

Inhibition of human leucocyte elastase by ursolic acid

Evidence for a binding site for pentacyclic triterpenes

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Several pentacyclic triterpenoid metabolites of plant origin are inhibitors of hydrolysis of both synthetic peptide substrates and elastin by human leucocyte elastase (HLE). Ursolic acid, the most potent of these compounds, has an inhibition constant of 4–6 μM for hydrolysis of peptide substrates in phosphate-buffered saline. With tripeptide and tetrapeptide substrates, the inhibition is purely competitive, whereas with a shorter dipeptide substrate the inhibition is non-competitive, suggesting that ursolic acid interacts with subsite S_3 of the extended substrate-binding domain in HLE, but not with subsites S_1 and S_2 . The carboxy group at position 28 in the pentacyclic-ring system of the triterpenes contributes to binding to HLE, since replacement of this group with a hydroxy group, as in uvaol, the alcohol analogue of ursolic acid, reduces the potency of inhibition. The inhibitory potency of ursolic acid is also reduced by addition of 1 M-NaCl, further supporting a postulated electrostatic interaction between the negative charge on the triterpene and a positively charged residue on the enzyme, which we assign to the side chain of Arg-217, located in the vicinity of subsites S_4 and S_5 in HLE. These observations are consistent with a binding site for ursolic acid which extends from S_3 towards S_4 and S_5 on the enzyme. Other triterpenes, including oleanolic acid, erythrodiol, hederagenin and 18 β -glycyrrhetic acid, can also interact with this binding site. On the basis of these results we conclude that the extended substrate-binding domain of HLE can accommodate a variety of hydrophobic ligands, including not only such molecules as fatty acids [Ashe & Zimmerman (1977) *Biochem. Biophys. Res. Commun.* **75**, 194–199; Cook & Ternai (1988) *Biol. Chem. Hoppe-Seyler* **369**, 629–637], but also polycyclic molecules such as the pentacyclic triterpenoids.

INTRODUCTION

Human leucocyte elastase (HLE, EC 3.4.21.37) is a lysosomal proteinase stored in high quantities in the azurophilic granules of polymorphonuclear leucocytes (Dewald *et al.*, 1975). For decades the enzyme has been the subject of intensive study because of its potential for tissue destruction in many disease states, such as pulmonary emphysema and rheumatoid arthritis (Janoff, 1985). Administration of exogenous elastase inhibitors has been proposed as a possible means of protecting tissues from proteolytic attack (Powers, 1983; Groutas, 1987). A number of inhibitors have been isolated from natural sources or synthesized *de novo* (Groutas, 1987; Bode *et al.*, 1989); some of these have been proved to be effective in animal models (Powers, 1983; Schnebli, 1985; Soskel *et al.*, 1986). In an effort to find naturally occurring substances which may be utilized as prototype compounds for synthetic inhibitors, we have identified several plant triterpenoids with considerable inhibitory activity towards HLE. Ursolic acid, a pentacyclic triterpene acid, has the greatest potency in this class of compounds. The present paper provides evidence that the inhibition of HLE by the triterpenes is due to reversible binding to a site which lies within a portion of the extended substrate-binding domain of the enzyme.

MATERIALS AND METHODS

Chemicals

Ursolic acid and other pentacyclic triterpenes, except uvaol, were from Carl Roth (Karlsruhe, Germany), and were all of

h.p.l.c. standard grade. Uvaol was from Sigma (St. Louis, MO, U.S.A.). The structures of these triterpenes are given in Fig. 1. Boc-Leu-ONp, MeO-Suc-Ala-Ala-Pro-Val-pNA, Suc-Ala-Ala-Ala-pNA and Suc-Ala-Ala-Pro-Phe-pNA were also from Sigma. Bz-Ile-Glu-Gly-Arg-pNA (S-2222) and pGlu-Pro-Val-pNA (S-2484) were from Kabi (Franklin, OH, U.S.A.). Elastin from bovine neck ligaments was from Elastin Products (Pacific, MO, U.S.A.) and was labelled with [^{14}C]formaldehyde according to the procedure of Yu & Yoshida (1979) to a specific radioactivity of 160 nCi/mg. Dulbecco's modified phosphate-buffered saline [DPBS (per litre: KCl, 0.2 g; KH_2PO_4 , 0.2 g; NaCl, 8 g; and $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 2.171 g; pH 7.2 at 25 °C; Hazleton Biologics, Lenexa, KS, U.S.A.)] was used as the buffer throughout the study.

