Pseudoperoxidase activity of 5-lipoxygenase stimulated by potent benzofuranol and *N*-hydroxyurea inhibitors of the lipoxygenase reaction

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The purified 5-lipoxygenase from porcine leukocytes was found to catalyse the degradation of lipid hydroperoxides in the presence of potent inhibitors of the lipoxygenase reaction. Derivatives of diphenyl-*N*-hydroxyureas, 4-hydroxybenzofurans and 5-hydroxydihydrobenzofurans all stimulated the 5-lipoxygenase-mediated destruction of 13-hydroperoxyoctadecadienoic acid (13-HPOD). The reaction was dependent on inhibitor and hydroperoxide concentrations (1–10 μ M) and could not be detected using heat-inactivated enzyme, when ATP and Ca^{2+} were omitted or when the hydroperoxide was replaced by the corresponding alcohol. The stability of the inhibitors during this pseudoperoxidase reaction was investigated by measuring the recoveries of 5-hydroxy-2-phenethyl-6-(3-phenoxypropyl)-2,3-dihydrobenzofuran and *N*-(4-chlorophenyl)-*N*-hydroxy-*N'*-(3-chlorophenyl)urea from the reaction mixtures using reverse-phase h.p.l.c. By using an equimolar concentration of 13-HPOD and inhibitor (10 μ M) and under conditions where 50% of the 13-HPOD was consumed, the concentration of the benzofuranol decreased by 30%, whereas the *N*-hydroxyurea derivative could be completely recovered from the reaction mixture. A stimulation of the pseudoperoxidase reaction could be detected only with very effective inhibitors of leukotriene B_4 biosynthesis by human leukocytes [IC_{50} (concn. causing 50% inhibition) < 100 nM], but not with closely related structural analogues of lower potency or other inhibitors such as nordihydroguaiaretic acid, quercetin or the hydroxamate A-64077. These results demonstrate that 5-lipoxygenase possesses a pseudoperoxidase activity and indicate that potent inhibitors in both *N*-hydroxyurea and benzofuranol series can function as reducing agents for the enzyme.

INTRODUCTION

The enzyme 5-lipoxygenase catalyses the dioxygenation of arachidonic acid at the C-5 position as a first step in the leukotriene-biosynthesis pathway [1]. Considering the potential therapeutic applications of leukotriene-biosynthesis inhibitors in the treatment of asthma and inflammatory diseases, extensive studies on structure–activity relationships of 5-lipoxygenase inhibitors have been published on flavonoids, catechols, aminopyrazolines, benzofuranols and hydroxamate derivatives [2–6]. Much of the information available on the mechanism of inhibition of lipoxygenases by these classes of compounds has come from studies using soybean 5-lipoxygenase as a model [7–13]. It has been reported that the enzymic reaction can be inhibited by chelation of the non-haem iron atom [8,9], trapping of radical intermediates [10] and by reductive inactivation [11–13]. In the reductive-inactivation model, compounds inhibit by reducing the ferric form of the enzyme to an inactive ferrous form. This mechanism is supported by direct e.s.r. and fluorescence measurements of enzyme reduction and by observations on inhibitor oxidation catalysed by the enzyme in the presence of either lipid hydroperoxides [11–13] or linoleic acid [14]. Since the reduced lipoxygenase can be reoxidized by hydroperoxides without inactivation, the soybean enzyme also catalysed the degradation of hydroperoxides (pseudoperoxidase activity) in the presence of reducing inhibitors. Reductive inactivation has

been suggested to account for the inhibition of soybean lipoxygenase by *N*-alkylhydroxylamines [11], catechols [12] and pyrazoline derivatives [13], but has not yet been applied to the mechanism of 5-lipoxygenase inhibition.

