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Intracellular Protein Degradation in a Mesophile and a Thermophile of the Genus *Bacillus*

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INTRACELLULAR PROTEIN DEGRADATION IN A MESOPHILE AND A THERMOPHILE OF THE GENUS *BACILLUS*

by

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INTRACELLULAR PROTEIN DEGRADATION IN A MESOPHILE AND A THERMOPHILE OF THE GENUS *BACILLUS*

Cameron C. Hoover, M.A.

Western Michigan University, 1987

^{14}C-leucine was incorporated into the proteins of the mesophile *Bacillus licheniformis* and the thermophile *Bacillus stearothermophilus*. Aliquots of the cell suspension were treated with chloramine-T in order to decarboxylate the ^{14}C-leucine that was released as the proteins degraded. The $^{14}CO_2$ was trapped and counted. This allowed for the determination of the extent of intracellular protein degradation at various temperatures and at various stages of the growth curve. For the mesophile, the extent of degradation increased with temperature from 30° C to 55° C. For the thermophile, no significant amount of degradation occurred until about 30 hours had passed, at which time the extent of degradation increased for the next 24 hours. The results show that slow turnover may be an important characteristic of thermophily.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Intracellular Protein Degradation

Much research has been done on protein turnover (synthesis and degradation) since the 1950's but most of that work was concerned with synthesis which, as a result, is well understood. Protein degradation, which is the breakdown of protein into its constituent amino acids, received little attention until the late 1960s, at least partially because before then it was thought to be relatively unimportant biologically (1). Once degradation was studied in more depth it was found to be much more complicated and important than previously thought, although our knowledge of it is still far behind that of synthesis.

The following, based mainly on *Escherichia coli* and mammalian studies, are believed to be true about intracellular protein degradation in general:

1. It is random (2), which means that a newly synthesized protein is as likely to be degraded as an old one of the same type.

2. It is ongoing. Proteins are continually being broken down and resynthesized (3). However, it should be pointed out that in bacterial species, a majority of the proteins are not subject to degradation (4,5) or at least have very long half-lives (6).

3. It accelerates during starvation (7).

4. It has an energy requirement (2). Protein degradation can be reduced or completely blocked with inhibitors of energy metabolism (8,9).

5. Once a protein begins to degrade, it is quickly broken down into its amino acids. There is little evidence for the presence of partially degraded proteins in vivo (10).

6. The proteins of a cell exhibit a marked heterogeneity in half-lives (1,11).

Several plausible ideas have been presented and supported that explain what a cell might gain by doing something as energetically expensive as continuously breaking its proteins down.

One advantage to the cell is the capacity to exert more control over protein levels. The cell often needs to make changes in its protein levels as it develops or in response to environmental changes. Obviously, synthesis can only increase the protein levels, so a process that can decrease the levels is important. Also, the more rapidly that turnover of a protein occurs, the more quickly its level can be adjusted to meet changing physiological needs (1).

The ability to survive starvation periods is another advantage that the cell gains from degradation of its proteins. During starvation, the amino acids needed for synthesis come from degradation of pre-existing proteins (3,12). The cell can break down "luxury" proteins and use the released amino acids for more necessary proteins (2). This helps explain the long known fact that bacterial cells increase their rate of protein degradation up to several fold when deprived of a nitrogen or carbon source or a required amino acid (3).

Another advantage of degradation seems to be the removal of abnormal proteins. Once an abnormal protein appears, whether as a result of chemical aging, incorrect synthesis, or some other process, it is very susceptible to degradation (1,13). Pine (13) and Goldberg (14) showed that, in *E. coli*, proteins that contain certain amino acid analogues are degraded more rapidly than the normal proteins. Goldschmidt (15) showed that prematurely terminated *B*-galactosidase fragments produced from nonsense mutants of *E. coli* are rapidly degraded whereas the wild type enzyme is very stable under the same conditions. Platt et al. (16), using *E. coli* with a deletion mutation, showed that the resulting altered lac repressor protein also is degraded rapidly as

opposed to that of the stable wild type protein. Evidence suggests that the enhancement in susceptibility to degradation is related to the conformation of the abnormal protein (17) since the abnormality (incorrect synthesis, incorporation of an amino acid analogue, etc.) probably caused a difference in conformation from that of the normal protein (11). Conformation is likely to be related to the degradation of normal proteins as well. This will be discussed further below.

The wide variations in half-lives of different proteins are probably due to differences in protein structure. There are many types of evidence for this conclusion (1):

1. The increase in degradation rates of abnormal, as compared to normal, proteins as discussed above. Additionally, it is well known that denatured polypeptides are broken down proteolytically much more quickly than the native proteins (18,19).

2. The decrease in degradation rates after the addition of certain ligands, presumably because the ligands induce conformational changes upon binding that are more favorable for protein stability (1).

