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Characterization of an L-serine dehydratase activity in permeabilized cells of *Brevibacterium linens* ATCC 9175

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SUMMARY

Brevibacterium linens *ATCC 9175 produces large quantities of ammonia and pyruvate from L-serine. This reaction occurs via an L-serine dehydratase (EC.4.2.1.13), whose activity is maximal at the end of exponential growth on rich medium and which abruptly decreases at the beginning of the stationary phase. It is not possible to extract the soluble form of the enzyme without a total loss of activity. Subcellular localization studies after creating stable protoplasts with lysozyme have shown that a part of the activity is bound to cell membranes. A stable form of activity has been obtained by treating the cells with Triton X-100 and the main properties of the enzyme have been studied with this insoluble form. The apparent Michaelis constant is 50 mM and the Vmax is 200 nmol of pyruvate formed per minute and per mg of dry extract. The optimum pH is included between 8.5 and 9.4 and activity is very stable between 8.0 and 9.4. The enzyme is slightly thermostable. The exclusive substrate is L-serine. D-serine and 2-aminoethanol are competitive inhibitors. L-serine dehydratase activity is strongly inhibited by o-phenanthroline. Pyridoxal phosphate is required for maximum activity.*

Key words : *Brevibacterium linens* - L-serine dehydratase - Serine catabolism.

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RESUME

CARACTERISATION D'UNE ACTIVITE L-SERINE DESHYDRATASE DANS DES CELLULES PERMEABILISEES DE BREVIBACTERIUM LINENS ATCC 9175

Brevibacterium linens ATCC 9175 produit de grandes quantités d'ammoniac et de pyruvate à partir de la L- sérine. Cette réaction est due à une enzyme de type L- sérine déshydratase (E.C. 4.2.1.13) dont l'activité est maximum en fin de croissance exponentielle dans un milieu complexe. Elle décroît ensuite rapidement dès le début de la phase stationnaire de croissance. Il n'a pas été possible d'extraire l'enzyme sous une forme soluble sans avoir une perte totale de son activité. Des essais de localisation cellulaire de l'activité ont été tentés après obtention par action du lysozyme de protoplastes stables. Il est montré qu'une partie de l'activité est liée à la fraction membranaire de la cellule. Par contre, une forme stable de l'activité enzymatique était obtenue par traitement des cellules au Triton X 100. Les principales propriétés de cette enzyme ont donc pu être étudiées sous cette forme insoluble.

La constante apparente de Michaelis est de 50 mM et la vitesse maximum apparente de 200 nmoles de pyruvate formé par minute et par mg d'extrait sec cellulaire. Le pH optimum est compris entre 8,5 et 9,4. L'activité est très stable entre 8,0 et 9,4. La température optimale d'action est comprise entre 26 et 35° C. Les cinétiques d'inactivation thermique montrent que l'enzyme est stable jusqu'à 30° C mais qu'elle s'inactive rapidement à 40° C. L'enzyme est très spécifique puisque seule la L- sérine est substrat. La D- sérine et le 2- aminoéthanol sont des inhibiteurs compétitifs. D'autre part, cette activité L- sérine déshydratase est fortement inhibée par l'ortho- phénanthroline. L'E.D.T.A. entraîne au contraire un effet stimulant de l'activité aux concentrations inférieures à 10^{-3} M, puis devient inhibiteur aux concentrations supérieures. Le pyridoxal phosphate est exigé pour obtenir une activité maximum.

Mots clés : *Brevibacterium linens* - L-sérine déshydratase - Catabolisme de la sérine.

I. INTRODUCTION

Among the microorganisms which play a role in cheese ripening, the coryneform bacteria and especially *Brevibacterium linens* are involved (Boyaval and Desmazeaud, 1983). Their quantitative importance has in fact been shown on the surface of a large number of cheeses including Camembert (Richard and Zadi, 1983), Livarot, Munster, Maroilles, Gruyere, Trappist, etc. (Ades and Cone, 1969; El Erian, 1972; Veisseyre, 1975; Accolas *et al.*, 1978; Brandl, 1978). The

general metabolism of *B. linens*, however, remains poorly understood. Although these proteolytic and lipolytic activities have been studied in the past (Thomasow, 1950; Friedman *et al.*, 1953; Hosono, 1968; Sorhaug and Ordal, 1974; Foissy, 1978 a, b, c), the catabolism of amino acids in this species and its effect on the appearance of flavor constituents is only beginning to be understood. It has been shown that serine is deaminated to a considerable extent by different strains of *B. linens* (Hemme *et al.*, 1982). Ammonia, which is present in all ripened rinds but at varying concentrations is, according to Dumont and Adda (1978) the only volatile amine to play a genuine role in cheese flavoring.

