

## Research Paper

# Reaction of 5-Aminosalicylic Acid with Peroxyl Radicals: Protection and Recovery by Ascorbic Acid and Amino Acids

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**Purpose.** The aims of the study are to analyze the interaction between 5-aminosalicylic acid (5-ASA) and peroxyl radicals and to evaluate the effect of some endogenous compounds such as ascorbic acid and amino acids on the oxidation of 5-ASA induced by 2,2'-azo-bis(2-amidinopropane) dihydrochloride.

**Methods.** The consumption and/or the recovery of 5-ASA (7.6  $\mu$ M) exposed to a peroxyl radical source [2,2'-azo-bis(2-amidinopropane)] was followed by techniques such as spectrofluorescence, high-performance liquid chromatography, and differential pulse voltammetry.

**Results.** 5-Aminosalicylic acid was found to readily react with peroxyl radicals at micromolar concentrations and to protect c-Phycocyanin in a very similar fashion to that shown by Trolox. Exposure of 5-ASA to peroxyl radicals led to its oxidation into the corresponding quinone-imine. Disappearance of 5-ASA was prevented by tryptophan, cysteine, glutathione, and ascorbic acid. Furthermore, some of these compounds induced the partial (cysteine and glutathione) or total (ascorbic acid) recovery of 5-ASA when added after its almost total consumption.

**Conclusions.** 5-Aminosalicylic acid is a very efficient peroxyl radical scavenger. The 5-ASA oxidation by peroxyl radicals was prevented by ascorbic acid, cysteine, and glutathione. In addition, 5-ASA can be regenerated by these endogenous compounds, which would be a valuable mechanism to preserve 5-ASA in tissues undergoing oxidative stress conditions.

**KEY WORDS:** amino acids; ascorbic acid; peroxyl radicals; 5-aminosalicylic acid.

## INTRODUCTION

The anti-inflammatory drug 5-aminosalicylic acid (5-ASA) has risen to considerable prominence within the medical and pharmacological communities through its use in the treatment of chronic bowel diseases, such as Crohn's disease and ulcerative colitis, and in the maintenance of their clinical remission (1). 5-Aminosalicylic acid, the functionally active component of sulfapyridine congeners, may act by blocking the production of prostaglandins and leukotrienes, inhibiting bacterial peptides-induced neutrophil chemotaxis, scavenging reactive oxygen metabolites, and, possibly, inhibiting the activation of nuclear factor- $\kappa$ B (2).

Incubation of 5-ASA with activated human mononuclear cells, as well as its direct exposure to the major respiratory burst components, leads to the formation of a variety of metabolites, including salicylate and gentisate (2,5-dihydroxy benzoate) (3). 5-Aminosalicylic acid is an efficient scavenger of peroxyl radicals and a highly effective molecule in protecting membranes against lipid peroxidation (4-6). Depending on the free radical source employed, 5-ASA has been proposed to be either less (7) or more reactive

(8) than ascorbic acid toward peroxyl radicals. 5-Aminosalicylic acid has also been shown to readily react with hypochlorous acid (HOCl) (8), singlet oxygen (9), and hydroxyl radicals (10). In the latter case, the reaction involves the formation of a hydroxylated 5-ASA derivative (10). On the other hand, a quinone-imine 5-ASA derivative has been shown to be generated during the electrochemical oxidation of 5-ASA (11).

In the present study, we have evaluated the interaction between 5-ASA and peroxyl radicals, in terms of the disappearance of 5-ASA and the formation of its major metabolite (quinone-imine; Fig. 1), and investigated the ability of ascorbic acid and certain amino acids to protect 5-ASA against such oxidation. We provide data showing that antioxidants presenting a reducing capacity, such as ascorbic acid, cysteine, and glutathione, not only can afford protection against peroxyl radical-induced 5-ASA oxidation, but also "regenerate" 5-ASA from its quinone-imine metabolite.