The oxidation of arachidonic acid by 5-lipoxygenases appears to proceed through mechanisms similar to those described for the soybean enzyme, although the mammalian enzyme is less stable, requires ATP and Ca^{2+} as cofactors [15,16] and is subject to strong inhibition by compounds which are very poor inhibitors of the other lipoxygenases [2–6]. Studies on the mechanism of 5-lipoxygenase inhibition have been limited by lack of information on the native enzyme, such as its content of metal ions, mainly due to the high enzyme lability. By using various cell-free preparations, it has been shown that the 5-lipoxygenase reaction is inhibited by reducing agents [17–19] and iron chelators [5,6] and that the inhibition potency of various compounds correlates with antioxidant properties [20]. Hydroperoxyeicosatetraenoic acids (HPETEs) have been shown to activate the reaction [18,19], to increase the rate of 5-lipoxygenase inactivation [21] and to inactivate irreversibly the soybean lipoxygenase in the presence of certain 5-lipoxygenase inhibitors [22]. Although all the above observations tend to support the assumption that the reactions catalysed by the two lipoxygenases are analogous, there is direct experimental evidence for neither the existence of different states of oxidation of 5-lipoxygenases nor for the reduction of the enzyme by inhibitors.

Abbreviations used: BW 755C, 3-amino-1-[3-(trifluoromethyl)phenyl]-2-pyrazoline; DTT, dithiothreitol; HPETE, hydroperoxyeicosatetraenoic acid; 13-HOD, (13*S*)-hydroxyoctadeca-(9*Z*,11*E*)-dienoic acid; 13-HPOD, (13*S*)-hydroperoxyoctadeca-(9*Z*,11*E*)-dienoic acid; IC_{50} , concentration of inhibitor causing 50% inhibition; NDGA, nordihydroguaiaretic acid; PC, phosphatidylcholine; LTB_4 , leukotriene B_4 .

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In the present paper we demonstrate that 5-lipoxygenase possesses a pseudoperoxidase activity and we report the identification of benzofuranols and *N*-hydroxyurea derivatives as novel and efficient stimulators of pseudoperoxidase reactions catalysed by lipoxygenases. We have also examined the quantitative relationship between inhibitory potency and efficiency of stimulation of the pseudoperoxidase reaction for several structural analogues. The results provide a basis for the evaluation of the redox properties of 5-lipoxygenase inhibitors and for further investigations on the regulation of the enzyme by oxidoreduction processes and its potential role in co-oxidation reactions.

MATERIALS AND METHODS

Materials

Arachidonic acid, ATP (99–100%), L- α -phosphatidylcholine (type III-E, from egg yolk), quercetin and nordihydroguaiaretic acid (NDGA) were obtained from Sigma Chemical Co. (13*S*)-Hydroxyoctadeca-(9*Z*,11*E*)-dienoic acid (13-HPOD) and 12-hydroperoxyeicosatetraenoic acid (12-HPETE) were from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). The structures of the various lipoxygenase inhibitors used in this study are given in Fig. 1. These compounds were synthesized at the Department of Medicinal Chemistry, Merck Frosst Centre for Therapeutic Research, according to previously described procedures: **I**, 5-hydroxy-2-phenethyl-2,3-dihydrobenzofuran [23]; **II**, 5-hydroxy-2-phenethyl-6-(3-phenoxypropyl)-2,3-dihydrobenzofuran or L-670,630 [23]; **III**, 7-chloro-4-hydroxy-2-[(4-methoxyphenyl)methyl]-3-methylbenzofuran [3]; **IV**, 7-chloro-4-hydroxy-2-[(4-methoxyphenyl)methyl]-3-methyl-5-propylbenzofuran or L-656,224 [3]; **V**, *N*-(4-chlorophenyl)-*N*-hydroxy-*N'*-(3-chlorophenyl)urea [24]; **VI**, *N*-(3,4-dichlorophenyl)-*N*-hydroxy-*N'*-(3-chlorophenyl)urea [24]; **VII**, *N*-methyl-2-[4-(2,4,6-trimethylphenyl)phenyl]propenehydroxamic acid [6]; **VIII**, *N*-[(*E*)-3-(3-phenoxyphenyl)prop-2-enyl]acetohydroxamic acid or

Fig. 1. Chemical structures of 5-lipoxygenase inhibitors from various classes

I and **II**, 5-hydroxy-2,3-dihydrobenzofurans; **III** and **IV**, 4-hydroxybenzofurans; **V** and **VI**, *NN'*-diphenyl-*N*-hydroxyureas; **VII** and **VIII**, hydroxamic acids; **IX**, *N*-hydroxyurea A-64077.

