no change in the cytochrome *a*-α-band region (607 nm), showing that no measurable reduction of cytochrome *a* occurs. A subsequent cycle of anoxia causes the appearance of the cyto-

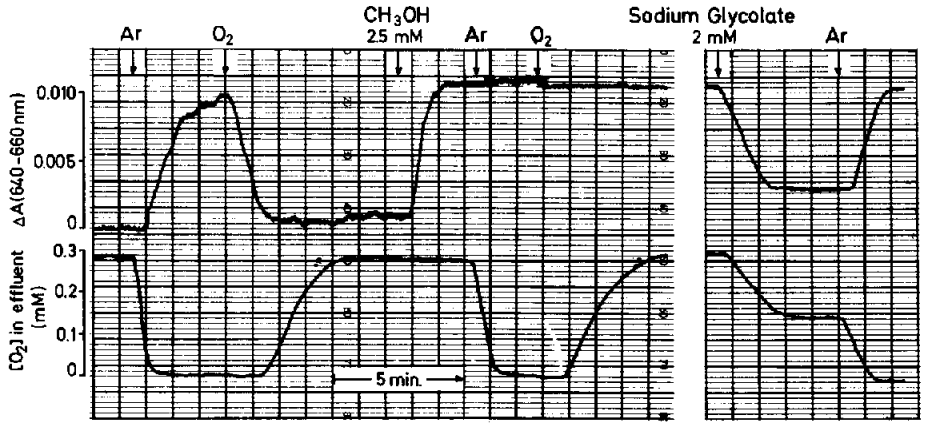

Fig. 1. Dual-wavelength reading of absorbance difference Δ A (640–660) from perfused rat liver (upper trace) and simultaneous recording of oxygen concentration in effluent perfusate (lower trace). Catalase compound I decomposition during a cycle of anoxia and subsequent methanol response is demonstrated by increase of Δ A (640–660) due to decreased absorbance at 660 nm; compare absolute spectrum (fig.2). *Right part* : methanol is present at a concentration of 1 mM. Glycolate then causes substantial decrease of Δ A (640–660) toward the initial level and an increase of oxygen uptake.

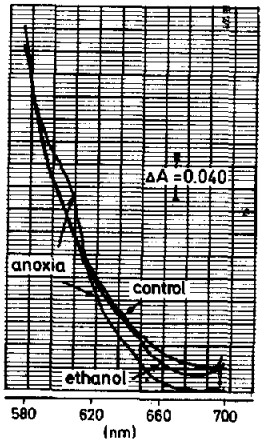

Fig. 2. Absorbance spectra of perfused liver. A control spectrum was recorded first. Then, ethanol (8.5 mM) was added, resulting in a decrease in the catalase compound I region. A subsequent anoxia spectrum demonstrates appearance of reduced cytochrome *a*, peaking at 607 nm.

chrome *a*-α-band and a further decrease in the long wavelength region.

It is of interest to note that the addition of methanol has no effect on oxygen uptake (fig.1) or on reduced pyridine nucleotide surface fluorescence (not shown), whereas ethanol increases both of these parameters.

3.2. *Hydrogen peroxide generation*

Glycolate is an effective reactant for endogenous H_2O_2 production within rat liver peroxisomes [2]. Addition of sodium glycolate to the perfusate did not cause changes of Δ A (640–660) when only endogenous substrates were present. However, when exogenous hydrogen donor had been added and Δ A (640–660) was increased, glycolate caused the return of Δ A (640–660) toward the initial level (fig. 1, right part). The effect was greater when compound I was only partially depleted, e.g. when methanol was present at a concentration of 1 mM (fig. 1, right part), as might be expected due to the balance between H_2O_2 production and H_2O_2 depletion. Other reactants for H_2O_2 production such as xanthine and urate (0.1 mM) gave similar but less pronounced effects. Exogenously added H_2O_2 reacted accordingly; again, ΔA (640–660) was not affected when only endogenous substrate was present. Here, the oxygen concentration in the effluent increased due to decomposition of H_2O_2.

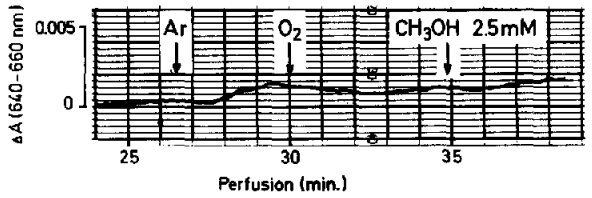

Fig. 3. Dual-wavelength reading of absorbance difference ΔA (640–660) from perfused liver of a rat treated with 1 g of aminotriazole per kg body weight 1 hr prior to perfusion. Cycle of anoxia and subsequent addition of methanol. Compare untreated control (fig. 1).

Ethyl hydrogen peroxide (0.1–0.3 mM) caused ΔA (640–660) to increase like the hydrogen donors. This finding is unexpected because ethyl hydrogen peroxide is known to increase the compound I level *in vitro* as well as in bacterial cells [3] by further binding to catalase hematin.

