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Title ___THE EFFECT OF TEMPERATURE AND HYDROSTATIC PRES-___

___SURE ON DEAMINATION OF L-SERINE BY VIBRIO MARINUS, AN___

___OBLIGATE PSYCHROPHILE_____

Abstract approved ████████████████████████
                        (Major professor)

Vibrio marinus MP-1 (ATCC 15381) deaminated nine of seventeen

amino acids tested with L-glutamine being deaminated to the greatest

and L-serine to the second greatest extent in one hour. The optimum

pH for the L-serine deamination was 8.4.

The response of washed cells to temperature on the deamination

of L-serine depended upon the growth temperature of V. marinus

MP-1. Cells grown at 15 C had an optimum of deamination activity

at 40 C, and a shoulder at 15 C, while 4 C grown cells had greatest

activity at 38 and 11 C.

It is suggested that these peaks in deamination of L-serine at

different temperatures might be due to several different enzymes

deaminating the amino acid or the result of loss of permeability con-

trol above the maximum growth temperature of the organism.

Hydrostatic pressure stimulated or suppressed L-serine

deamination by washed cells depending upon the temperature at which the cells were grown and the incubation temperature of the reaction mixture. Cells grown at 15 and 4 C had deamination stimulated under hydrostatic pressure in the following cases: (1) cells grown at 15 C and tested for deamination at 15 C, (2) cells grown at 4 C and tested at 4 C and (3) cells grown at 4 C and tested at 15 C. When cells were grown at 15 C and tested at 4 C no stimulation of deamination activity due to hydrostatic pressure was observed.

These results adequately show that a very important part of the nitrogen cycle, the deamination of an amino acid by a marine bacterium, can occur in the ocean down to hydrostatic pressures of 400 atm and at temperatures below 20 C.

THE EFFECT OF TEMPERATURE AND HYDROSTATIC PRESSURE
ON DEAMINATION OF L-SERINE BY VIBRIO MARINUS,
AN OBLIGATE PSYCHROPHILE

by

LAWRENCE JOHN ALBRIGHT

A THESIS

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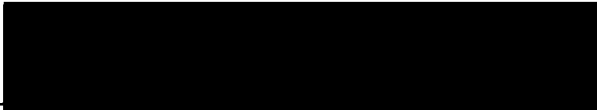in partial fulfillment of
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THE EFFECT OF TEMPERATURE AND HYDROSTATIC PRESSURE ON DEAMINATION OF L-SERINE BY VIBRIO MARINUS, AN OBLIGATE PSYCHROPHILE

INTRODUCTION

There have been few studies performed on the effect of microbial activities on the nitrogen and carbon cycles in the ocean depths. The few studies which have been done (16, 18, 19) were attempts to relate these cycles, as studied and elucidated in the terrestrial environment, to the ocean. The physical conditions of the oceans, however, are vastly different from conditions in the terrestrial environment. The temperature of the marine environment ranges from -1.9 C (the freezing point of sea water having a salinity of 35 ‰) to about 30 C in the tropics (16). By volume, more than 90 percent of the marine environment is colder than 5 C (16), with little temperature fluctuations except near the surface and where upwelling occurs. The hydrostatic pressure varies from one atm at the surface of the oceans to 1,100 atm in the deepest known part of the oceans, the Challenger Deep. The terrestrial environment, on the other hand has large temperature fluctuations in certain regions of the world and the atmospheric pressure is nearly constant at one atm. The following question then presents itself. With these large differences in physical environment, do bacteria act on any or part of the nitrogen cycle, as it is presently understood, at the ambient temperatures and hydrostatic pressures

found in the ocean depths.

This study was therefore initiated to determine if one part of the nitrogen cycle, the deamination of an amino acid by a marine micro-organism, could operate in the ocean depths at the in situ temperatures and pressures from which the organism was isolated as well as to obtain some data on the temperature and/or pressure effects on the system.

REVIEW OF LITERATURE

Little work has been done on cellular reactions under conditions approximating the physical environment of the oceans. Until recent times meaningful studies could not be undertaken due to the lack of a suitable marine bacterium capable of producing sufficient cell yields at conditions similar to those of the ocean depths. These bacteria would have to grow well at temperatures from -1 to 20 C and hydrostatic pressures from 1 to 1,100 atm.

ZoBell and Morita (19) demonstrated that bacteria are present and growing at all depths of the oceans. They found that many different genera of bacteria existed at all depths of the oceans. Generally there was a difference between bacteria found near the surface of the oceans and those from the Deeps with respect to their hydrostatic pressure tolerance. From 700 meters downwards barophiles predominated over non-barophilic bacteria. These investigators also demonstrated the presence of several physiological types at different depths by inoculating suitably diluted core samples at isobaric and isothermic conditions equivalent to the depths from which they were taken. In this manner starch hydrolyzers, nitrate reducers, ammonifiers, and sulfate reducers were found to be present in the Philippine Trench, the Sudan Deep and the Weber Deep.

Although the oxygen, carbon, sulfur and nitrogen cycles have

been thought to operate in the oceans much the same as in the terrestrial environments, very little work has been done on these cycles using bacteria which are able to proliferate at the extremes of temperature and hydrostatic pressure found in the oceans. This investigation was initiated to determine if the deamination of an amino acid by a marine bacterium proceeds under conditions of salinity, temperature and hydrostatic pressure found in the oceans.

The organism used in this study was a marine vibrio isolated from the North Pacific Ocean by Morita and Haight (10) in 1963. Subsequently it was established by Colwell and Morita (2) to be a reisolate of Vibrio marinus, first isolated by Russell (14) in 1891 from the Bay of Naples. V. marinus grows over a temperature and hydrostatic pressure range of -1 to 20 C and 1 to 400 atm respectively (8).

METHODS AND MATERIALS

Organism

The strain of V. marinus used in this investigation was designated MP-1.

Medium

The growth medium used throughout this investigation consisted of succinic acid (Baker and Adamson reagent) 10 gm; proteose peptone (Difco) 10 gm; yeast extract (Difco) 3 gm; and Rila marine mix (Rila Products Company, Teaneck, New Jersey) 35 gm. These compounds were dissolved in a liter of distilled water, boiled, cooled and filtered. The pH was adjusted to 7.2 with 10 N NaOH before sterilization. The medium was then dispensed in appropriate test tubes or flasks and autoclaved at 15 psi for 20 minutes. After sterilization the pH was 7.2. The medium, whether used in test tubes, Erlenmeyer, or Fernbach flasks was cooled to either 15 or 4 C before use.

Preparation of Washed Cell Suspensions

The stock culture of V. marinus MP-1 was kept at 15 C in 20 x 150 mm screw capped test tubes, each containing 10 ml of medium. The culture was transferred each day by inoculating 1 ml from a previously grown 24 hour culture into another test tube containing the

same medium. To obtain enough 15 C grown cells for experimental purposes 5 ml was inoculated into a Fernbach flask containing 500 ml of medium at 15 C. This flask was then incubated for 24 hours at 15 C in a Psychro-Therm Controlled Environmental Incubator (New Brunswick Scientific Company, Inc., New Brunswick, New Jersey) at a shaking rate of 200 strokes per minute with a 2.54 cm stroke.