Prior work (Hamouy, 1983) has shown that L-serine is catabolized by *B. linens* ATCC 9175 by an L-serine dehydratase (EC.4.2.1.13). This enzyme degrades L-serine to ammonia and pyruvate and thus plays a key role in serine and glycine metabolism (Morris, 1963). L-serine dehydratase and L-threonine dehydratase had long been considered to be the same enzyme. However, since the work of Benziman *et al.* (1960) and Carter and Sagers (1972), who proved the existence of an L-serine dehydratase specific for L-serine in *Clostridium acidiurici*, similar activities have been reported in extracts of other microorganisms, such as *Arthrobacter globiformis* (Bridgeland and Jones, 1965; Gannon *et al.*, 1977), *Escherichia coli* (Alföldi *et al.*, 1968), *E. coli* K12 (Isenberg and Newman, 1974), *Bacillus cereus*, *Salmonella typhimurium* (Rasko *et al.*, 1969), *Pseudomonas cepacia* (Wong and Lessie, 1979), *Bacillus alvei* (Griffiths and De Moss, 1970), and *Corynebacterium spp.* (Morikawa *et al.*, 1974). In *Brevibacterium linens*, on the other hand, the existence of this enzyme has not yet been demonstrated. The present report described the general properties of the L-serine dehydratase extracted from *B. linens* ATCC 9175.

II. MATERIALS AND METHODS

A. Organism

The strain utilized was *Brevibacterium linens* ATCC 9175.

B. Cell growth and harvest

The following culture medium was used: 3 g soy peptone (Difco), 7 g bactotryptone (Difco), 5 g yeast extract (Difco), 5 g of 60 % sodium DL-lactate (Sigma, grade IV), 5 g NaCl (Prolabo), 2.5 g K_2HPO_4 (Prolabo), distilled water q.s. 1000 ml. The pH was adjusted to 7.0 before autoclaving. The medium was inoculated at 3 % (v/v) with a preculture containing 10^9 cells/ml. Cells were grown at 26° C with agitation (120 rpm) and were harvested by centrifuging at 5000 *g* at 4° C for 10 minutes. The pellet was washed with a saline solution containing 0,25 % K_2HPO_4 and 0.5 % NaCl, pH 7.0.

C. Enzyme extraction

Permeabilization with Triton X-100

The technique of Miozzari *et al.* (1978) was used. Cells were harvested, washed and then resuspended in 50 mM TRIS-HCl, pH 9, containing 0.05 % (v/v) Triton X-100. The treated suspension contained 13 mg dry extract/ml. After freezing at — 20° C for at least 15 h, the cells were thawed at 26° C. The resulting cell suspension was used directly as the enzyme extract.

Permeabilization with toluene

The techniques used have been described by Gannon *et al.* (1977) and Serrano *et al.* (1973).

Solubilization with sodium deoxycholate (DOC)

The technique used has been described by Regnier and Thang (1979).

Sodium dodecyl sulfate (SDS) solution

Cell extracts were solubilized with SDS at a final concentration of 0.2 % (w/v) for 10 min at 26° C.

Lysis with egg white lysozyme

A suspension at 2.6×10^{12} cells/ml was treated with 40,000 U of lysozyme/ml (Sigma) at a final concentration of 40 µg/ml. Lysis was carried out at 26° C, either in 50 mM TRIS-HCl, pH 9, or in the same buffer containing 2 M (final) sucrose. Protoplasts were recovered from hypertonic medium by centrifuging at 1000 *g* at 4° C for 1 h. An aliquot was then suspended in 50 mM TRIS-HCl, pH 9, in the presence of DNase and RNase (Sigma), each at a final concentration of 40 µg/ml, in order to lyse the protoplasts by osmotic shock.

Lysis with glass beads

Cells were suspended in 50 mM TRIS-HCl, pH 9, containing 5×10^{-5} M pyridoxal phosphate (PLP) at a cell concentration of 13 mg dry extract/ml. They were ground at 4° C with a Vibrogen grinder (Edmund Buhler, Tubingen, G.F.R.).

