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STUDY OF THE MECHANISM OF METABOLIC ACTIVATION OF CHLORAMPHENICOL BY RAT LIVER MICROSOMES

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Abstract—The mechanism of the metabolic activation of chloramphenicol (CAP) by a cytochrome P-450 enzyme system in liver microsomes of rats has been studied by measuring the covalent binding to microsomal protein of specifically labeled [^{14}C] and [^{3}H] derivatives of CAP. The lack of binding of dichloroacetic acid, CAP base, and the acetamido and trifluoroacetamido derivatives of CAP indicates that the dichloroacetamido group of CAP is required for activation. The binding of dichloroacetamide supports this conclusion. Moreover, the C—H bond of the dichloromethyl carbon of CAP appears to be broken in the activation process since the hydrogen is lost in covalent binding. These results are consistent with the view that CAP is activated by hydroxylation of the dichloroacetamido group followed by spontaneous dehydrochlorination to an oxamyl chloride which acylates microsomal protein. The possible pharmacologic and toxicologic importance of this oxidative dehalogenation process is discussed.

Chloramphenicol (CAP) is a broad spectrum antibiotic which is indicated in severe salmonellae infections, and is regarded as an alternate agent for pneumococcal, meningococcal and Haemophilus influenza meningitis [1]. This agent has also been reported to be effective in serious Bacteriodes infections [1]. However, bone marrow depression has been associated with the use of this valuable antibiotic. This toxicity is usually reversible if CAP treatment is discontinued, but approximately 1 in 20,000 patients develops aplastic anemia, a bone marrow disease which is often irreversible and fatal [2]. Several groups of investigators have attempted to elucidate the mechanism of this toxicity. The results of their studies suggest that CAP may produce this blood disorder by inhibiting directly bone marrow mitochondrial protein synthesis [3–5] or DNA synthesis [5]. Other findings suggest that the bone marrow depression is an immunological disease [6–9]. Since this toxicity has not been produced in animals, however, these or other potential mechanisms have not been unequivocally established.

The rare incidence of CAP-induced aplastic anemia suggested to us that a minor metabolite may be involved in the induction of the toxicity. This possibility seemed reasonable since the toxicity [10–14] and carcinogenicity [11, 15–17] of other drugs and environmental chemicals were known to be related to the formation of electrophilic metabolites, which reacted with tissue macromolecules. CAP seemed to be particularly susceptible to metabolic activation since it contained both nitrophenyl and dichloromethyl groups. The metabolic reduction of a nitro aromatic group is known to activate other compounds into electrophilic toxic agents [17]. Similarly, the halocarbons, carbon tetrachloride [18–20] and chloroform [21], are biotransformed into reactive intermediates that produce toxicity. Consequently we have attempted to determine if a minor reactive metabolite of CAP is formed in the body.

Previous studies with CAP labeled with [^{14}C] in the 1 and 2 positions of the dichloroacetamido moiety revealed that this compound is bound irreversibly to protein when it is incubated with rat liver microsomes [22, 23]. The binding appeared to be related to the formation of an unknown reactive metabolite which was produced by a cytochrome P-450 monooxygenase in liver microsomes of phenobarbital-treated rats. Moreover, when [^{14}C]CAP was given to rats, the [^{14}C] label was found to bind irreversibly to tissue protein in various organs, including the bone marrow [22, 23].

In the present investigation, we have studied the nature of the metabolic activation process by conducting binding studies *in vitro* with specifically labeled derivatives of CAP [24].

MATERIALS AND METHODS

Materials

[1,2-^{14}C]dichloroacetic acid, 2.42 mCi/m-mole; [^{14}C]chloramphenicol [(1R,2R)-(+)-1-*p*-nitro-phenyl-2-[1,2-^{14}C]dichloroacetamido-1,3-propane-diol], 4.87 mCi/m-mole; [^{14}C]fluoramphenicol [(1R,2R-1-*p*-nitrophenyl-2-[2-^{14}C]trifluoroacetamido-1,3-propanediol], 4.20 mCi/m-mole; [^{3}H]sodium borohydride, 185 mCi/m-mole; and [^{3}H]water, 1.8 mCi/m-mole were purchased from New England Corp., Boston, MA, Nuclear Chemical Co., Milwaukee, WI. Dichloroacetic acid, dichloroacetamide, acetic anhydride, *N*,*N*′-carbonyldiimidazole, and deuterated water (99 per cent) were purchased from Aldrich. NADP, NADH, glucose 6-phosphate, nicotinamide, CAP, CAP base [(1R,2R-(−)-1-*p*-nitrophenyl-2-amino-1,3-propanediol], and *N*-bromosuccinimide were purchased from Sigma Biochemicals, St. Louis, MO. Glucose 6-phosphate dehydrogenase was pur-

