Biochemical Pharmacology, Vol. 34, No. 10, pp. 1835–1837, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

CONVERSION OF HEPATIC MICROSOMAL CYTOCHROME P-450 TO P-420 UPON PHOSPHORYLATION BY CYCLIC AMP DEPENDENT PROTEIN KINASE

HISAAKI TANIGUCHI, WALTER PYERIN* and ANTON STIER

Max-Planck-Institut für Biophysikalische Chemie, Abt. Spektroskopie, D-3400 Göttingen, and *Deutsches Krebsforschungszentrum, Institut für Experimentelle Pathologie, D-6900 Heidelberg, Federal Republic of Germany

(*Received* 22 *August* 1984; *accepted* 20 *November* 1984)

Abstract—Cytochrome P-450, purified from liver microsomes of phenobarbital-induced rabbits, was phosphorylated by the catalytic subunit of cyclic AMP-dependent protein kinase. Upon phosphorylation P-450 was found to be converted to its denatured form, P-420, as verified spectroscopically from the CO-bound form of the reduced cytochrome. The conversion was dependent on both kinase and ATP. Thus, cyclic AMP may regulate the biotransformation system through the control of the degradation rate of microsomal P-450 *in vivo*.

Almost two decades ago it was found that catecholamines depressed the hepatic metabolism of drugs [1]. Although the injection of N^6,O^2-dibutyryl cyclic AMP (cAMP), an analogue of cAMP, was later shown to decrease the content of microsomal cytochrome P-450 (P-450) in liver *in vivo* [2], the mechanism of the hormonal action, which is apparently mediated by the cellular level of cAMP, on the biotransformation system remained unclear. Recent experiments demonstrated that a major inducible form of P-450, purified from phenobarbital-pretreated rabbit liver microsomes, was phosphorylated by the catalytic subunit of cAMP-dependent protein kinase, and the phosphorylation of P-450 was accompanied by a decrease in the reconstituted monooxygenase activity [3, 4]. This suggests that the hormonal action is mediated by direct phosphorylation of protein components of the microsomal biotransformation system. In the present report, we describe further studies on the mechanism of phosphorylation-induced decrease in monooxygenase activity by examining the spectral properties of P-450. Phosphorylation was found to cause conversion of P-450 to its denatured form, P-420. The phosphorylation of P-450 may, therefore, regulate the biotransformation system through the control of the half-life of P-450.

MATERIALS AND METHODS

P-450 was purified from liver microsomes of phenobarbital-pretreated rabbits as described [5]. Several minor contaminants, which were detected in SDS gel electrophoresis by silver staining, were removed by passing through a small column of aminooctyl Sepharose 4B equilibrated with 10 mM potassium phosphate buffer (pH 7.3) containing 20% glycerol and 0.2% Emulgen 913. The final preparations had specific contents of 18–20 nmole/mg protein and were free from detergents (less that 1 mole per mole P-450) [5]. The catalytic subunit of cAMP-dependent protein kinase was purified as described previously [6]. Phosphorylation of P-450 was performed as described [3, 4]. 0.8 nmole of P-450 was incubated at 37° with 65 μl of 60 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate buffer (pH 7.4) containing 6 mM $MgCl_2$, 0.35 mM ATP and 0.2 μM kinase. Aliquots of the incubation mixture taken at indicated times were diluted with 180 mM potassium phosphate buffer (pH 7.3) containing 20% glycerol and 0.2% Emulgen 913. The solution was first bubbled with CO and reduced with a few grains of dithionite. The concentrations of P-450 and P-420 were determined from absolute spectra of the CO-bound form of the reduced cytochrome assuming millimolar extinction coefficients 109 at 451 nm and 30 at 416 nm for P-450, 24 at 451 nm and 150 at 416 nm for P-420. All spectral measurements were performed in a Shimadzu UV-240 spectrophotometer. Protein concentration was determined by the method of Lowry *et al.* [7] using bovine serum albumin as a standard. All chemicals and biochemicals were of the highest quality available commercially.