Ultrasonic lysis

A cell suspension containing 13 mg of dry extract/ml in 50 mM TRIS-HCl, pH9, containing 5×10^{-5} M PLP was chilled and sonicated for a total of 2 minutes. An M.S.U. 157 Ultrasonic (Meaux, France) apparatus was used, operating at 0.7 A for 30 s periods, followed by a 1 min cooling. The sonication tube was immersed in crushed ice during the operation.

Crude extracts (CE) arising from these various operations were separated into a pellet (P) and supernatant (S) by centrifuging at 25,000 *g* for 1 h at 4° C.

D. Enzyme assay

L-serine dehydratase activity was determined by assaying the pyruvate produced, based on the method of Friedemann and Haugen (1943). 100 µl of enzyme extract were added to the following reaction mixture 500 µl of buffer, 100 µl of PLP (5×10^{-4} M), 100 µl of L-serine, 200 µl of distilled water (the nature of the buffer, its pH and the L-serine concentration are defined for each experiment). Incubation was generally carried out at 26° C and the reaction was stopped by adding 500 µl of 10 % (w/v) trichloroacetic acid (TCA). The mixture was held in an ice bath for 30 min and was then centrifuged at 5000*g* for 10 min at 4° C. 500 µl of 0.1 % (w/v) 2-4-dinitrophenylhydrazine (DNPH) in 2 N HCl were then added to 500 µl of supernatant. After 2 min at ambient temperature, 2 ml of 2 N NaOH were added and the «claret» color was determined at 520 nm.

The activity of intact resting cells was determined by using the Nessler reagent to assay the ammonium ions produced. After development of the color at alkaline pH, absorbance changes were measured at 425 nm. A standard range was realized with ammonium sulfate solutions.

The specific activity of L-serine dehydratase is expressed as nmoles of pyruvate formed per minute and per mg of dry extract or per mg of protein. The protein content was determined with the method of Herbert *et al.* (1971) or that of Wang and Smith (1975) (modified Lowry assay for proteins in the presence of Triton X-100). Bovine serum albumin was used as standard.

III. RESULTS

A. Influence of physiological stage of growth

The specific activity of L-serine dehydratase increased during exponential growth reaching a maximum at the end of this phase. It then decreased abruptly at the onset of the stationary phase (fig. 1). In order to verify that this decrease was related uniquely to the physiological state of the cells and not to a decreased permeabilization of coccoid forms by Triton (since the cell wall is different from that of the rod form), a protein assay was performed on cells at 24, 48, 72 and 96 h of growth, after treating the samples with Triton X-100. Total proteins and extracted proteins were then assayed and it was found that the detergent extracted the same quantities of protein from 24 and 72 hour-old cells. The decrease of specific activity is thus

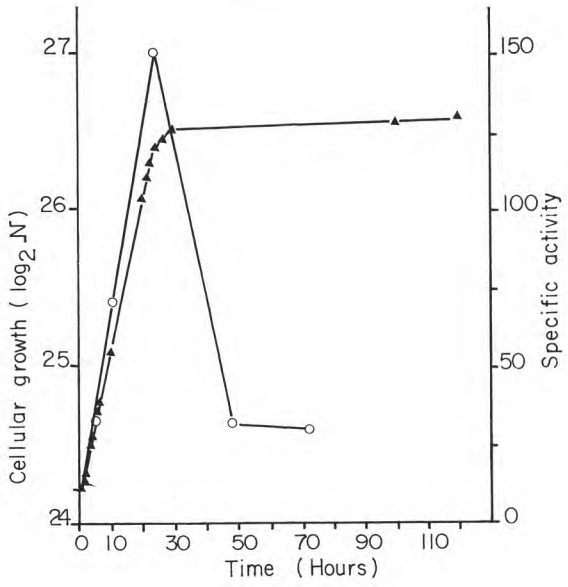

fig. 1

Influence of physiological stage on the specific activity of L-serine dehydratase

▲—▲ : cell growth expressed as $\log_2$ of number of cells/mL.
○—○ : specific of L-serine dehydratase.

Influence de l'âge physiologique des cellules sur l'activité spécifique de la L-sérine déshydratase

▲—▲ : *croissance cellulaire exprimée en $\log_2$ du nombre de cellules/mL.*
○—○ : *activité spécifique de la L-sérine déshydratase.*

not related to a decreased permeabilization by Triton X-100. The remainder of the study was carried out with cells harvested at the end of the exponential phase.

