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The role of 4- and 5-aminosalicylic acids in a riboflavin-photosensitised process

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Abstract

A kinetic study on the effect of the therapeutic anti-oxidative drugs 4- and 5-aminosalicylic acids (ASA) in a Vitamin B2 (riboflavin (Rf))-photosensitised process was performed. Employing Rf 0.01–0.02 mM and ASA 0.5–1 mM both in the excited singlet and triplet states of the pigment are quenched by ASA with rate constant values slightly lower than the diffusional ones. From the latter states singlet molecular oxygen and $Rf^{\bullet -}$ are generated and subsequently scavenged with the concomitant degradation of ASA, mainly by means of superoxide radical anion. As a consequence, the photodegradation of the vitamin is impeded due to the electron transfer process from Rf^{-} to ground state dissolved oxygen.

Keywords: Aminosalicylic acids; Photo-oxidation; Photosensitisation; Riboflavin; Singlet molecular oxygen; Superoxide ion

1. Introduction

A natural daylight-absorbing pigment of particular interest is Vitamin B2 or riboflavin (Rf; Scheme 1) which has been postulated as a possible sensitiser for the photooxidative degradation of numerous relevant natural substrates present in different classes of living organisms [1]. Rf produces the highly reactive species $O_2(^1\Delta_g)$ with a quantum yield of 0.48 in MeOH [2]. Also innumerable cases of $(O_2^{\bullet -})$-mediated reactions towards different substrates of biological and environmental interest, sensitised by Rf, have been reported [3]. Nevertheless, the scavenging action of oxygen active species by certain biologically-relevant endogenous or externally added compounds can either inhibit or promote photodamage in natural media. In this context, an important class of these potentially interactive Rf, in humans, may be the externally administered drugs namely aminosalicylic acids (ASA; Scheme 1) [4–6]. The antioxidant action of ASA operates by different mechanisms, including the scavenging of activated electrophilic forms, such as peroxy radicals, hydroxy radicals [7] and $O_2(^1\Delta_g)$, this one recently demonstrated in vitro in our laboratory [8]. Nevertheless, the potential properties, as a redox intermediary of ASA in the presence of Rf as a visible-light sensitiser, to our knowledge, has not been investigated. This is the aim of the present kinetic and mechanistic study, employing the visible-light transparent 4- and 5-ASA.

2. Materials and methods

4-Aminosalycilic acid (4-ASA) and 5-aminosalycilic (5-ASA) acid, riboflavin (Rf), sodium azide (NaN_3) and superoxide dismutase (SOD) were purchased from Sigma (USA). In all cases, the solvent employed was the mixture of water–MeOH 1:1 (v/v). Water was triply distilled and MeOH (HPLC quality) was provided by Sintorgan (Argentina).

Ground state absorption spectra were registered in a Hewlett-Packard 8452A diode array spectrophotometer.

The laser flash photolysis apparatus, the time-resolved and stationary fluorimeters and the irradiation devices for static photolysis have been already described [9].

The Rf-sensitised photo-oxygenation rates of ASA (0.4 mM) and Rf (0.02 mM) were determined by evaluation of the initial slopes of oxygen consumption versus irradiation time, employing an specific oxygen electrode (Orion 97-08). Anaerobic photo-decomposition rates of Rf were determined by evaluation of the initial slopes of Rf consumption (decrease of absorbance at 446 nm) versus irradiation time.

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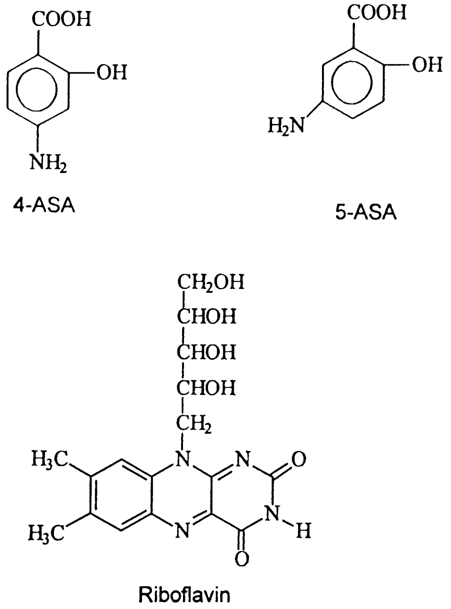


Scheme 1. Chemical structures of 4- and 5-aminosalicylic acids and riboflavin.

