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Scavenging ability on ROS of alpha-lipoic acid (ALA)

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Abstract

The scavenging ability of alpha-lipoic acid (ALA) towards superoxide anions, hydroxyl radicals and hydrogen peroxide was evaluated by means of chemiluminescence (CL). In neutral or acid conditions, ALA could scavenge superoxide anions and its scavenging efficiency depended on its concentration. In neutral or alkaline conditions, the CL intensity of the reaction of ALA with hydroxyl radical (OH• radicals) decreased after the first 10 min and then increased with time, suggesting that ALA could scavenge OH • radicals at the beginning, but yielded unknown intermediate products that could lead to the increase of CL intensity at later stages. The increase of CL intensity in the reaction of hydrogen peroxide with ALA may also be due to these products.

Keywords: Alpha-lipoic acid; Reactive oxygen species; Superoxide anions; Hydroxyl radical; Hydrogen peroxide; Chemiluminescence

1. Introduction

Alpha-lipoic acid (ALA) is a unique short chain fatty acid with two sulphur atoms. In its reduced form, dihydrolipoic acid (DHLA), the two sulphur atoms are converted to sulfhydryl (SH) groups (Sudesh, Linda, & Pawan, 2002). Being a thiosulfinate of ALA, beta-lipoic acid (BLA) is an oxidized form of it (Perricone, Nagy, Horva'th, Dajko, Uray, & Nagy, 1999). The chemical structures of ALA and its related compounds are shown in Fig. 1.

There is increased interest in study of ALA and related compounds in biochemistry, food and clinical chemistry. ALA is a naturally occurring compound that can be synthesized in the body of animals and humans, functioning as a cofactor in several mitochondrial multienzyme complexes involved in energy production (Hande, Hilal, Serdar, & Nuran, 1999). In our diet, it is present mainly in meat and liver but is undetectable in vegetables (Hiroyuki, 1998). Some studies showed that ALA could have diverse biological functions when used as a food supplement (Sudesh, Linda, & Pawan, 2002). For example, dietary supplementation with ALA may improve carbohydrate metabolism, lower blood pressure and normalize associated biochemical and histopathological changes (Sudesh, Linda, & Pawan, 2002). Administration of ALA decreases oxidative injury in the kidney, and is associated with a significant improvement of renal function (David, Radhi, Dave, & Jackson, 2001). ALA supplementation has been found to be beneficial in preventing neurovascular abnormalities in diabetic neuropathy (Sushil, Jain, & Gideon, 2000). It displays a protective effect in various models against age-dependent cognitive deficits. In addition, ALA also proves to be useful in topical applications against aging signs of the skin (Perricone, Nagy, Horva'th, Dajko, Uray, & Nagy, 1999).

''Reactive oxygen species'' (ROS) is a collective name for a group of oxygen-containing species such as superoxide anion, hydroxyl radical and hydrogen peroxide. ROS are widely considered to induce cancer, aging and some other chronic diseases. The biological functions of ALA described above may be due to its scavenging ability on ROS. The radical-scavenging ability of ALA has been investigated in some biological models using some measures such as ESR. However, reports on scavenging activity of ALA with the CL method are few.

CL is a simple, direct and effective method for ROS and antioxidant studies, which has been proven to be a sensitive assay for tracing the reaction process of antioxidant scavenging of ROS. In this paper, the scavenging ability of ALA towards superoxide anions, hydroxyl

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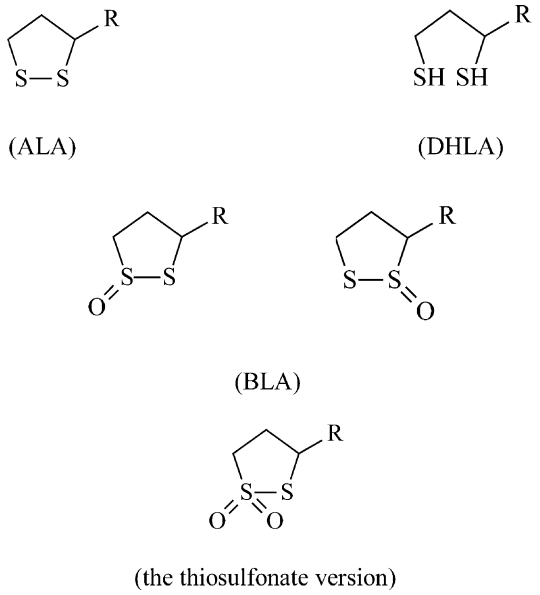

