Purification and characterization of a heat-stable esterase from the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*

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A heat-stable esterase has been purified 1080-fold to electrophoretic homogeneity from *Sulfolobus acidocaldarius*, a thermoacidophilic archaebacterium; 20% of the starting activity is recovered. The purified enzyme shows a specific activity of 158 units/mg, based on the hydrolysis of *p*-nitrophenyl acetate. The esterase hydrolyses short-chain *p*-nitrophenyl esters, aliphatic esters and triacylglycerols. It is strongly inhibited by paraoxon and phenylmethanesulphonyl fluoride, but only weakly by eserine. From sedimentation-equilibrium data and molecular sieving in polyacrylamide gels, the M_r of the esterase is estimated to be 117 000–128 000. SDS/polyacrylamide-gel electrophoresis reveals a single band of protein, of M_r 32 000. The purified esterase crystallizes in the presence of poly(ethylene glycol) in short rods. The enzyme is inactivated only on prolonged storage at temperatures above 90 °C.

INTRODUCTION

Esterases are widely distributed enzymes. Several esterases from mammalian sources have been purified and studied in detail (Krisch, 1971). Ester-cleaving enzymes from other eukaryotic sources and from eubacteria have also been described, including the esterases from *Aspergillus* (Iwai *et al.*, 1983) and bacteria such as *Escherichia coli* (Pacaud, 1982), *Pseudomonas* species (Shum & Markovetz, 1974*a,b*; Ohkawa *et al.*, 1979), *Mycobacterium* (Akao *et al.*, 1981) and members of the family of Bacillaceae (Higerd & Spizizen, 1973; Matsunaga *et al.*, 1974). We found an esterase activity in *Sulfolobus acidocaldarius*, a thermoacidophilic archaebacterium, which grows at 70 °C and pH 2. Archaebacteria represent a third form of life, in addition to the well-known eubacteria and eukaryotes (Woese, 1982). So far no esterases have been described from archaebacteria. In the present paper we report on the purification and some properties of this remarkably heat-stable enzyme.

EXPERIMENTAL

Materials

Sulfolobus acidocaldarius (DSM 639) was grown in 50-litre batches as described previously (Grossebüter & Görisch, 1985). Isoelectric-focusing and SDS/polyacrylamide-gel-electrophoresis calibration kits, Q-Sepharose, benzamidine-Sepharose 6B and Mono Q were purchased from Pharmacia (Uppsala, Sweden). *p*-Nitrophenyl esters, α -naphthyl acetate, Fast Blue BB, eserine (physostigmine), paraoxon (diethyl *p*-nitrophenyl phosphate), 5,5'-dithiobis-(2-nitrobenzoic acid), *p*-hydroxymercuribenzoate, ovalbumin and Tris were from Sigma (Taufkirchen, Germany), and phenylmethanesulphonyl fluoride and bovine serum albumin were from Calbiochem (Frankfurt, Germany). Poly(ethylene glycol) (PEG) 6000 and 20 000, Coomassie Brilliant Blue R-250 and triacylglycerols were purchased from Serva (Heidelberg, Germany). Ultrafiltration membranes (Diaflo, YM 10) and Centricon RM 10 were obtained from Amicon (Witten, Germany). Yeast alcohol de-

hydrogenase was from Boehringer (Mannheim, Germany). Hydroxyapatite was prepared as described by Atkinson *et al.* (1973). All other organic and inorganic chemicals were of analytical reagent grade.

Enzyme assay

In the standard test, esterase activity was measured with *p*-nitrophenyl acetate (0.9 mM) as substrate in 50 mM-Tris/HCl, pH 8.0 at 25 °C, as described by Heymann & Mentlein (1981). The reaction was started by the addition of enzyme, and the release of *p*-nitrophenol was monitored at 405 nm continuously in a Zeiss model PMQ III spectrophotometer equipped with a recorder. A molar absorption coefficient of 16 400 M⁻¹·cm⁻¹ was used (Heymann & Mentlein, 1981). In the range of enzyme concentrations used (10–120 munits), the reaction rate is a linear function of the protein concentration. One unit of esterase is defined as that amount releasing 1 μ mol of *p*-nitrophenol/min from *p*-nitrophenyl acetate at 25 °C.