Enzymes

HLE from azurophilic granules was from Athens Research and Technology (Athens, GA, U.S.A.). α -Chymotrypsin (EC 3.4.21.1) and tosylphenylalanylchloromethane ('TPCK')-treated trypsin (EC 3.4.21.4) were from Sigma. Porcine pancreatic elastase (EC 3.4.21.11) was from Elastin Products. The active centre of HLE was titrated with Z-Ala-Ala-Pro-azaAla-ONp (Enzyme Systems Products, Livermore, CA, U.S.A.) as described by Powers *et al.* (1984).

Enzyme assays with synthetic substrates

The activity of HLE was routinely measured with substrate MeO-Suc-Ala-Ala-Pro-Val-pNA (Bieth *et al.*, 1974) in DPBS

Abbreviations used: Boc-Ala-Ala-Pro-Ala-pNA, *N*-t-butyloxycarbonyl-L-alanyl-L-alanyl-L-prolyl-L-alanine *p*-nitroanilide; Boc-Leu-ONp, *N*-t-butyloxycarbonyl-L-leucine *p*-nitrophenyl ester; Bz-Ile-Glu-Gly-Arg-pNA, *N*-benzoyl-L-isoleucyl-L-glutamylglycyl-L-arginine *p*-nitroanilide; pGlu-Pro-Val-pNA, L-pyroglutamyl-L-prolyl-L-valine *p*-nitroanilide; DMSO, dimethyl sulphoxide; DPBS, Dulbecco's modified phosphate-buffered saline; MeO-Suc-Ala-Ala-Pro-Val-pNA, *N*-methoxysuccinyl-L-alanyl-L-alanyl-L-prolyl-L-valine *p*-nitroanilide; Suc-Ala-Ala-Ala-pNA, *N*-succinyl-L-alanyl-L-alanyl-L-alanine *p*-nitroanilide; Suc-Ala-Ala-Pro-Phe-pNA, *N*-succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine *p*-nitroanilide; Z-Ala-Ala-Pro-azaAla-ONp, *N*-carbobenzoxycarbonyl-L-alanyl-L-alanyl-L-prolyl- α -aza-L-alanine *p*-nitrophenyl ester; HLE, human leucocyte elastase.

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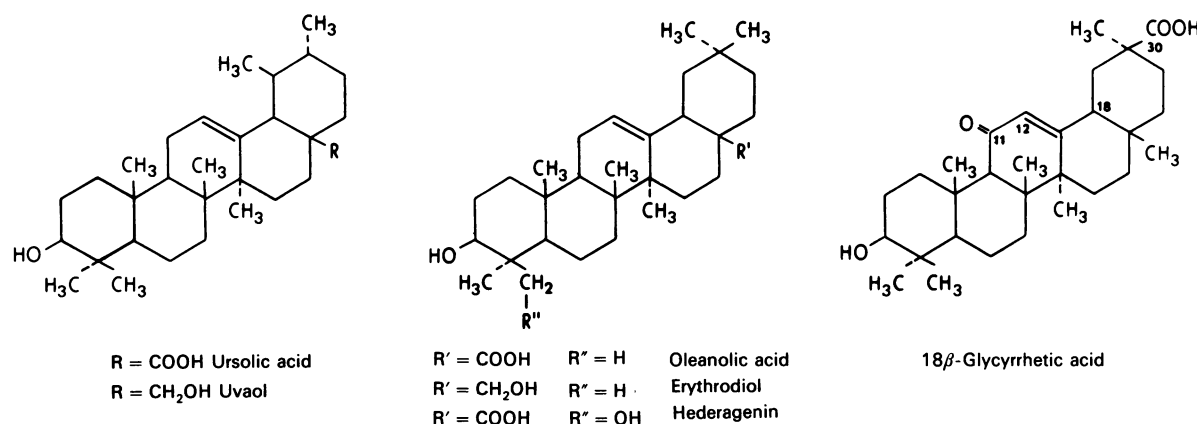


