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IDENTIFICATION OF A COMPONENT OF RAT MONONUCLEAR CELL SRS AS LEUKOTRIENE D

Michael K. Bach and John R. Brashler,
Dept. of Hypersensitivity Diseases Research,
The Upjohn Company, Kalamazoo, Michigan 49001, USA

and

Sven Hammarström and Bengt Samuelsson,
Dept. of Chemistry, Karolinska Institutet
S-104 01 Stockholm, Sweden

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SUMMARY

Slow reacting substance (SRS), produced by rat peritoneal mononuclear cells after stimulation with ionophore A23187, consists of two main components (Bach, M.K. et al. (1979) J. Immunol. 122, 160-165). One of these components was recently identified as leukotriene C-1. The other component has now been identified as leukotriene D.

INTRODUCTION

Rat peritoneal mononuclear cells produce a slow reacting substance (SRS^{ri}) when treated with ionophore A23187 (1,2). SRS^{ri} separates into two components upon high performance liquid chromatography on Florisil (3). Recently, the more polar component was shown to consist mainly of leukotriene (LT) C-1 (4). LTC-1 is a slow reacting substance from murine mastocytoma cells (5). It is a 5-hydroxy-6-S-glutathionyl derivative of arachidonic acid with three conjugated ($\Delta^{7,9,11}$) and one isolated (Δ^{14}) double bonds (5,6). The stereochemistry of LTC-1 has been elucidated by comparison with compounds obtained by total organic syntheses (7). Recently, an SRS from rat basophilic leukemia (RBL) cells has been characterized (8). The RBL SRS which has the same basic structure as LTC-1 but lacks the glutamyl residue of the peptide substituent (Fig. 1) has been named leukotriene D (8).

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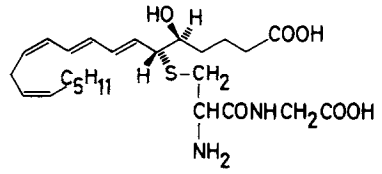


Leukotriene D

Fig. 1. Structure of leukotriene D.

The present report shows that the less polar component of SRS^{ri} is identical to LTD.

MATERIALS AND METHODS

Rat mononuclear cell SRS (3)

Rat peritoneal mononuclear cells were isolated and SRS^{ri} was produced by ionophore A23187 challenge in the presence of 10 mM cysteine. The purification scheme consisted of alkali treatment, chromatography on Amberlite XAD-7 and Florisil HPLC. Two components, named peak I (less polar, 33% of the biological activity) and peak II (67% of the biological activity) were obtained.

Leukotrienes

LTC-1 was generated by ionophore A23187 treatment of murine mastocytoma cells and purified as described before (5). LTD was prepared from LTC-1 by incubation with γ-glutamyl transpeptidase (8).

High performance liquid chromatography

Reverse phase HPLC was performed on C_{18} Polygosil (10 x 500 mm, 5 µm particles, Machery-Nagel Co., Düren, Germany) using a model 6000 A pump and a U6K injector (Waters Associates Inc., Milford, Ma, USA) and a variable wavelength ultraviolet light absorption detector (Spectromonitor II, Laboratory Data Control, Riviera Beach, Florida, USA). Methanol/water 7:3 (v/v) containing 0.1% acetic acid and adjusted to pH 5.4 with ammonium hydroxide was used as mobile phase at a flow rate of 4.5 ml/min.

Analytical methods

Leukotrienes (2-3 nmoles) were hydrolyzed with 6 N HCl/0.5% (v/v) phenol in evacuated ampoules (110°C for 21 hr). Norleucine (5 or 10 nmoles) was added as internal reference prior to amino acid analyses on a Beckman 121 M instrument.

Sequence analyses were performed with the dansyl Edman procedure (9,10). Dansyl amino acids were identified by thin-layer chromatography on polyamide layers in four systems (11).