BW A4C(5); and **IX**, *N*-(1-benzo(*b*)thien-2-ylethyl)-*N*-hydroxyurea or A-64077 [25].

Purification and assay of 5-lipoxygenase

The 5-lipoxygenase was purified from porcine leucocytes by immunoaffinity chromatography as previously described [16]. The enzyme was eluted in 50 mM-sodium carbonate, pH 10, containing 0.2% sodium deoxycholate, 0.5 mM-dithiothreitol (DTT), 1 mM-EDTA and was mixed with $\frac{1}{8}$ vol. of 0.5 M-Tris/HCl, pH 7.4, to lower the pH of the eluting fractions. Enzyme activity was measured by using a spectrophotometric assay from the optimal rate of conjugated diene formation (A_{234}) after incubation of an enzyme aliquot with 20 μ M-arachidonic acid in the presence of ATP, Ca²⁺ and PC, as previously described [26].

Measurement of pseudoperoxidase activity of 5-lipoxygenase

The pseudoperoxidase activity was determined from the inhibitor-dependent decrease in 13-HPOD concentration catalysed by 5-lipoxygenase at room temperature using the variation in A_{234} or reverse-phase h.p.l.c. to monitor 13-HPOD levels. Typical reaction mixtures contained 10 μ M-13-HPOD and inhibitor, 0.2 mM-ATP, 0.4 mM-CaCl₂ and PC (24 μ g/ml) in 0.05 M-Tris/HCl or potassium/sodium phosphate buffer, pH 7.4. Inhibitors were added as 500-fold-concentrated solutions in ethanol or dimethyl sulphoxide, and the reactions were initiated by the addition of enzyme (3–7 μ g of protein in a final volume of 0.5 ml). Initial rates of the reaction were estimated from the decrease in A_{234} at zero time, assuming a first-order process of decay. The variation in the levels of 13-HPOD during the pseudoperoxidase reaction was also monitored by reverse-phase h.p.l.c. Reactions were stopped at the indicated time by mixing the incubation solution with 1 vol. of methanol and aliquots were injected on a Nova-Pak C₁₈ column (Millipore) using a mixture of acetonitrile/water/acetic acid (6500:3500:1, by vol.) as solvent system and a flow rate of 2 ml/min. The concentrations of 13-HPOD were determined by comparison of the u.v.-absorbance peak area at 235 nm (eluted at 2.2 min) with a known amount of standard.

Reverse-phase h.p.l.c. analysis of 13-HOD and inhibitors **II** and **V**

Pseudoperoxidase assays were performed using inhibitors **II** or **V** and the reaction products obtained after an incubation of 4 min were analysed by reverse-phase h.p.l.c. as described above. The retention times of inhibitors **II** and **V** were 3.9 min and 1.4 min respectively. The concentrations of inhibitors were determined for reactions stopped at zero time and 4 min from comparison of the u.v.-absorbance peak area with known amounts of standards using wavelengths of 308 nm for inhibitor **II** and of 260 nm for inhibitor **V**. Samples for 13-HOD analysis were injected on a Nova Pak C₁₈ column at a flow rate of 2 ml/min using acetonitrile/water/acetic acid (550:450:1, by vol.) as eluant, where (13*S*)-hydroxyoctadeca-(9*Z*,11*E*)-dienoic acid (13-HOD) (retention time 3.5 min) is resolved from 13-HPOD (retention time 4.1 min). Elution of these compounds was monitored by A_{235} .

