Short Communication

Heme Release from Rabbit Liver Microsomal Cytochrome P-450 LM_2

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Cytochrome P-450 represents a family of hemoproteins with absorbance maxima of their Fe(II) · CO complex at about 450 nm. Upon denaturation this band usually shifts to about 420 nm. Binding of heme to apoprotein may be the rate-limiting event in the formation of functionally active cytochrome.[1] Heme is revesibly transferred between different molecular species of cytochrome P-450 in rat liver.[2] It hence appears that a fraction of cytochrome P-450 exists in its apoform in vivo. During purification of the membrane-bound cytochrome P-450's in the presence of detergents, a variable and often extensive loss of heme occurs. The extent depends upon the isozyme in question, and as a result the final preparation often contains 20–60 % apoenzyme. The release of heme from a hemoprotein can be optically monitored using another apohemoprotein as a recipient. Apomyoglobin is suitable for this purpose.[3] The on-reaction is several orders of magnitude faster than the off-reaction which makes the concentration of free heme negligible. In the present communication we examine under what conditions cytochrome P-450 LM_2 will release heme at any appreciable rate. It is concluded that the loss of heme from cytochrome P-450 requires the presence of detergent.

Materials and Methods. Cytochrome P-450 LM_2 was purified to electrophoretic homogeneity from liver microsomes of phenobarbital-treated rabbits essentially according to the method of Haugen and Coon,[4] as described previously.[5] The protein used contained 14.5 nmol of heme per mg or protein, giving heme/protein ~0.7. The enzyme (18 μM) was stored in 59 mM potassium phosphate buffer, pH 7.4, containing 20 % glycerol and 1 mM EDTA. The cytochrome P-450 and cytochrome P-420 contents were measured according to Omura and Sato.[6] Apomyoglobin was prepared by acid acetone treatment (−20 °C) of sperm whale myoglobin (main fraction from Sigma type III, gradient chromatographed on CM-52 in 10–100 mM sodium phosphate, pH 6.0) and dialyzed against water, 1.5 mM sodium bicarbonate and 50 mM sodium phosphate, 1 mM EDTA buffer, pH 7.0.[7] The heme transfer was measured at the wavelength of the Soret band maximum of myoglobin, 409 nm, using a thermostated Beckman DU-7 spectrophotometer. Horseradish peroxidase isoenzyme A2 was prepared as previously described.[8]

Results and discussion. When cytochrome P-450 LM_2 (5 μM, freed from glycerol by dialysis) was mixed with a tenfold excess of apomyoglobin at 35 °C in 50 mM sodium phosphate, 1 mM EDTA, pH 7.0, no significant increase in A_{409} occurred within 10 h. At the end of this time, almost all cytochrome P-450 had been converted into cytochrome P-420. Thus the rate of heme loss from either of the two forms of the hemoprotein was negligible under the conditions used; <1 pmol of enzyme lost its heme per h. Addition of horseradish peroxidase A2 to give a final concentration of 10 μM caused a gradual increase in A_{409}, comparable in extent to the results previously presented.[3]

In other experiments P-450 as above but with or without 3 % (v/v) glycerol was treated with cholate at a final concentration of 0.5 % (w/v).

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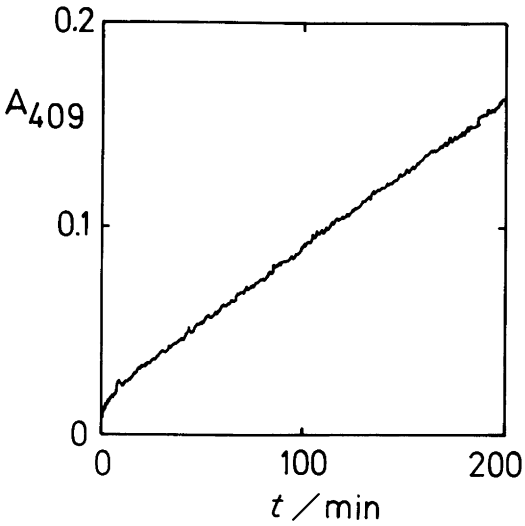

Fig. 1. Heme loss from cytochrome P-420 in the presence of 0.5 % sodium cholate as monitored by the formation of holomyoglobin at 409 nm.

Within 5 min this treatment caused an almost quantitative conversion of P-450 into P-420. As judged from the spectrum P-420 was stable. However, when apomyoglobulin was added an extensive transfer of heme from P-420 to apomyoglobin occurred at zero order kinetics (Fig. 1). The rate of heme transfer was 450 nmol of hemoprotein per h.

In conclusion, no measurable heme loss occurred from cytochrome P-450 LM_2 or from cytochrome P-420, generated during storage of P-450 for prolonged periods at 35 °C. By contrast, cytochrome P-420 in the presence of cholate readily lost heme, exhibiting a much smaller affinity for heme. It appears plausible that hydrophobic interactions between heme and apoenzyme are perturbed in the presence of detergent and also that the loss of heme from cytochrome P-450 requires the presence of detergent. This fact appears to be of importance during purification of membrane-bound forms of this hemoprotein.

References

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