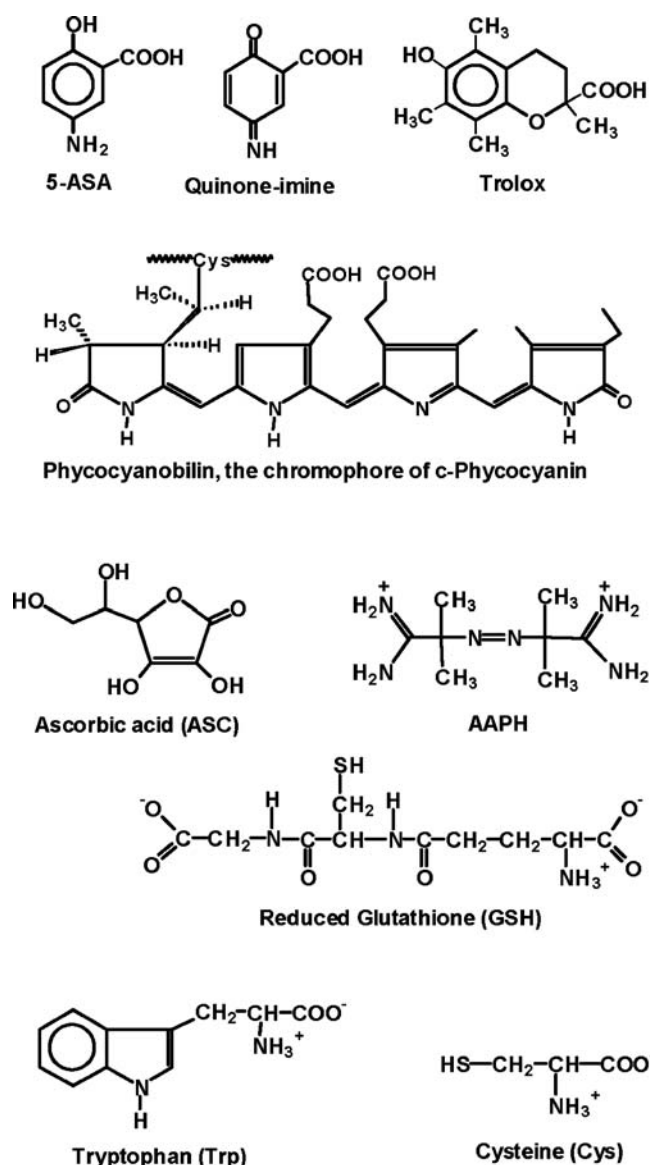
## MATERIALS AND METHODS

### Chemicals

2,2'-Azo-bis(2-amidinopropane) dihydrochloride (AAPH), 5-aminosalicylic acid (5-ASA), ascorbic acid (ASC), c-Phycocyanin (c-Pc), Trolox, glutathione (GSH)

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**Fig. 1.** Chemical structures of the compounds used in the present study: 5-aminosalicylic acid (5-ASA), quinone-imine, Trolox, Phycocyanobilin, the chromophore of c-Phycocyanin, ascorbic acid (ASC), 2,2'-azo-bis(2-amidinopropane) dihydrochloride (AAPH), reduced glutathione, tryptophan, and cysteine.

(Fig. 1), and all tested amino acids (AA) (L-isomers of cysteine, tryptophan, histidine, hydroxyproline, aspartic acid,  $\beta$ -alanine, isoleucine, valine, proline, threonine, glycine, phenylalanine, methionine, tyrosine, glutamine, arginine, leucine, lysine, asparagine, serine, and glutamic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

#### Protection of c-Phycocyanin by 5-Aminosalicylic Acid

The consumption of c-Pc, elicited by its incubation (0–20 min, 37°C) in the presence of 10 mM AAPH (prepared in sodium phosphate buffer, pH 7.0), was evaluated by monitoring the decrease in fluorescence intensity (excitation at 620 nm, emission at 640 nm) (12). Experiments were carried out in the absence and presence of 5-ASA and Trolox.

#### Consumption of 5-Aminosalicylic Acid Mediated by Peroxyl Radicals and Its Inhibition by Amino Acids

2,2'-Azo-bis(2-amidinopropane) dihydrochloride solutions (2 mM) were incubated (during 60 min at 37°C) in the presence of 5-ASA (7.6  $\mu$ M). Considering a rate constant of decomposition of AAPH of  $1.36 \times 10^{-6} \text{ s}^{-1}$  (13,14), we have assumed that under the present experimental conditions, the rate of peroxyl radical formation was near constant. The time course of the reaction between 5-ASA and peroxyl radicals was assessed through the decrease in 5-ASA-dependent fluorescence (332 and 492 nm as excitation and emission wavelengths, respectively). No significant changes in the shape of the fluorescence band were observed up to 70% of 5-ASA disappearance. Control 5-ASA solutions, incubated in the absence of AAPH, revealed no changes in the original fluorescence intensity. The incubation of 5-ASA in the presence of AAPH resulted in a steady (0–60 min) decrease in fluorescence intensity. After 60 min of incubation, the decrease in the initial fluorescence (ascribed a value of 1.0 at time zero) was, on average, around 75%. The capacity of different AA to protect 5-ASA against AAPH-induced disappearance was evaluated by adding them at a concentration of 120  $\mu$ M, comparing the fluorescence intensity at time zero and after 60 min of incubation. The % of inhibition (protection) of fluorescence decrease due to the addition of a given AA was estimated according to Eq. (1):

$$\% \text{ inhibition} = \left[ 1 - \frac{\Delta F_{5\text{-ASA} + \text{AA}}}{\Delta F_{5\text{-ASA}}} \right] \times 100 \quad (1)$$