3. Results

Results are interpreted and discussed on the basis of Scheme 2. $^1Rf^*$ and $^3Rf^*$ represent the excited singlet and triplet states of Rf.

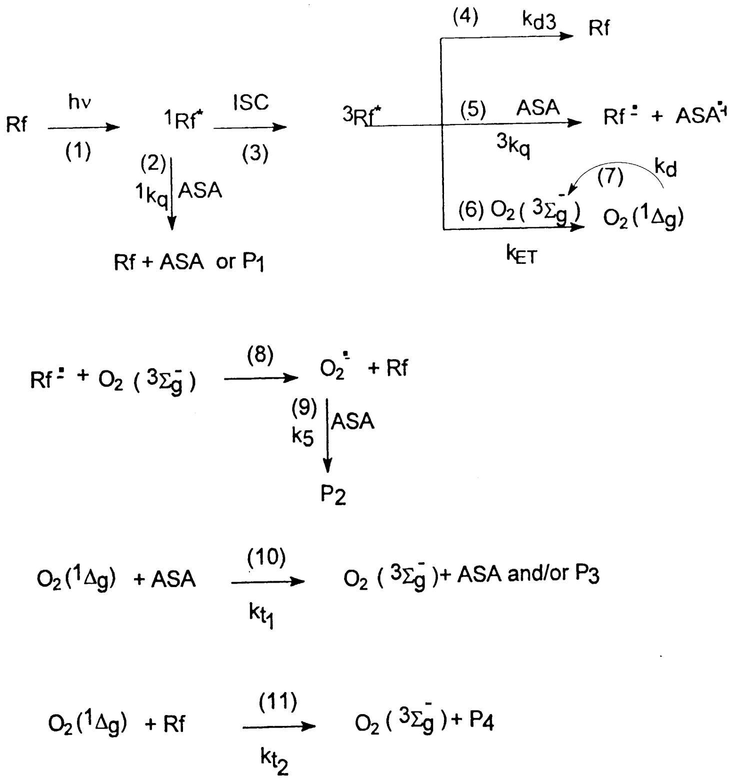
When the system ASA/Rf/oxygen (air)/H_2O–MeOH was illuminated with light of wavelength higher than 400 nm, spectral changes were observed in the UV/visible absorption spectrum of the mixture, with simultaneous oxygen consumption. No changes in the rate of oxygen consumption could be observed in the presence of the known $O_2(^1\Delta_g)$ quencher NaN_3 2 mM [10], whereas, in the presence of 1 mg/100 ml superoxide dismutase (SOD), an employed superoxide radical anion scavenger [11–14], the rate of oxygen consumption was slightly increased.

Comparative irradiations of N_2-saturated aqueous solutions of Rf in the presence of ca. 0.05 mM 4- and 5-ASA showed a decrease in the rate of Rf consumption, as monitored by the evolution of the 445 nm absorption band. This fact suggests a process of electronic excited singlet and/or triplet Rf ($^1Rf^*$ and $^3Rf^*$, respectively) [15] represented by processes (2) and (5) in Scheme 2, being ^1k_q and ^3k_q the respective quenching rate constants.

The quenching of Rf fluorescence (process (2)), was detected through time-resolved methods and evaluated through the classical Stern–Volmer treatment. The ^1k_q values are shown in Table 1.

The lifetime of $^3Rf^*$ markedly decreases in the presence of ASA in the mM concentration range (process (5)). As before, a Stern–Volmer treatment of the triplet quenching, yielded the bimolecular rate constants ^3k_q (Table 1).

The transient absorption spectrum of Rf, immediately after the laser pulse is similar to the reported one for the Rf



Scheme 2. Possible reaction steps in a riboflavin (Rf) photosensitised process in the presence of 4- and 5-aminosalicylic acids (ASA). P_1, P_2, P_3 and P_4 represent hypothetical photoproducts.