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chased from Cal-Biochem. 3-Methylcholanthrene was purchased from Eastman Chemicals, Rochester, NY. The scintillation mixture, which consists of a mixture of 0.4% BBOT [2,5-bis(5-tert-butyl-benzooxazoyl) thiophene], 0.8% naphthalene, and 40% methylcellusolve in toluene, was purchased from Yorktown Research, Hackensack, NJ. Scintillation counting was performed with a Tri-Carb model 3375 spectrophotometer, using 15 ml of scintillation mixture. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected.

Methods

Thin-layer chromatography. Thin-layer chromatography (t.l.c.) was performed using Eastman 6060 Silica gel plates. The chromatograms were usually developed 15 cm with one of the following three solvent systems: solvent system 1, chloroform–methanol (100:15); solvent system 2, benzene–methanol–acetic acid (45:8:4); and solvent system 3, isopropanol–ammonium hydroxide–water (85:5:15). Thin-layer chromatography analysis with these three solvent systems indicated that all the radiochemicals were at least 99 per cent pure.

Synthesis of [1-^{3}H]CAP, (1R,2R)-1-p-nitrophenyl-2-dichloroacetamido-1,3-[1-^{3}H]propanediol. This compound was synthesized using essentially the procedure of Levai *et al.* [25], which Kutter and Machleidt [26] used for the synthesis of [1-^{2}H]CAP. The procedure was modified as follows: tritiated sodium borohydride (102 mg, 2.70 m-mole, 500 mCi) was added to absolute ethanol (6 ml) and the resulting solution was stirrred magnetically, protected from water in the atmosphere with a drying tube, and cooled to $-30 \pm 3°$ in a bath of dry ice and bromobenzene. A solution of $CaCl_2 \cdot 2H_2O$ (178 mg, 1.21 m-mole in 8 ml of absolute ethanol) was then added dropwise (3 min) and the reaction mixture was magnetically stirred for 80 min at $-30 \pm 3°$. Then a solution of CAP ketone, (R)-(+)-1-*p*-nitrophenyl-2-dichloro-acetamido-3-hydroxyl-1-propanone (593 mg, 1.85 m-mole, in 7 ml of absolute ethanol), which was prepared by the method of Kutter and Machleidt [26], was added dropwise (4 min). The reaction mixture was stirred for an additional 3.5 hr, while the temperature of the cooling bath slowly increased. Acetone (5 ml) was then added to the reaction mixture to consume the excess calcium borohydride. After 30 min of mixing at room temperature, 3 N hydrochloric acid (1.2 ml) was added, followed by the addition of unlabeled CAP (600 mg, 1.85 m-mole). The reaction mixture was evaporated (N_2) and the resulting pale yellow oil was allowed to crystallize. The product was then recrystallized from water twice and ethylene dichloride twice to yield white needle crystals, 517 mg, sp. act. 23.4 mCi/m-mole, m.p. 149–52° (lit. [27] 150–51° for unlabeled CAP). The specific activity was constant after further recrystallization. Radiochemical purity (99 per cent) was confirmed by t.l.c. The tritium label was established to be present in the benzylic position of CAP (>99 per cent) by oxidizing CAP back to CAP ketone with *N*-bromosuccinimide [26].

Synthesis of [1-^{3}H]CAP base hydrochloride ([1-^{3}H]CAP base), (1R,2R)-1-p-nitrophenyl-2-amino-1,3-[1-^{3}H]propanediol. The method of Rebstock *et al.* [28] was followed for the preparation of this compound. [1-^{3}H]CAP (9.1 mg, 23.4 mCi/m-mole) was diluted with unlabeled CAP (31.6 mg) and hydrolyzed with 1 N hydrochloric acid (1 ml) at $100 \pm 2°$ for 2 hr. The resulting reaction solution was extracted with ethyl ether (3 ml, four times), and the acidic aqueous phase was lyophilized to yield a pale yellow solid, 17 mg (53 per cent yield); 5.3 mCi/m-mole; radiochemical purity (99 per cent) was confirmed by t.l.c.