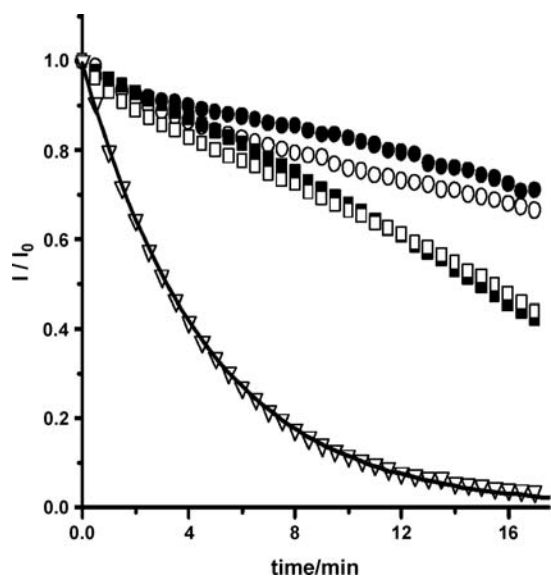
where  $\Delta F_{5\text{-ASA} + \text{AA}}$  is the decrease in fluorescence (from 0 to 60 min) elicited by the incubation of 5-ASA in presence of AAPH and AA and  $\Delta F_{5\text{-ASA}}$  is the decrease in fluorescence (from 0 to 60 min) elicited by the incubation of 5-ASA in presence of AAPH.

For those AA that showed a protection equal to or greater than 15%, additional experiments were conducted by adding them at time zero over a wide concentration range. For those AA showing the greatest capacity to protect 5-ASA, additional experiments were conducted, adding them 30 min after initiating the 5-ASA–AAPH reaction. In these experiments, the changes in fluorescence were monitored continually thereafter (30–60 min).

In experiments aimed at evaluating a possible 5-ASA regenerating effect, the tested compounds (AA and ASC) were added after a substantial or almost complete disappearance of the initially added 5-ASA had taken place. The changes in fluorescence were registered immediately after their addition.

#### Disappearance of 5-Aminosalicylic Acid and Appearance of Its Metabolites as Monitored by High-Performance Liquid Chromatography

Chromatograms were obtained using an Agilent 1100 Series high-performance liquid chromatography (HPLC), equipped with a LiChrosphere RP-18 column, and a fluorescence G1321A Agilent 1100 Series detector (332 and 492 nm as excitation and emission wavelengths, respectively). Buffer phosphate ( $\text{KH}_2\text{PO}_4$ , 0.1 M, adjusted to pH 4.4 with orthophosphoric acid)/methanol, 95:5 mixture, was employed as mobile phase. The flow rate was 0.8 mL/min. To evaluate

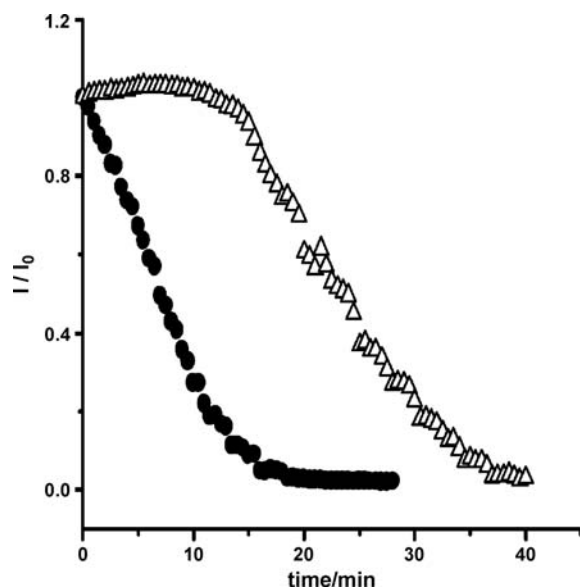


**Fig. 2.** c-Phycocyanin (0.001 mg/mL) fluorescence decay resulting from its incubation in the presence of AAPH (10 mM), in phosphate buffer (10 mM), pH 7.0, at 37°C. ( $\nabla$ ) Control; ( $\blacksquare$ ) Trolox 5  $\mu$ M; ( $\square$ ) 5-ASA 5  $\mu$ M; ( $\bullet$ ) Trolox 10  $\mu$ M, and ( $\circ$ ) 5-ASA 10  $\mu$ M.

the interaction between 5-ASA and peroxy radicals, 5-ASA (7.6  $\mu$ M) and AAPH (2 mM) (in 10 mM phosphate buffer, pH 7.0) were incubated (37°C) under continuous stirring. Aliquots were taken every 5-min interval, cooled immediately after, and injected in the chromatograph. Control solutions of 5-ASA incubated in the absence of AAPH showed no changes in the chromatographic profile. Results were analyzed with the HPLC-computer-coupled ChemStation LC 3D program.