Four C grown cells were obtained by inoculating a 500 ml Erlenmeyer flask containing 100 ml of medium with 10 ml of a V. marinus MP-1 culture from a previous 24 hour, 4 C grown culture. After this inoculated flask had incubated at 4 C for 24 hours, 5 ml was inoculated into 500 ml of medium in a Fernbach flask at 4 C. This flask was incubated, with shaking, in a Psychro-Therm for 24 hours at 4 C, as previously described.

After 24 hours growth the 15 and 4 C grown cells were harvested in a Sorvall RC-2 Superspeed Refrigerated Centrifuge at 15 and 4 C respectively, and at 6,000 x g for five minutes. The cells were then suspended and washed twice in sea water (35 gm of Rila marine mix per liter of distilled water, pH 7.6) at the appropriate temperature. It was found that the pH of the medium after growth had occurred was 7.6 - 7.8. The third suspension was in sea water + 0.1 M tris-HCl (pH 7.6 or 8.4). The concentration of this third suspension was adjusted so that a 1:3 dilution in sea water + 0.1 M tris-HCl gave an absorbance of 1.0 at 525 mμ using one-half inch cuvettes in a Bausch

and Lomb Spectronic 20 spectrophotometer. These washed cells were used immediately and kept at the appropriate temperature at all times.

By varying the temperature of growth of cells, type and concentration of amino acid, pH, temperature, and hydrostatic pressure, the influence of each variable was studied.

Assay for Deamination

After the incubation period of the reaction mixtures, the reactions were stopped by the addition of 0.5 ml of 10 percent trichloroacetic acid. The reaction mixtures were then centrifuged at 6,000 x g for five minutes and the resulting supernatants were tested for ammonia by nesslerization. To 1 ml of the clarified reaction mixture was added 4 ml of Nessler's reagent followed by 5 ml of 3 N NaOH. The samples were mixed and allowed to stand for ten minutes. The absorbances of each were then determined using a Bausch and Lomb Spectronic 20 at 450 mμ. The resulting absorbances, corrected for controls, were then converted to μmoles of ammonia by comparison with a standard curve prepared with reagent grade ammonium sulfate. The ammonia determinations were always read in the linear range.

Amino Acid Deamination Studies

Each amino acid was dissolved to a concentration of 20 μmoles per ml of sea water + 0.1 M tris-HCl (pH 7.6). Two tenths of a ml

of each amino acid solution was added to two ml of cell suspension.

For each amino acid used the following format was employed.

Tube No.	ml of Cells	ml of Amino Acid Solution	ml of Sea Water tris-HCl
1	2 ml	0.2 ml	- - -
2	2 ml	- - -	0.2 ml
3	- - -	0.2 ml	2 ml

Tubes 2 and 3 respectively, were controls for the ammonia produced
endogenously and for the ammonia produced from the amino acids
which may result from the high pH used in the Nessler determination
of ammonia.

Optimum pH Studies

The standard cell suspension was divided into suitable portions
and each was adjusted to pH values of 6.8, 7.2, 7.6, 8.0, 8.4, 8.8,
9.2, 9.6, and 10.0. An L-serine solution (Sigma grade or Sigma
grade II) was made to a concentration of 200 μmoles per ml of sea
water + 0.1 M tris-HCl, divided into nine aliquots and adjusted to the
various pH levels. The reaction mixture contained 1 ml of the cell
suspension plus 1 ml of the L-serine preparation at each pH. The
final L-serine concentration in each reaction mixture was 100
μmoles per ml of solution.

Substrate Saturation Studies

Eleven L-serine solutions were made up in sea water + 0. 1 M tris-HCl to concentrations of 0, 5, 10, 15, 20, 40, 60, 80, 100, 200, and 500 μmoles per ml of solution, all at pH 8. 4. Each reaction mixture contained 1 ml of each of these solutions plus 1 ml of the cell suspension. The reaction mixtures were allowed to incubate at 15 C for 1 hour at which time each solution was tested for ammonia as previously described. The substrate saturation studies, using 4 C grown cells, were done exactly as above except that L-serine concentrations of 0, 5, 10, 20, 40, 60, 80, 100, and 200 μmoles per ml of sea water + 0. 1 M tris-HCl were used. An optimum pH of 8. 4 was tacitly assumed for the deamination of L-serine by 4 C grown cells.

Temperature Effect on Ammonia Production

A polythermostat, similar to that used by Oppenheimer and Drost-Hansen (11) and constructed by Morita and Haight (10), was used with the temperature range adjusted from -1 to 60 C. At various temperatures, for the 15 C grown cells, the reaction mixture contained 1 ml of washed cells + 1 ml of sea water + 0. 1 M tris-HCl containing 120 μmoles of L-serine at pH 8. 4. For each temperature control tubes were also used containing 1 ml of washed cells plus 1 ml of buffered sea water.

For 4 C grown cells the protocol was similar with the exception that the L-serine concentration used was 20 μmoles per ml of sea water + 0. 1 M tris-HCl.

Hydrostatic Pressure Effect on Ammonia Production

One and a half ml of the standard cell suspension (15 or 4 C grown cells) was mixed with 1. 5 ml of the standard L-serine solution (120 μmoles of L-serine per ml of sea water + 0. 1 M tris-HCl for the 15 C grown cells and 20 μmoles for the 4 C grown cells), placed in a 10 x 75 mm test tube, closed with a number 000 neoprene stopper and immediately pressurized to the desired hydrostatic pressure by the method described by ZoBell and Oppenheimer (20). In this manner 15 C grown cells were pressurized at 15 and 4 C while 4 C grown cells were pressurized at 4 and 15 C. The four different reaction systems were incubated for one hour at their respective temperatures, de-pressurized and the ammonia concentration determined. Control tubes were also used at each temperature and pressure containing 1. 5 ml of washed cells plus 1. 5 ml of buffered sea water. The effect of hydrostatic pressure on washed cells to deaminate L-serine was determined at 1, 100, 200, 300, 400, 600, 800, and 1, 000 atm for each of the four systems.

RESULTS

V. marinus MP-1 was found to deaminate nine of the seventeen amino acids tested. The results are summarized in Table I. L-glutamine was found to be deaminated to the greatest extent in one hour but was also found to be highly unstable in alkaline solution. For this reason L-serine, which was deaminated to the second greatest extent, was selected for subsequent study.

The ability of washed cells to deaminate L-serine was maximal at pH 8.4 with enzymic activity decreasing at other pH values (Figure 1). There was very little enzymic activity at pH 10.0, however, at 6.8 there was still substantial activity.

At pH 8.4 washed cells grown and tested at 15 C were saturated with an L-serine concentration of 50 μmoles/ml of reaction mixture (Figure 2). On the other hand cells which were grown and tested at 4 C showed substrate saturation of 3 μmoles/ml of reaction mixture (Figure 3). This was a 17 fold difference between substrate saturation levels of the washed cells depending upon which temperatures the cells were grown.

When 15 C grown cells were tested for L-serine deamination as a function of temperature, there was enzymic activity from -1 to 52 C (Figure 4). Figures 4 and 5 show that there was a peak in activity at 40 C and a shoulder at approximately 15 C.