B. Localization of activity

In order to determine if the enzyme is soluble or bound to the cell envelope, assays of L-serine dehydratase activity were performed on crude extracts (CE), supernatants (S) and pellets (P) obtained from suspensions of cells permeabilized with Triton X-100 or with toluene. These assays were also performed with cells lysed with lysozyme, glass beads or by sonication (Table 1). The various supernatants tested were devoid of activity (Table 2) and only certain pellets, membrane fractions and protoplasts were active towards L-serine (Tables 2 and 3). Every time it was attempted to solubilize

TABLE 1 — TABLEAU 1

Extraction yields of cell proteins with different methods of permeabilization or grinding of cells

Rendements d'extraction des protéines cellulaires par différentes méthodes de perméabilisation ou de broyage

	Triton X-100	Sonication	Toluene	Toluene/ ethanol	Glass beads	Lysozyme (hypotonic medium)
Crude extract	507±50(a)	500±50	113±11	508±50	348±35	510±51
Supernatant	167±17	382±38	12±1	100±10	220±22	377±38
Pellet	340±34	117±12	100±10	407±41	126±13	133±13

(a) Results are expressed as μg of proteins (means of 4 experiments).

Note : different values in the crude extract result from the fact that the experiments were performed with different batches of bacterial suspensions.

TABLE 2 — TABLEAU 2

Extraction yields of L-serine dehydratase with the different permeabilization and grinding methods used. The results are the means of 4 experiments.

Rendements d'extraction de l'activité par différentes méthodes de perméabilisation ou de broyage. Les résultats sont les moyennes de 4 expériences.

	Triton X-100			Lysosyme (hypotonic medium)			Glass beads			Sonication			Toluene		
	CE[b]	P	S	CE	P	S	CE	P	S	CE	P	S	CE	P	S
Specific activity[a]	392 ±40	583 ±6	0	5 ±1	8 ±1	0	31 ±30	16 ±2	0	22 ±2	21 ±2	0	0	0	0
Yield : % of total activity	100	100	0	1	1	1	8	2	0	6	1	0	0	0	0

(a) Specific activity expressed as nmol of pyruvate or NH_4^+ produced per min and per mg of protein.

(b) CE = crude extract, P = pellet, S = supernatant.

TABLE 3 — TABLEAU 3

Subcellular localization of L-serine dehydratase activity after lysozyme treatment in hypertonic medium followed by osmotic shock.

Localisation cellulaire de l'activité L-sérine déshydratase après traitement en milieu hypertonique par le lysozyme, suivi d'un choc osmotique.

	Crude extract	Protoplast supernatant	Protoplasts	Membranes	Intracellular medium
Proteins extracted (μg)	510±50	193±20	316±30	83±10	233±20
Specific activity[a]	390±40	0	202±20	120±10	0
Total activity	198±20	0	64±5	10±1	0
Yield of activity %	100	0	32	5	0

[a] Specific activity is expressed as nmol of pyruvate produced per min and per mg protein. The results are the means of 4 experiments.

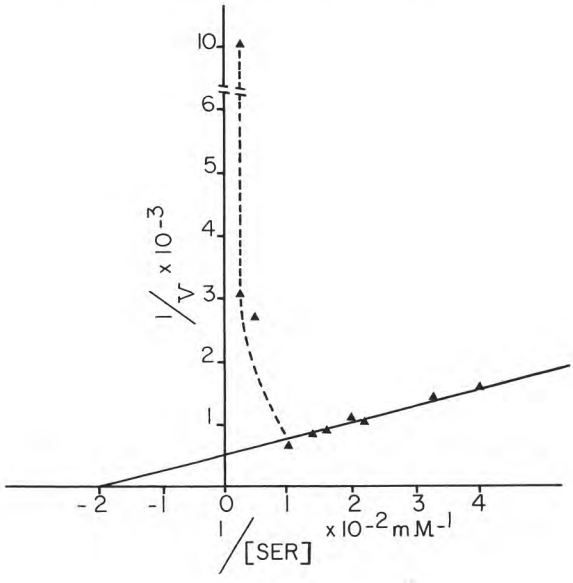

fig. 2

Graphic determination (Lineweaver-Burk) of kinetic constants of L-serine dehydratase activity.