Synthesis of [1-^{3}H]acetamphenicol [(1R,2R)-1-p-nitrophenyl-2-acetamido-1,3-[1-^{3}H]propanediol]. [1-^{3}H]CAP base hydrochloride (10.4 mg, 0.042 m-mole, 5.3 mCi/m-mole) was partially dissolved in a solution of saturated sodium bicarbonate (100 µl). Acetic anhydride (7.5 µl, 0.080 m-mole) was added rapidly and the reaction mixture was allowed to stand at room temperature for 30 min. The resulting reaction solution was acidified with 1 N hydrochloric acid (50 µl, four times) and extracted with ethyl acetate (150 µl, four times). The combined ethyl acetate extract was evaporated (N_2) to yield a pale yellow viscous oil, 5.7 mg (53 per cent yield). The product co-chromatographed on t.l.c. (solvent systems 1–3) with unlabeled acetamphenicol, which was synthesized by this method and by the method of Rebstock *et al.* [28], and was radiochemically pure (99 per cent).

Synthesis of ([α-^{3}H]CAP), (1R,2R)-1-p-nitrophenyl-2-[^{3}H]dichloroacetamido-1,3-propanediol. CAP (1 g, 3.1 m-moles) was dissolved in dimethyl sulfoxide (5 ml) and added to a solution of sodium carbonate (106 mg, 1.0 m-mole) in tritiated water (1 g, 100 mCi, 1.8 mCi/m-mole). The reaction mixture was stirred magnetically at room temperature for 1 hr, transferred to a screw-top centrifuge tube, and acidified with 3 N hydrochloric acid (1.5 ml) to give a pale yellow solution. Saturated sodium chloride solution (10 ml) was added to the acidic solution, which was extracted with ethyl acetate (15 ml, three times). The combined ethyl acetate extracts were evaporated under vacuum to give a viscous oil, which began to crystallize upon addition of water. The white solid product was recrystallized from water five times to yield white needle crystals, 300 mg, sp. act. 0.71 mCi/m-mole. Radiochemical purity (99 per cent) was determined by t.l.c. The specificity of labeling was confirmed by performing the exchange reaction with deuterated instead of tritiated water. The n.m.r. (60 MHz, DMSO-d_6, tetramethyl silane internal standard) of the deuterated product indicated that only the dichloromethine proton [δ (ppm) 6.51] had exchanged. The tritium label was found to exchange slowly (approximately 6 per cent) when [α-^{3}H]CAP was dissolved and heated (80–90°) in water for approximately 10 min. However, when [α-^{3}H]CAP was incubated at 37° for 20 min at pH 7.4, under incubation conditions (refer to covalent binding section *in vitro*), only 1 per cent exchange was observed.

Synthesis of [1,2-^{14}C]dichloroacetamide. [1,2-^{14}C]-dichloroacetic acid (18 mg, 0.14 m-mole, 2.42 mCi/m-mole) was dissolved in tetrahydrofuran (0.5 ml), and *N,N'*-carbonyldiimidazole (25 mg, 0.15 m-mole) was added rapidly to the reaction mixture, which was then stirred at room temperature for 6 hr. Ammonia (anhydrous) was bubbled into the reaction mixture for 15 min, and after standing an additional 30 min, the reaction mixture was evaporated (N_2) to yield a vis-

cous oil. The product was dissolved in ethyl acetate, and washed with 1 N hydrochloric acid (0.5 ml, three times) and saturated sodium bicarbonate solution (0.5 ml, three times). The washed product (45 per cent yield; in ethyl acetate) co-chromatographed on t.l.c. (solvent systems 1–3) with unlabeled dichloroacetamide, which was synthesized by this method, and was radiochemically pure (99 per cent).

Preparation of microsomes. Male Sprague–Dawley rats (160–200 g) obtained from Hormone Assay Laboratories (Chicago, IL) were used in the experiments. Animals were allowed free access to water and food (Purina Lab Rat Chow). Rats were pretreated with phenobarbital (80 mg/kg, in saline, i.p.) 72, 48 and 24 hr before the experiment or with 3-methylcholanthrene (20 mg/kg in corn oil, i.p.) 72, 60 and 48 hr prior to the experiment. The rats were killed by decapitation and the livers were removed immediately and washed in 0.9% saline. The livers of three rats were combined, homogenized in 3 vol. of 0.25 M sucrose and the microsomes were isolated by the calcium aggregation method [29, 30]. The microsomal pellet was resuspended in 1.15% KCl–0.02 M Tris–HCl, pH 7.4 (Tris–KCl), and the concentration of protein was determined by the method of Lowry *et al.* [31].