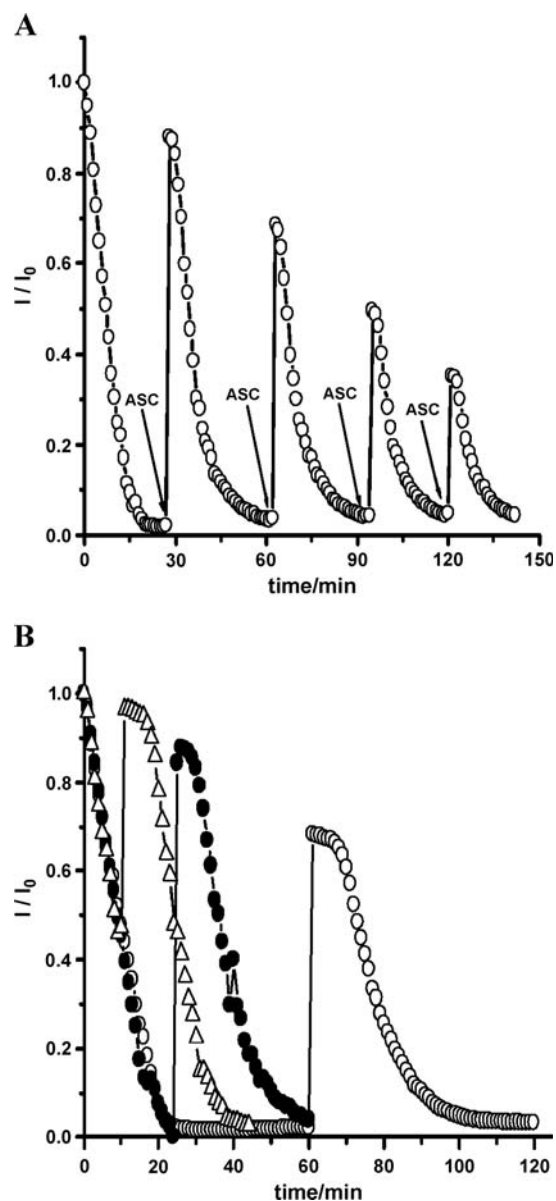
#### Changes in Electrochemical Parameters Associated with 5-Aminosalicylic Acid-Induced Modification by AAPH

The time course of the reaction between 5-ASA (100  $\mu$ M) and AAPH (20 mM) was followed by differential pulse



**Fig. 3.** Effect of ASC on 5-ASA (7.6  $\mu$ M) fluorescence decay resulting from its incubation in the presence of AAPH (10 mM), in phosphate buffer (10 mM), pH 7.0, 37°C. ( $\bullet$ ) Control; ( $\triangle$ ) ASC (15  $\mu$ M).

voltammetry (dpv). Under the experimental conditions used in the present study, the oxidation peak of 5-ASA was observed at 202 mV. Differential pulse voltammetry was performed using a BAS (Bioanalytical systems, West Lafayette, IN, USA) CV50 assembly. A glassy carbon stationary electrode was employed as working electrode, and a platinum wire was used as counter electrode. All potentials were measured against an Ag/AgCl electrode. Operating conditions were as follows: pulse amplitude, 40 mV; potential scan, 4 mV s<sup>-1</sup>; voltage range, 0–1,000 mV; current range, 5–25  $\mu$ A; temperature, 37°C. Control solutions prepared in the absence of AAPH revealed no changes in the peak current of 5-ASA during 120 min. To study the effect of AA (0.3–5.0 mM) on the peak current of 5-ASA, the former were added



**Fig. 4.** Effect of ASC addition on the fluorescence intensity of 5-ASA (7.6  $\mu$ M) incubated in the presence of AAPH (10 mM), in phosphate buffer (10 mM), pH 7.0, at 37°C. (A) To a given sample, ASC (15  $\mu$ M) was added at 27-, 62-, 94-, and 120-min incubation times. (B) To independent samples, ASC (15  $\mu$ M) was added after 9-, 24-, or 60-min incubation times.

**Table I.** Protection Against 2,2'-Azo-bis(2-Amidinopropane) Dihydrochloride (AAPH)-Induced 5-Aminosalicylic Acid (5-ASA) Disappearance Afforded by Amino Acids

Amino acid	Inhibition (%)	Amino acid	Inhibition (%)	Amino acid	Inhibition (%)
Cysteine	67 ± 2	Valine	< 5	Glutamine	< 5
Tryptophan	19 ± 1	Proline	< 5	Arginine	< 5
Histidine	7 ± 0.6	Threonine	< 5	Leucine	< 5
Tyrosine	6 ± 0.5	Glycine	< 5	Lysine	< 5
Aspartic acid	< 5	Phenylalanine	< 5	Asparagine	< 5
β-Alanine	< 5	Methionine	< 5	Serine	< 5
Isoleucine	< 5	Hydroxi proline	< 5	Glutamic acid	< 5

5-ASA (7.6 μM); amino acids (120 μM); AAPH (2 mM); buffer phosphate (10 mM), pH 7.0, 37°C.

30 min after initiating the 5-ASA–AAPH reaction, and dpv measurements were performed every 5 min thereafter.

## RESULTS

### Protection of c-Phycocyanin by 5-Aminosalicylic Acid

The addition of 5-ASA (5 and 10 μM) decreased the rate of c-Pc bleaching induced by peroxyl radicals generated during AAPH (10 mM) thermolysis. Typical results, depicted in Fig. 2, show that the addition of 5-ASA led to a concentration-dependent decrease in the rate of c-Pc bleaching. Such effect was very similar to that observed after the addition of equivalent micromolar concentrations of Trolox.

### Consumption of 5-Aminosalicylic Acid Induced by Peroxyl Radicals

Incubation of 5-ASA (7.6 μM) in the presence of AAPH (2 mM) led to a progressive decrease of its fluorescence (data not shown). These data reveal that 5-ASA traps peroxyl radicals according to a zero-order kinetics, at least within the range of concentrations of 5-ASA down to near 25% of the initial concentration.