Détermination graphique (selon Lineweaver-Burk) des constantes cinétiques de la L-sérine déshydratase.

the enzyme by the action of DOC or SDS on membrane fractions or cells permeabilized with Triton X-100, there was a total loss of activity. This was true in the absence or presence of protease inhibitors, such as 1 mM para-methylsulfonyl fluoride (PMSF) or parachrloromercuribenzoate (PCMB). It was not possible either to solubilize membranes by increasing the pH, since the enzyme was denatured at alkaline pH values.

C. Stability: effect of freezing

The enzymatic activity of cell suspensions supplemented with 5×10^{-5} M PLP and treated with Triton was not affected by two freeze-thaw cycles, although there was a 40 % loss at the fourth cycle. A single cycle, on the other hand, was sufficient to cause a total loss of activity from protoplasts, membrane extracts, sonicates and glass bead or lysozyme lysates. When 50 % (v/v) glycerol was added to these extracts, residual activity after freezing accounted for one-third of initial activity. Triton-permeabilized cells were thus used for the remainder of the experiments.

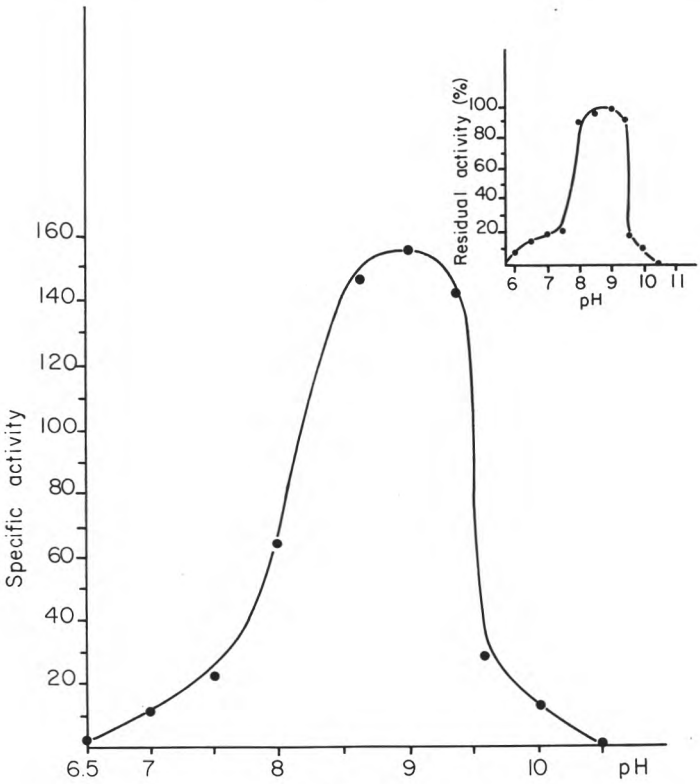

fig. 3

Influence of pH on the stability and activity of L-serine dehydratase.

Influence du pH sur la stabilité et l'activité de la L-sérine déshydratase.

D. Determination of kinetic parameters Km_{app} and Vm_{app}

The quantity of pyruvate produced by the oxidative deamination of L-serine was proportional to cell concentrations used (data not shown). In the presence of 100 mM L-serine and 1.3 mg of dry extract, pyruvate formation was linear for 30 minutes. The value of the apparent Km was high at 50×10^{-3} M and the Vm was 200 nmoles of pyruvate formed per min and per mg dry extract. An inhibition by excess substrate was also observed (fig. 2).

E. Effects of temperature and pH

Pyruvate production was optimal in the pH range of 8.5 to 9.4 with a maximum at 9.0 (fig. 3). The enzyme was also stable between pH 8.0 and 9.4 (inset, fig. 3). L-serine dehydratase activity was optimal