Fig. 1. The chemical structure of ALA and related compounds (R symbolizes pentanoic acid) (Perricone, Nagy, Horva'th, Dajko, Uray, & Nagy, 1999).

radicals and hydrogen peroxides is measured by means of CL. The results show how the scavenging effect of the ALA on ROS changes with time and how the concentration of ALA and pH affect the scavenging ability of ALA. The results of this paper may be helpful in the application of ALA as an antioxidant food supplement.

2. Materials and methods

2.1. Chemicals

Alpha-lipoic acid was purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Pyrogallol was purchased from Beijing Chemical Co. Ltd. (Beijing, China). 1,10-phenanthroline (Phen) and luminol were from Sigma Chemical Co. (St Louis, MO, USA). CuCl, HCl and NaOH were purchased from Yonghua Special Chemical Reagent Factory (Shanghai, China). H_2O_2 was from Shanghai Taopu Chemical Factory (Shanghai, China). All chemicals were of analytical grade. Phosphate buffer (pH = 5.29, 7.38, 9.18) and distilled water were prepared in our laboratory.

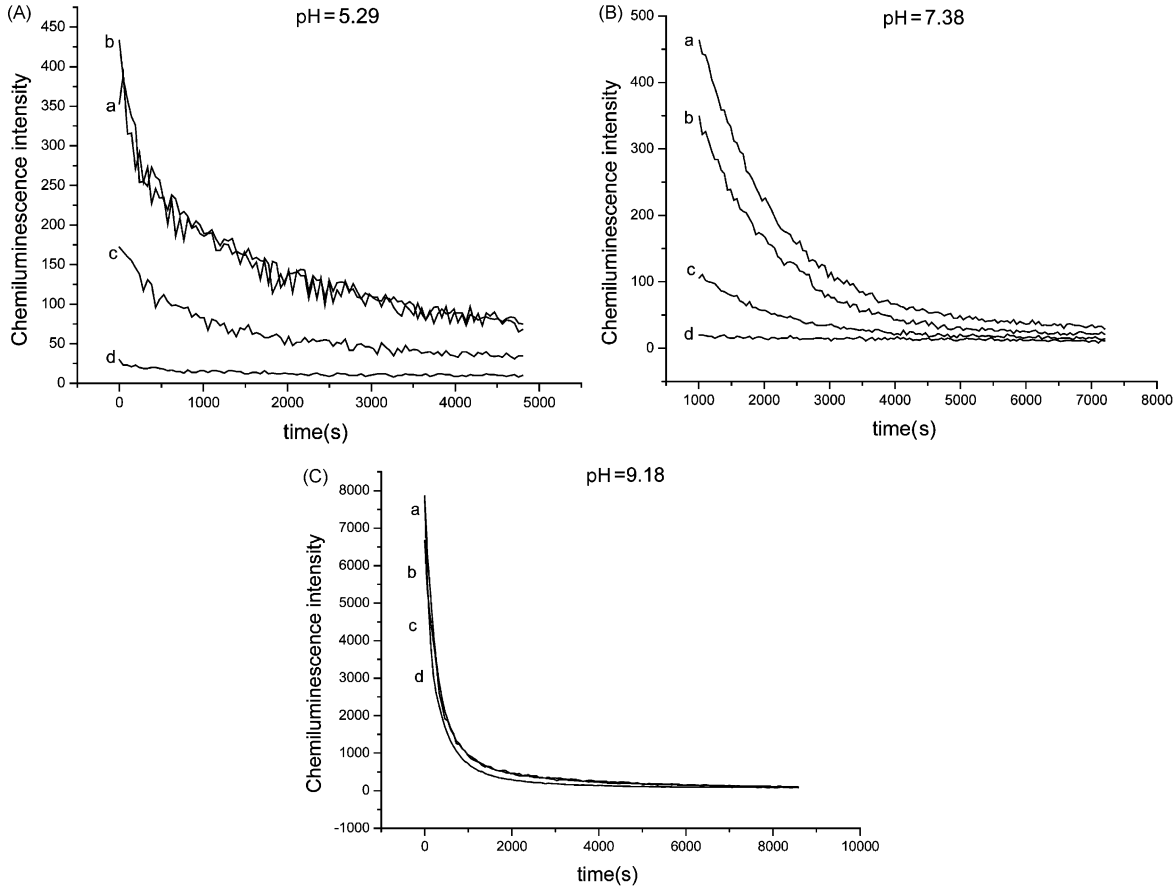

Fig. 2. The changes of CL intensity in the reaction of superoxide anion with ALA at different concentrations (a: 0 M; b: 1×10^{-4} M; c: 1×10^{-3} M; d: 1×10^{-2} M) (A: pH = 5.29; B: pH = 7.38; C: pH = 9.18).

2.2. Instrument

