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Protective Effects of Suprofen and its Methyl Ester Against Inactivation of Rabbit Kidney Carbonyl Reductase by Phenylglyoxal

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Suprofen (SP) was little reduced by rabbit kidney carbonyl reductase, whereas its methyl ester (SPM) was an efficient substrate of the enzyme. To account for the differential catalytic activities for SP and SPM, the protective effects of these compounds against the inactivation of the enzyme by phenylglyoxal (PGO) were compared. Since the carboxyl group of SP is negatively charged and one essential arginine residue is known to be located in the NADPH-binding site of the enzyme, the protection of SP against the inactivation of the enzyme by PGO is expected to be more effective than that of SPM lacking a carboxyl group. However, the protective effects of SP and SPM were very similar. These results suggest that in spite of evidence for the binding of SP to the coenzyme-binding site, the carboxyl group of SP fails to interact with one essential arginine residue located in the site.

Keywords: Suprofen; Suprofen methyl ester; Carbonyl reductase; Phenylglyoxal; Inactivation; Protective effect

INTRODUCTION

Carbonyl reductase (EC 1.1.1.184) is an enzyme responsible for the NADPH-dependent reduction of ketone-containing drugs to the corresponding alcohol metabolites.[1–3] This enzyme is widely distributed in various tissues of mammalian species.[4–9] We have purified a carbonyl reductase from the cytosolic fraction of rabbit kidney using acetohexamide, an oral antidiabetic drug, as a substrate.[10] The purified enzyme (rabbit kidney carbonyl reductase) effectively catalyzes the ketone-reduction of many drugs including acetohexamide,[10] but has little or no ability to reduce the ketone group of nonsteroidal anti-inflammatory drugs such as suprofen (SP), ketoprofen and fenbufen.[10,11]

We have demonstrated that one essential arginine residue located in the coenzyme-

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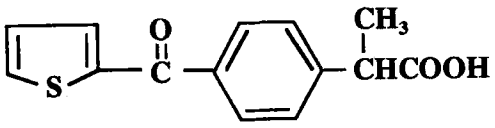
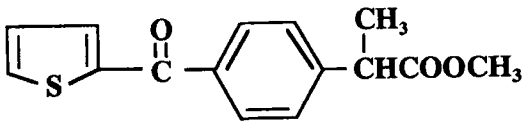
binding site plays an important role in the binding of NADPH to rabbit kidney carbonyl reductase.[12] The nonsteroidal anti-inflammatory drugs described above possess not only a ketone group, but also a carboxyl group within their chemical structures. Thus, the negatively charged carboxyl group of nonsteroidal anti-inflammatory drugs may interact with the arginine residue located in the coenzyme-binding site,[12–14] suggesting that the nonsteroidal anti-inflammatory drugs interfere with the binding of NADPH to the enzyme.

The present study was undertaken to elucidate why the ketone group of nonsteroidal anti-inflammatory drugs cannot be reduced by rabbit kidney carbonyl reductase. For this purpose, we examined whether or not the carboxyl group of SP interacts with one essential arginine residue located in the coenzyme-binding site, by comparing the protective effects of SP and its methyl ester (SPM) (Fig. 1) against the inactivation of the enzyme by a chemical modifier for the arginine residue, phenylglyoxal (PGO).



SP



SPM

FIGURE 1 Chemical structures of SP and SPM.

MATERIALS AND METHODS

Materials

Carbonyl reductase was purified from the cytosolic fraction of rabbit kidney using acetohexamide (Shionogi Co., Osaka, Japan) as a substrate, as described by us.[10] The purified enzyme was confirmed to be a homogeneous protein by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. SP was supplied by Nihon Chemiphar (Tokyo, Japan). SPM was synthesized by methylation of SP according to the method of Maruyama *et al.*[15] PGO and 4-acetylpyridine were purchased from Tokyo Kasei (Tokyo, Japan). NADPH and $NADP^+$ were obtained from Oriental Yeast (Tokyo, Japan). All other chemicals were of reagent grade.