Covalent binding to microsomal protein in vitro. Incubations were performed in triplicate; each incubation vial usually contained 2 mg of microsomal protein, 0.10 mM NADH, 2.00 mM nicotinamide, 0.20 mM NADP, 2.00 mM glucose 6-phosphate, and 1 unit of glucose 6-phosphate dehydrogenase in a total volume of 1 ml of Tris–KCl buffer (pH 7.4). The mixture was preincubated at 37° for 1 min and the reaction was started by the addition of substrate in 10 μl dimethylformamide. A substrate concentration of 0.10 mM was routinely employed, since in a previous study with [^{14}C]CAP, the apparent K_m of binding to microsomal protein was 0.05 mM [22]. The mixture was usually incubated for 8 min at 37° under air and the reaction was stopped by the addition of 10% trichloroacetic acid (1 ml) or absolute ethanol (3 ml). The reaction mixture was centrifuged and the resulting pellet (precipitated protein) was washed with methanol–ether (3:1, 5 ml, ten times). After aspiration of the final wash, which contained virtually no radioactivity, the protein was dissolved in 1 ml of 1 N sodium hydroxide. The covalently bound radioactivity was determined by counting an aliquot (0.5 ml) of the alkaline solution [22, 23]. Protein was determined by the method of Lowry *et al.* [31]. Nonspecific binding was determined by performing incubations with heat-denatured microsomal preparations. The amount of binding from these incubations (approximately 10 per cent of the binding from active microsomes) was subtracted from the binding results to obtain corrected values.

RESULTS

Effect of incubation conditions on the binding of [^{14}*C*]- *and* [1-^{3}*H*]*CAP*

When the incubations were conducted with microsomes from control or 3-methylcholanthrene-pretreated rats, no binding of either label was detected (Table 1). However, when microsomes from phenobarbital-pretreated rats were utilized, considerable binding of both labels was found. Moreover, the amounts of binding of the [^{14}C] and [^{3}H] labels were nearly identical. When NADPH was excluded from the incubation mixture, approximately a 78 per cent inhibition in the binding of both labels was observed. A similar decrease was also observed when the incubations were conducted in atmospheres of N_2 and $CO:O_2$ or in the presence of SKF 525-A, cysteine and glutathione (GSH) (Table 1).

The incubation with microsomes from phenobarbital-pretreated rats was also performed for various times and at different concentrations of microsomal protein. The rates of binding of both labels were nearly identical and were linear with both time for 8 min (Fig. 1) and microsomal protein concentration up to 4 mg/ml.

Table 1. Effect of various incubation conditions on the covalent binding *in vitro* of [^{14}C]- and [1-^{3}H]CAP to protein from liver microsomes of rat*

Incubation condition	Covalent binding† (pmoles/mg protein/8 min)		Inhibition (%)
	[1-^{3}H]CAP	[^{14}C]CAP	
Control microsomes	0	0	
3-MC microsomes‡	0	0	
PB microsomes‡	413 ± 27	444 ± 24	
− NADPH	83 ± 8	104 ± 20	78
+ N_2	168 ± 12	193 ± 5	58
+ $CO:O_2$ (9:1)	207 ± 15	209 ± 15	52
+ Cysteine (1.0 mM)	263 ± 25	333 ± 30	30
+ GSH (1.0 mM)	81 ± 20	88 ± 26	80
+ SKF 525-A (0.1 mM)	142 ± 5	160 ± 2	65
(1.0 mM)	10 ± 3	12 ± 3	97

* A mixture of [^{14}C]CAP (5.2 dis./min/pmole) and [1-^{3}H]CAP (25.9 dis./min/pmole), 0.10 mM, was incubated with microsomes for 8 min as outlined in Methods with deletions, additions or alterations of atmospheres as noted.

† Results are expressed as means ± S. E. of two experiments, which were each performed in triplicate on separate days.

‡ 3-MC microsomes were obtained from 3-methylcholanthrene-treated rats and PB microsomes were obtained from phenobarbital-treated rats as outlined in Methods.