The influence of the chain length of the acyl group of *p*-nitrophenyl esters on enzyme activity was determined by the same method, but the buffer contained 4.5% (v/v) acetonitrile and 0.85% (v/v) Triton X-100. Initial velocities were determined as a function of substrate concentrations, and Michaelis constants and maximal velocities were obtained by plotting the data as described by Hanes (1932). The activity of esterase as a function of pH was tested in the standard assay mixture, but with Tris/HCl buffer replaced by a buffer containing 25 mM-acetate, phosphate and borate (Britton & Robinson, 1931) with pH values between pH 4.0 and 10.5. The rate of hydrolysis was measured at 348 nm, the isosbestic wavelength of *p*-nitrophenol and its anion, *p*-nitrophenolate. A molar absorption coefficient of 5150 M⁻¹·cm⁻¹ was used (Lombardo & Guy, 1981).

Determination of hydrolysis with an autoburette

Hydrolysis of aliphatic esters and triacylglycerols was determined with an autoburette (pH-stat system; Metrohm, Herisau, Switzerland) at 25 °C. The reaction mixture (15 ml) contained 0.3 ml of substrate in 50 mM-

Tris/HCl (pH 8.0)/0.9% Triton X-100. The reaction was started by the addition of a suitable amount of partially purified enzyme (3–40 units), and the released acid was continuously titrated to pH 8.0 with 10 mM-NaOH. One unit of enzyme corresponds to the release of 1 μ mol of acid/min under the assay conditions.

Activity staining

Esterase-active bands after polyacrylamide-gel electrophoresis were detected by a modification of the staining technique described by Urakami & Komagata (1981). The gel was soaked in 100 ml of 50 mM-Tris/HCl, pH 8.0, to which was added 4.6 mg of α -naphthyl acetate dissolved in 0.5 ml of ethoxyethanol. The esterase-active bands were developed by the dropwise addition of 5 ml of Fast Blue BB solution (2 mg/ml of water).

Protein determination

Protein was estimated by the method of Groves *et al.* (1968); 224 nm and 235 nm were used as isoabsorbance wavelengths, with bovine serum albumin as standard.

Gel electrophoresis

Analytical polyacrylamide-gel electrophoresis under non-denaturing conditions was performed at pH 8.9 in a horizontal 7.5%-polyacrylamide gel with a LKB 2117 Multiphor apparatus, as described by Fehrström & Moberg (1977). SDS/polyacrylamide-gel electrophoresis was performed as described by Weber & Osborn (1969). For the determination of the subunit M_r of esterase, 7.5%- and 10%-acrylamide gels were used. A low- M_r kit (Pharmacia) was used as standard. After electrophoresis, protein was stained with Coomassie Brilliant Blue R-250.

Determination of the pI

Analytical isoelectric focusing was carried out in thin-layer polyacrylamide gels with Ampholines, pH range 3.5–9.5. A Pharmacia isoelectric-focusing calibration kit was used as standard.

Molecular sieving

The molecular sieving effect in polyacrylamide gels was used for M_r determinations as described by Hedrick & Smith (1968). Gels were prepared with acrylamide concentrations of 5.0, 6.25, 7.5, 10.0 and 12.5%. The gels were stained for protein and esterase activity as described above. A plot of log mobility versus percentage acrylamide concentration in the gel gave a negative slope for each marker protein used. A calibration curve was established by plotting the negative-slope values against M_r . The protein standards used were ovalbumin (M_r 43000), bovine serum albumin (M_r 67000) and yeast alcohol dehydrogenase (M_r 150000).

Centrifugation procedures

Sedimentation experiments were performed in a Beckman-Spinco model E analytical ultracentrifuge equipped with a photoelectrical scanner. Before centrifugation, the protein was dialysed against 5 litres of the appropriate buffer for 24 h. The dialysis buffer was used as reference solution in the ultracentrifugation run. Boundary-sedimentation studies were performed in 50 mM-potassium phosphate buffer, pH 7.0, at 20 °C with a 12 mm double-sector charcoal-filled Epon centrepiece at 261 000 *g*, with a protein concentration of 0.5 mg/ml.

Sedimentation-equilibrium studies by the meniscus-depletion method were performed in 5 mM-potassium phosphate buffer, pH 7.0, containing 0.17 mg of protein/ml at 16.6 °C in a six-channel charcoal-filled Epon centrepiece at 23 500 *g* for 48 h. The protein distribution was recorded at 280 nm. The M_r was calculated as described by Yphantis (1964), assuming a partial specific volume $\bar{v} = 0.732$ cm³/g.