Incubations with soybean lipoxygenase

Two nmoles of leukotriene in 1 ml of Tyrode's buffer were treated with soybean lipoxygenase (10 µg, Sigma Chemical Company, Type I). Ultraviolet spectra were recorded before addition of enzyme and after 10 min at 20°C, using a Cary 219 instrument.

Bioassay

Bioassay was performed on the isolated guinea pig ileum (4) in Tyrode's buffer containing atropine sulfate (1 µm) and mepyramine maleate (1 µM). The SRS antagonist FPL 55712 (kindly provided by Fisons Pharmaceutical Laboratories, Loughborough, England) added at maximal contraction (10 ng/ml) caused an immediate relaxation of the ileum. Leukotrienes were usually added as 2.5 µM solutions in methanol/water 7:3 (v/v) plus 0.1% acetic acid adjusted to pH 5.4 with ammonium hydroxide.

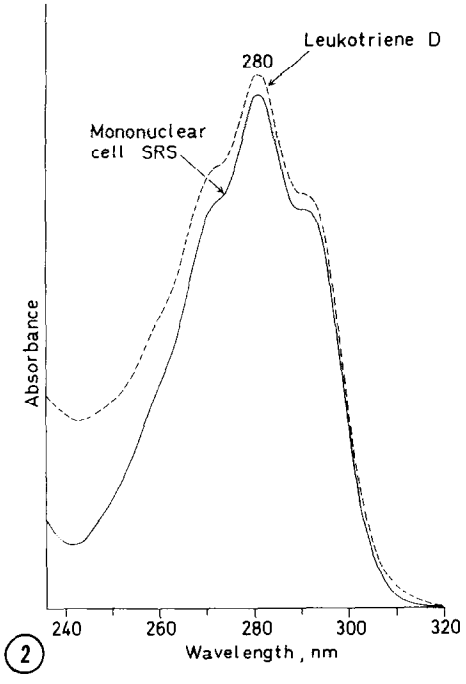
RESULTS

The less polar fraction of SRS^{ri} (peak I; 3.0 x 10^6 units) was subjected to reverse phase HPLC. A minor component (peak I:1; 1.7 nmoles) was eluted after 24 min. Peak I:1 cochromatographed with LTC-1 on HPLC and had the same UV spectrum and identical biological activity as LTC-1. A major component (peak I:2; 11 nmoles) was eluted in the same region where LTD elutes (39 min, c.f. (8)).

The ultraviolet spectrum of peak I:2 (Fig. 2) showed the characteristic triene absorption of LTD and LTC-1 (5) with absorption maximum at 280 nm and shoulders at 270 and 292 nm. When treated with soybean lipoxygenase a spectral change indicating the formation of a conjugated tetraene was observed (Fig. 3).

Peak I:2 induced a more rapid contraction of the guinea pig ileum than LTC-1 as has previously been reported for peak I compared to peak II (3) and for LTD compared to LTC-1 (8). Peak I:2 also gave a dose-response curve which was indistinguishable from that of LTD in the bioassay (Fig. 4) and was more potent than LTC-1.

When chromatographed with an equal amount of LTD on HPLC, peak I:2 gave a single symmetrical peak with the same elution



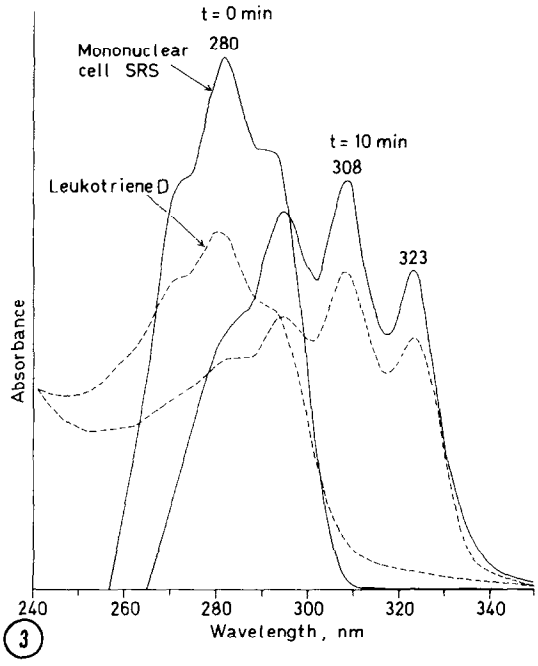


Fig. 2. Ultraviolet spectra of rat monocyte SRS (peak I:2) and LTD.