3. The increase in degradation rate with increase in protein size in eukaryotes (20). This may be due to the likelihood of larger proteins having more sites than smaller ones that are susceptible to an initial, rate-limiting attack by a protease (1) (see the last paragraph of this section concerning the initiating event). An even simpler explanation could be that a large protein merely presents a larger target for a protease to bump into.

4. The correlation between the degradation rate of proteins in vivo and their susceptibility to proteases in vitro (12,17,21).

From the above, one can conclude that the underlying factor of protein structure that influences protein stability is the conformation (12). It may be that normal, long-lived proteins share conformational features that protect them from degradation, and that changes in the conformation are responsible for an increase in susceptibility to proteases (12). If this is true, then it follows that abnormal proteins must be in unfavorable

conformations and that normal but short-lived proteins must either exist in unfavorable conformations or convert to them easily. McLendon and Radany (22), among others, presented a slight twist to this conformation theory. Their idea is that it is not the native conformational state of the protein that determines its susceptibility to proteases, but rather how much time the protein spends in a reversibly unfolded, susceptible condition. They found a correlation between in vitro thermostability and in vivo turnover rates of nine intracellular proteins that supports this view.

The mechanism of intracellular protein degradation is still not understood. As stated earlier, there is an energy requirement, which implies that the mechanism is not strictly proteolysis since proteolysis per se is exergonic (2,23). The mechanism consists, most likely, of a coupling of proteolysis with one or more energy requiring processes (2,14). Furthermore, the degradation of normal and abnormal proteins seems to be independently regulated and probably follows a different or partially different pathway (14,24,25).

The initiating event of the mechanism is not known either. One possibility is that a highly specific protease makes the initial nick cleaving one peptide bond and therefore opening up the protein to attack by more general proteases (7). However, the initial event may not be proteolytic in nature. It may involve labeling of the protein for degradation by chemical or enzymic modification (2). Alternatively, the initial event may be the activation of a protease or the unfolding of the protein.

Thermophily

Most organisms have optimum growth temperatures between 30^{o} C and 45^{o} C and are called mesophiles (26,27). Those which thrive best at higher temperatures are known as thermophiles (27).

Three main theories (28) have been proposed to explain thermophily, or growth

at high temperature.

One theory, the lipid protection theory (29), maintains that the presence of high melting point lipids in the cells of thermophiles helps to protect their proteins and subcellular structures against heat denaturation. Indeed, many researchers found that mesophiles and thermophiles have different lipid compositions (30,31,28). Moreover, by growing a bacterial species at different temperatures, it has been shown that there is a correlation between the growth temperature and the percentage of high melting point fatty acids present in both mesophiles (32) and thermophiles (33,34).

Another theory, the kinetic theory (35), attributes thermophily to a special metabolic state characterized by high turnover rates. A high turnover rate would serve to quickly remove and replace macromolecules that have been denatured by the high temperature environment of thermophiles. Bubela and Holdsworth, in a frequently cited paper (36), reported a higher rate of protein and nucleic acid turnover at 40^{o} C for the thermophile *Bacillus stearothermophilus* than for the mesophile *E. coli*. However, it is important to note that they used a very short protein labeling period and so were looking at only the most labile proteins (see Results & Discussion: Methods). After reviewing available data (up to 1978), Amelunxen and Murdock (26) concluded that rapid turnover is probably not a major factor in thermophily.

The third theory, the macromolecular theory, states that thermophily is made possible by physical-chemical differences in the macromolecules of thermophiles compared to those of mesophiles (37,38,39). It is well established that thermophilic proteins are more thermostable than their mesophilic counterparts (27,40,41) even though they are structurally very similar (26,42). This thermostability appears to be intrinsic to the protein molecules (26,27, 42) and, if so, must be due to small but important differences between the structures of corresponding mesophilic and thermophilic proteins (27,42). Likewise, the information transfer systems of thermophiles have been compared to

those of mesophiles, again with greater thermostability being shown for the thermophiles. This extends to both DNA (43) and RNA (44,45) where greater guanine plus cytosine (G + C) contents have been shown for thermophiles as well as correlations between G + C content and maximum growth temperatures of the organisms (46,45,47). The G + C content usually correlates with nucleic acid thermostability since the more guanine-cytosine pairs there are, the greater the extent of hydrogen bonding holding the two strands of the double helix together. Thermophilic ribosomes have been shown to be more heat stable than mesophilic ones (27,48,49) and the melting out temperatures of the ribosomes have been correlated with the maximum growth temperatures of the organisms (46,45).

The Present Study

The present study deals both with the problem of protein degradation and the problem of thermophily. Specifically, the extent of intracellular protein degradation, in both a mesophile and a thermophile, was determined at various growth stages and at various temperatures. The purpose was to find out how the extent of degradation would be affected by changes in temperature and to investigate the applicability of the kinetic theory of thermophily by comparing the degradation rates of the two bacteria.