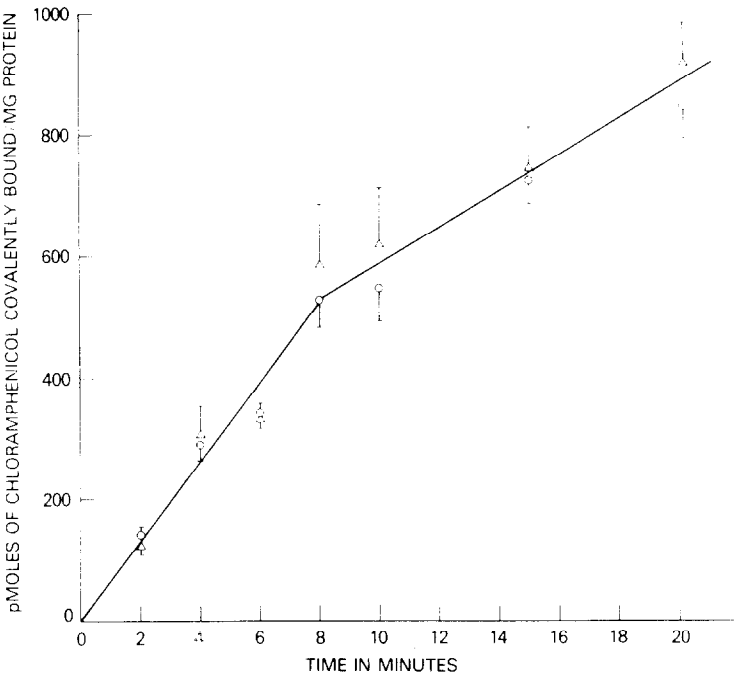


Fig. 1. Covalent binding *in vitro* of [^{14}C]- and [1-^{3}H]CAP to protein from liver microsomes of rat as a function of time. A mixture of [^{14}C]CAP (5.2 dis./min/pmole) and [1-^{3}H]CAP (25.9 dis./min/pmole), 0.10 mM, was incubated with microsomes from phenobarbital-pretreated rats for 2, 4, 6, 8, 10, 15 and 20 min as outlined in Methods. Values are expressed as means ± S. E. of two experiments, which were each performed in triplicate on separate days. An open circle represents the [^{14}C]CAP results whereas an open triangle represents the [1-^{3}H]CAP results.

Effect of structure on covalent binding

[^{14}C]- and [1-^{3}H]CAP were bound to microsomal protein to the same extent when they were incubated with microsomes from phenobarbital-pretreated rats (Fig. 2). When incubations were conducted with radiolabeled dichloroacetic acid, CAP base, and the acetamido, trifluoroacetamido and [α-^{3}H]derivatives of CAP, no binding of radiolabel occurred. However, the [^{14}C] label of dichloroacetamide did bind to microsomal protein, when this compound was incubated at a concentration of 1.0 mM.

DISCUSSION

In this investigation, we have attempted to define more clearly the mechanism of the metabolic activation of chloramphenicol by rat liver microsomes. The results of this study have led to a better understanding of this process and are consistent with the mechanism presented in Fig. 3. The involvement in this process of a cytochrome P-450 monooxygenase, which is induced by phenobarbital, is supported by the binding results of previous studies [22, 23] with [^{14}C]CAP and the results given in Table 1. The inhibition of binding in an atmosphere of CO:O_2 or in the absence of NADPH or in the presence of SKF 525-A suggests the involvement of a cytochrome P-450-activating system. The inhibition in the presence of an atmosphere of N_2 is consistent with an oxygen-dependent process.

The binding of [^{14}C]- and [^{3}H]CAP in a ratio of 1:1 (see Table 1 and Fig. 1) under various conditions indicates that the intact molecule is involved in the activation and binding processes. The lack of binding of [^{14}C]dichloroacetic acid and [1-^{3}H]CAP base (see Fig. 2), which could result from CAP by enzymatic hydrolysis [32], further suggests that the intact molecule is involved in the metabolic activation. The lack of binding of the acetamido and trifluoroacetamido derivatives of CAP (see Fig. 2) reveals that the dichloromethyl group and not the nitrophenyl of CAP is the site of metabolic activation. This observation is confirmed by the binding of [^{14}C]dichloroacetamide (see Fig. 2). The absence of binding by [^{14}C]dichloroacetic acid, which contains a dichloromethyl group, is likely due to the low lipid solubility of this strong acid, which would be virtually ionized at pH 7.4.