Leukotriene B₄ (LTB₄) production by human leucocytes

The potency of compounds as inhibitors of LTB₄ biosynthesis was determined by using Ca²⁺-ionophore-activated leucocytes [27]. Inhibitors were preincubated for 2 min at 37 °C with freshly isolated polymorphonuclear leucocytes from human blood (by Dextran and Ficoll sedimentation) at a concentration of 5 $\times$ 10⁵ cells/ml. Inhibitors were added as 500-fold concentrated solution

in dimethyl sulphoxide. After preincubation, the cells were stimulated with Ca^{2+} ionophore A23187 ($10\ \mu\text{M}$ final concn.) and the level of LTB_4 produced after 5 min (37°C) was measured by radioimmunoassay (typically $20\text{--}40\ \text{pmol}/5 \times 10^5$ cells). The concentration of inhibitor causing 50% of LTB_4 release was derived from non-linear-regression analysis of five-point titrations for a minimum of three independent determinations.

RESULTS AND DISCUSSION

Inhibitor-dependent degradation of hydroperoxide by 5-lipoxygenase

Using the immunoaffinity-purified 5-lipoxygenase from porcine leucocytes, we have shown that the presence of inhibitors extends the initial lag phase of the reaction and increases the dependence of the initiation of the reaction on exogenous hydroperoxides [26]. Such an effect had also been observed during inhibition of the soybean lipoxygenase reaction by compounds that reduce the non-haem iron of the enzyme [11,12], suggesting that enzyme reduction may also occur on 5-lipoxygenase. This possibility was further studied using compounds II and V (Fig. 1) as models for the benzofuranol and *N*-hydroxyurea structural classes of inhibitors.

Fig. 2 shows that the incubation of 5-lipoxygenase with either compound II or V ($10\ \mu\text{M}$) in the presence of 13-HPOD ($10\ \mu\text{M}$) caused a time-dependent decrease in the hydroperoxide levels that can be followed by changes in A_{234} . For both compounds, the change in absorbance followed an apparent first-order decay, with the initial rate of the reaction increasing with the amount of inhibitor up to concentrations of $10\text{--}15\ \mu\text{M}$. No reaction could be detected either in the absence of inhibitor or using heat-inactivated enzyme.

The reaction was dependent on the presence of a lipid hydroperoxide, and similar levels of activity were observed with $10\ \mu\text{M}$ -13-HPOD, -5-, -12- or -15-HPETE. As illustrated in Fig. 3 for the reaction supported by compound II, decreasing the concentration of 13-HPOD resulted in lower activity, and no reaction could be detected when ATP and Ca^{2+} were removed from the incubation mixture or when 13-HOD was used instead of 13-HPOD. The results from Figs. 2 and 3 indicated that 5-lipoxygenase catalyses the degradation of hydroperoxides in the presence of compounds II and V. The strong requirement of the pseudoperoxidase reaction for very potent inhibitors of the 5-lipoxygenase activity (see below) also supports the conclusion that the reaction is catalysed by this enzyme. By using concentrations of $10\ \mu\text{M}$ for 13-HPOD and inhibitor, the initial rates of hydroperoxide consumption for various enzyme preparations ranged from 0.14 to 0.30 and from 0.24 to $0.54\ \mu\text{mol}/\text{min}$ per mg for compounds II and V respectively. The specific activity of the pseudoperoxidase reaction with compound V under these conditions corresponds to about 30% of that of the 5-oxygenase reaction using arachidonic acid as substrate [26].

The changes in A_{234} during the pseudoperoxidase reaction are consistent with a loss of the conjugated system of 13-HPOD, rather than a reduction of 13-HPOD to the corresponding alcohol (13-HOD), since both compounds have the same absorption coefficient at this wavelength [28]. Direct measurement of 13-HOD levels by reverse-phase h.p.l.c. after reactions with either inhibitor II or V showed that less than 0.5% of 13-HOD can be detected under conditions where 20% of 13-HPOD was consumed (results not shown). This result indicates that 13-HPOD reduction to 13-HOD does not occur to a significant extent during incubations with 5-lipoxygenase and inhibitors.