CHAPTER II

MATERIALS AND METHODS

Reagents

L-[1-^{14}C]-leucine (52 mCi/mmole) was obtained from ICN; L-leucine was obtained from Nutritional Biochemicals Corp.; chloramine-T (N-chloro-p-toluene-sulfonamide, sodium salt) was obtained from Sigma Chemical Co.; ethanolamine (2-aminoethanol) was obtained from J.T. Baker Chemical Co.; scintillation grade 1,4-dioxane and citric acid monohydrate were obtained from Fisher Scientific. All percent concentrations are in terms of w/v.

Bacterial Strains and Media

Two strains of the genus *Bacillus* were used for this study; a mesophile, *Bacillus licheniformis* (NRS 243), and a thermophile, *Bacillus stearothermophilus* 10. The liquid growth medium consisted of 1.0% Trypticase (BBL) and 0.2% yeast extract (DIFCO). The slants contained, in addition, 2.0% Bacto-Agar (DIFCO). The media were always brought to the appropriate temperature before using.

Outline of Procedure

Bacterial cells were grown overnight in a liquid medium that contained ^{14}C-leucine in order to incorporate the labeled amino acid into the proteins of the cells. In the morning, the cells were harvested by filtration and then resuspended in fresh liquid medium that contained a large excess of unlabeled leucine. The cells were allowed to continue to grow while duplicate aliquots of the culture were taken as a function of time. Each aliquot was added to a special reaction flask that was equipped with a CO_2 trap

and that contained chloramine-T and citric acid. Chloroamine-T reacts with free amino acids by decarboxylating the #1 carbon (50). Amino acids tied up as residues in the proteins will not react with chloramine-T. Because of this, the $^{14}CO_2$ that was released and trapped represented the ^{14}C-leucine that was released upon degradation of the proteins. Therefore, the extent of protein degradation is equal to $^{14}CO_2$/total ^{14}C (i.e., ^{14}C in the form of both free leucine and protein incorporated leucine) per unit volume. Details of the individual steps of the procedure are given below.

Growth and Labeling of Bacteria

Bacterial cells were transferred in a sterile fashion from a slant to 16 ml of medium in a 125 mL Erlenmeyer flask. The culture was then incubated at the optimum growth temperature (37° C for the mesophile; 55° C for the thermophile) in a rotary incubator-shaker at a setting of 125 rpm.

After approximately 5 hours of incubation, 8.0 mL of the culture were pipetted in a sterile fashion into 12 mL of growth medium in a 125 mL Erlenmeyer flask. The 12 mL of medium contained 10 uL of ^{14}C-leucine (52 mCi/mmole). The final incubation mixture of 20 mL, therefore, contained ^{14}C-leucine at a concentration of 0.96 uM or 0.05 uCi/mL (51). The culture was incubated overnight in the incubator-shaker as described.

Harvesting and Resuspension of Bacteria

The labeled bacterial cells were harvested by filtration on 0.45 um cellulose disks (Amicon Corp.). The cells were then washed with 5 to 10 mL of medium in order to remove any remaining free ^{14}C-leucine.

Filtration was stopped when just enough liquid was left to keep the cells moist. The cells were removed from the filter disk with a small spatula and resuspended in a

125 mL Erlenmeyer flask that contained growth medium that was 4.6 mM in unlabeled L-leucine (51,6). The volumes of this resuspension medium were 24 mL and 34 mL for the mesophile and thermophile, respectively.

Degradation

Incubations for the degradation studies were also done in the rotary incubator-shaker set at 125 rpm. The incubation temperatures used were 30°, 37°, 45°, 55°, 65° for the mesophile and 45°, 55°, 65° for the thermophile.

Duplicate 1.00 mL aliquots for decarboxylation and a corresponding 0.20 mL "total" aliquot were taken over a 24 hour and 54 hour period for the mesophile and thermophile, respectively. The 0.20 mL "total" aliquot was not decarboxylated but instead was pipetted directly onto a filter paper in a scintillation vial. The filter paper was allowed to dry before adding 8 mL of scintillation fluid (composition given below) to the vial.

Decarboxylation

Each duplicate 1.00 mL aliquot was added to the main part of a 15 mL Warburg flask (a small Erlenmeyer flask modified with a sidearm and a 1 mL center well). The main part of the flask also contained 1.0 mL of water and 0.10 mL of 75% citric acid (51,52). The sidearm contained 0.5 mL of 17% chloramine-T (51) and the center well contained 0.1 mL of ethanolamine and a small piece of filter paper (Whatman 40).