Enzyme Assay

Enzyme activity was assayed spectrophotometrically by monitoring NADPH oxidation at 340 nm.[10] SP, SPM and acetohexamide were used as the substrates. The reaction mixture, in a total volume of 1.0 ml, consisted of 0.1 M sodium potassium phosphate buffer (pH 6.0), 0.25 mM NADPH, 0.1 mM substrate and the purified enzyme. The reaction was started by addition of the enzyme. One unit of the enzyme activity was defined as the amount causing a decrease in absorbance of 340 nm corresponding to the oxidation of 1 μmol of NADPH/min at 30°C. Protein concentrations were determined with bovine serum albumin as the standard by the method of Lowry *et al.*[16]

Chemical Modification

Chemical modification with PGO was performed according to standard techniques.[12] The purified enzyme was incubated with 10 mM PGO in 0.25 ml of 0.1 M sodium potassium phosphate buffer (pH 7.4) at 30°C. The reaction was started by addition of the

enzyme after pre-incubating the reaction mixture for 3 min. At intervals, aliquots were removed from the incubation mixture. The aliquots were diluted with 0.1 M sodium potassium phosphate buffer (pH 6.0) to minimize the influence of the modifier on the enzyme reaction. Conditions of the enzyme assay were the same as those described above, except that 4-acetylpyridine at a concentration of 1.0 mM was used as the substrate. Compounds ($NADP^+$, SP and SPM), which were used to test the protective effect against the inactivation of the enzyme by PGO, were included in the pre-incubation mixture. The residual activity was expressed as percentage of the initial activity.

RESULTS

Catalytic Activities for SP, SPM and Acetohexamide

The catalytic activities of rabbit kidney carbonyl reductase for SP, SPM and acetohexamide were compared. As expected, SP was little reduced by rabbit kidney carbonyl reductase (Fig. 2). On the other hand, SPM and acetohexamide were efficient substrates of the enzyme (Fig. 2).

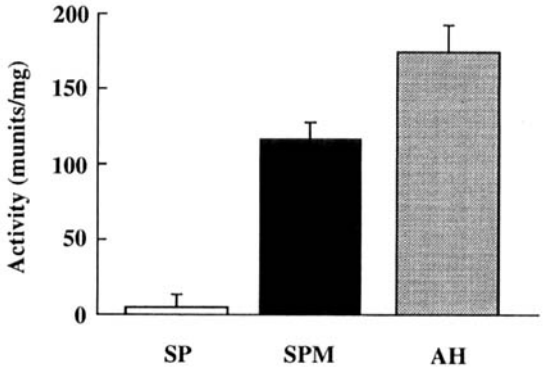


FIGURE 2 Substrate specificities of rabbit kidney carbonyl reductase for SP, SPM and acetohexamide (AH). Each bar represents the mean ± S.D. of three experiments.

Protective Effect of $NADP^+$ Against Inactivation by PGO

Figure 3 shows the protective effect of $NADP^+$ against inactivation of rabbit kidney carbonyl reductase by PGO, which is a chemical modifier of the arginine residue. The enzyme was rapidly inactivated by PGO, indicating that one arginine residue is essential for the enzyme activity as pointed out in our paper.[12] Furthermore, the inactivation of the enzyme by PGO was protected in the presence of $NADP^+$. Thus the arginine residue was confirmed to be located in the coenzyme-binding site of the enzyme.

Protective Effect of SP and SPM Against Inactivation by PGO

It was examined whether or not SP and SPM protected the inactivation of rabbit kidney carbonyl reductase by PGO. As shown in Fig. 4, the inactivation of the enzyme by PGO was protected in the presence of SP at a concentration of 0.5 mM. A similar protective effect was

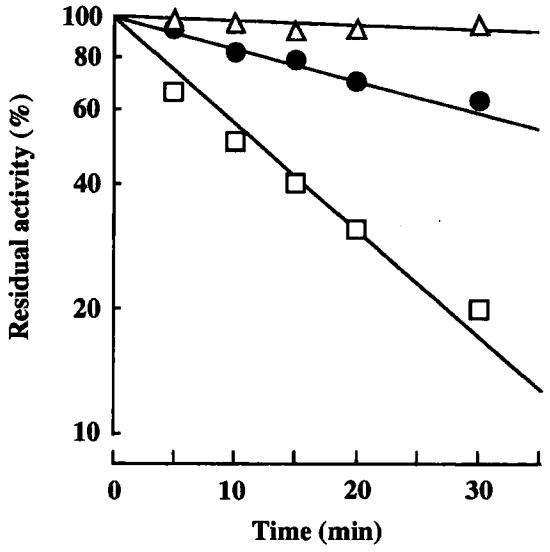


FIGURE 3 Protective effect of $NADP^+$ against the inactivation of rabbit kidney carbonyl reductase by PGO. The enzyme was incubated with: 0 mM PGO (△), 10 mM PGO (□) and 10 mM PGO+0.02 mM $NADP^+$ (●).