Active-enzyme centrifugation was performed at 20 °C with a charcoal-filled Vinograd-type double-sector centrepiece at 261 000 *g* as described by Cohen & Mire (1971). The substrate was dissolved in 50 mM-potassium phosphate buffer, pH 7.0, whereas the enzyme (1.9 munits) was in 10 μ l of 5 mM-potassium phosphate buffer, pH 7.0. The formation of *p*-nitrophenol was observed at 405 nm.

Crystallization of esterase

Crystallization was achieved by the hanging-drop method (McPherson, 1976) in 20 mM-Tris/HCl, pH 8.0. Samples (10 μ l) containing 3.6 mg of esterase/ml and 15 or 17.5% (w/v) PEG 6000 or PEG 20000 were vapour-equilibrated against the same buffer containing 30 or 35% PEG 6000 or PEG 20000 respectively.

Inhibitor studies

Irreversible inhibition by paraoxon, eserine and phenylmethanesulphonyl fluoride was studied by incubating 4.7 units of the esterase preparation/ml with various inhibitor concentrations for 10 min at 25 °C. The residual activity was determined by the standard assay.

Effect of temperature on stability

Thermal stability of the enzyme was tested by incubation of 3.9 units/ml in 20 mM-Tris/HCl, pH 7.5, containing 0.1% Triton X-100 at different temperatures for 1 h in sealed vessels. After chilling the samples in ice, the enzyme activity was determined immediately by the standard assay.

Enzyme purification

I: Crude extract. Wet frozen cells (240 g) were thawed at room temperature in 240 ml of 0.1 M-Tris/HCl, pH 7.5. All subsequent steps were carried out at 4 °C. Cells were disrupted by passing the suspension through a French pressure cell at a pressure of 19 000 lb/in² (131 MPa). Cell debris was removed by centrifugation at 48 400 *g* for 1 h. The resulting supernatant was dialysed overnight against 10 litres of 20 mM-Tris/HCl, pH 7.5, and was termed 'crude extract'.

II: Q-Sepharose chromatography. The crude extract was applied to a Q-Sepharose column (8 cm $\times$ 13 cm) equilibrated with 20 mM-Tris/HCl, pH 7.5. The enzyme was eluted with a linear gradient of 0–1 M-NaCl in 5200 ml of 20 mM-Tris/HCl, pH 7.5. The flow rate was maintained at 300 ml/h, and 19 ml fractions were collected. To the active fractions (72–115) eluted from the Q-Sepharose column, solid (NH₄)₂SO₄ was added with constant stirring to give a final saturation of 60%. After centrifugation at 30 000 *g* for 30 min, the resulting precipitate was dissolved in 50 ml of 20 mM-potassium phosphate buffer, pH 6.8, and dialysed overnight against 2 $\times$ 5 litres of the same buffer.

Table 1. Purification of esterase from *S. acidocaldarius*

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification (fold)	Yield (%)
Crude extract	24820	3670	0.147	1	100
Q-Sepharose	3253	2814	0.86	5.9	76
Hydroxyapatite	637	2653	4.1	28.9	72
Benzamidine-Sepharose 6B	12.9	1730	134.1	912	47
Mono Q	4.6	726	158	1080	20

III: Hydroxyapatite chromatography. The dialysed enzyme solution was loaded on a hydroxyapatite column (5 cm × 14 cm) equilibrated with 20 mM-potassium phosphate buffer, pH 6.8. Esterase was eluted with a linear gradient of 0.02–0.5 M-potassium phosphate buffer, pH 6.8, in a total volume of 2000 ml at a flow rate of 140 ml/h; 10.6 ml fractions were collected.

IV: Benzamidine-Sepharose chromatography. To the combined fractions 47–80 from the hydroxyapatite column, solid $(\text{NH}_4)_2\text{SO}_4$ was added with constant stirring to give 20% saturation. This solution was applied to a benzamidine-Sepharose 6B column (2.5 cm × 4 cm) equilibrated with 20 mM-potassium phosphate buffer, pH 6.8, which contained 20%-satd. $(\text{NH}_4)_2\text{SO}_4$. The column was washed with the same buffer until no protein could be detected in the eluate as judged from the A_{224} . Esterase was eluted from the column with 20 mM-potassium phosphate buffer, pH 6.8, at a flow rate of 60 ml/h; 10 ml fractions were collected. The active fractions were pooled (230 ml) and concentrated to 10 ml with an Amicon Diaflo ultrafiltration apparatus with a YM 10 membrane. The final concentration to 1.4 ml was performed with a Centricon TM 10 apparatus. The enzyme solution was dialysed overnight against 5 litres of 20 mM-Tris/HCl, pH 8.0.