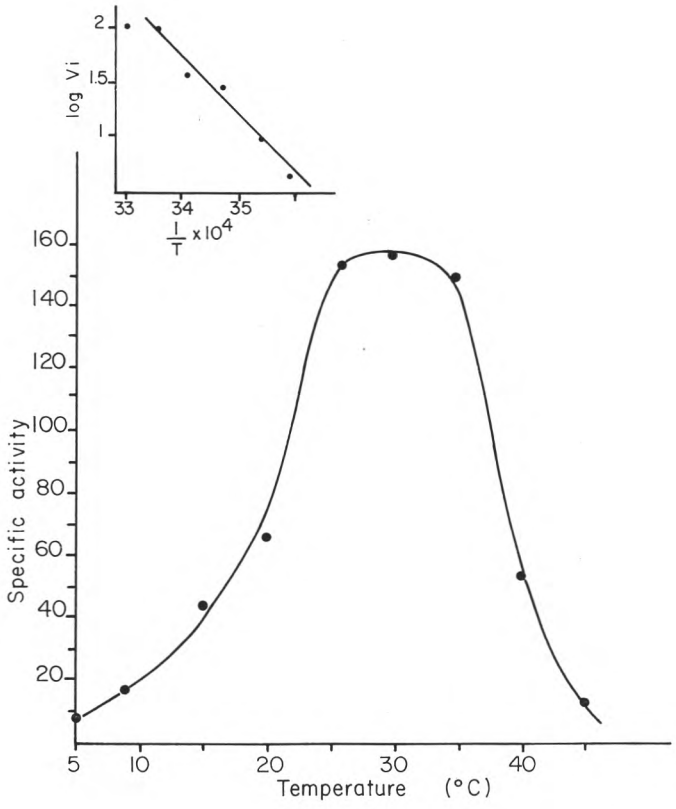

fig. 4

Influence of temperature on the activity of L-serine dehydratase.

Influence de la température sur l'activité de la L-sérine déshydratase.

between 26 and 35° C (fig. 4). Thermal inactivation kinetics showed that the enzyme was stable up to 30° C with a notable loss of activity at 40° C (75 % loss in 15 min (fig. 5). The apparent activation energy of the activation reaction, calculated with an Arrhenius plot, is 99.5 kJoules/mole (inset, fig. 4).

F. Inhibition of activity and specificity of L-serine dehydratase

Action of substrate analogues and small peptides

The only active substrate among those tested was L-serine: there was no activity with L-threonine. D-serine and 2-aminoethanol were competitive inhibitors (curves not shown). L-cysteine was a non-

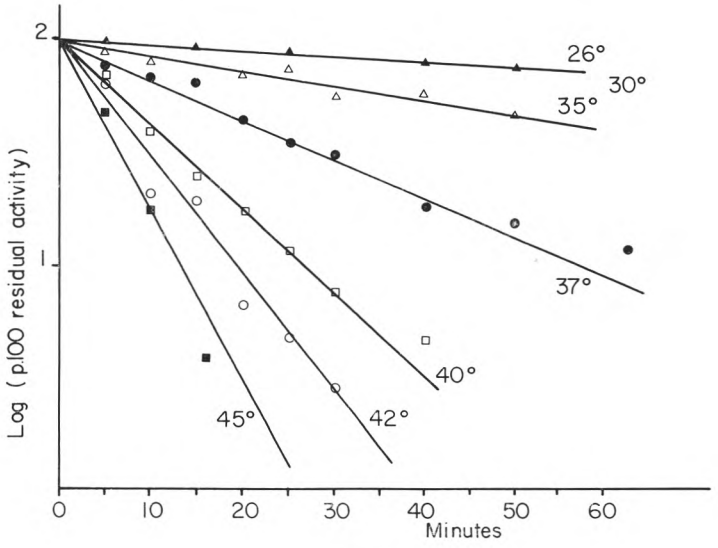

fig. 5

Thermal stability of L-serine dehydratase activity.

Stabilité thermique de la L-sérine déshydratase.

TABLE 4 — TABLEAU 4

Effects of substrate analogues on serine dehydratase activity.

Effets des analogues de substrats sur l'activité L-sérine déshydratase.

D-serine	competitive inhibition
2-aminoethanol	competitive inhibition
L-cysteine	non-competitive inhibition
D-cysteine	non-competitive inhibition
Serine amide	uncompetitive inhibition
Serine-0-sulfate	
DL-isoserine or	
beta-2-thienylserine	inhibition (type not determined)
Ser-Gly, Ser-Met or	
$(Ser)_3$	inhibition (type not determined)
L-threonine or	
gamma-aminobutyrate	no inhibition

TABLE 5 — TABLEAU 5

Influence of various ions on serine dehydratase activity.

Influence de différents ions sur l'activité L-sérine déshydratase.

