

Albumin stabilizes 14,15-leukotriene A₄

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14,15-Leukotriene A₄ is a pivotal biosynthetic intermediate in 15-lipoxygenase initiated leukotriene biosynthesis. This compound hydrolyzes instantaneously in phosphate buffer at pH 7.4. However, addition of human or bovine albumin to otherwise identical buffer solutions increases its stability. Intact 14,15-leukotriene A₄ then decomposes by first-order kinetics with rate constants inversely proportional to the albumin concentration. Stabilization of 14,15-leukotriene A₄ under certain conditions may influence its proportionate transformation by enzymatic vs non-enzymatic processes.

14,15-Oxido-5,8,10,12-eicosatetraenoic acid
Bovine serum albumin

14,15-leukotriene A₄ *Hydrolytic stability*
Human serum albumin

1. INTRODUCTION

In certain cells 15-hydroperoxy-5,8,10,13-eicosatetraenoic acid (15-HPETE) is a lipoxygenase metabolite of arachidonic acid [1,2]. Dehydration of 15-HPETE generates 14,15-oxido-5,8,10,12-eicosatetraenoic acid, designated 14,15-leukotriene A₄ (14,15-LTA₄) [3]. The latter compound is a transient biosynthetic precursor for other leukotrienes such as isomeric 8,15-dihydroxy-5,9,11,13-eicosatetraenoic acids and 14,15-dihydroxy-5,8,10,12-eicosatetraenoic acids [4,5]. 14,15-LTA₄ and its metabolites resemble, in some respects, the leukotrienes formed via 5-lipoxygenase metabolism of arachidonic acid [6]. However, in contrast to 5,6-LTA₄,

less is known about 14,15-LTA₄ and some dissimilarities exist between the corresponding systems of products derived from these compounds. For instance, some enzymatic features of the metabolism of 14,15-LTA₄ are uncertain [3,5,7,8]. Since 14,15-LTA₄ contains an allylic epoxide susceptible to rapid non-enzymatic hydrolysis we evaluated its stability under selected conditions. The results indicate that human and bovine serum albumin prolong its half-life to a significant degree under conditions that otherwise favor its rapid hydrolysis.

2. EXPERIMENTAL

2.1. Materials

PRP-1^R polymer reversed phase HPLC columns were obtained from Hamilton, Reno, NE. Bovine serum albumin (BSA), essentially fatty acid free (Sigma, St Louis) or crystalline (Armour Pharmaceutical, Eastbourne, England); and human serum albumin (HSA), crystalline (Kabi, Stockholm), >98% pure were used as received. 15-Hydroperoxyeicosatetraenoic acid (15-HPETE) was prepared as in [3]. 14,15-Oxido-5,8,10,12-eicosatetraenoic acid (14,15-LTA₄) was chemically synthesized using 15-HPETE methyl ester as a starting material [9].

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Abbreviations: 5,6-LTA₄, 5(S),5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; 14,15-LTA₄, 14,15-oxido-5,8,10,12-eicosatetraenoic acid; 15-HPETE, 15-hydroperoxy-5,8,10,13-eicosatetraenoic acid; TXA₂, thromboxane A₂; PGI₂, prostacyclin; HSA, human serum albumin; BSA, bovine serum albumin; HPLC, high performance liquid chromatography; RP-HPLC, reversed phase high performance liquid chromatography

2.2. Stability determination procedure

Phosphate buffer (0.10 M, pH 7.4) containing HSA or BSA (1–10 mg/ml) was equilibrated at 4 or 25°C; then 2.0 ml were mixed with 14,15-LTA₄ (10 µg) dissolved in tetrahydrofuran (5 µl). Samples (200 µl) were withdrawn at appropriate intervals for immediate RP-HPLC quantitation of intact 14,15-LTA₄.

2.3. Quantitation of intact 14,15-leukotriene A₄

Intact 14,15-LTA₄ was determined by RP-HPLC [10]. Samples (100 µl) were chromatographed on a PRP-1^R polymer reversed phase column, eluted at 1.0 ml/min with 0.01 M acetonitrile (pH 9.5)/sodium borate (35:65, v/v). Quantitation was based on spectrophotometric detection at 280 nm. Intact 14,15-LTA₄ eluted with an approximate retention time of 7 min. Dihydroxy-leukotrienes formed by non-enzymatic hydrolysis of 14,15-LTA₄ had retention times between 2 and 4 min and did not interfere with the analysis. The half-life of 14,15-LTA₄ in the mobile phase exceeded 60 min at 25°C; therefore, decomposition was less than 3% during its chromatographic transit. Half-life and rate constants were based on peak height measurements.

3. RESULTS

When the allylic epoxide 14,15-LTA₄ was added to 0.10 M phosphate buffer (pH 7.4) it degraded instantaneously. Within 15 s no intact epoxide was detected by HPLC. When human albumin was added to the same buffer the stability of 14,15-LTA₄ (5 µg/ml) was greatly increased. Fig.1 shows its half-lives at 4 and 25°C as a function of albumin concentration. Significant stabilization occurred with as little as 1 mg/ml HSA. For instance, half-lives increased to 7.0 ± 1.4 and 62.4 ± 8.3 min at 25 and 4°C, respectively. The stabilizing effect reached a plateau at 10 mg/ml HSA. It is notable that these concentrations of albumin are below normal plasma levels of 35–50 mg/ml. In all experiments 14,15-LTA₄ decomposed by first-order kinetics with rate constants inversely proportional to the albumin content and proportional to the temperature (fig.2). There was no qualitative difference in the hydrolysis products formed from 14,15-LTA₄ in the presence or absence of albumin. At a constant albumin concentration and tempera-