Fig. 1. Chemical structures of the pentacyclic triterpenes used in the present study

containing 5% (v/v) dimethyl sulphoxide (DMSO), pH 7.2. Assays were performed on a ThermoMax Kinetic Microplate Reader (Molecular Devices Co., Palo Alto, CA, U.S.A.) in 96-well microplates at 25 °C. For a typical assay, 50 μ l of the substrate in DPBS was first mixed with inhibitor in 50 μ l of DPBS containing 10% DMSO. Controls were diluted with DPBS/DMSO alone. The mixture was equilibrated at 25 °C for 5 min before initiation of amidolysis by the addition of 50 μ l of HLE in DPBS containing 5% DMSO. Release of *p*-nitroaniline was monitored at 405 nm. The rate of substrate hydrolysis was linear during the first 1 min of recording. Concentrations of *p*-nitroaniline released were calibrated with equal volumes of standard solutions of *p*-nitroaniline (Sigma) in the same buffer. HLE activity was also assayed with substrates pGlu-Pro-Val-pNA (Kramps *et al.*, 1983) and Boc-Leu-ONp. Esterolysis of Boc-Leu-ONp was monitored on a computer-controlled LKB Ultraspec-4050 spectrophotometer equipped with reaction-rate software (Pharmacia-LKB, Piscataway, NJ, U.S.A.). The reaction mixtures contained 850 μ l of DPBS and 25 μ l each of the appropriate concentrations of substrate and inhibitor in DMSO in polystyrene cuvettes at 24 °C, to which 100 μ l of HLE in DPBS was added to start the reaction. Release of *p*-nitrophenol was monitored at 405 nm after a 1 min delay to allow the reaction to reach a steady state. After this lag, the reaction rate remained linear. Absorbance was recorded over the next 1 min and was converted into concentration of product according to Beer's law, by using an absorption coefficient (ϵ_{405}) of $1.27 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$, determined experimentally from a calibrated stock solution (Sigma).

Other serine proteinases were assayed with the following substrates: α -chymotrypsin, Suc-Ala-Ala-Pro-Phe-pNA (Del-Mar *et al.*, 1979); trypsin, Bz-Ile-Glu-Gly-Arg-pNA (Aurell *et al.*, 1977); and porcine pancreatic elastase, Suc-Ala-Ala-Ala-pNA (Bieth *et al.*, 1974). The buffer used was 0.05 M-Tris/HCl containing 0.15 M-NaCl and 10% DMSO, pH 8.0.

Enzyme assays with [¹⁴C]elastin

Elastolysis was determined by using a method modified from that of Yu & Yoshida (1979). A volume of 100 μ l of a 10 mg/ml suspension of elastin prepared by diluting our stock of [¹⁴C]elastin with unlabelled protein to give a final specific radioactivity of 32 nCi/mg was added to a 1.5 ml microcentrifuge tube. The suspension was diluted with 800 μ l of DPBS and mixed with inhibitor in 50 μ l of DMSO. Elastolysis was started by addition of 4.5 μ g of HLE in 50 μ l of DPBS. The reaction was carried out with constant gentle agitation at 37 °C for 2 h, and quenched by addition of 400 μ l of 25% (w/v) trichloroacetic acid. After

centrifugation, labelled peptides in supernatants were counted for radioactivity.