The SHG-D Biochemistry Chemiluminescence Meter (BCM) is produced by Shanghai Measurement Equipment Factory, Shanghai, China. The BCM is composed of three parts: an automatically rotating sample support in which 12 sample cells can be placed, a chemiluminescence monitor, and a data processor. The sample cells are fed into the monitor, successively, and held there according to a predetermined programme. The chemiluminescence (CL) intensity in each cell is recorded by the data processor (Yu, Zhao, Xue, Jin, & Wang, 2001).

2.3. Experimental methods

2.3.1. Superoxide anion

Superoxide anions were generated by pyrogallol autoxidation (Sudesh, Linda, & Pawan, 2002). The reaction mixture contained 100 μl of pyrogallol (1×10^{-3} M), 100 μl of luminol (1×10^{-3} M), and 700 μl of phosphate buffer. A series of concentrations of ALA samples (100 μl) (0 M, 1×10^{-4} M, 1×10^{-3} M, 1×10^{-2} M) were injected into the mixture. The final volume was always the same (1 ml) for all assays. Because ALA is practically insoluble in water, it is first dissolved with 5% NaOH, then mixed with distilled water. The sample cells were immediately placed in the BCM. The CL intensity was simultaneously recorded by the processor, every 6 s. The sample without ALA was also recorded for a blank comparison. The test was repeated at least three times.

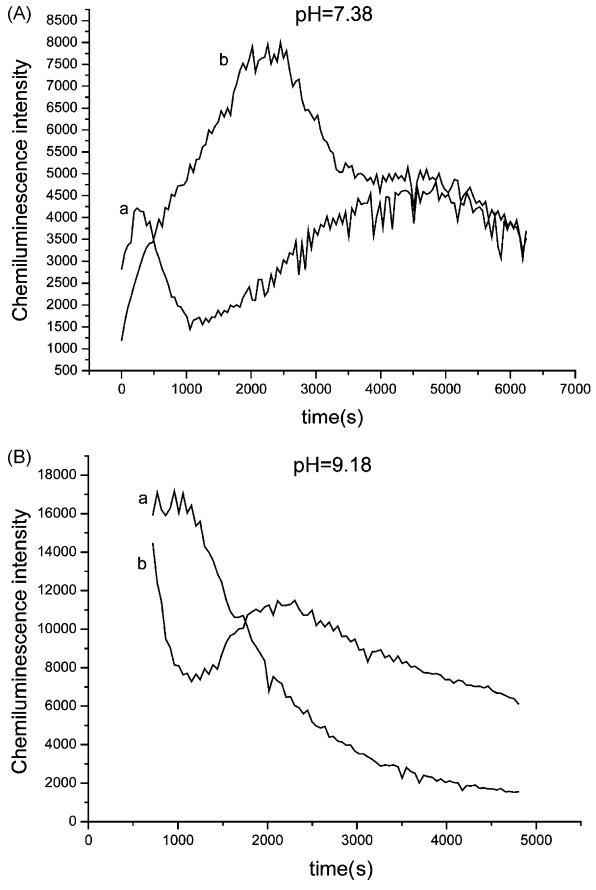

Fig. 3. The changes of CL intensity in the reaction of hydroxyl radical with ALA at different concentrations (a: 0 M; b: 1×10^{-2} M) (A: pH = 7.38; B: pH = 9.18).