RESULTS AND DISCUSSION

As shown in Fig. 1, the incubation of P-450 with catalytic amounts of kinase and ATP resulted in a slight red shift of the Soret band in the oxidized state. When either ATP or kinase was omitted from the incubation mixture, no significant change was detected. When the same preparation was reduced under saturating CO, more drastic changes were revealed; the major Soret peak shifted from 451 to 416 nm, indicating the conversion of P-450 to its denatured form, P-420 (Fig. 2b). The wavelength of the peak maximum observed was around 416 nm, shorter than that reported for P-420 formed by several denaturating agents [8]. This is simply due

Abbreviations: cAMP, 3',5'-cyclic AMP; P-450, cytochrome P-450; kinase, catalytic subunit of cyclic AMP-dependent kinase.

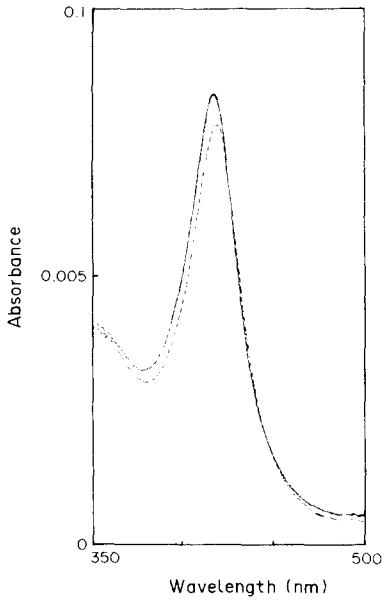

Fig. 1. Effect of phosphorylation on the absolute spectrum of P-450 in the oxidized state. P-450 was incubated with kinase as described in Materials and Methods. After the incubation for 1 hr, the reaction mixture was diluted with 1 ml of 180 mM potassium phosphate buffer containing 20% glycerol and 0.2% Emulgen 913. A solid line represents the spectrum of P-450 before incubation and that of P-450 incubated with kinase in the absence of ATP. The two spectra fall on the same line. A broken line represents the spectrum of P-450 incubated in the presence of both ATP and kinase.

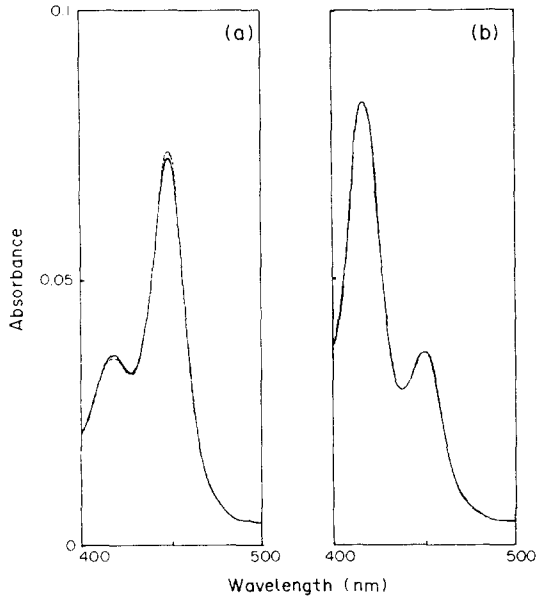

Fig. 2. Effect of phosphorylation on the absolute spectrum of P-450 in the CO-bound reduced state. The same preparations shown in Fig. 1 were bubbled with CO and reduced with dithionite. (a) A solid line represents the absolute spectrum of P-450 incubated with kinase in the absence of ATP. A broken line represents that of P-450 before incubation. (b) P-450 incubated with kinase and ATP.

to the interaction between P-420 and the non-ionic detergent, Emulgen 913, which was included in the medium to prevent the conversion of P-450 to P-420 during the spectral measurements. P-420, which was prepared by other techniques, namely by the addition of cholate in the absence of glycerol, showed essentially the same spectrum when measured in the presence of 0.2% Emulgen 913 (data not shown). The conversion of P-450 to P-420 was dependent on both kinase and ATP. P-450 incubated with either kinase or ATP did not show any significant spectral change both in oxidized and CO-bound reduced forms, and showed essentially the same spectra to those of P-450 before incubation (Figs. 2 and 3). The conversion observed, therefore, is clearly caused by the catalytic activity of the kinase.

With incubation time, the concentration of P-450 decreases continuously, while that of P-420 increases until 60 min (Fig. 3). Further incubation led to a decrease of the total heme protein concentration, indicating heme detachment from P-420. The time course of the decrease of P-450 corresponds very well to that of the extent of phosphorylation measured under the same conditions as reported [3]. After a 1 hr incubation, about 60% of the P-450 was converted to P-420. From these observations, it can be concluded that the decrease of the reconstituted monooxygenase activities upon phosphorylation [4] is caused by conversion of P-450 to its denatured form, P-420.