The absence of binding of [α-^{3}H]CAP (see Fig. 2) is consistent with the C—H bond of the dichloromethyl carbon being broken in a hydroxylation process (see Fig. 3). The α-carbon hydroxylation of acetamido moieties is a documented metabolic process which has been observed with *N*-arylacetamides in studies in rabbit [33, 34] and man [34]. In these cases, since no halogens were attached to the α-carbon, the glycolamide metabolites were stable and thus could be isolated and identified. However, in the case of CAP, the hydroxylated metabolite would be expected to spontaneously dehydrochlorinate to produce an oxamyl chloride derivative (see Fig. 3). This reactive metabolite could either react with water to produce CAP oxamic acid or with microsomal protein. Cysteine and GSH would be expected to react with the

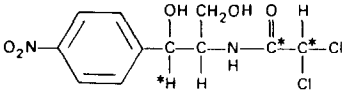
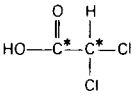
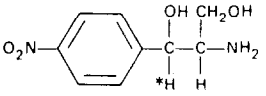
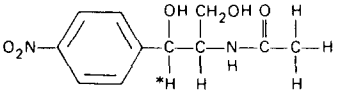
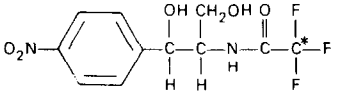
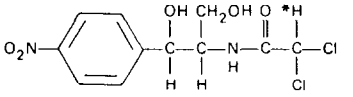
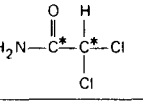
Substrate	Concentration	Covalent Binding (pmoles/mg protein/8min)
[^{14}C] + [1-^{3}H] CAP 	0.1 mM	440
[^{14}C] Dichloroacetic acid 	0.1 mM 1.0 mM	0 0
[1-^{3}H] CAP base 	0.1 mM 1.0 mM	0 0
[1-^{3}H] Acetamphenicol 	0.1 mM 1.0 mM	0 0
[^{14}C] Fluramphenicol 	0.1 mM 1.0 mM	0 0
[α-^{3}H] CAP 	0.1 mM	0
[^{14}C] Dichloroacetamide 	0.1 mM 1.0 mM	0 56

Fig. 2. Covalent binding *in vitro* of derivatives of CAP to protein from liver microsomes of phenobarbital-pretreated rats. Incubations were conducted for 8 min as outlined in Methods with the following substrates: a mixture of [^{14}C]CAP (5.2 dis./min/pmole) and [1-^{3}H]CAP (25.9 dis./min/pmole), 0.1 mM; [^{14}C]dichloroacetic acid (5.4 dis./min/pmole), 0.1 and 1.0 mM; [1-^{3}H]CAP base (11.6 dis./min/pmole), 0.1 and 1.0 mM; [1-^{3}H]acetamphenicol (11.6 dis./min/pmole), 0.1 and 1.0 mM; [^{14}C]fluoramphenicol (9.3 dis./min/pmole), 0.1 and 1.0 mM; [α-^{3}H]CAP (1.5 dis./min/pmole), 0.1 and 1.0 mM; and [^{14}C] dichloroacetamide (5.4 dis./min/pmole), 0.1 and 1.0 mM. The asterisk indicates the position of the [^{14}C] and [^{3}H] labels. Results are expressed as means of two experiments, which were each performed in triplicate on separate days.

oxamyl chloride and, therefore, inhibit covalent binding to protein (see Table 1). These two thiol compounds have previously been shown to block the covalent binding of other compounds *in vitro* by reacting with electrophilic intermediate metabolites [35, 36].

The isolation and characterization of CAP oxamic acid [24, 37], as a metabolite of CAP, from liver microsomes of phenobarbital-pretreated rats support the proposed intermediate role of the oxamyl chloride reactive metabolite (see Fig. 3). Importantly, the oxamic acid was not detected when the incubations were conducted with normal microsomes [37]. Since no binding of CAP was observed under these conditions, the covalent binding correlates with the formation of CAP oxamic acid. Moreover, CAP oxamic acid was also isolated from the base hydrolyzate of radiolabeled microsomal protein [37]. This finding suggests that the reactive oxamyl chloride intermediate acylates microsomal protein (see Fig. 3) because a thioester, ester or amide bond of the conjugate would be expected to hydrolyze in sodium hydroxide to yield CAP oxamic acid.