2.3.2. Hydroxyl radical

Hydroxyl radicals were generated from the CuCl–Phen–H_2O_2 system. The reaction mixture contained 100 μl of CuCl (1×10^{-3} M), 100 μl of 1,10-phenanthroline (1×10^{-3} M), 100 μl of H_2O_2 (0.6%), and 700 μl of phosphate buffer. A series of concentrations of ALA samples (100 μl) (0 M, 1×10^{-2} M) was injected into the mixture. The final volume was always the same (1 ml) for all assays. The test procedure was similar to that for the superoxide anion assay. The test was repeated at least three times.

2.3.3. Hydrogen peroxide

The reaction mixture contained 100 μl of H_2O_2 (30%) and 700 μl of phosphate buffer. A series of concentrations of ALA samples (100 μl) (0 M, 2×10^{-3} M, 4×10^{-3} M, 8×10^{-3} M, 1×10^{-2} M) were injected into the mixture. The final volume was always the same (1 ml) for all assays. The test procedure was similar to that for the superoxide anion assay. The test was repeated at least three times.

3. Results and discussion

3.1. Scavenging ability towards superoxide anion

The changes of CL intensity in different ALA concentrations with time are shown in Fig. 2 when pH was 5.29, 7.38 and 9.18.

As shown in Fig. 2A and B, a marked decrease of CL intensity was observed when the ALA concentration was 1×10^{-2} M, and the CL intensity of 1×10^{-4} M was similar to that of 0 M. As Fig. 2C shows, the CL intensity of all concentrations were very similar. Evidently ALA could scavenge superoxide anions effectively when pH was 5.29 and 7.38, and the scavenging ability decreased with ALA concentration. When ALA concentration was less than 1×10^{-4} M, it almost showed no scavenging effect. ALA had no scavenging effect on superoxide anions when pH was 9.18.

3.2. Scavenging ability towards hydroxyl radical

The changes of CL intensity in different ALA concentrations with time were studied when pH was 5.29, 7.38 and 9.18, respectively. We do not consider the result with pH = 5.29 because, in that case, the CL intensity of all ALA concentrations was very low (less than 20). An interesting phenomenon was observed