TABLE I.   THE DEAMINATION OF VARIOUS AMINO ACIDS BY
           WASHED CELLS OF V. MARINUS MP-1.

| Amino Acid* | μmoles of Ammonia/ml of Reaction Preparation | |
	1 Hour	3 Hours
L-alanine	0.30	0.85
L-asparagine	0.22	0.70
L-aspartate	0.475	1.65
L-arginine	0.15	1.00
L-cysteine	0.22	0.20
L-glutamate	0.00	0.00
L-glutamine	0.70	1.50
L-glycine	0.313	0.70
L-histidine	0.00	0.00
L-isoleucine	0.00	0.00
L-leucine	0.00	0.00
L-lysine	0.00	0.00
L-methionine	0.00	0.00
L-proline	0.00	0.00
L-serine	0.63	1.65
L-threonine	0.14	0.38
L-valine	0.00	0.00

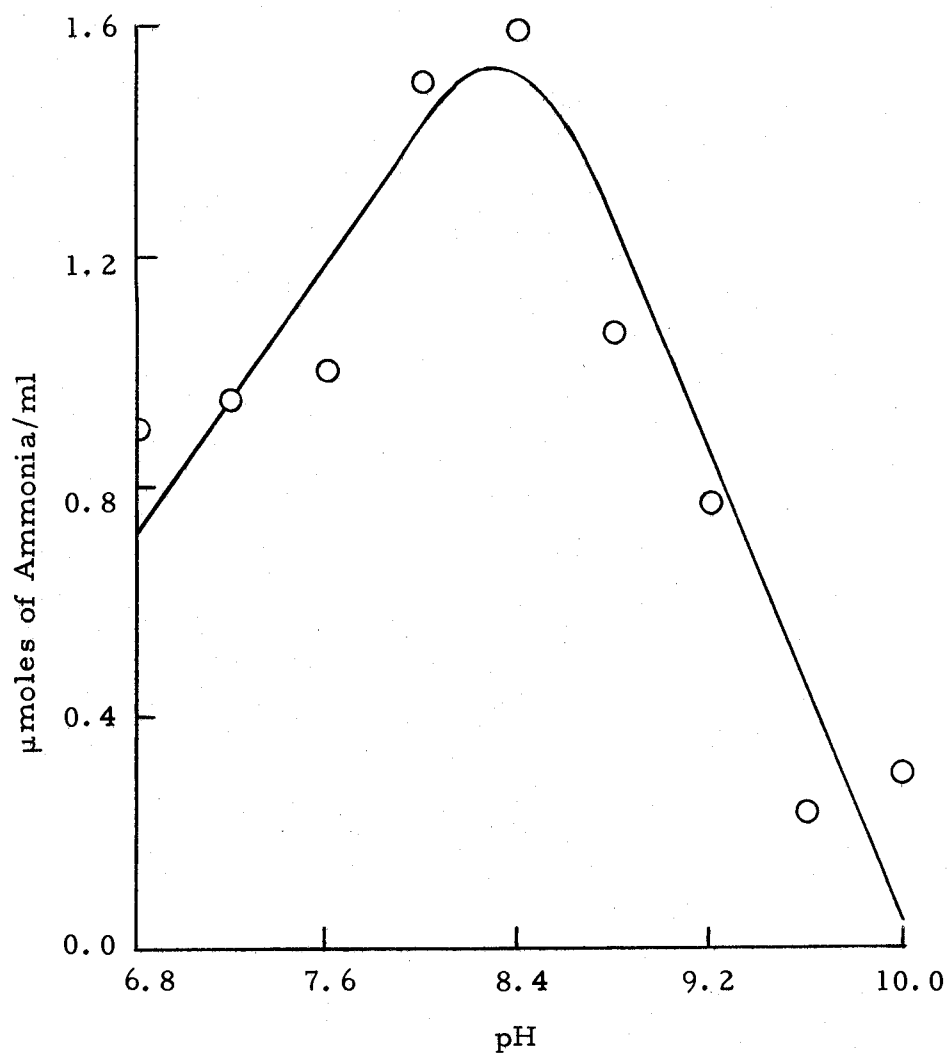*Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

Figure 1. The effect of pH on L-serine deamination by washed cells of V. marinus. Each reaction mixture contained 1 ml of washed 15 C grown cell suspension and 1 ml of L-serine solution (200 μmoles/ml) at various pH levels. Incubation period was 1 hour at 15 C.

Figure 2.   The effect of L-serine concentration on deamination
by 15 C grown washed cells of V. marinus. Each
reaction mixture contained 1 ml of washed cell sus-
pension and 1 ml of L-serine solution at various con-
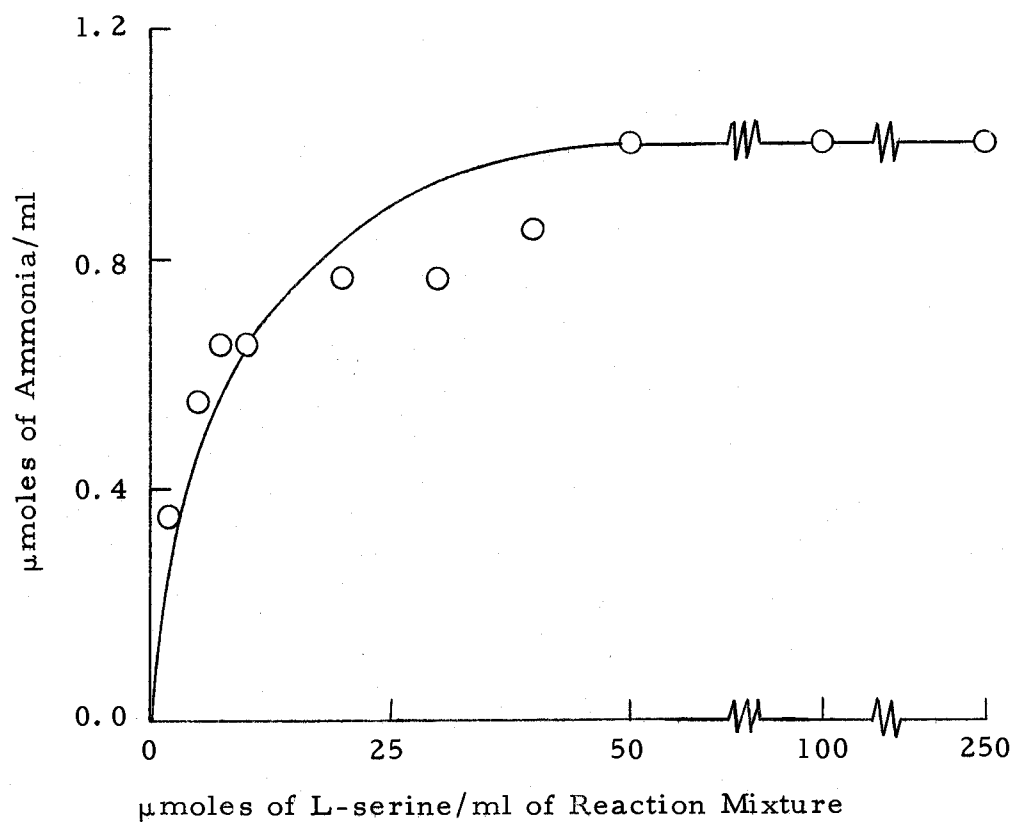centrations at pH 8. 4. Incubation period was 1 hour
at 15 C.