Kinetic analysis

Kinetic data were analysed by Dixon (1953) and, when appropriate, Cornish-Bowden (1974) plots, which were generated by non-linear least-squares regression of the raw velocity data and transformation of the best fits into the appropriate forms (Enzfitter; Elsevier-BIOSOFT, Cambridge, U.K.).

RESULTS

Inhibitory activity

Effects of ursolic acid on the amidolytic activity of HLE were first tested with MeO-Suc-Ala-Ala-Pro-Val-pNA as the substrate. Fig. 2 shows a Cornish-Bowden plot obtained for several different ursolic acid concentrations. Ursolic acid can be described as a competitive inhibitor, with a computed inhibition constant, K_i , of 4 μ M. Interaction between ursolic acid and HLE appears to be rapid, since a linear rate of amidolysis had already been achieved within the instrumentation's 30 s dead time between mixing and commencement of data collection. Inhibition is reversible, as judged by partial recovery of amidolytic activity upon dilution of the enzyme-inhibitor mixture. More complete removal of the inhibitor by gel filtration on Sephadex G-25 resulted in full restoration of amidolytic activity. Since the inhibitor and the enzyme appear to form a reversibly dissociable complex which cannot progress further towards the catalytic step, the inhibition constant we have determined, 4 μ M, can be considered as a true dissociation constant for ursolic acid from a binding site on HLE.

We next examined the inhibitory activity of ursolic acid using the insoluble substrate [¹⁴C]elastin. Fig. 3 shows that ursolic acid can effectively protect against degradation of [¹⁴C]elastin by HLE. Addition of 100 μ M-ursolic acid inhibits elastolysis more than 90%; complete inhibition is observed with 200 μ M-triterpene. However, this concentration exceeds the solubility of ursolic acid in the buffer we have employed, and the inhibitory mechanism may be complicated by the precipitation of the compound on the surface of the insoluble substrate.

The inhibitory mechanism

Previous kinetic and X-ray-crystallographic studies have established an extended substrate-binding domain in HLE which interacts with at least five substrate residues on the proximal side,

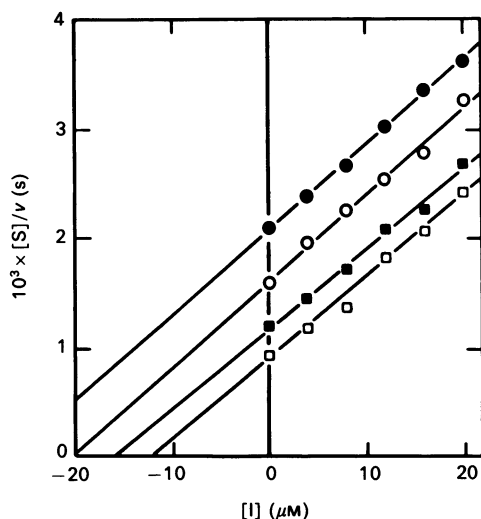


Fig. 2. Cornish-Bowden plot of the inhibition of HLE by ursolic acid with MeO-Suc-Ala-Ala-Pro-Val-pNA as the substrate

The assays were performed in DPBS/5% DMSO, pH 7.2, at 25 °C. The enzyme concentration was 11 nM. The substrate concentrations were 243 μM (●), 172 μM (○), 114 μM (■) and 80 μM (□).