	Final concentration (mM)	Residual activity (%) in comparison to controls without salt
NaCl or KCl, or $FeCl_2$ or $MgCl_2$	1	100
	10	100
LiCl	1	65
	10	65
$CaCl_2$	1	90
	10	70
$FeCl_3$	1	90
$FeSO_4$	1	80
$ZnCl_2$	1	90
$HgCl_2$	1	10
$MnCl_2$	1	30
NH_4Cl	50	100
	1 + 50	150
KCl + NH_4Cl	1 + 15	115
KCl + NH_4Cl + NaCl	1 + 50 + 15	140

competitive inhibitor and this action could have resulted from chelation by its sulfhydryl group. D-cysteine was thus tested as substrate and inhibitor and no serine dehydratase activity could be demonstrated with this amino acid as substrate. In the presence of both L-serine and D-cysteine, on the other hand, the enzyme was greatly inhibited (Table 4). This would tend to prove that inhibition is independent of cysteine stereochemistry. This amino acid would thus act by chelating an ion required for enzymatic catalysis. These results show that the enzyme is highly specific. The specificity of the enzyme was also shown by the fact that all the serine derivatives tested in the presence of L-serine as substrate caused inhibition, while threonine and gamma-aminobutyrate, structuraly similar, did not inhibit serine dehydratase activity (Table 4).

TABLE 6 — TABLEAU 6

Effects of chelators on serine dehydratase activity.

Effets des chélateurs sur l'activité L-sérine déshydratase.

EDTA		o-phenanthroline	
Final concentration (M)	Residual activity (%)	Final concentration (M)	Residual activity (%)
0	100	0	100
10^{-5}	115	10^{-7}	80
10^{-4}	125	10^{-6}	80
10^{-3}	385	10^{-5}	60
10^{-2}	0	10^{-4}	45
10^{-1}	0	10^{-3}	25

Effects of ions, chelators and coenzyme (PLP)

These results do not take into account the concentrations of inorganic ions in the preparation, which were not determined. Among the ions tested, only $HgCl_2$, $MnCl_2$ and to a lesser extent LiCl decreased activity notably. Ions such as K^+, NH^+_4 and Na^+, combined in pairs or all three together, stimulated activity, although they had no effect individually (Table 5).

Ortho-phenanthroline was a potent inhibitor of L-serine dehydratase (Table 6). In the presence of 1 mM inhibitor, enzyme activity decreased by 75 %. Activity was stimulated to a considerable extent in the presence of EDTA, providing its concentration did not exceed 10^{-3} M (Table 6). Inhibition was total at higher concentrations.

The effect of exogenous PLP on activity is striking. At a final concentration of 10^{-5} M, activity was increased in comparison to a reaction mixture which did not contain the cofactor (data not shown). Exogenous PLP was unable to reactivate activity lost after freezing.

IV. DISCUSSION

The detection of L-serine dehydratase activity was optimal in cells permeabilized by Triton X-100. This technique was also used

by Moses (1972) to render *E. coli* cells permeable to macromolecules and by Schnaitman (1971) to demonstrate a selective solubility of a large proportion of membrane proteins. Even though the enzyme is bound to the cell envelope, treatment with this mild detergent is the most adequate means for protecting L-serine dehydratase and stabilizing it. A cell support is in fact absolutely necessary, since all attempts at solubilizing the enzyme were ineffective for preserving activity. To our knowledge this is the first report of a membrane-bound L-serine dehydratase.

The specific activity contained in membranes obtained from lysed protoplasts is 16 times greater than that of the pellet of cells treated with lysozyme in isotonic medium. In this case, it is possible that lysozyme acts differently than in hypertonic medium: it may interact with certain membrane components, as reported by Metcalf and Diebel (1973) for enterococci. The differential specific activity between protoplasts and membranes could be explained by a partial release of the enzyme in the course of osmotic shock (Neu and Heppel, 1965).

Coryneform bacteria are characterized by their particular cycle of development (Komagata *et al.*, 1969; Veldkamp, 1970; Crombach, 1974). The transformation of the bacilloid form to the coccoid form of *B. linens* is characterized by a decrease in L-serine dehydratase activity which is not due to the action of Triton X-100.