Table 1
Rate constants (k_{t_2}) for the quenching of $O_2(^1\Delta_g)$, for the quenching of excited singlet riboflavin (^1k_q) and for the quenching of excited triplet Rf (^3k_q) by 4- and 5-aminosalicylic acids (ASA) all in H_2O–MeOH 1:1 (v/v)

Compound	$k_{t_2} \times 10^7$ $(\mathrm{M\,s})^{-1} \pm 10\%$	$^1k_q \times 10^9$ $(\mathrm{M\,s})^{-1} \pm 5\%$	$^3k_q \times 10^9$ $(\mathrm{M\,s})^{-1} \pm 10\%$
4-ASA	3.0[a]	3.0	5.0
5-ASA	20.0[a]	5.7	5.7

[a] Ref. [8].

neutral triplet state in MeOH [16]. The corresponding spectral trace recorded at 40 μs after the laser pulse, under identical experimental conditions as the formerly described, but in the presence of 5 mM 5-ASA, possesses a shape similar to those habitually identified with the presence of the well known Rf radical anion ($\mathrm{Rf}^{\bullet -}$) [2,16,17].

4. Discussion

Experimental evidence indicates the participation in the photoprocesses of reactive oxygen species generated directly or indirectly by $^1\mathrm{Rf}^*$ and/or $^3\mathrm{Rf}^*$. In spite of the high value—close to the diffusion limit—of the rate constant for the interaction $^1\mathrm{Rf}^*$/ASA (^1k_q, process (2)), concentrations of ASA, much higher than those employed in our photolysis experiments, are necessary to prevent Rf triplet state population. $^3\mathrm{Rf}^*$ in solution generates both $O_2(^1\Delta_g)$ and $O_2^{\bullet -}$ upon visible light irradiation with reported quantum yields of 0.48–049 and 0.009, respectively [2,18]. Hence, in comparative terms, the direct generation of $O_2^{\bullet -}$ by electron transfer from $^3\mathrm{Rf}^*$ to O_2 must be considered negligible. Regarding the participation of $O_2(^1\Delta_g)$ as responsible for oxygen consumption, in a previous paper [8], we demonstrated that ASA are merely physical quenchers of $O_2(^1\Delta_g)$ in H_2O, with overall rate constants for process (10) in the order of 10^7–10^8 $(\mathrm{M\,s})^{-1}$ (Table 1). In other words, neither spectral changes nor oxygen uptake should be observed as a consequence of process (10), in a Rf-sensitised irradiation of ASA.

On the other hand, although Rf has been reported as a chemical quencher of $O_2(^1\Delta_g)$ with $k_{t_2} = 6 \times 10^7$ $(\mathrm{M\,s})^{-1}$ [2], under work conditions most of the reactive oxygen species generated should be physically scavenged by ASA given that $k_{t_1}[\mathrm{ASA}] \gg k_{t_2}[\mathrm{Rf}]$. Furthermore, the experiments in the presence $\mathrm{NaN_3}$ confirm the above postulations.

The predominance of a given process will depend on the competition between O_2 and ASA for the quenching of $^3\mathrm{Rf}^*$. Employing literature information for the involved rate constants and $O_2(^3\sum_g^-)$ concentration [19–21], it arises that process (5) is the preferred form of $^3\mathrm{Rf}^*$ deactivation, and considering the previous discussion, oxygen consumption in the Rf-sensitised photo-oxidation of ASA should be ascribed to a $O_2^{\bullet -}$-mediated mechanism. Nevertheless, the results of the experiments of stationary photo-irradiation in the presence of SOD constitute, in principle, evidence against this proposition: kinetic inhibition in a $O_2^{\bullet -}$-mediated reaction has been frequently interpreted as a confirmation of the said mechanism. Nevertheless, a relatively high value of 2.7×10^9 $(\mathrm{M\,s})^{-1}$ for the rate constant of interaction with $O_2(^1\Delta_g)$ has been reported [22] for SOD in H_2O. This argumentation suggest that, under work conditions, a slow increase in the rate of oxygen uptake in Rf/ASA/SOD solutions is not sufficient evidence to discard a pure $O_2^{\bullet -}$-mediated reaction. Furthermore, the direct evidence for $\mathrm{Rf}^{\bullet -}$ generation under photo-irradiation, strongly implies the formation of $O_2^{\bullet -}$ working in an air-saturated solution. A rate constant value of 1.4×10^8 $(\mathrm{M\,s})^{-1}$ has been recently reported for the generation of $O_2^{\bullet -}$ from $\mathrm{Rf}^{\bullet -}$ [18] (process (8)), a reaction that at the same time regenerates ground state Rf, a crucial step in living organisms in which it is well known that $O_2^{\bullet -}$ is a key intermediate in the oxygen redox chemistry [23].

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