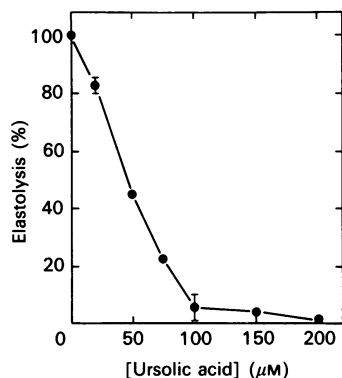


Fig. 3. Inhibition of HLE-mediated solubilization of [^{14}C]elastin by ursolic acid

The assays were performed in DPBS/5% DMSO, pH 7.2, at 37 °C. The substrate concentration was 1 mg/ml, with a specific radioactivity of 32 nCi/mg. The enzyme concentration was 4.5 $\mu\text{g}/\text{ml}$.

and two residues on the distal side, of the scissile peptide bond (Nakajima *et al.*, 1979; Wei *et al.*, 1988; Navia *et al.*, 1989; reviewed by Bode *et al.*, 1989). The tetrapeptide substrate, MeO-Suc-Ala-Ala-Pro-Val-pNA, is bound to five subsites within this extended binding domain, designated S_1 – S_5 . Since ursolic acid displays pure competitive inhibition with this substrate, we can assume that the binding site for the triterpene lies within these five subsites. In order to localize the binding site for ursolic acid more precisely, two shorter peptide substrates, namely pGlu-Pro-Val-pNA and Boc-Leu-ONp, were used. The interactions of these small peptides with the extended substrate-binding domain have not been previously reported. However, it is reasonable to assume that pGlu-Pro-Val-pNA interacts with subsites S_1 , S_2 , and S_3 , and Boc-Leu-ONp with S_1 and S_2 . Table 1 shows the effect of substrate length on the kinetics of inhibition by ursolic acid. With pGlu-Pro-Val-pNA as substrate, ursolic acid is still a competitive inhibitor. With Boc-Leu-ONp as a substrate, however, the mode of inhibition changes to non-competitive (Fig. 4).

Table 1. Inhibition constants for ursolic acid

The Table shows inhibition by ursolic acid of the HLE-catalysed hydrolysis of substrates of different lengths. Assays were performed in DPBS/5% DMSO, pH 7.2, at 25 °C for the amidolytic substrates and at 24 °C for Boc-Leu-ONp, as described in text. The results are expressed as means \pm s.d., with the numbers of determinations shown in parentheses.

Substrate	K_i (μM)	Mode of inhibition
MeO-Suc-Ala-Ala-Pro-Val-pNA	4.4 ± 0.7 (3)	Competitive
pGlu-Pro-Val-pNA	6.6 ± 1.8 (4)	Competitive
Boc-Leu-ONp	5.7 ± 1.0 (3)	Non-competitive

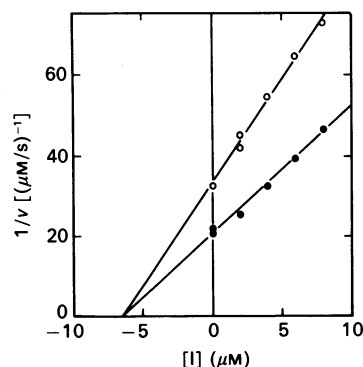


Fig. 4. Dixon plot of the inhibition of HLE by ursolic acid with Boc-Leu-ONp as the substrate

The assays were carried out in DPBS/5% DMSO, pH 7.2, at 24 °C. The enzyme concentration was 56 nM. The substrate concentrations were 55 μM (●) and 20 μM (○).

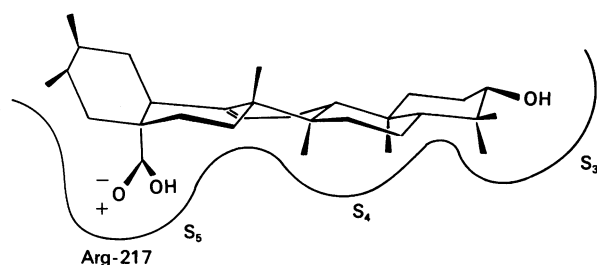
This transformation points to the critical role of S_3 for binding of ursolic acid to HLE. The simplest explanation for the results is that substrates which occupy S_3 block ursolic acid from this binding site. The amino acid components of S_3 have been identified to be Val-216 and Phe-192 (Wei *et al.*, 1988; Navia *et al.*, 1989; Bode *et al.*, 1989). It is likely that the binding site for ursolic acid and the S_3 subsite for substrate binding share at least some common elements of these residues.