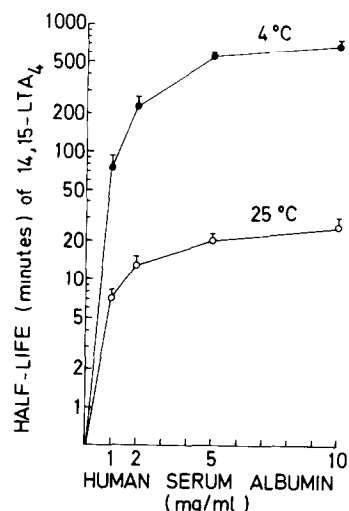


Fig.1. Effect of HSA on 14,15-LTA₄ stability. Values represent half-life (mean $\pm$ SD for ≥ 3 experiments) at 4°C (●—●) and 25°C (○—○) as a function of the albumin content of 0.10 M phosphate buffer (pH 7.4).

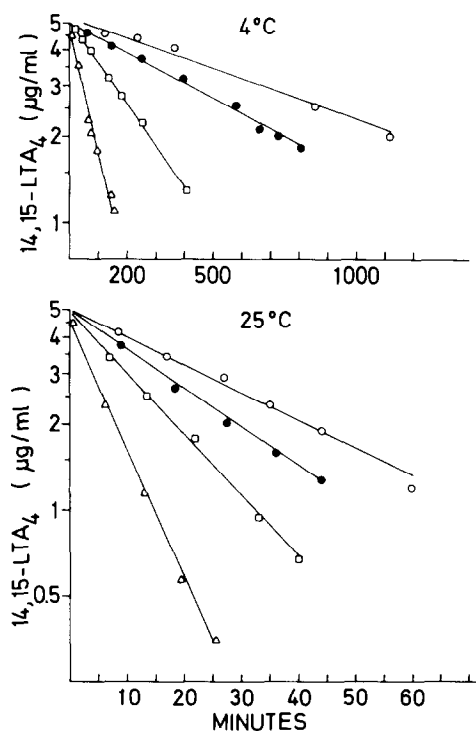


Fig.2. Decomposition kinetics of 14,15-LTA₄ at 4°C (top panel) and 25°C (lower panel). Values shown are representative experiments for albumin concentrations of 1 (△—△), 2 (□—□), 5 (●—●) and 10 (○—○) mg/ml.

Table 1
Effect of human and bovine albumin on the stability of 14,15-leukotriene A₄

Albumin concentration (mg/ml)	HSA		BSA	
	4°C	25°C	4°C	25°C
1	62.4 ± 8.3	7.0 ± 1.4	72.4 ± 2.4	11.0 ± 2.9
2	232.0 ± 35.6	12.9 ± 2.0	173.3 ± 4.0	16.9 ± 2.4
5	574.0 ± 19.0	20.4 ± 2.5	298.1 ± 10.3	19.5 ± 1.3
10	700.0 ± 70.7	25.8 ± 4.6	311.6 ± 9.8	22.9 ± 2.7

Values represent half-lives (mean ± SD, $n \geq 3$ experiments) of 14,15-LTA₄, initial concentration 5 µg/ml

ture the stabilizing effect also increased with pH. For example, at 4°C in 0.20 M buffers containing 1 mg/ml HSA the half-lives of 14,15-LTA₄ were 9.0, 27.6, 62.4, 127.8 and 704.7 min at pH 6, 7, 7.4, 8 and 9, respectively.

Several drugs reduced the stabilizing effect of albumin to varying degrees. Preincubation of HSA (1 mg/ml) in 0.10 M phosphate buffer (pH 7.4) with phenylbutazone, nordihydroguaiaretic acid, aspirin and warfarin for 1 h at 37°C at a ligand : albumin mole ratio of 10:1 reduced the half-life of 14,15-LTA₄ from 62.4 ± 8.3 to 37.5, 28.1, 43.4, 35.5 min, respectively. Atropine sulfate had no effect.

Stabilization of 14,15-LTA₄ was evident with albumin from different species. Table 1 compares its half-lives in the presence of HSA and BSA (1–10 mg/ml) at 4 and 25°C.

4. DISCUSSION

We show here that an unstable allylic epoxide, 14,15-oxido-5,8,10,12-eicosatetraenoic acid, an intermediate in the leukotriene cascade is stabilized by albumin, especially HSA. This stabilizing effect was pH, concentration and temperature dependent. A similar effect of albumin has previously been described for several other unstable arachidonic acid metabolites [10–13]. Certain drugs reduced the stabilizing capacity of HSA. Most effective were phenylbutazone, warfarin and nordihydroguaiuretic acid. Acetylsalicylic acid had a small effect and atropine did not reduce the stability at all. These results are in accordance with previous data found for TXA₂ and 5,6-LTA₄ and they provide further evidence for the hypothesis that

the binding site of albumin for drugs such as phenylbutazone and warfarin could be involved in this stabilizing effect.

Many of the important compounds in the arachidonic acid metabolic cascade are chemically unstable. These unstable compounds can have either a direct biological activity, like TXA₂ or PGI₂, or they can act as precursors for other biologically important products as is the case with LTA₄. Therefore, factors that influence the stability of these compounds are important because they can change the relative amounts of different metabolites and enhance or prolong their biological impact. Our data provide further evidence that albumin might play such a role in arachidonic acid metabolism. From a practical perspective, the stabilization of 14,15-LTA₄ with albumin has been useful to trap this compound, in its intact form, momentarily during experiments to elucidate alternative pathways of its biosynthetic origin [14].

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