L-serine dehydratase is a Michaelis type enzyme with a high apparent Km (50 mM), although this value is not exceptional. Thus, the enzyme from other species, such as *Pseudomonas cepacia* (Wong and Lessie, 1979) and *E. coli* K12 (Newman and Kapoor, 1980) has a Km on the same order of magnitude or even higher, respectively 2×10^{-2} M and 42×10^{-2} M. The same is true of eukaryotes (rat liver cells): 5×10^{-2} M to 8×10^{-2} M (Suda and Nakagawa, 1971; Selim and Greenberg, 1959). Newman and Kapoor (1980) formulated a hypothesis according to which this determination of the Km with *E. coli* K12 was determined with an inactive or partially inactivated form of the enzyme. In the case of *B. linens* 9175, treatment with Triton X-100 is not responsible for this type of inactivation. The L-serine dehydratase activity (determined by assaying NH^+_4 ions) of intact non-permeabilized cells exhibits the same Km values (Hamouy, 1983).

In the system of permeabilized cells which we used in this study, the enzyme is stable to freezing. This had been reported by Miozzari *et al.* (1978) for other enzymes such as threonine dehydratase. Moreover, L-serine dehydratase activity in *B. linens* 9175 is independent of the rate at which the cell suspension is frozen. Samples frozen in liquid nitrogen or in a home freezer (— 20° C) exhibited identical activities. This system of permeabilized cells presents a

number of advantages, of which the two most important are the high stability of enzymes and the possibility of measuring activity *in situ* without inactivation or the dissociation of enzyme complexes (Hobot *et al.*, 1982; Weitzman, 1973).

The pH optimum is high. The pH optimum of serine dehydratases is generally slightly alkaline with a mean value close to 8. Thus, it is 8 to 9 for *Corynebacterium sp.* (Morikawa *et al.*, 1974), 8 to 9 for *E. coli* (Alföldi *et al.*, 1968), 8 to 8.4 for *Cl. acidi-urici* (Sagers and Carter, 1971), 6 to 9 for *Klebsiella aerogenes* (Vining and Magasanik, 1981) and 6.5 to 9.5 for *Pseudomonas cepacia* (Wong and Lessie, 1979).

L-serine dehydratase is specific for L-serine. The non-competitive inhibition by L-cysteine is interesting since this amino acid is generally cited as being a competitive inhibitor of the enzyme (Alföldi *et al.*, 1968; Suda and Nakagawa, 1971; Morikawa *et al.*, 1974; Gannon *et al.*, 1977). In the case of *B. linens*, it would appear that cysteine acts as a chelator. This is an isolated event, while reducing agents such as hydrosulfite, glutathione, 2-mercaptoethanol, dithiothreitol and cysteine are required for activity in *Cl. acidi-urici* (Benziman *et al.*, 1960; Suda and Nakagawa, 1971). D-serine is a competitive inhibitor in *B. linens* 9175, while it has no effect in *Cl. acidi-urici* (Benziman *et al.*, 1960) and *S. cerevisiae* (Ramos and Wiame, 1982). L-threonine inhibits in *A. globiformis* (Gannon *et al.*, 1977) and *E. coli* (Newman and Kapoor, 1980), but has no inhibitory effect in *B. linens* 9175.

Results obtained with o-phenanthroline, L-cysteine and $HgCl_2$ would tend to show that a metal ion, probably Fe^{2+} participates in the enzymatic catalysis; this has been shown in other cases (Benziman *et al.*, 1960; Carter and Sagers, 1972; Newman and Kapoor, 1980). In addition, Fe^{2+} is absolutely necessary for L-serine dehydratase activity in *Cl. acidi-urici* (Carter and Sagers, 1972). The stimulation of activity by combinations of KCl, NaCl and NH_4Cl, has not been reported for other bacterial L-serine dehydratases. Stimulation of activity by EDTA (10^{-3} M) is not clearly explained. In *E. coli* K12, on the other hand, this chelator has no stimulatory or inhibitory effect (Newman and Kapoor, 1980). The effect of exogenous PLP on activity is notable, although it has no effect in *Cl. acidi-urici* (Sagers and Carter, 1981) or in *A. globiformis* (Gannon *et al.*, 1977). PLP has no stabilizing role in *B. linens* 9175: it cannot reactivate the enzyme, as it can in *S. cerevisiae* (Ramos and Wiame, 1982) and in rat hepatocytes (Selim and Greenberg, 1959).

ACKNOWLEDGEMENTS

Part of this work was supported by Contract No. 81 C 1298 TAA 046, from the Industry and Research Ministry.

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