### Protection of 5-Aminosalicylic Acid by Ascorbic Acid

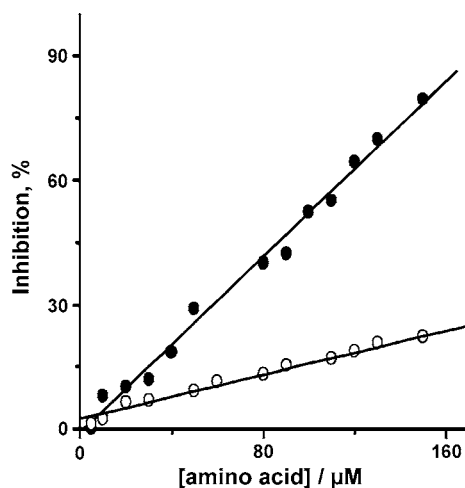
Figure 3 depicts the decreases in fluorescence intensity of 5-ASA (7.6 μM) induced by AAPH in the presence or absence of ASC (15 μM). In the presence of ASC, a clear induction time of approximately 15 min was evident before any decay in fluorescence was observed. It is worth noting that the rate at which 5-ASA fluorescence decayed thereafter was very similar to that seen in the control condition. Figure 4 shows the changes in 5-ASA fluorescence elicited prior to and after the successive (Fig. 4A) or the single addition (Fig. 4B) of ASC. Figure 4A reveals that although the first addition of ASC led to an almost total recovery of 5-ASA fluorescence, the successive addition of equal concentrations of ASC led to proportionally smaller recoveries. In all cases, however, the rates of fluorescence decay were always apparently the same. Presumably, each addition of ASC leads to a fast reduction of the available quinone-imine, with quinone-imine molecules undergoing continuous and irreversible hydrolysis during the incubation. To test this possibility, the effect of ASC, at different times (on independent 5-ASA–AAPH solutions), was evaluated (Fig. 4B). Results show that the extent of the recoveries decreased

considerably with the time elapsed between the start of 5-ASA consumption and the time of ASC addition.

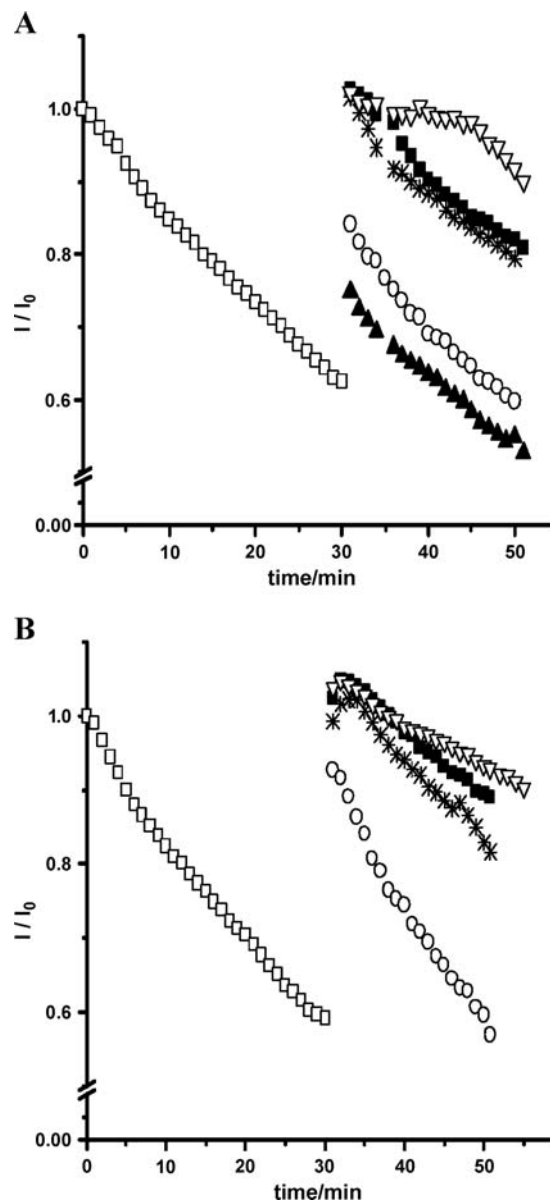
### Protection of 5-Aminosalicylic Acid by Amino Acids

To assess if other endogenous antioxidants are also able to protect and/or regenerate 5-ASA (7.6 μM) from its oxidation product(s), we analyzed the effect of a series of AA. The protection exerted by the tested AA (120 μM) on the loss of 5-ASA induced by AAPH (2 mM) was evaluated after 60 min of incubation (see Materials and Methods). As shown in Table I, among the 21 studied AA, a significant protection was observed only with Cys (67 ± 2%) and Trp (19 ± 1%). A considerably lesser protection (6–7%) was seen with Tyr and His, whereas no protection was evidenced in the presence of any of other tested AA. The protection provided by Cys and Trp was concentration-dependent and lineal along the range of studied concentrations (Fig. 5).