It has been proposed that 13-HPOD is degraded by soybean lipoxygenase to a mixture of products via the alkoxide radical

formed by one-electron reduction of the hydroperoxide [11,12]. The alkoxide radical would decompose via (1) cyclization to an unstable epoxyallylic radical, yielding a mixture of hydroxy derivatives that do not absorb in the near-u.v. [29], and (2) β -cleavage to give 13-oxo-octadeca-9,11-dienoic and 13-oxo-

Fig. 2. Dependence of the pseudoperoxidase activity of 5-lipoxygenase on inhibitor concentration

The decrease in A_{234} was measured after incubation of the enzyme with $10\ \mu\text{M}$ -13-HPOD and the indicated concentration of compounds II and V, under the conditions of the pseudoperoxidase assay described in the Materials and methods section. The continuous lines show the fitted curve obtained from non-linear-regression analysis of the data points assuming first-order kinetics [26].

Fig. 3. Hydroperoxide-dependence of the pseudoperoxidase reaction catalysed by 5-lipoxygenase

The variation in A_{234} was monitored with time under the conditions of the pseudoperoxidase assay using $10\ \mu\text{M}$ -inhibitor II. Incubation mixtures contained $10\ \mu\text{M}$ -13-HPOD (○), $7.5\ \mu\text{M}$ -13-HPOD (●), $4\ \mu\text{M}$ -13-HPOD (△), $10\ \mu\text{M}$ -13-HOD instead of 13-HPOD (▲) and $10\ \mu\text{M}$ -13-HPOD in the absence of ATP and Ca^{2+} (□).

Fig. 4. Change in u.v. spectra during the pseudoperoxidase reaction

Differential spectra between incubation mixtures analysed at zero time and after incubation for 4 min were determined for pseudoperoxidase reactions with inhibitors II (a) and V (b). The dotted line shows the result of control experiments performed under identical conditions using heat-inactivated enzyme.

Fig. 5. Degradation of 13-HPOD and inhibitors during the pseudoperoxidase reaction

The concentrations of 13-HPOD (□) and inhibitor (▨) were determined by reverse-phase h.p.l.c. analysis of the reaction mixtures after incubation of the compounds (10 μM each) with 5-lipoxygenase (5 nmol of arachidonic acid oxidized/min) or heat-inactivated (70 °C, 30 min) enzyme. Results are expressed as the decrease in the concentration of the indicated compound after 4 min relative to the control, for which the reaction was stopped at zero time (mean ± S.D., $n = 3$).

trideca-9,11-dienoic acids as minor products that absorb at 280–285 nm [29–31]. Consistent with this interpretation are the u.v.-spectral changes measured during the pseudoperoxidase reaction of 5-lipoxygenase with inhibitors II and V (Fig. 4), showing that the decrease in $A_{234-236}$ of the conjugated diene hydroperoxide is accompanied by a relatively smaller increase at 280–290 nm, presumably corresponding to the mixture of oxodienoic acids and accounting for a maximum of 20% of 13-

HPOD consumption. This result is in excellent agreement with observations for the degradation of 13-HPOD catalysed by soybean lipoxygenase in the presence of alkylhydroxylamine inhibitors [11].