We have considered the role of the carboxy group at position 28 of the ursolic ring system for binding to HLE. The activity of ursolic acid was assayed in the presence of high salt to probe the contribution of electrostatic interactions involving this group. Addition of up to 1 M-NaCl to the assay system does not change the mode of inhibition, but raises the value of K_i from 4 μM to 13 μM . These results might be explained by a conformational distortion of the binding site caused by high salt. To exclude this possibility, we tested the inhibitory activity of uvaol, a pentacyclic triterpene alcohol with a structure identical with that of ursolic acid, except for a hydroxy group instead of a carboxy group at position 28 (Fig. 1). Uvaol is also a competitive inhibitor, with an inhibition constant, K_i , of 16 μM , a value comparable with that of ursolic acid in high salt solution (Table 2). These results demonstrate that electrostatic interactions between the 28-carboxy group and a positively charged group on the enzyme contribute to binding. At the pH employed in this study, arginine residues are the only positively charged amino acid residues in the enzyme (Sinha *et al.*, 1987). Their spatial distribution has been carefully determined by X-ray-crystallographic analysis

Table 2. Effect of ionic strength on inhibition of HLE by triterpenes

The Table shows the effect of increasing salt concentration and reduction of the 28-carboxy group on inhibition of HLE by ursolic acid. The substrate MeO-Suc-Ala-Ala-Pro-Val-pNA was used in the determination of the inhibition constants as described in text. With this substrate, both ursolic acid and uvaol display competitive inhibition. The results are expressed as means \pm s.d., with the numbers of determinations indicated in parentheses.

Inhibitor	K_i (μM)
Ursolic acid (12-ursen-3 β -ol-28-oic acid)	
In DPBS/5% DMSO, pH 7.2	4.4 \pm 0.7 (3)
In DPBS/5% DMSO, pH 7.2, containing 1 M-NaCl	13.3 \pm 0.6 (3)
Uvaol (12-ursene-3 β ,28-diol)	
In DPBS/5% DMSO, pH 7.2	15.7 \pm 1.5 (3)

**Fig. 5. Diagrammatic representation of the binding of ursolic acid to the extended substrate-binding domain of HLE****Table 3. Inhibition constants for various triterpenes**

Inhibition constants for pentacyclic triterpenes other than ursolic acid against HLE were determined as described in text. The assays were performed with MeO-Suc-Ala-Ala-Pro-Val-pNA as the substrate in DPBS/5% DMSO, pH 7.2, except for hederagenin, for which DPBS/10% DMSO, pH 7.2, was used. With this substrate, all the triterpenes display competitive inhibition. The results are expressed as means \pm s.d., with the numbers of determinations indicated in parentheses.

Triterpene		K_i (μM)
Trivial name	Systematic name	
Oleanolic acid	12-Oleanen-3 β -ol-28-oic acid	6.4 \pm 1.0 (3)
Erythrodiol	12-Oleanen-3 β ,28-diol	17.3 \pm 0.6 (3)
Hederagenin	12-Oleanen-3 β ,23-diol-28-oic acid	62 \pm 5.5 (3)
18 β -Glycyrrhetic acid	12-Oleanen-3 β -ol-11-one-30-oic acid	185 \pm 5 (3)

(Wei *et al.*, 1988; Navia *et al.*, 1989). If an ursolic acid molecule interacts with HLE at subsite S_3 , the only arginine residue which would be accessible to the carboxy group at position 28 would be Arg-217. This residue has been identified as a component of subsites S_4 and S_5 (Wei *et al.*, 1988; Navia *et al.*, 1989; Bode *et al.*, 1989).