V: Mono Q chromatography. The last purification step was performed at room temperature in a fast protein liquid chromatography system (Pharmacia). In two separate runs, 0.7 ml of the enzyme solution (containing 6.45 mg of protein) was applied to a Mono Q column (HR 5/5) equilibrated with 20 mM-Tris/HCl, pH 8.0; 1 ml fractions were collected, and the esterase was eluted with a linear gradient of 0–100 mM-NaCl in 40 ml of 20 mM-Tris/HCl, pH 8.0, at a flow rate of 1 ml/min. The enzyme was eluted in 7 ml at a NaCl concentration of about 70 mM.

RESULTS

Purification

Table 1 shows the results of the purification procedure of esterase from *S. acidocaldarius*. The enzyme is purified 1080-fold, and 20% of the starting activity is recovered. The purified enzyme shows a specific activity of 158 units/mg of protein when catalysing the hydrolysis of *p*-nitrophenyl acetate. On the basis of four catalytically active subunits, a catalytic-centre activity of 85 is calculated.

Fig. 1. Polyacrylamide-gel electrophoresis of purified esterase from *S. acidocaldarius* under non-denaturing conditions

Polyacrylamide gels (7.5%) were prepared as outlined by Fehrström & Moberg (1977). Each lane contained 30 μg of protein. The left lane was stained for protein with Coomassie Blue, and the right lane was stained for activity as described in the Experimental section. The electrophoresis was performed from bottom to top.

Homogeneity

The purified enzyme shows a single protein band on polyacrylamide-gel electrophoresis under both non-denaturing and denaturing conditions. When stained for esterase activity, the band of the native enzyme coincides with the protein band. Two other very faint esterase-active bands are observed, but these bands cannot be detected when stained for protein (Fig. 1). By the method of Hedrick & Smith (1968), the additional bands are found to show M_r values which are multiples of the M_r of the native esterase. In boundary-sedimentation experiments in the analytical ultracentrifuge, the enzyme moves as a homogeneous protein, with a sedimentation coefficient, $s_{20, w}$, of 6.4 S.

M_r and subunit structure

From SDS/polyacrylamide-gel electrophoresis, the subunit M_r was determined to be 32000 (Fig. 2). The M_r of the native esterase was determined by meniscus-depletion sedimentation equilibrium in the analytical ultracentrifuge, and was found to be 117000. By the

Fig. 2. SDS/polyacrylamide-gel electrophoresis

Left lane, homogeneous preparation of 6 μ g of esterase. Right lane, M_r marker proteins. A calibration curve for M_r determination was obtained with phosphorylase *b* (M_r , 94 000), bovine serum albumin (M_r , 67 000), ovalbumin (M_r , 43 000), carbonic anhydrase (M_r , 30 000), soya-bean trypsin inhibitor (M_r , 20 100) and lactalbumin (M_r , 14 400). The electrophoresis was performed from bottom to top.

method of Hedrick & Smith (1968) an M_r of 128 000 was found for the native enzyme. The esterase therefore is a tetramer with an M_r of 117 000–128 000, and consists of four subunits of identical size. In active-enzyme sedimentation experiments at very low protein concentrations and in the presence of substrate, conditions that correspond to those used in steady-state experiments, the esterase from *S. acidocaldarius* moves as a single species with a sedimentation coefficient, $s_{20,w}$, of 6.4. Under the conditions used, a more slowly moving species would have been detected if its activity amounted to more than 5 or 10 % of the total activity of the sample. Thus the catalytically active species of the esterase is the tetramer, of M_r 128 000.

Isoelectric point

Analytical isoelectric focusing reveals a single protein band; the pI was determined to be 4.9.

Crystals of esterase

Crystals of esterase were obtained by vapour diffusion in hanging drops as described in the Experimental section. The enzyme crystallizes in rods of length

Fig. 3. Crystals of purified esterase from *S. acidocaldarius*

The enzyme was crystallized by vapour equilibration in hanging drops. Left, crystals obtained from 30 % (w/v) PEG 6000; right, a crystal obtained from 35 % (w/v) PEG 20000. The bar represents 0.1 mm.

0.05–0.45 mm (Fig. 3). A droplet containing crystals was layered on 2 ml of 20 mM-Tris/HCl, pH 8.0, with 60 % (w/v) PEG 6000 and centrifuged until pellet formation was observed. The pellet of crystals was redissolved in 20 mM-Tris/HCl, pH 8.0. The redissolved crystals were enzymically active.