To gain further understanding on the possible mechanisms by which Cys and Trp afford such protection, these AA were added 30 min after initiating the incubation, namely, at a time where a substantial part of 5-ASA had already been consumed. The addition of Cys to such system, rather than changing the kinetics of fluorescence decay, led to a fast and



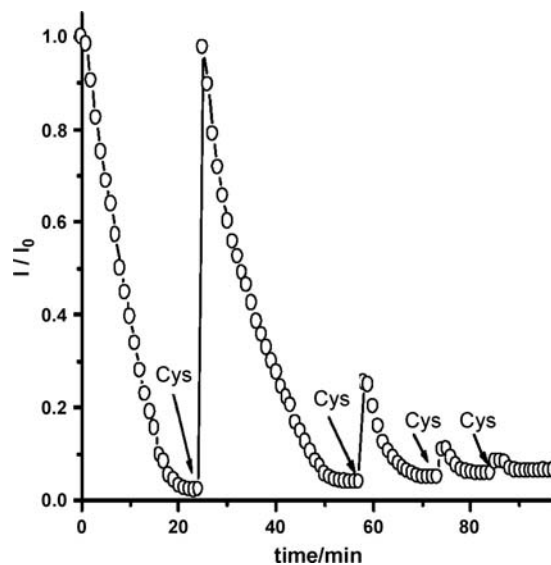
**Fig. 5.** Effects of cysteine and tryptophan on the consumption 5-ASA (7.6 μM) induced by AAPH (2 mM). The ordinate represents the inhibition (expressed as %) of the oxidative consumption of 5-ASA afforded by increasing concentrations of (●) Cysteine and (○) Tryptophan. Incubations were carried out in phosphate buffer (10 mM), pH 7.0, 37°C. The percentage of inhibition was calculated according to Eq. (1) (described in Materials and Methods).



**Fig. 6.** Effect of thiols addition upon the fluorescence intensity from a 5-ASA (7.6  $\mu\text{M}$ ) sample incubated in presence of AAPH (2 mM), in phosphate buffer (10 mM), pH 7.0, at 37°C. Thiols were added after 30 min of incubation. (A) Effect of cysteine addition. ( $\square$ ) Control; cysteine: ( $\blacktriangle$ ) 1  $\mu\text{M}$ , ( $\circ$ ) 3  $\mu\text{M}$ , ( $*$ ) 5  $\mu\text{M}$ , ( $\blacksquare$ ) 10  $\mu\text{M}$ , and ( $\nabla$ ) 30  $\mu\text{M}$ . (B) Effect of reduced glutathione addition. ( $\square$ ) Control; GSH: ( $\circ$ ) 1  $\mu\text{M}$ , ( $*$ ) 3  $\mu\text{M}$ , ( $\blacksquare$ ) 5  $\mu\text{M}$ , and ( $\nabla$ ) 10  $\mu\text{M}$ .

concentration-dependent increase in the fluorescence intensity (Fig. 6A). A similar effect was seen when, instead of Cys, GSH was added (Fig. 6B). In turn, the addition of Trp to the same system did not lead to an increase in the fluorescence intensity, but only resulted in a small change in the slope of the fluorescence decay curve (data not shown).

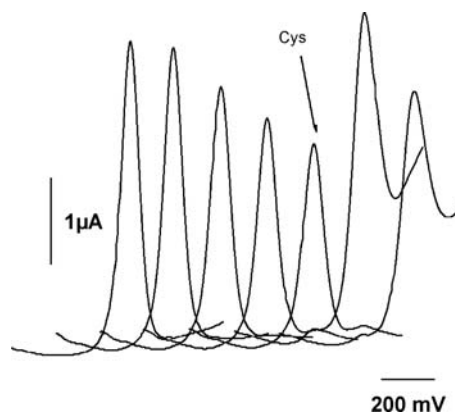
Figure 7 shows the effect of successive additions of Cys on the recovery of 5-ASA. These data reveal that while the first addition of Cys led to an almost instantaneous and total recovery of the fluorescence, the extent of the recovery markedly decreased with successive additions. Thus, the effect of Cys closely resembles that previously described for ASC.



**Fig. 7.** Effect of cysteine (20  $\mu\text{M}$ ) addition on the fluorescence intensity of a 5-ASA (7.6  $\mu\text{M}$ ) sample incubated in the presence of AAPH (10 mM), phosphate buffer (10 mM), pH 7.0, 37°C. The arrows indicate the times at which cysteine (Cys) was added.

To investigate whether the recovery of the fluorescence seen after Cys addition resulted in the actual regeneration of 5-ASA, we monitored directly 5-ASA concentrations using HPLC and dpv techniques. Chromatographic analysis of a mixture of 5-ASA (7.6  $\mu\text{M}$ ) plus AAPH (2 mM), to which Cys (3  $\mu\text{M}$ ) was added 30 min after the initiation of the reaction, reveals the presence of a 5-ASA peak that is 35% greater than that quantified in samples from equal mixtures to which no Cys had been added (data not shown). The chromatogram data showed to us that the addition of Cys resulted not only in the recovery of 5-ASA as such, but also in the appearance of four new peaks that share fluorescence characteristics with 5-ASA but whose identity remains to be established.