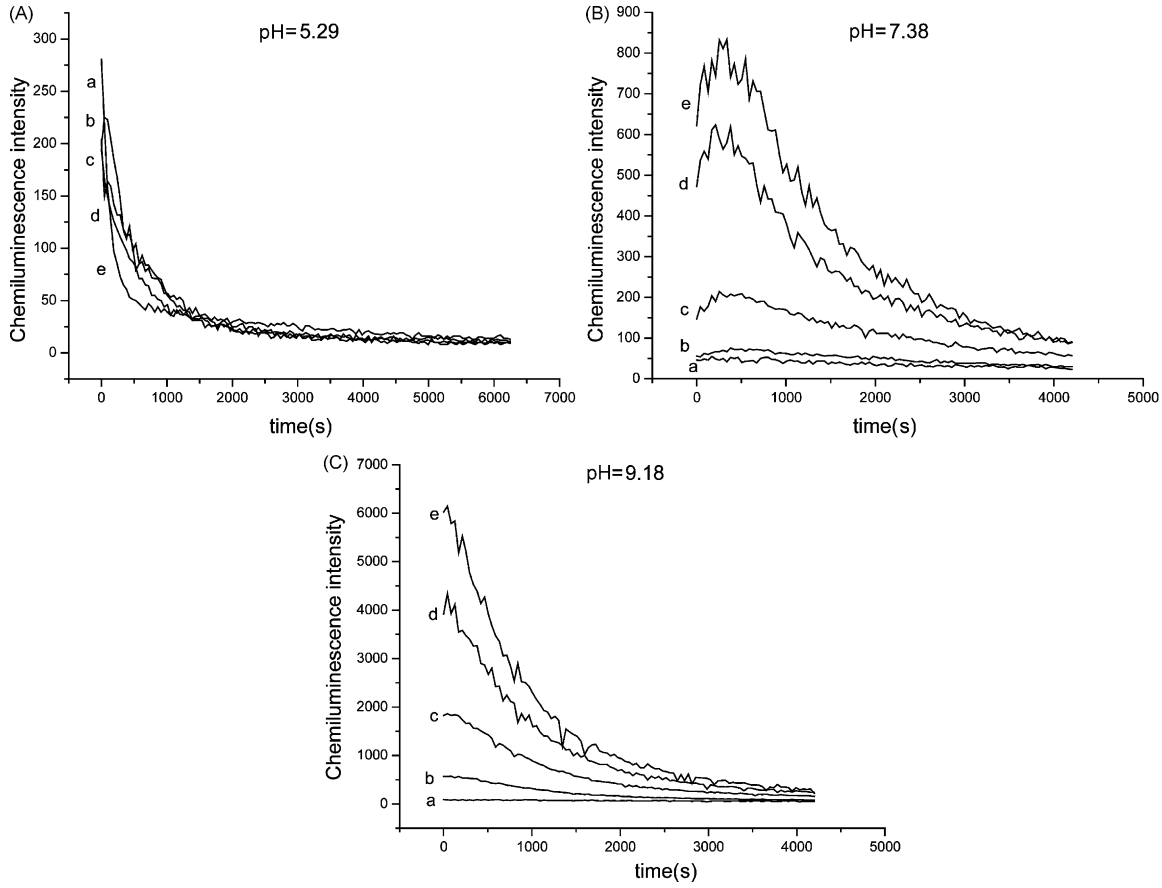

Fig. 4. The changes of CL intensity in the reaction of hydrogen peroxide with ALA in different concentrations (a: 0 M; b: 2×10^{-3} M; c: 4×10^{-3} M; d: 8×10^{-3} M; e: 1×10^{-2} M) (A: pH = 5.29; B: pH = 7.38; C: pH = 9.18).

from the results of pH = 7.38 and pH = 9.18, which was obvious when the ALA concentration was 1×10^{-2} M, as shown in Fig. 3.

After the first 10 min, there was a decrease of CL intensity when the ALA concentration was 1×10^{-2} M, as shown in Fig. 3A. Then the CL intensity showed a marked increase with time. There is a similar phenomenon in Fig. 3B. It has been reported that the interaction of the OH• radical with ALA takes place on the disulfide group of ALA, yielding thiosulfinate or thiosulfonate (Perricone, Nagy, Horva'th, Dajko, Uray, & Nagy, 1999). Evidently at the beginning, ALA scavenged most of the OH• radical and yielded thiosulfinate or thiosulfonate which led to the later increase of CL intensity. Identification of the resulting compounds will be done in the future.

3.3. Scavenging ability towards hydrogen peroxide

A number of studies have demonstrated that ALA could effectively neutralize free radicals such as the OH• radical, but not hydrogen peroxide (Perricone, Nagy, Horva'th, Dajko, Uray, & Nagy, 1999). Here we have also studied the CL of reaction of ALA with hydrogen peroxide. The changes of CL intensity in different ALA concentrations with time are shown in Fig. 4 when pH was 5.29, 7.38 and 9.18.

The CL intensities of all concentrations were very similar to that of 0 M when pH was 5.29, as Fig. 4A shows. In B and C, CL intensity increased with concentration increase. The extent of increase was determined by the ALA concentration. There was a marked increase when ALA concentration was 1×10^{-2} M, and when ALA concentration was less than 2×10^{-3} M, the increase could hardly be seen. This phenomenon was similar to that observed in Fig. 3A and B. Hydrogen peroxide could produce a small amount of OH• radicals, so this result was consistent with the supposition that the increase of CL intensity was due to the product resulting from the interaction of the OH• radicals with ALA.

4. Conclusion

In this study, ALA was found to scavenge superoxide anions effectively in a neutral or acid environment. The scavenging ability was related to its concentration and pH. Moreover, in neutral or alkaline conditions, the CL intensity of the reaction of hydroxyl radical with ALA decreased after the first 10 min and then increased with

time, suggesting that ALA scavenged most of the OH• radical at the beginning but yielded some compounds which could lead to an increase of CL intensity. The CL intensity also increased in the reaction of hydrogen peroxide with ALA in neutral or alkaline conditions. Since hydrogen peroxide could produce a small amount of OH• radicals, the increase of CL intensity may also be due to the product resulting from the interaction of OH• radicals and ALA. However, the mechanisms of the effect of pH on ALA's scavenging ability and how the reaction product increases the CL intensity are unclear and should be studied further.

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