Figure 3. The effect of L-serine concentration on deamination by 4 C grown washed cells of V. marinus. Each reaction mixture contained 1 ml of washed cell suspension and 1 ml of L-serine solution at various concentrations at pH 8.4. Incubation period was 1 hour at 4 C.

Figure 4.   The effect of temperature on deamination of L-serine by 15 C grown washed cells of V. marinus. Each reaction mixture contained 1 ml of washed cell suspension and 1 ml of L-serine solution (120 μmoles/ml) at pH 8.4. Incubation period was 1 hour at various temperatures.
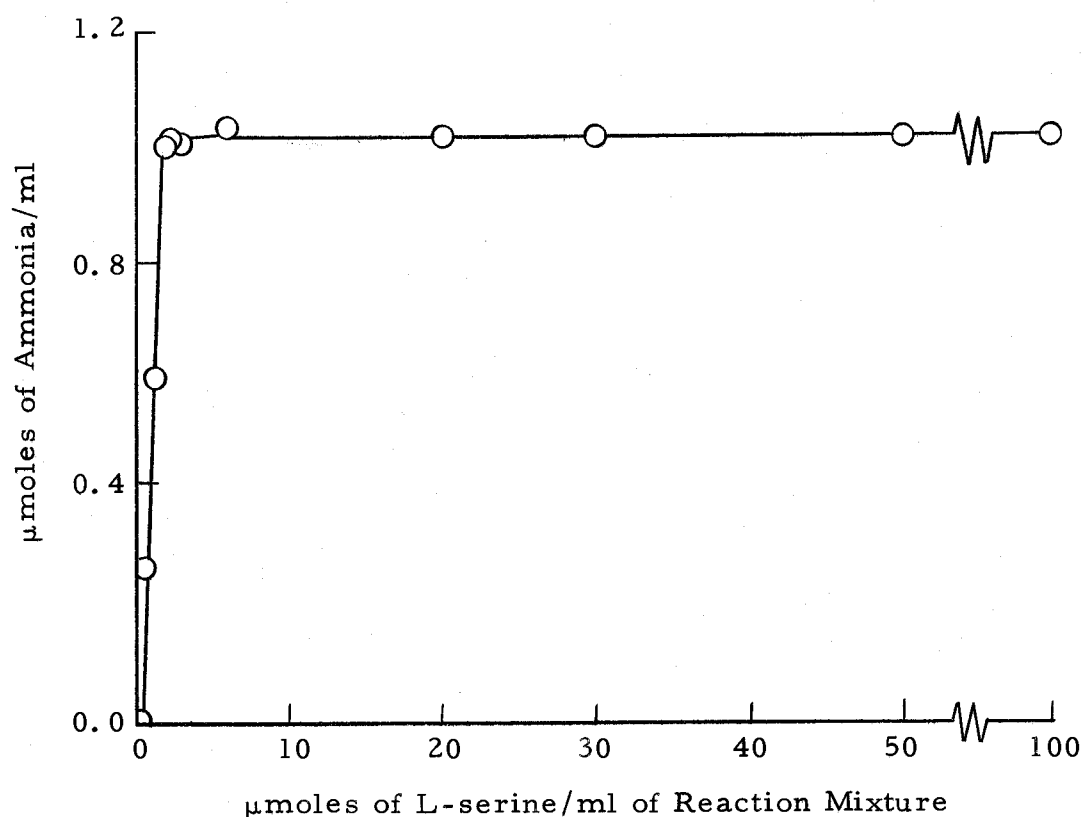
Figure 5.   The effect of temperature on deamination of L-serine
by 15 C grown washed cells of V. marinus.   Reaction
mixtures and incubation period identical to that in
Figure 4.

Four C grown cells also showed deamination activity from -1 to 50 C with two peaks in activity at 11 and 35 C (Figure 6). No shoulders were observed in deamination activity, as a function of temperature, with 4 C grown cells.

When 15 C grown washed cells were tested at 15 C for L-serine deamination in response to hydrostatic pressure over the range of 1 to 1,000 atm, a peak in deamination activity occurred at 300 atm, with very little deamination at 1,000 atm (Figure 7). If however, the 15 C grown cells were tested for L-serine deamination at 4 C in response to hydrostatic pressure there was no peak in activity (Figure 7), but a linear and progressive decrease in activity with pressure until there was very little activity at 800 atm and no activity at 1,000 atm.

