Albumin stabilizes 14,15-leukotriene A_4

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14,15-Leukotriene A_4 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 A_4 then decomposes by first-order kinetics with rate constants inversely proportional to the albumin concentration. Stabilization of 14,15-leukotriene A_4 under certain conditions may influence its proportionate transformation by enzymatic *vs* non-enzymatic processes.

14,15-Oxido-5,8,10,12-eicosatetraenoic acid *14,15-leukotriene A_4* *Hydrolytic stability*
Bovine serum albumin *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 A_4 (14,15-LTA$_4$) [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-LTA$_4$ and its metabolites resemble, in some respects, the leukotrienes formed via 5-lipoxygenase metabolism of arachidonic acid [6]. However, in contrast to 5,6-LTA$_4$, less is known about 14,15-LTA$_4$ 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-LTA$_4$ are uncertain [3,5,7,8]. Since 14,15-LTA$_4$ 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.

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Abbreviations: 5,6-LTA$_4$, 5(S),5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; 14,15-LTA$_4$, 14,15-oxido-5,8,10,12-eicosatetraenoic acid; 15-HPETE, 15-hydroperoxy-5,8,10,13-eicosatetraenoic acid; TXA_2, thromboxane A_2; PGI_2, prostacyclin; HSA, human serum albumin; BSA, bovine serum albumin; HPLC, high performance liquid chromatography; RP–HPLC, reversed phase high performance liquid chromatography

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-LTA$_4$) was chemically synthesized using 15-HPETE methyl ester as a starting material [9].

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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-LTA_4 (10 μg) dissolved in tetrahydrofuran (5 μl). Samples (200 μl) were withdrawn at appropriate intervals for immediate RP–HPLC quantitation of intact 14,15-LTA_4.

2.3. *Quantitation of intact 14,15-leukotriene A_4*

Intact 14,15-LTA_4 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-LTA_4 eluted with an approximate retention time of 7 min. Dihydroxy-leukotrienes formed by non-enzymatic hydrolysis of 14,15-LTA_4 had retention times between 2 and 4 min and did not interfere with the analysis. The half-life of 14,15-LTA_4 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-LTA_4 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-LTA_4 (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-LTA_4 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-LTA_4 in the presence or absence of albumin. At a constant albumin concentration and tempera-

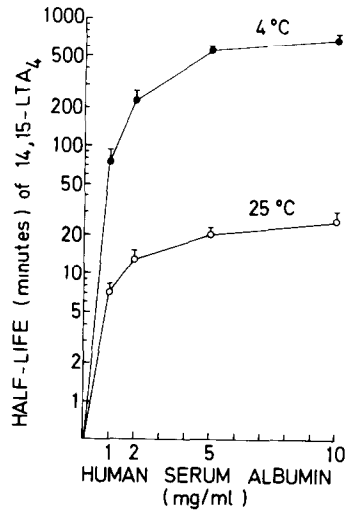

Fig.1. Effect of HSA on 14,15-LTA_4 stability. Values represent half-life (mean ± SD for ≥3 experiments) at 4°C (●—●) and 25°C (○—○) as a function of the albumin content of 0.10 M phosphate buffer (pH 7.4).

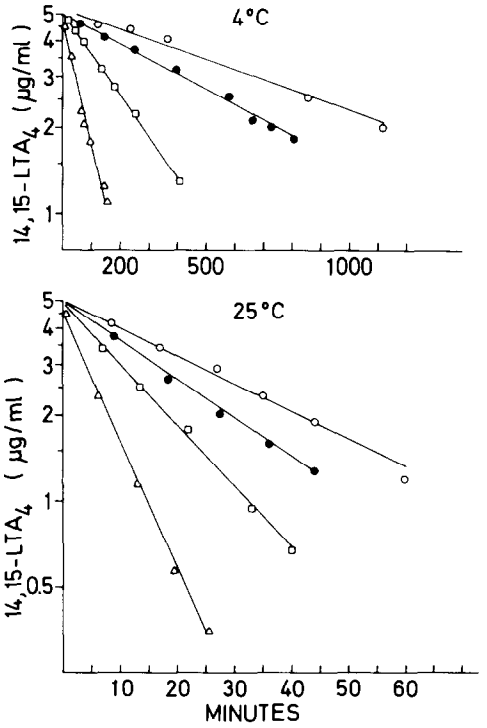

Fig.2. Decomposition kinetics of 14,15-LTA_4 at 4°C (top panel) and 25°C (lower panel). Values shown are representative experiments for albumin concentrations of 1 (△—△), 2 (□—□), 5 (●—●) and 10 (○—○) mg/ml.

Table 1

Effect of human and bovine albumin on the stability of 14,15-leukotriene A_4

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-LTA_4, 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-LTA_4 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-LTA_4 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-LTA_4 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. Acetylsalicyclic acid had a small effect and atropine did not reduce the stability at all. These results are in accordance with previous data found for TXA_2 and 5,6-LTA_4 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 TXA_2 or PGI_2, or they can act as precursors for other biologically important products as is the case with LTA_4. 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-LTA_4 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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