Degradation of inhibitor during the pseudoperoxidase reaction

The above results are consistent with the proposed model of reductive inactivation of soybean lipoxygenase by catechols and *N*-alkylhydroxylamines where both the inhibitor and hydroperoxide are consumed during several cycles of enzyme reduction and reoxidation [11,12]. Since this model would predict that inhibitors should be oxidized during these reactions, the recoveries of compounds II and V were measured after pseudoperoxidase assays (Fig. 5). Analysis of the reaction mixtures by reverse-phase h.p.l.c. confirmed the result of the spectrophotometric assays on 13-HPOD degradation and showed that compound II was also consumed, in contrast with samples incubated under identical conditions with inactivated enzyme. The phenoxyl radical of compound II would be the first expected product of oxidation, in agreement with the reductive inactivation model and recently obtained e.s.r.-spectroscopy data providing evidence for the formation of free radicals from phenol and catechol inhibitors of the soybean lipoxygenase in the presence of hydroperoxides [32]. The stoichiometry of inhibitor II to 13-HPOD consumption was about 0.6, which could be explained by an irreversible reaction between the phenoxyl radical of II and the hydroperoxide to yield non-radical products, as observed during chemical trapping of peroxy radicals by phenolic antioxidants [33]. Unsuccessful attempts were made to characterize the oxidation products of another benzofuranol (compound IV), which appeared as a complex mixture, including an alkylated enzyme and a major product which further decomposes after isolation by reverse-phase h.p.l.c. (C. A. Rouzer, unpublished work).

Fig. 5 also shows that no significant change in the level of compound V could be detected after the pseudoperoxidase reaction, even under conditions where the inhibitor caused degradation of 50% of the 13-HPOD. However, we have found that this inhibitor could be converted into other unidentified species by soybean lipoxygenase in a hydroperoxide-dependent reaction with a stoichiometry of 0.5 mol of compound V to 13-HPOD (C. A. Rouzer, unpublished work). This result indicates that inhibitor V can serve as an electron donor to the enzyme, in agreement with previous observations on the reductive properties of this class of compounds [34]. The high recovery of inhibitor after pseudoperoxidase assay with 5-lipoxygenase seems to be due to the presence of DTT used to stabilize the enzyme, since this reducing agent (0.2 mM) completely protects the degradation of inhibitor V by the soybean enzyme without inhibiting 13-HPOD consumption (J. P. Falgoutret, F. Gervais & D. Riendeau, unpublished work). It thus seems likely that the free-radical form of compound V is generated during the pseudoperoxidase reactions and that the reduction of the radical by DTT regenerates the parent *N*-hydroxyurea molecule. This interpretation is consistent with the behaviour of hydroxyurea in model systems in which transient long-lived species can be detected by e.s.r. spectroscopy under oxidizing conditions in aqueous solutions [35]. The results in Figs. 2–5 support the hypothesis that the benzofuranol II and the hydroxyurea V can act as reductants for lipoxygenases, with recycling of the hydroxyurea inhibitor in the presence of DTT.

Pseudoperoxidase activity and inhibitor potency

The relationship between the efficiency of various inhibitors in stimulating the pseudoperoxidase activity and in inhibiting the

Fig. 6. Relationship between inhibitory potency and stimulation of pseudoperoxidase activity

IC₅₀ values correspond to the inhibitor concentration causing a 50% decrease in LTB₄ production by human leucocytes. The relative level of pseudoperoxidase activity was determined from the initial rate of decrease in A₂₃₄ after incubation of a fixed amount of 5-lipoxygenase (5 nmol of arachidonic acid oxidized/min) with 10 μ M-13-HPOD and 10 μ M-inhibitor (mean $\pm$ S.E.M., $n = 3-5$). Inhibitors labelled I-IX correspond to those given in Fig. 1. Inhibitors X, XI and XII are NDGA, BW 755C and quercetin respectively. The IC₅₀ values ($\pm$ S.E.M., $n = 3-5$ using different cell preparations) for inhibitors I-XII are: I, 0.08 ± 0.01 μ M; II, 6 ± 2 nM; III, 0.13 ± 0.01 μ M; IV, 6 ± 2 nM; V, 10 ± 1 nM; VI, 20 ± 7 nM; VII, 0.05 ± 0.01 μ M; VIII, 17 ± 6 nM; IX, 1.5 ± 0.5 μ M; X, 44 ± 6 nM; XI, 2.6 ± 0.6 μ M; XII, 0.7 ± 0.1 μ M.