On the basis of the data described above, we propose that the

binding site for ursolic acid extends from a C-terminal limit at S_3 , covers S_4 and S_5 , and reaches to Arg-217 in the N-terminal direction. Binding of ursolic acid to this site should be responsible for the observed inhibition. Fig. 5 shows a diagrammatic representation of the binding. The conformer of ursolic acid is drawn by inference from the X-ray-crystallographic structure of hederagenin (Roques *et al.*, 1978), a closely related pentacyclic triterpene acid (Fig. 1).

Specificity

The effect of ursolic acid on amidolysis catalysed by α -chymotrypsin, trypsin and porcine pancreatic elastase was examined by using substrates Suc-Ala-Ala-Pro-Phe-pNA, Bz-Ile-Glu-Gly-Arg-pNA and Suc-Ala-Ala-Ala-pNA respectively. With these chromogenic oligopeptide analogues as substrates, ursolic acid behaves as a simple competitive inhibitor of α -chymotrypsin, trypsin and pancreatic elastase, but with much lower potency than that seen with HLE. The inhibition constants were determined to be 37.0 \pm 4.6 μM for α -chymotrypsin ($n = 3$), 157 \pm 8 μM for trypsin ($n = 3$), and 180 \pm 8 μM for pancreatic elastase ($n = 3$). These results indicate that the specificity of inhibition by ursolic acid is not strictly restricted to HLE, and it can be expected that other serine proteinases might also be inhibited by this compound.

Activity of other triterpenes

Table 3 lists the inhibition constants of other pentacyclic triterpenes against HLE. The four compounds in Table 3 are members of the β -amyrin group of triterpenes, ursolic acid and uvaol belonging to the α -amyrin group. Since these triterpenes all have closely related structures (Fig. 1), it is reasonable to assume that they are bound at the same site on HLE as ursolic acid. The contribution of electrostatic interactions to the overall free energy of binding of these inhibitors is apparent from comparison of the relative activities of oleanolic acid and erythrodiol, which differ only in the replacement of a carboxy group by a hydroxy group. The results in Table 3 show that oleanolic acid is a stronger inhibitor than erythrodiol, supporting the conclusion we have already drawn from comparison of the two corresponding α -amyrins, ursolic acid and uvaol, that binding of triterpene acids to HLE is strengthened through formation of a salt bridge involving the carboxy group of the inhibitor.

DISCUSSION

Ursolic acid can function either as a competitive inhibitor or as a non-competitive inhibitor of hydrolysis of peptide substrates by HLE, depending on the length of the substrate (Table 1). On the basis of this dependence on substrate size, we have concluded that the binding site for triterpenes overlaps subsite S_3 of the extended substrate-binding domain of the enzyme. It is conceivable that the binding site lies some distance from the extended substrate-binding domain, but occupation of this site induces a conformational change which extends to S_3 . An allosteric mechanism of this sort requires an appropriate set of structural interactions. There have been no reports of such allosteric interactions extending beyond the substrate-binding domain. However, there is both kinetic and structural evidence for an allosteric mechanism by which interactions of substrates or inhibitors with subsite S_3 on the enzyme affect the catalytic function. Stein *et al.* (1987) have shown that interactions between subsite S_3 on the enzyme and the residue at position P_3 on a peptide substrate markedly affect the kinetics of acylation. The structural basis for the communication between subsite S_3 and

the catalytic centre has been attributed to sharing of the side chain of Val-216 by both subsites S_1 and S_3 (Bode *et al.*, 1989). Aside from these interactions between subsites S_1 and S_3 , no other obvious conformational interactions can be deduced from the published X-ray-crystallographic data.