The mechanism described in Fig. 3 can be considered an oxidative dehalogenation process. This pathway of biotransformation could occur potentially with other halocarbons, if a given carbon is bonded to hydrogen and one or more halogens. In fact, metabolic studies *in vitro* performed with chloroethanes [38, 39] and halomethanes [40, 41] indicate that these compounds are oxidatively dehalogenated by a similar cytochrome P-450 monooxygenase which is induced by phenobarbital treatment but not appreciably changed by 3-methylcholanthrene treatment. The

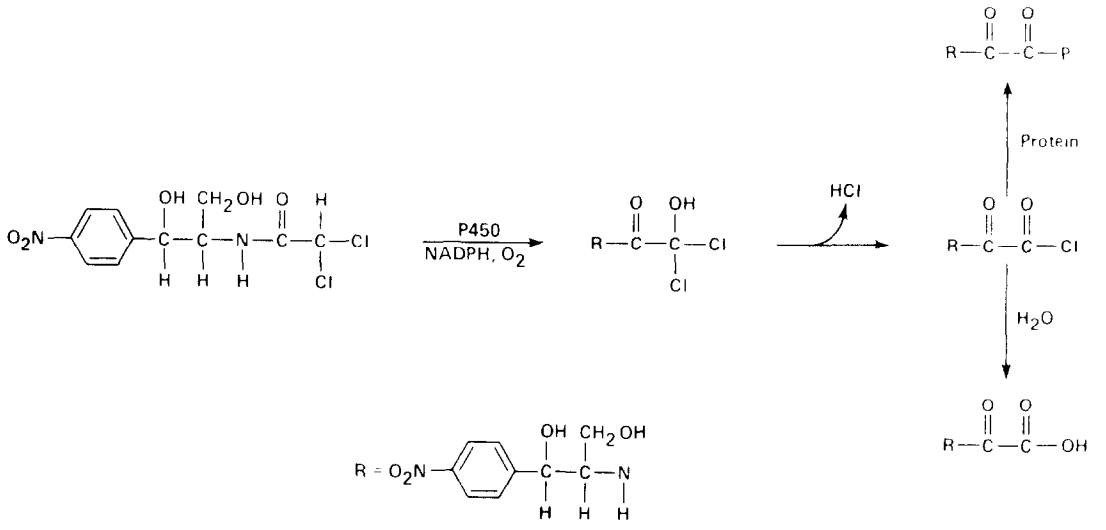


Fig. 3. Proposed mechanism for the metabolic activation of CAP by liver microsomes from phenobarbital-pretreated rats.

structure of the organic metabolite resulting from these reactions will depend upon the number of halogens bonded to carbon. If one halogen is bounded to carbon as in the case of chloroethane, the expected product after spontaneous dehydrohalogenation would be an aldehyde. In the case of di- and tri-halo substituted carbons, acyl halides would be an anticipated product. These reactive products could either hydrolyze to an acid or react with tissue macromolecules (see Fig. 3). In the case of the halomethanes, the acyl halide, formyl halide, would also be expected to spontaneously dehydrohalogenate to yield carbon monoxide [42], a major product of the oxidative dehalogenation of these compounds [40, 41].

The activation of chloroform may be related to this process. Chloroform binds covalently to liver tissue protein both *in vivo* [21, 43] and *in vitro* [21, 43, 44]. The binding *in vitro* to mouse [21], rabbit [43] and rat [44] microsomal protein is catalyzed by a P-450 monooxygenase, which is induced by phenobarbital. Moreover, the binding of [^{14}C]chloroform by liver microsomes from phenobarbital-pretreated rats can be blocked by cysteine; concomitantly a new metabolite which was identified as 2-oxothiazolidine-4-carboxylic acid is formed.* This product presumably resulted from the reaction of phosgene, $COCl_2$, with cysteine. Phosgene could also hydrolyze to yield carbon dioxide, which is known to be formed from chloroform in rat liver homogenates [45]. A similar process may be involved in the activation of the general anesthetic, halothane, which contains a bromochloro-substituted carbon [43, 46–48].

Although the mechanism for metabolic activation of CAP by rat liver microsomes is now more clearly understood, the pharmacologic and toxicologic relevance of this finding remains to be determined.

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