If, on the other hand, cells were grown at 4 C and tested for deamination at 4 and 15 C, there were peaks in deamination activity in response to hydrostatic pressure. A peak in activity occurred at about 275 atm when the cells were tested at 15 C (Figure 8) and 130 atm when the cells were tested at 4 C (Figure 9).
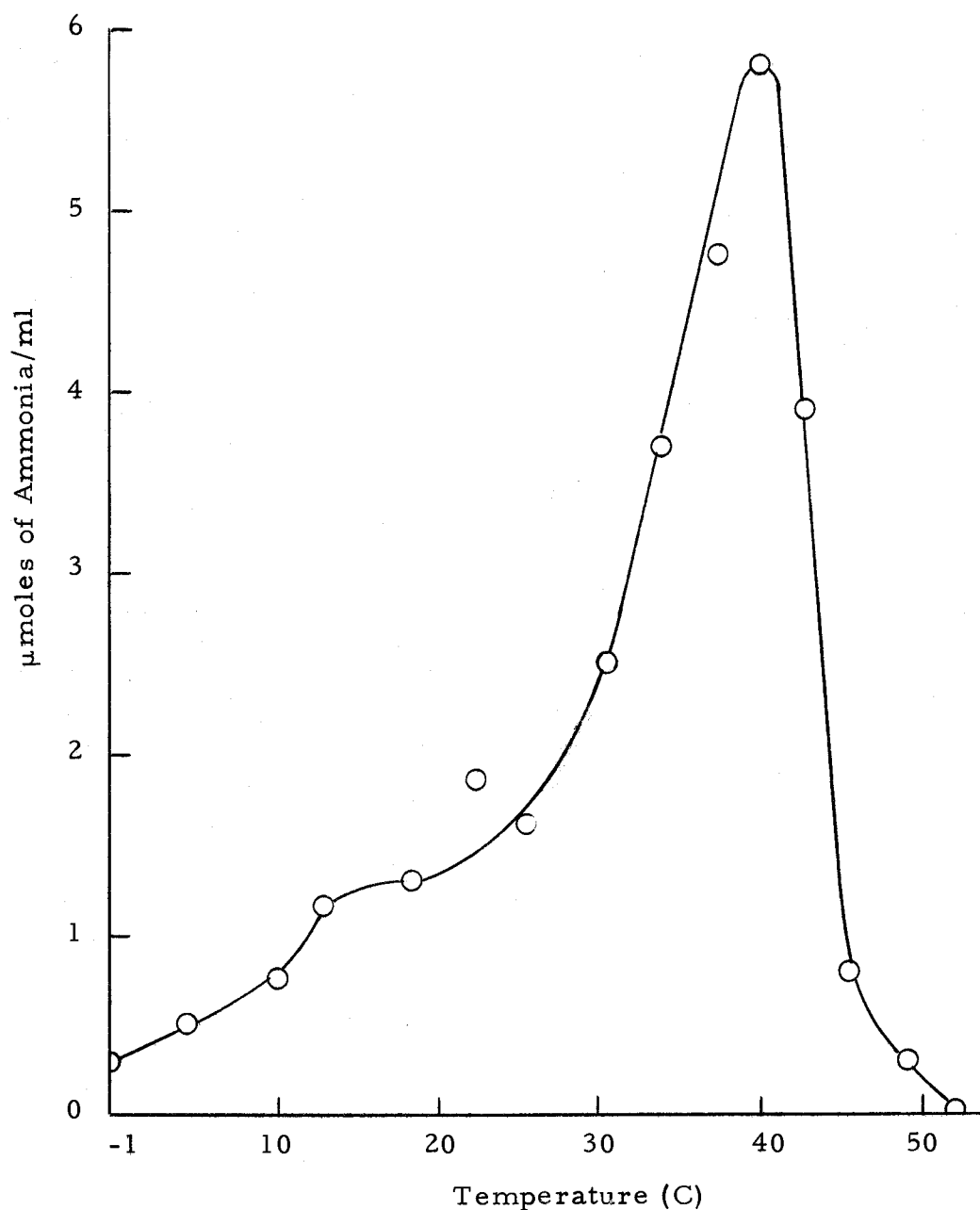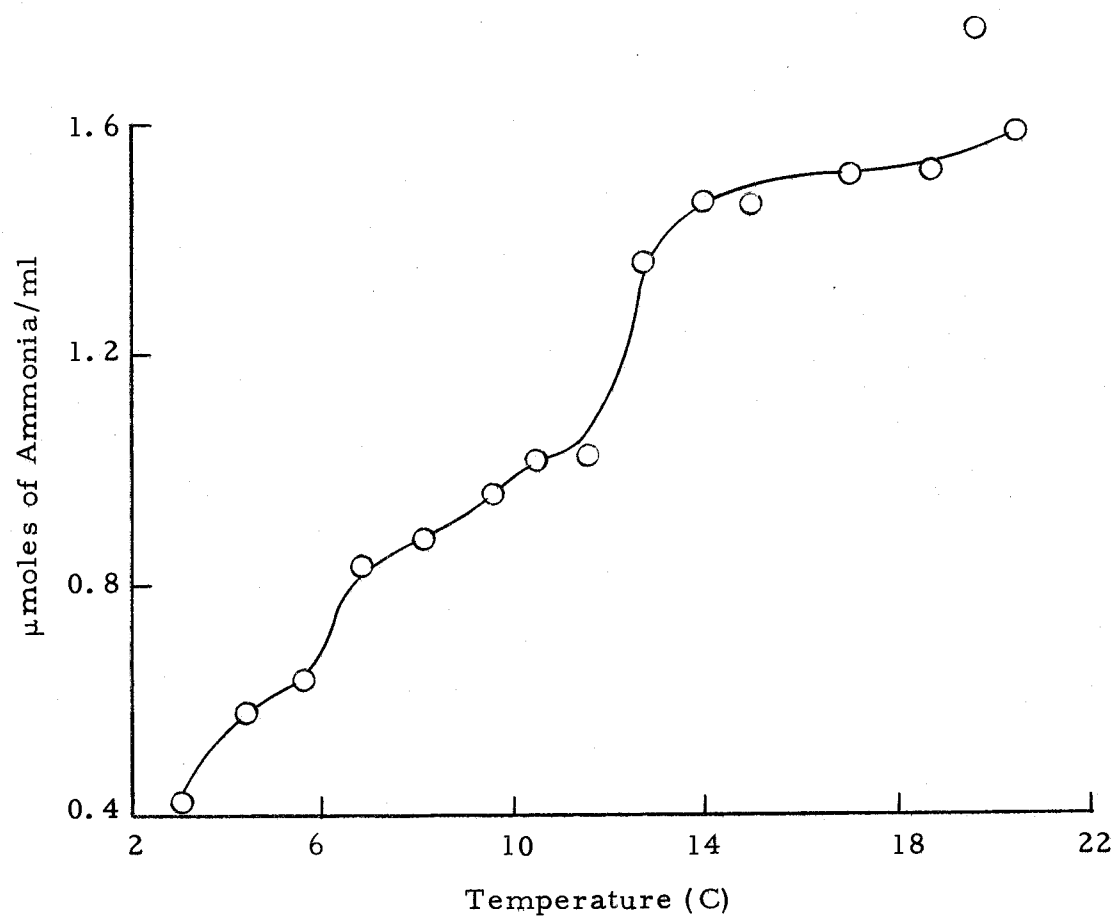
Figure 6.   The effect of temperature on the deamination of
L-serine by 4 C grown washed cells of V. marinus.
Each reaction mixture contained 1 ml of washed cell
suspension and 1 ml L-serine solution (20 μmoles/ml)
at pH 8.4.   Incubation period was 1 hour at various
temperatures.

Figure 7. The effect of hydrostatic pressure on deamination of
L-serine by 15 C grown washed cells of V. marinus.
Each reaction mixture contained 1.5 ml of washed
cell suspension and 1.5 ml of L-serine solution (120
μmoles/ml) at pH 8.4. Incubation period was 1 hour
at various hydrostatic pressures.