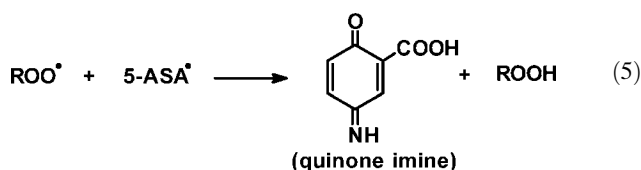
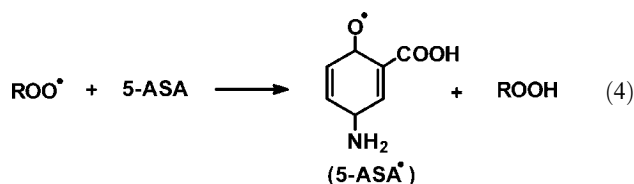
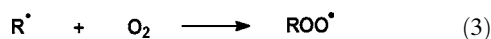
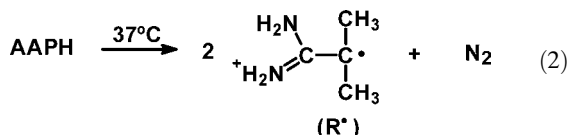
Finally, using dpv, a total recovery of the 5-ASA oxidation peak (202 mV) was also seen when Cys was added to a partially exhausted 5-ASA plus AAPH sample (Fig. 8). Assuming that the recovery of such peak involves only a partial regeneration of 5-ASA, the above-referred HPLC results indicated that the compounds comprised in the HPLC peaks would share the electrochemical properties of 5-ASA.



**Fig. 8.** Effect of cysteine (Cys) (1 mM) addition on the current intensity associated with the 5-ASA (100  $\mu\text{M}$ ) oxidation peak, after its incubation with AAPH (20 mM) at pH 7.0, 37°C, during 30 min.

## DISCUSSION

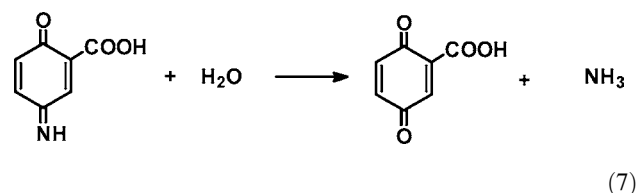
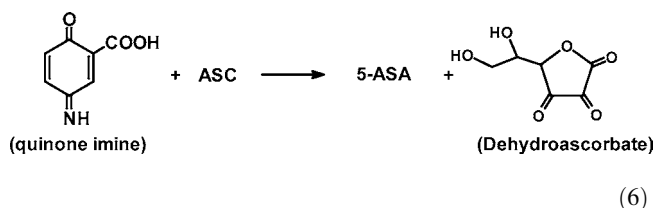
Establishing the ability of 5-ASA to interact with free radical species is fundamental toward defining the actual mechanisms by which this agent exerts its protective actions against the oxidative events contributing to the damage featured by inflammatory bowel diseases (2,8). Our data substantiate the ability of 5-ASA to react with peroxyl radicals. This ability was evidenced, firstly, by showing the capacity of 5-ASA to protect c-Pc against AAPH-induced loss of fluorescence (12). The activity of 5-ASA was similar to that displayed by Trolox, a water-soluble vitamin E analog, priorly proven to trap peroxyl radicals very efficiently (15). The above results were complemented by experiments in which the reactivity of 5-ASA against peroxyl radicals was assessed by the loss of 5-ASA-associated fluorescence. Results from the latter studies indicate that 5-ASA would trap peroxyl radicals very efficiently, and that the consumption of 5-ASA would follow zero-order kinetics. Under such conditions, most of the AAPH-derived radicals would be trapped by 5-ASA (15). The high reactivity of 5-ASA toward peroxyl radicals has been ascribed by previous workers to the presence of the amino group in its structure (see Fig. 1) (4). We have estimated that the rate of 5-ASA consumption would be close to  $0.095 \mu\text{M}/\text{min}$ . Assuming a rate of peroxyl radical production of  $0.16 \mu\text{M}/\text{min}$  (13,14), it can be estimated that, when present at low micromolar concentrations, each 5-ASA molecule would be able to remove nearly 1.7 peroxyl radicals. Accordingly, we explained the interaction between 5-ASA and AAPH-derived peroxyl radicals in terms of the main reactions (2)–(5):



where 5-ASA<sup>•</sup> is the semioxidized 5-ASA derived radical.