Although the mechanism of induction of the biotransformation activity in liver endoplasmic reticulum by drugs and chemical carcinogens is well known [9], the control of degradation of the same system has drawn less attention. After the initial observation by Fouts, which showed that catecholamines depress biotransformation by liver microsomes [1], dibutyryl-cAMP was shown to increase sleeping time after hexobarbital administration [10], indicating that control of the microsomal biotransformation system by these transmitters is also mediated by cAMP as is the well known cellular control of many enzymes [11]. Hutterer *et al.* [2] have shown further that the administration of dibutyryl cAMP decreases the content of microsomal P-450, which results in a decrease of the biotransformation activities, both in phenobarbital-induced and in uninduced rats. Our present results explain these obser-

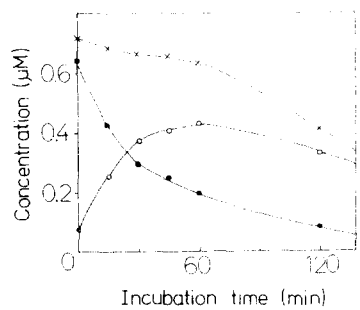

Fig. 3. Time course of the conversion of P-450 to P-420 by phosphorylation. Concentration of P-450 (●) and that of P-420 (○) were calculated as described in Materials and Methods. Total heme protein concentration (×) is also presented.

vations on the molecular level. Since P-420, unlike the native P-450, loses heme easily to form the apoprotein (see Fig. 3), the conversion of P-450 to P-420 may well be the rate-limiting step in the degradation pathway of P-450. Thus, phosphorylation of P-450 may, together with induction mechanisms, regulate the cellular content of P-450. Phosphorylation of P-450, in fact, has been shown to occur *in vivo* [12].

Since the conversion of P-450 to P-420 by detergents and lipases was first demonstrated by Omura and Sato [13], many substances of quite different nature have been added to the list of denaturing agents of P-450 [8]. Because all the substances so far reported are not present *in vivo*, the conversion of P-450 to P-420 has been considered to be an *in vitro* artifact. The present study, however, suggests for the first time a physiological function for the conversion.

Acknowledgements—The excellent technical assistance of Ms. T. Eisbein is appreciated. This work was supported in part by a grant from National Foundation of Cancer Research (to H. T. & A. S.).

REFERENCES

1. J. R. Fouts, (1962) *Fed. Proc.* **21**, 1107 (1962).
2. F. Hutterer, K. Dressler, H. Greim, P. Czygan, F. Schaffner and H. Popper, in *Cytochromes P-450 and b_5* (D. Y. Cooper, O. Rosenthal, R. Snyder and C. Witmer, eds.), pp. 117–126. Plenum Press, New York (1975).
3. W. Pyerin, C. R. Wolf, V. Kinzel, D. Kübler and F. Oesch, *Carcinogenesis* **4**, 573 (1983).
4. W. Pyerin, H. Taniguchi, A. Stier, F. Oesch and C. R. Wolf, *Biochem. biophys. Res. Commun.* **122**, 620 (1984).
5. Y. Imai, C. Hashimoto-Yutsudo, H. Satake, A. Girardin and R. Sato, *J. Biochem.* **88**, 489 (1980).
6. W. Pyerin, M. Gagelmann, W. Kübler and V. Kinzel, *Z. Naturforsch.* **34c**, 1186 (1979).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
8. Y. Imai in *Cytochrome P-450* (Eds. R. Sato and T. Omura), pp. 37–72. Kodansha-Academic Press, Tokyo (1978).
9. D. W. Nebert, H. J. Eisen, M. Negishi, M. A. Lang, L. M. Hjelmeland and A. B. Okey, *Ann. Rev. Pharmac. Toxicol.* **21**, 431 (1981).
10. M. Weiner, G. G. Buterbaugh and D. A. Blake, *Res. Commun. Chem. Path. Pharmac.* **3**, 249 (1972).
11. P. Cohen, *Nature* **296**, 613 (1982).
12. R. N. Sharma, M. Behar-Bannelier, F. S. Rolleston, and R. K. Murray *J. biol. Chem.* **253**, 2033 (1978).
13. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).