15 C (o)
4 C (Δ)

Figure 8. The effect of hydrostatic pressure on the deamination of L-serine by 4 C grown washed cells of V. marinus. Each reaction mixture contained 1.5 ml of washed cell suspension and 1.5 ml of L-serine solution (20 μmoles/ml) at pH 8.4. Incubation period was 1 hour at 15 C at various hydrostatic pressures.
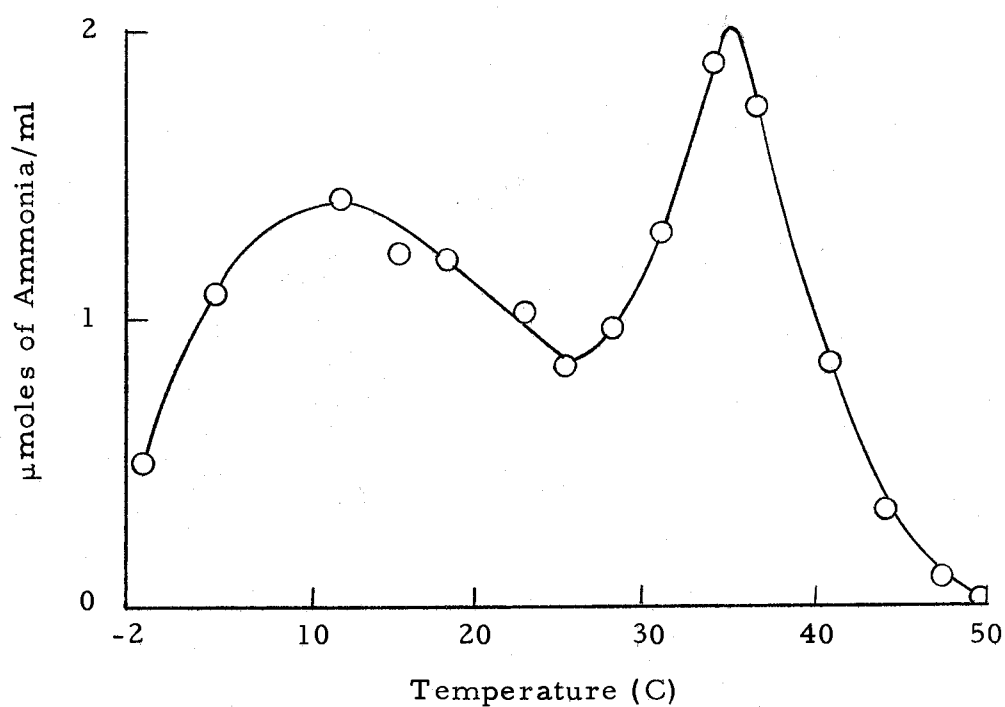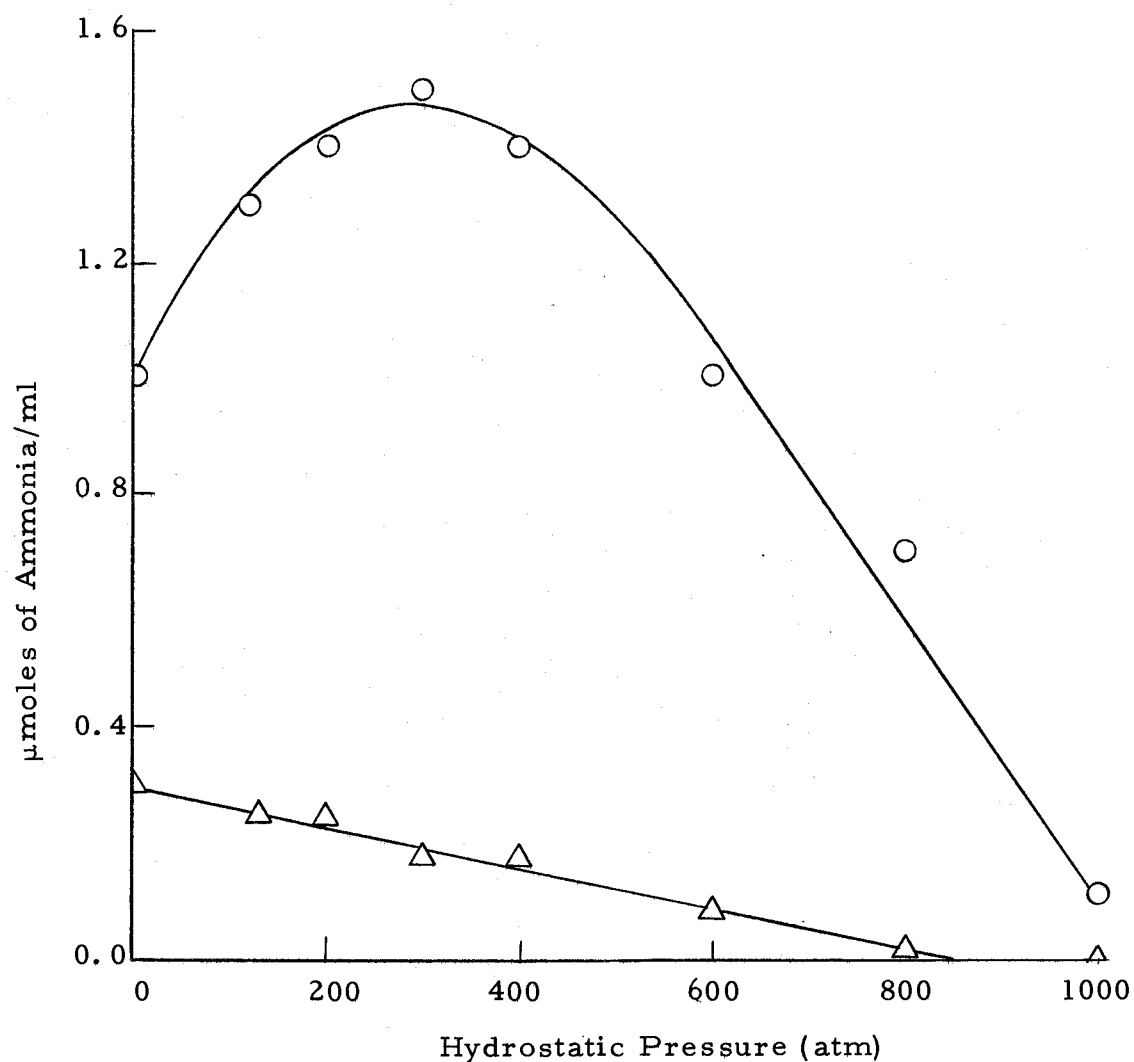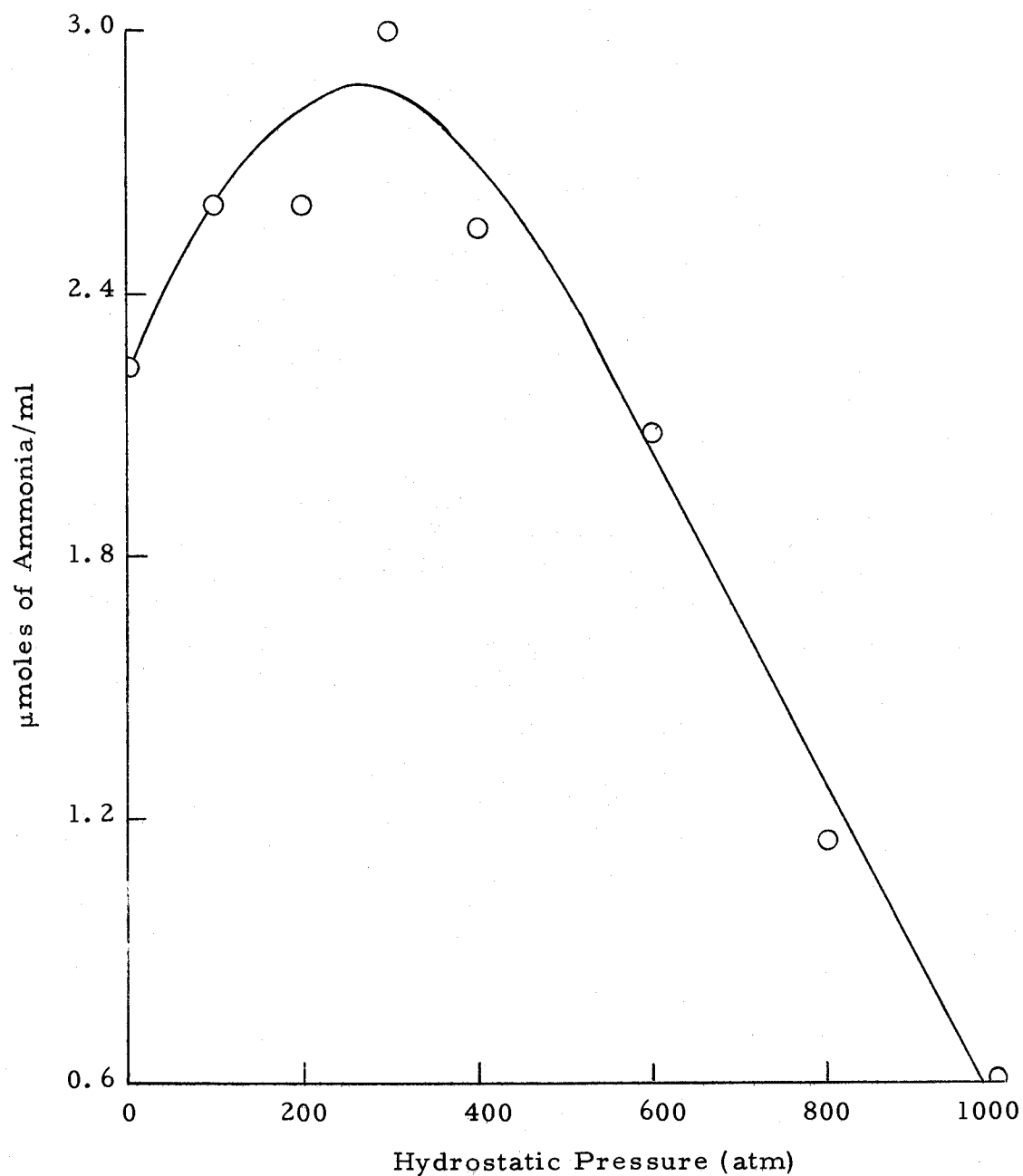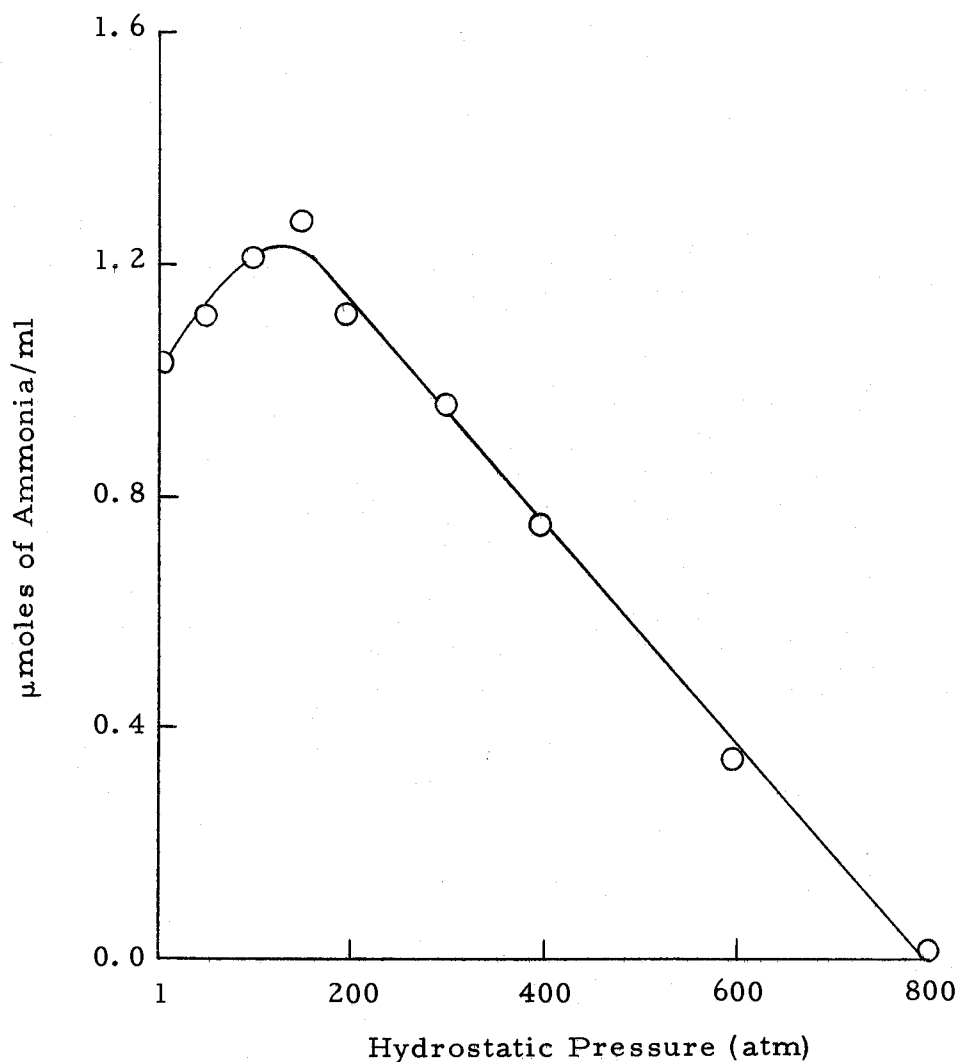
Figure 9. The effect of hydrostatic pressure on the deamination of L-serine by 4 C grown washed cells of V. marinus. Each reaction mixture contained 1.5 ml of washed cell suspension and 1.5 ml of L-serine solution (20 μmoles/ml) at pH 8.4. Incubation period was 1 hour at 4 C at various hydrostatic pressures.