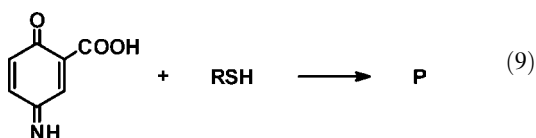
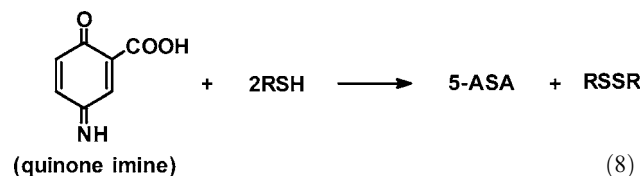
Taking advantage of the possibility of studying the consumption of 5-ASA through monitoring its fluorescence, we evaluated the ability of ASC and that of several amino acids to protect 5-ASA against AAPH-induced oxidation. The data obtained clearly indicate that ASC is able both to effectively prevent the loss of 5-ASA and to regenerate

5-ASA from a media where most of it had been oxidatively consumed. Regarding the mechanisms underlying the protecting and regenerating actions of ASC, the observation that the addition of ASC (at initial incubation time) results in a clear lag time suggests that ASC can competitively trap the peroxyl radicals generated and needs to be fully consumed before any loss of 5-ASA occurs. Yet, the possibility exists that ASC could also simultaneously repair 5-ASA<sup>•</sup> radicals and/or swiftly reduce the molecules of quinone-imine generated. Data presented here reveal that the addition of ASC to a media where most of 5-ASA has been oxidatively consumed leads to an almost total recovery of 5-ASA fluorescence, and that the extent of the recoveries decreased considerably with the time elapsed between the start of 5-ASA consumption and the time of ASC addition. These results are compatible with the occurrence of reactions (6) and (7) (shown below) and suggest that a slow hydrolysis, with a pseudo-first-order rate constant of approximately  $1.4 \times 10^{-4} \text{ s}^{-1}$ , to produce 2-carboxy-1,4-benzoquinone (16) would take place.



On the other hand, although most of the tested AA showed a poor ability to react with peroxyl radicals, Cys and Trp seem to be particularly effective in preventing 5-ASA oxidation. For these two AA, a relatively similar reactivity was previously seen when tested in the protection of lysozyme against its inactivation promoted by peroxyl radicals (17) and in their reaction against ABTS radical cation (18). Interestingly, the present study shows that in addition to protecting 5-ASA against peroxyl radicals, Cys also has the ability to partially regenerate 5-ASA from its oxidation products (quinone-imine). Results from our HPLC studies reveal that, simultaneously with promoting the partial recovery of 5-ASA peak, the addition of Cys to a 5-ASA plus AAPH system leads to the appearance of four new peaks that share fluorescence properties with 5-ASA but whose identity remains to be established. Thus, the presence of these fluorescent compounds could contribute and possibly explain the "total" recovery of the fluorescence elicited by the addition of a thiol to a system where an almost total 5-ASA depletion has taken place. Results from experiments in which 5-ASA recovery (resulting from the single or repeated Cys addition) was assessed by spectrofluorescence and HPLC techniques indicate that these fluorescent compounds would also be bleached by peroxyl radicals, but that the products resulting from such interaction would not recover their fluorescence by further

thiol addition. The processes associated with the interaction of quinone-imine with thiols could then be represented by the following competitive reactions:



where P products involve the addition of the thiol to the aromatic ring. These (fluorescent) products would be irreversibly modified by further interaction with peroxy radicals. A similar type of reactions has been previously proposed for the interaction between 5-ASA and hypochlorous acid (8). Interestingly, the ability of Cys to regenerate 5-ASA was shared by GSH but not Trp. The latter suggests that while a hydrogen-donating capacity would be needed for preventing peroxy radicals from damaging 5-ASA (e.g., Cys and Trp), a reducing capacity such as that associated with the thiol moiety of Cys and GSH would be needed to regenerate 5-ASA [reactions (8) and (9)]. In this regard, Rosen *et al.* (19) have shown that the quinone-imine produced during the oxidation of acetaminophen reacts with thiols via two pathways: (a) a simple reduction and (b) a conjugation of the thiol group in which this moiety would act as nucleophile. A similar reaction mechanism would be compatible with the fluorescence, HPLC, and dpv data obtained in the here studied 5-ASA plus AAPH system.

The above-mentioned reactions suggest that—under oxidative stress conditions—Cys, GSH, and ASC could extend the antioxidant life span of 5-ASA, defined as the number of peroxy free radicals removed by each molecule introduced into the system. Finally, it is also interesting to point out that, independently of the relative reactivity of 5-ASA and ASC toward peroxy radicals, the occurrence of a reaction such as that depicted in Eq. (6) could explain the consumption of ASC reportedly faster than that of 5-ASA (7). Thus, to the extent to which such proposed repair mechanism occurs, it would seem reasonable to suggest that a joint administration of 5-ASA and ASC could significantly extend the actual pharmacological life span of 5-ASA.

## CONCLUSION

It can be concluded that 5-ASA is a good peroxy radical scavenger. The reaction of 5-ASA with peroxy radicals was effectively prevented by ASC and thiol derivatives (Cys and GSH). From the recovery of 5-ASA fluorescence and HPLC studies, we postulate that 5-ASA can be regenerated by ASC and thiols. Thus, the ability of these endogenous compounds to regenerate 5-ASA could constitute a valuable mechanism to preserve 5-ASA in tissues undergoing oxidative stress conditions.

## ACKNOWLEDGMENT

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