Some further understanding of the mechanism of inhibition of HLE by ursolic acid may be obtained by comparing the inhibition with that of the fatty acids. Cook & Ternai (1988) have recently proposed a binding site for oleic acid and related *cis*-unsaturated fatty acid analogues which lies within the extended substrate-binding domain of HLE. They suggest that the double bond of oleic acid interacts with subsite S_3 , whereas the distal portion of the alkyl chain of the fatty acid extends to S_1 and the carboxy group is pointed towards the side chain of Arg-217. This putative binding site is very similar to the site we have described for ursolic acid, although we find no evidence for extension of the triterpenes towards S_2 and S_1 on the enzyme. Regardless of the precise structural basis of the interactions of these inhibitors with HLE, the present data show that the extended substrate-binding domain of this proteinase has the ability to bind hydrophobic ligands with very different structures. The functional significance of the ability of HLE to accommodate a diversity of hydrophobic structures is uncertain, but it is well known that the enzyme is able to hydrolyse a broad spectrum of proteins, from the major components of the extracellular matrix to many plasma proteins (Bieth, 1989). To bind and degrade various protein substrates, the extended substrate-binding domain should be able to accommodate molecules with different conformations. It seems reasonable to conclude that there is a common structural basis for the broad substrate specificity of HLE and its ability to bind a wide range of hydrophobic ligands which are not substrates, but which do affect substrate binding or catalysis. We implicate Arg-217 as playing a critical role in contributing to the free energy of binding of the negatively charged inhibitors to HLE, but the relative contributions of binding of such inhibitors to the S_3 subsite and their electrostatic interactions with Arg-217 to the detailed molecular mechanism for inhibition remain to be elucidated.

Ursolic acid is widely distributed in the plant kingdom (Simonsen & Ross, 1957). The compound is present in great abundance in the wax-like coatings of apples, pears and other fruits (Sando, 1923; Markley *et al.*, 1935; Markley & Sando, 1937; Croteau & Fagerson, 1971). From the data of Fernandes *et al.* (1964) we calculate that one mature Bramley apple, for example, may contain over 50 mg of ursolic acid in its peel. Ursolic acid has also been isolated from many medicinal plants, and has a variety of pharmacological activities attributed to it, including anti-arthritis, anti-ulcer and anti-inflammatory activities (Iwu & Ohiri 1980; Gupta *et al.*, 1981; Kosuge *et al.*, 1985; Hirota *et al.*, 1990). The reported anti-inflammatory activities of this compound prompted us to investigate its potential as an inhibitor of HLE. The present data demonstrate that the compound is an effective agent in protecting several synthetic peptide substrates and insoluble elastin against hydrolysis by HLE. Ursolic acid is even effective as an inhibitor of HLE-mediated degradation of ^3H , ^{35}S -labelled extracellular matrix (Q.-L. Ying, A. R. Rinehart, S. R. Simon & J. C. Cheronis, unpublished work). However, these '*in vitro*' results do not necessarily confirm that the pharmacological effects observed *in vivo* can be attributed to elastase inhibition. In the published studies employing animal models, the triterpene was administered orally (Iwu & Ohiri, 1980; Gupta *et al.*, 1981; Kosuge *et al.*, 1985). Given the low solubility of ursolic acid in aqueous solution and the value of the inhibition constant, this compound may not reach a sufficiently high concentration at an inflammatory focus to inhibit the levels of HLE released by large numbers of

recruited neutrophils (Bieth, 1984). Alternative mechanisms of action may contribute to the observed pharmacological effects. Such alternative mechanisms are even more likely to account for the efficacy of oleanolic acid, hederagenin and 18 β -glycyrrhetic acid, which have also been reported to be effective anti-inflammatory agents (Finney & Somers, 1958; Gupta *et al.*, 1969; Bhargava *et al.*, 1970; Chaturvedi *et al.*, 1976; Takagi *et al.*, 1980). However, as a class of readily available natural products of limited toxicity, pentacyclic triterpenes can serve as parent compounds for a new class of HLE inhibitors with greater potency.

This work was supported by grants from the U.S. Public Health Service (HL-14262), New York State Office of Science and Technology (Biotechnology Center, State University of New York at Stony Brook) and Cortech, Inc. to S.R.S.

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Received 29 October 1990/21 January 1991; accepted 30 January 1991