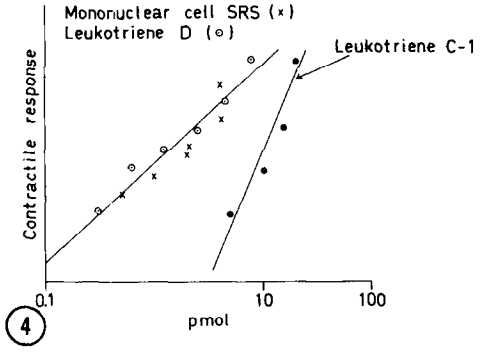
Fig. 3. Ultraviolet spectra of rat monocyte SRS (peak I:2) and LTD before (t = 0 min) and after treatment with soybean lipoxygenase (t = 10 min). Spectra were recorded in Tyrode's buffer.

time and shape as LTD chromatographed alone (Fig. 5).

Amino acid analyses after acid hydrolyses showed that peak I:2 contained approximately 1 mol of glycine per mol of leukotriene. Smaller amounts of 1/2 cystine (approximately 0.2 mol/mol) were also found whereas glutamic acid and other residues were not detected. Glycine was obtained after one step of Edman degradation.

DISCUSSION

Peak I of rat mononuclear SRS contained a small amount of LTC-1 as judged by cochromatography and bioassay. The major component (peak I:2) however, eluted later in reverse phase HPLC. Peak I:2 was identical with leukotriene D as judged by ultra-



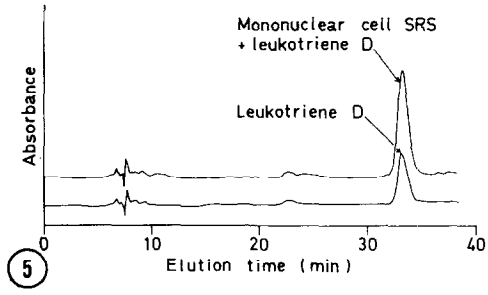


Fig. 4. Bioassay on guinea pig ileum of rat monocyte SRS (peak I:2 (x)), LTD (⊙) and LTC-1 (●).

Fig. 5. Cochromatography of rat monocyte SRS (peak I:2) and LTD on reverse phase high performance liquid chromatography (C_{18} polygosil, 4.6 x 250 mm; methanol/water 7:3 v/v + 0.1% acetic acid adjusted to pH 5.4 with NH_4OH; 4.5 ml/min). The absorbance at 280 nm was continuously recorded at the column effluent.

violet spectroscopy (Fig. 2), spectral changes observed after incubations with soybean lipoxygenase (Fig. 3), biological activity on the isolated guinea pig ileum (Fig. 4), cochromatography on reverse phase HPLC (Fig. 5), and amino acid and sequence analyses.

Evidence for a precursor-product relationship between SRS^{ri} peaks II (precursor) and I has recently been reported (12). It has also been shown that LTC-1 can be enzymatically converted to LTD (8). Our previous demonstration that peak II of SRS^{ri} consists mainly of LTC-1 and the present identification of peak I as LTD are consistent with these observations.

It is interesting to note that immunologically released SRS from human lung corresponded to peak I of SRS^{ri} (3) as judged by Florisil HPLC and bioassay. This suggests that human SRS-A may be identical with leukotriene D.

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