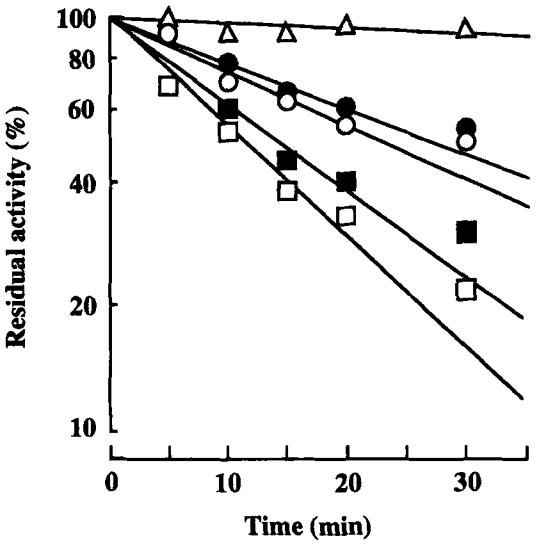


FIGURE 4 Protective effects of SP and SPM against the inactivation of rabbit kidney carbonyl reductase by PGO. The enzyme was incubated with: 0 mM PGO (△), 10 mM PGO (□), 10 mM PGO+0.1 mM SP (■), 10 mM PGO+0.5 mM SP (●) and 10 mM PGO+0.5 mM SPM (○).

observed in the presence of SPM at a concentration of 0.5 mM.

DISCUSSION

Carbonyl reductases generally have a broad substrate specificity and contribute to the reductive metabolism of a variety of drugs including acetohexamide.[1–3] Rabbit kidney carbonyl reductase purified by us can also catalyze the ketone-reduction of acetohexamide and daunorubicin.[10] The present study demonstrated that SP is little reduced by rabbit kidney carbonyl reductase, whereas SPM, as well as acetohexamide, is an efficient substrate of the enzyme. SP possesses a carboxyl group within its chemical structure. Thus the negatively charged carboxyl group of SP may interact with one essential arginine residue located in NADPH-binding site of the enzyme,[12–14] as described in the introduction. We have also shown that the enzyme reaction follows an ordered Bi Bi mechanism,[17] in which NADPH binds to the enzyme first and $NADP^+$ leaves from the enzyme last. It is possible that because of its affinity for the NADPH-binding site, SP is little reduced by the enzyme. To elucidate the mechanism of the differential catalytic activities for SP and SPM, the protective effects of these compounds against the inactivation of the enzyme by PGO were compared. The protection of SP against the inactivation of the enzyme by PGO is expected to be more effective than that of SPM lacking a carboxyl group.[12–14] However, the protective effects of SP and SPM were very similar, even though the catalytic activities of the enzyme for SP and SPM were markedly different. These results suggest that in spite of evidence for the binding of SP to the NADPH-binding site, the carboxyl group of SP fails to interact with one essential arginine residue located in the coenzyme-binding site.

It has been reported that the partition coefficient of SPM is much larger than that of SP.[15] Thus, the reason that SPM is an efficient substrate of rabbit kidney carbonyl reductase is probably explained by a higher affinity of SPM than SP to the substrate-binding site. Furthermore, we have revealed the presence of a hydrophobic pocket located in the substrate-binding site of the enzyme.[10] The methyl ester moiety of SPM molecule may be inserted into the hydrophobic pocket whereas the carboxyl moiety of SP is charged under the assay conditions and would be repelled by this pocket. Further studies are necessary to elucidate the mechanism for the differential catalytic activities of the enzyme for SP and SPM.

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