3.3. *Effect of inhibitors*

Treatment of the rat with 1 g of 3-aminotriazole per kg body weight is known to irreversibly inhibit catalase activity by 90% or more within 1 hr [14]. This pretreatment resulted in the abolishment of the anoxia response and of the hydrogen donor response of ΔA (640–660) (fig. 3). On the other hand, the anoxia responses of cytochrome *a* and of the pyridine nucleotides were still present.

Infusion of sodium azide at low concentration (12 μM) was followed by a decrease of ΔA (640–660) (fig. 4). Subsequent addition of methanol gave a response of only ¼ of that in the controls, suggesting that coupled oxidation of methanol is largely suppressed under these conditions. A further increase of ΔA (640–660) was observed when oxygen was withdrawn; this is attributed to the decomposition of the inhibitory FeNO–catalase complex. The azide effect was reversible by washout with azide-free perfusate.

Inhibition of mitochondrial oxidations at the level of NADH dehydrogenase by amobarbital or by rotenone had no detectable effect on the anoxia or hydrogen donor responses of ΔA (640–660).

4. Discussion

The measurement of changes of the absorbance difference ΔA (640–660) in hemoglobin-free perfused rat liver was found to be highly selective for detection of changes in the state of catalase compound I. Several experimental conditions, e.g. cycle of anoxia, addition of hydrogen donors, of hydrogen peroxide generating substances, and of catalase inhibitors, were shown to affect the signal in an expected manner. Other wavelength settings, screened systematically at 10 nm intervals from 600–680 nm with Δλ of 20 nm, and the use of 720 nm as reference wavelength (not shown), provided results which were less selective for the catalase system. For example, the hydrogen donor response was smaller than the anoxia response when measured at such wavelength settings, reflecting interference from other pigments.

Assuming that a major part of the observed changes of ΔA (640–660) is due to catalase compound I, the following conclusions may be drawn: (a) A steady state level of catalase compound I is maintained during the endogenous steady state of isolated, hemoglobin-free perfused rat liver; (b) compound I is rapidly decomposed during the cycle of anoxia and replenished after restitution of oxygen supply; (c) the failure of added glycolate or H_2O_2 to cause a decrease of ΔA (640–660) in the endogenous steady state may be interpreted to indicate that compound I is saturated with hydrogen peroxide as was seen with *Micrococcus lysodeikticus* [3]. However, the source of endogenous hydrogen peroxide and hydrogen donor remains to be established. More detailed experiments will permit calculation of the intracellular concentration of H_2O_2 and its rate of formation [15]; (d) the observation that ethyl hydrogen peroxide apparently did not increase the compound I level is in contrast to findings with bacterial cells and with isolated catalase (see above). If the present finding is substantiated by absolute spectra, this would lead to the interesting conclusion that in intact liver all catalase hematin is saturated with H_2O_2, requiring appropriate modification of reactions 1, 2 above; (e) the catalase system in liver is adequately supplied with H_2O_2 for peroxidatic function. It was demonstrated here in a direct manner that catalase compound I takes part in methanol oxidation in liver, as was suggested earlier [1, 4], as well as in coupled oxidation

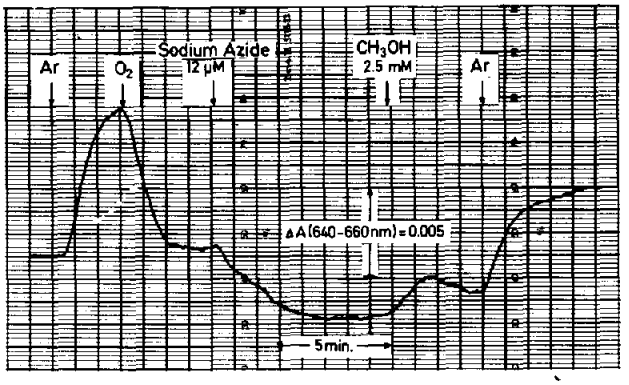

Fig. 4. Effect of sodium azide at low concentration on ΔA (640–660). The methanol response is substantially smaller than in the control (fig. 1).

of ethanol, nitrite [5], and formate [6].

The theoretically interesting role of catalase compound I in tissue alcohol metabolism may subsequently be also of practical interest. For example, the absence of adverse effects upon the cytosolic redox balance and biosynthetic pathways as are caused by the reaction of ethanol with ADH.NAD^+ [16, 17] speaks in favor of extensive use of the catalase pathway in ethanol toxicity with the help of a specific ADH inhibitor [18, 19] and a concomitant increase of H_2O_2 production by substances such as glycolate. Another example would be methanol metabolism. Provided that the spectral observations of fig. 4 correlate with the flux rates, excessive rates of methanol oxidation by catalase compound I could be controlled by very low concentrations of azide (1-5 μM) which have negligible effects on mitochondrial cytochromes, on the pyridine nucleotides, and on overall respiration [20].

Acknowledgement

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