DISCUSSION

The ability of V. marinus MP-1 to deaminate various amino acids is illustrated in Table I. Although L-glutamine was deaminated to the greatest extent in one hour, L-serine was selected for subsequent studies because L-glutamine was found to be highly unstable in alkaline solution.

The ability of washed cells to deaminate L-serine was maximal at pH 8.4 with enzymic activity decreasing at other pH values (Figure 1). Nevertheless, the data shows that the pH range is compatible with the pH range as found in the ocean. Since pH 8.4 was maximal for L-serine deamination, it was selected for further studies.

From the early literature (1, 15), it is well recognized that many marine organisms show better at temperatures from 10 to 20 C higher than the original environmental temperature of the organisms. The same holds true with V. marinus MP-1 as demonstrated in this laboratory by Morita and Haight (10), Morita and Albright (8) and Haight (3). Haight demonstrated that there were differences in cells of V. marinus MP-1 grown at 4 and 15 C in terms of their ability to respire.

This investigation also showed differences between cells grown at various temperatures. In terms of substrate saturation, the 15 C grown cells required approximately 50 μmoles of L-serine while the

4 C grown cells were saturated with 3 μmoles. This large difference in substrate saturation between the 4 and 15 C grown cells may be a matter of cell permeability differences. However, the amount of ammonia liberated in one hour by cells grown at two different temperatures was approximately the same, provided the cells were saturated with substrate. Further saturation of the systems does not bring about a decrease in activity.