5-lipoxygenase reaction was investigated. The relative level of pseudoperoxidase activity was estimated from the initial rate of decrease in A₂₃₄ induced by 10 μ M-inhibitor upon incubation of the enzyme with 10 μ M-13-HPOD. 5-Lipoxygenase inhibition potency was determined from the effect of the compounds on LTB₄ production by Ca²⁺-ionophore-stimulated leucocytes. This sensitive assay can be performed at a low cell concentration and allows for an estimation of the potency of compounds effective in the nanomolar range. The results for various inhibitors are summarized in Fig. 6, where the pseudoperoxidase activity is expressed as a function of IC₅₀ values for LTB₄ biosynthesis inhibition. Only very potent inhibitors (IC₅₀ < 100 nM) were able to support measurable levels of pseudoperoxidase activity. This observation held for benzofuranols of closely related structures, such as I and III, which corresponds to less potent analogues of compounds II and IV, respectively. Similarly, pseudoperoxidase activity was stimulated by the *N*-hydroxyureas V and VI and the hydroxamate BW A4C (compound VII), whereas the less potent inhibitor A-64077 (compound IX) had no effect. In addition, only traces of activity could be detected with NDGA and BW 755C, compounds which have been shown to be oxidized by soybean lipoxygenase [12,13]. Although the lack of activity may be accounted by the low sensitivity of the assay, these results indicate that the ability of the compounds to stimulate the pseudoperoxidase reaction is associated with a high potency of inhibition.

It is noteworthy that potent inhibitors from different structural series supported the pseudoperoxidase reaction. Although the reducing properties of benzofuranols are expected from the absolute requirement of the phenolic group for 5-lipoxygenase inhibition [27], the rationale behind the development of hydroxamate and *N*-hydroxyurea derivatives has been to inhibit the enzyme by chelation of a putative iron at the active site [5,6]. The present observations strongly suggest that the reducing properties of compounds in these series could contribute to 5-lipoxygenase inhibition potency. This situation appears anal-

ogous to that described for catechol inhibitors, which can either form stable complexes with, or reduce the iron of, the soybean enzyme, depending on the nature and the position of the substituents on the catechol ring [9].

The present observations suggest that the model of inhibition by reductive inactivation described for the soybean lipoxygenase [11,12] may apply to potent 5-lipoxygenase inhibitors. The two enzyme systems appear to differ, however, by the inactivation of the 5-lipoxygenase enzyme during the pseudoperoxidase reaction, which stopped before either 13-HPOD or inhibitor consumption was completed and could be re-initiated by addition of fresh enzyme (results not shown). Some loss of enzyme activity also occurred during incubation of 5-lipoxygenase with the hydroperoxide in the absence of inhibitor, in agreement with previous observations on partially purified enzyme preparations [21], but this loss of activity was much lower than that observed in the presence of inhibitor. It is possible that other reactions leading to enzyme inactivation also occurred during the assay. For example one of the benzofuranols used in this study, L-656,224 (compound IV), is able to react covalently with the 5-lipoxygenase from human leukocytes (C. A. Rouzer, unpublished work).

Compounds II, IV and V were also found to inhibit the lipoxygenase reaction and support the pseudoperoxidase activity of the soybean 15-lipoxygenase. Potentially these inhibitors could be of general use to identify and quantify the pseudoperoxidase activities of several types of lipoxygenases. The finding that the *N*-hydroxyurea V is not consumed in the presence of DTT makes this compound a useful substrate for measuring the pseudoperoxidase reaction with minimal interference of inhibitor-derived species and under a fixed inhibitor concentration. In addition, hydroxyurea analogues have proven to be useful in the characterization of the tyrosine free radical of the redox centre of ribonucleotide reductases [35]. It would be of interest to determine whether the *N*-hydroxyurea V or the other potent inhibitors described in the present study could also serve to identify essential amino acids on the active site of lipoxygenases.

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