Acyl-chain specificity

The enzymic activity with *p*-nitrophenyl esters is strongly influenced by the chain length of the acyl group. As shown in Table 2, the rate of hydrolysis increases as the chain length increases to C₅, and decreases as the chain length increases further. The highest rate of hydrolysis was observed with *p*-nitrophenyl valerate, whereas of all esters tested *p*-nitrophenyl laurate shows the lowest rate. In the buffer system used to solubilize the substrates, the specific activity of the esterase is decreased by acetonitrile to 126 units/mg for *p*-nitrophenyl acetate.

Hydrolysis of aliphatic esters and triacylglycerols

The activity of the esterase towards various esters and triacylglycerols was determined with an autoburette. As shown in Table 3, the activity of the esterase towards aliphatic esters is influenced by the chain length of the

Table 2. *p*-Nitrophenyl esters: influence of the chain length of the acyl group on the esterase activity

Enzyme activity was determined at 25 °C in 50 mM-Tris/HCl, pH 8.0, containing 4.5 % (v/v) acetonitrile and 0.85 % (v/v) Triton X-100. Initial-velocity data were analysed as described by Hanes (1932). Relative activity was determined at 0.9 mM substrate.

Carbon chain length of the acyl group...	2	3	4	5	6	8	10	12
Relative activity (%)	8.8	18	83	100	75.4	11.5	1.8	0.8
K_m (μ M)	21.5	33.8	128.7	151.7	103.6	60.6	—	—
Relative V_{max} (%)*	6.4	14.6	67.8	100	58.6	7.3	—	—

* 100 % = 1.97 μ mol/min per μ g.

Table 3. Hydrolysis of aliphatic esters and triacylglycerols

Hydrolysis was determined with an autoburette at 25 °C. The reaction mixture contained 0.3 ml of substrate in 15 ml of 50 mM-Tris/HCl (pH 8.0)/0.9% Triton X-100. The reaction was started by the addition of 3–40 units of partially purified esterase.

Substrate	Activity ($\mu\text{mol}/\text{min}$ per unit)
Pentyl valerate	2.36
Ethyl valerate	0.17
Hexyl acetate	0.27
Pentyl acetate	0.21
Ethyl acetate	0
Triacetin	2.84
Tributylin	3.83
Triolein	0

Table 4. Effect of inhibitors on esterase activity

Enzyme (4.7 units/ml) was incubated with various concentrations of inhibitor for 10 min at 25 °C. The residual activity was determined by the standard assay

Inhibitor	Concn. (mM)	Residual activity (%)
Eserine	10	81
	1	94
	0.1	100
Phenylmethanesulphonyl fluoride	0.1	0
	0.01	55
Paraoxon	0.2	0
	0.01	0
<i>p</i> -Hydroxymercuribenzoate	10	100
Dithiobis-(2-nitrobenzoate)	0.5	100
EDTA	10	95
	1	100
NaF	100	100

acyl group as well as the chain length of the alkyl group. The esterase showed a higher rate of hydrolysis with pentyl valerate than with pentyl acetate or with ethyl valerate. Of the triacylglycerols tested, short-chain acyl esters were readily hydrolysed, whereas triolein was not hydrolysed. These findings are in agreement with the results obtained from the hydrolysis of different *p*-nitrophenyl esters, where negligible hydrolysis occurs with long-chain acyl esters of *p*-nitrophenol. Water-insoluble substrates (e.g. pentyl valerate) are not hydrolysed in the absence of Triton X-100.

Inhibitors

The effects of paraoxon and other inhibitors on the esterase from *S. acidocaldarius* were studied. As shown in Table 4, the enzyme is strongly inhibited by paraoxon and phenylmethanesulphonyl fluoride. Esterase inactivated totally by paraoxon was dialysed against 2 × 2 litres of 0.1 M-Tris/HCl, pH 8, for 2 days. Only 0.3% of the original esterase activity could be detected. Eserine is

Fig. 4. Enzyme stability as a function of temperature

Purified esterase (3.9 units/ml) was incubated in 20 mM-Tris/HCl (pH 7.5)/0.1% Triton X-100 at the temperatures indicated. After 1 h the samples were chilled in ice and assayed for enzyme activity.

also inhibitory to the esterase, but to a lesser extent and only in high concentrations. Thiol-group reagents and EDTA do not inhibit the enzyme.