Maximal enzyme activity was observed at 40 C with cells grown at 15 C. Since this enzyme is not abnormally heat sensitive it probably does not contribute to the abnormally low maximum temperature of growth of this organism. In all probability, this enzymic reaction is likely to be more heat sensitive than that from a mesophilic bacterium whose optimum temperature is around 37 C. Rapid activity loss occurs above 50 C with very little activity remaining at 50 C. The shoulder of activity appearing at approximately 15 C may be the result of permeability factors within the organism or a shift in the organism's metabolic reactions, whereas the higher activity levels observed in Figure 4 could result from the metabolic permeability control mechanisms. Permeability control loss at temperatures above the maximum growth temperature has been demonstrated by Morita and Burton (9) using V. marinus PS 207 (optimum temperature for growth, 24 C; maximum, 30 C). These investigators found that malic dehydrogenase (MDH) was operating much below its

capacity in whole cells. If the cells were heated or treated with Triton X-100 (to lyse the cells) MDH activity increased with the enzyme being allowed to operate much nearer its observed capacity. They concluded that either heating the cells or treating with Triton X-100 apparently destroyed some regulatory factor(s) for MDH activity. They thought that this factor might be cell permeability.

On the other hand 4 C grown cells displayed a different pattern of enzymic activity with temperature. Two peaks of activity were observed; one at 11 C and the other at 35 C (Figure 6). The first peak may have been due to cellular integrity (i. e. permeability control, metabolic control mechanisms, etc.) whereas the second peak at 35 C may be the result of the loss of permeability and reflects the true enzymatic rate of the enzyme as if it were in a cell-free extract. However, it remains to be explained why the shift occurs in the peak toward a lower temperature (Figure 6 versus Figure 5) in 4 C grown cells. The level of activity between 4 and 15 C grown cells may represent the amount of enzyme in the cells grown at the two different temperatures.

There are two known enzymes which catalyze L-serine deamination. L-serine dehydrase which splits out water yielding alpha-aminoacrylic acid, the cofactor used being pyridoxal phosphate. This alpha-aminoacrylic acid is unstable and rearranges to alpha-iminoproprionic acid, which is hydrolyzed in water to yield ammonia

and pyruvic acid. L-amino acid oxidase uses oxygen to produce pyruvic acid, ammonia and hydrogen peroxide from L-serine. Perhaps these two enzymes are responsible for the shoulder and peak observed with 15 C grown cells and the two peaks observed with 4 C grown cells (Figures 4, 5 and 6). Further investigations employing purified enzyme preparations might help clarify this situation.

Generally, hydrostatic pressure stimulated deamination of L-serine by washed cells if the cells were tested for deamination at or above the temperature at which the cells were grown. These observations are seen in Figures 7, 8, and 9. As shown by Figure 7, cells were grown at 15 C and tested at 15 C under various hydrostatic pressures. Similarly, in Figures 8 and 9 the cells were grown at 4 and tested at 15 C and grown at 4 and tested at 4 C respectively. In all these cases hydrostatic pressures in the order of 130 to 300 atm caused stimulation of L-serine deamination. It should be noted that the 4 C grown cells when tested for L-serine as a function of hydro-static pressure at 4 C had an optimum of 130 atm. This is very close to the hydrostatic pressure (120 atm) and temperature (3. 25 C) from which this organism was isolated (10). Although this stimulation of deamination activity due to hydrostatic pressure was found at 130 atm for cells grown at 4 C and 1 atm, it is not known whether cells grown at 130 atm and 4 C would exhibit the same stimulation of deamination activity. This is impossible to check at the present time because,

due to methodology, sufficient cells cannot be grown at 130 atm and 4 C.

When cells, however, were grown at 15 C and tested at 4 C under various hydrostatic pressures for L-serine deamination, no pressure optimum was observed. This is in line with the hypothesis of Johnson, Eyring and Polissar (6) that a decrease in temperature along with an increase in hydrostatic pressure act concomitantly to cause a molecular volume decrease of the enzyme or enzymes involved. It is believed that this in turn caused a marked decrease in the activity of the enzyme(s) studied here. In the three other cases the temperature was either raised or kept the same as the growth temperature while the hydrostatic pressure was increased. Presumably there was sufficient thermal energy available to counteract the decrease in molecular volume brought about by increased hydrostatic pressure.

Haight and Morita (4) obtained results similar to the results of this study using a cell-free aspartase preparation of Escherichia coli. The cell-free enzyme preparation of E. coli was grown at 37 C and tested for ammonia production as a function of various temperatures and hydrostatic pressures. They found that hydrostatic pressures of from 1 to 1,000 atm increased aspartic acid deamination if the temperature of the enzyme preparation was kept above 50, 53, or 56 C. At 45 or 37 C there was no stimulation of deamination activity due to hydrostatic pressure; on the contrary there was diminution in

deamination activity with increased pressure.

As can be seen from the foregoing data, the deamination of L-serine as well as other amino acids can occur at low temperatures which exist in the ocean below the thermocline as well as at both polar regions. The liberation of ammonia from L-serine also takes place at various hydrostatic pressures as well. Unfortunately there have been no previous studies on the activities of obligate psychrophilic bacteria under conditions that exist in the oceans. The liberation of ammonia from amino acids has been demonstrated in this study. It is known that V. marinus MP-1 also possesses proteolytic enzymes (2). Since some strains of V. marinus can be isolated from fish (2), it should be expected that this organism can liberate ammonia from various types of dead organisms that fall below the thermocline.

It is unfortunate that other studies on the nitrogen cycle as affected by bacteria in the deeper portions of the oceans have not been made. The effects of hydrostatic pressure on nitrate reduction have been studied on a Pseudomonas sp by ZoBell and Budge (17) but the organism was a mesophile and the low temperature aspect was neglected.

Although this investigation definitely demonstrated that there are differences in the response of cells grown at 4 and 15 C to the deamination of L-serine, another important concept was brought into focus. This is that obligate psychrophiles possess enzymes like MDH

which are abnormally heat labile as shown by Langridge (7). Another

investigator in our laboratory (Miller, personal communication) re-

ports that glucose-6-phosphate is not abnormally heat labile as is

MDH. From the limited data available concerning obligate psychro-

philes, it appears that certain enzymes are not abnormally heat labile

and certain enzymes are. Rose (13) has suggested that TCA cycle

enzymes are probably the abnormally thermolabile enzymes. The

above may be a reflection of the evolution of psychrophiles in nature

which, in this case, would mean that certain enzymes have mutated to

become abnormally heat sensitive while others have not. If one en-

zyme in the metabolism of V. marinus MP-1 is abnormally heat

labile, then the master reaction scheme of Ingraham's for metabolism

comes into play (5). However, Ingraham's concept does not take into

consideration the possibility of an abnormally heat labile cell mem-

brane as evidenced by the work of Robison (12) and Haight (3).

SUMMARY

Washed cells of V. marinus MP-1 deaminated nine of seventeen amino acids tested with L-glutamine being deaminated to the greatest and L-serine to the second greatest extent in one hour. The optimum pH for L-serine deamination was 8.4.

Fifteen and 4 C grown cells were saturated with L-serine concentrations of 50 and 3 μmoles of L-serine respectively. Fifteen C grown cells showed a peak in deamination activity at 40 C and a shoulder at 15 C while 4 C grown cells showed peaks at 11 and 35 C.

Fifteen C grown cells had deamination of L-serine stimulated by various hydrostatic pressures at 15 C, with an optimum at 300 atm. When the 15 C grown cells were tested at 4 C at various hydrostatic pressures there was a marked and linear decrease in deamination activity as a function of increasing pressure. Four C grown cells had peaks in deamination activity at 275 and 130 atm pressure when tested at 15 and 4 C respectively.

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