Effect of pH on enzyme activity

The enzyme activity was determined at different pH values as described in the Experimental section. The esterase shows a broad optimum between pH 7.5 and 8.5.

Effect of temperature on enzyme stability

Esterase from *S. acidocaldarius* is not inactivated by heat treatment for 1 h at temperatures up to 80 °C. At 90 °C only 8% of activity is lost. Even after 1 h at 100 °C, 50% of the enzyme activity is recovered (Fig. 4).

DISCUSSION

An esterase from the thermoacidophilic archaebacterium *S. acidocaldarius* has been purified 1080-fold to electrophoretic homogeneity. For hydrolysis of *p*-nitrophenyl acetate the enzyme shows a catalytic-centre activity of 85. Analytical polyacrylamide-gel electrophoresis under non-denaturing conditions reveals a single band when stained for protein. However, when stained for esterase activity some weak bands are visible, in addition to the one strong band coinciding with the band obtained from protein staining. The number of

additional bands differs with the gel concentration. We detected four, three and two active bands respectively in 5, 7.5 and 12.5%-acrylamide gels. These additional bands were found to show M_r values which are multiples of that of the native esterase. Therefore it is assumed that partial aggregation of the enzyme occurs under the conditions used.

The archaeobacterial esterase shows an unspecific substrate spectrum, hydrolysing triacylglycerols, aliphatic and aromatic esters; however, only short-acyl-chain esters are hydrolysed. Classification of esterases is based on substrate specificity and sensitivity towards various inhibitors (Aldridge, 1953; Augustinsson, 1961). Aryl-esterases or A-esterases are not inhibited by organophosphates, such as paraoxon, and hydrolyse aromatic esters. Carboxylesterases or B-esterases (serine esterases) are inhibited by organophosphates and have a wide substrate specificity, hydrolysing aliphatic and aromatic esters. C-esterases are inhibited by organophosphates and eserine, and hydrolyse choline esters. However, esterases have been shown to exhibit overlapping substrate specificities (Augustinsson, 1961), leading to problems of identification and classification (Walker & Mackness, 1983). The esterase from *S. acidocaldarius* is strongly inhibited by phenylmethanesulphonyl fluoride and paraoxon. The weak inhibition by eserine at high concentration is not a significant effect. EDTA and thiol-group reagents do not inhibit the esterase from *S. acidocaldarius*. On the basis of this inhibition pattern, the enzyme from *S. acidocaldarius* is classified as a serine esterase. This classification is supported by the wide substrate specificity of the enzyme, which is typical for a serine esterase.

The native archaeobacterial esterase was found to be a tetramer, with subunits of M_r 32000. The catalytically active species of the esterase is the tetrameric form.

A number of serine esterases have been purified and characterized from a variety of eukaryotic and eubacterial sources. The serine esterases from mammalian liver show an M_r of about 160000 and consist of two identical subunits (Krisch, 1971). *Aspergillus niger* produces four kinds of serine esterases, with M_r values ranging from 23000 to 127000 (Iwai *et al.*, 1983). Multiple forms of esterases were also found in eubacteria. *Butyrivibrio fibrisolvens* possesses six different esterases (Lanz & Williams, 1973). One of the enzymes was further characterized as a serine esterase, with an M_r of 66000. Two monomeric isoenzymes of M_r 34000 were isolated and characterized from *Pseudomonas cepacia* (Shum & Markovetz, 1974a,b). A monomeric serine esterase of M_r 42000–47000 is present in *Bacillus stearothermophilus* (Matsunaga *et al.*, 1974), whereas two enzymes were separated in *Bacillus subtilis*. One has an M_r of 51000, whereas the other has an M_r of 160000 and a subunit M_r of 31000. The serine esterase from *S. acidocaldarius* differs in M_r from the liver esterases and most of the esterases of eubacteria. With respect to M_r , it seems to be similar to the larger esterases from *B. subtilis*, which possesses a subunit M_r of 31000. It is noteworthy that similarities have been observed for other enzymes of *Sulfolobus* and *Bacillus* species. Malate dehydrogenase

and citrate synthase of *S. acidocaldarius* represent the type of enzymes which occur in *Bacillus* (Hartl *et al.*, 1987; Grossebüter & Görisch, 1985), and glucose dehydrogenase from *S. solfataricus* resembles also the enzyme produced by *Bacillus* (Giardina *et al.*, 1986).

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