The flask was stoppered and the chloramine-T poured from the sidearm into the main part of the flask by tilting the flask. Decarboxylation of the ^{14}C-leucine was allowed to proceed for 2.0 hours in an oscillating water bath shaker set at 30° C and 50 oscillations per minute. The $^{14}CO_2$ was collected on the ethanolamine soaked filter paper (53).

At the end of the 2 hour decarboxylation, the filter paper was removed and placed in 6 mL of scintillation fluid in a counting vial. The center well of the flask was rinsed twice with 1 mL aliquots of scintillation fluid which were transfered to the counting vial by means of a disposable pipet. The vials were kept in the dark overnight before counting to minimize chemiluminescence (54).

Counting

All samples were counted in an ISOCAP/300 Liquid Scintillation System Model 6868 (Searle Analytic, Inc.). The scintillation fluid consisted of scintillation grade 1,4-dioxane containing 10% naphthalene, 0.4% 2,5-diphenyloxazole (PPO) and 0.005% 1,4-bis-(5-phenyloxazol-2-yl) benzene (POPOP). Corrections were made for quenching using the external standard channels ratio method (55).

Calculations

The extent of protein degradation, D, for each aliquot was calculated according to the equation:

$$\% D = [(C-Z)/5(T-B)] \times 100$$

where:

C = dpm due to $^{14}CO_2$ trapped, that is, dpm obtained from decarboxylation of 1.0 mL of cell suspension.

T = dpm contained in 0.2 mL of cell suspension ("total ^{14}C").

Z = C at time zero, that is, immediately after resuspending the labeled cells in the medium.

B = dpm of background.

CHAPTER III

RESULTS AND DISCUSSION

Methods

The long overnight labeling period was used for two reasons. First, for the purposes of this study, it was desired that all proteins, both short- and long-lived, be as uniformly labeled as possible. The only way to get any uniformity is by long-term labeling since a short labeling period will preferentially label proteins with short half-lives (56,57,58). Second, the overnight labeling was needed in order to incorporate enough ^{14}C-leucine into the cellular protein to allow accurate scintillation counting. Of the ^{14}C-leucine available in the medium, an average of 3.9 % and 7.2 % was incorporated into the proteins by morning for the mesophile and the thermophile, respectively. After filtration and resuspension, this resulted in an average count of about 3600 dpm/mL of cell suspension for the mesophile and about 4700 dpm/mL for the thermophile. This difference in incorporation could be due to differences in leucine transport across the cell membrane and/or due to differences in leucine content of cellular proteins and/or due to differences in protein synthesis and degradation between the two organisms.

Filtration of the cells, after labeling, was done on 2 or 3 filters simultaneously in order to shorten the filtration and washing time to 10 or 20 minutes. Washing the cells on the filters before resuspension was usually successful in rinsing away free ^{14}C-leucine, thus reducing zero time dpm due to collected $^{14}CO_2$ to nearly background. However, this was not essential since the zero time aliquot was standardized to zero dpm anyway.

The high concentration of unlabeled leucine in the resuspension medium (4.6 mM)

was needed in order to keep reincorporation of ^{14}C-leucine into the protein to a minimum (59). That that was a sufficient excess can be seen by the fact that, even with as much as 40% degradation (which was reached in only one out of the eight experiments), the ^{14}C-leucine concentration in the medium would only be about 1.2×10^{-5} mM.

A greater volume of resuspension medium was used for the thermophile than for the mesophile (34 mL vs. 24 mL). The greater volume was needed because of greater evaporation of the medium in the case of the thermophile since experiments involving the thermophile were conducted over longer time periods than those involving the mesophile. Longer degradation periods were used for the thermophile because the thermophilic proteins were degraded at a much slower rate than the mesophilic ones. The thermophilic proteins were degraded to only a negligible level during the first 30 hours whereas the mesophilic proteins were degraded to a significant extent within 2 hours at all but the lowest temperature (30°).

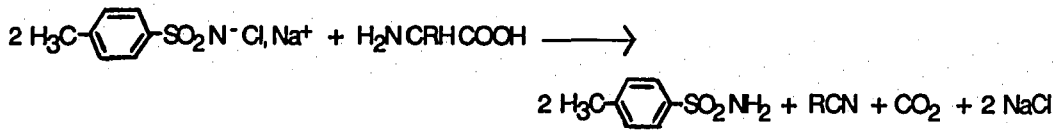
The cultures were incubated at different temperatures so that the effect of temperature on degradation could be determined. The incubation temperatures were chosen so that comparisons could be made within a strain (i.e., mesophile or thermophile) above, below, and at its optimum growth temperature. It should be noted that, by using a mesophile and a thermophile of the same genus, the possibility that observed differences are due to intergeneric differences between the organisms is eliminated.

In all experiments, for each pair of duplicate aliquots taken for decarboxylation, a "total" aliquot was also taken. The "total" aliquot was not decarboxylated so it represented all of the ^{14}C present, whether as free leucine or as leucine residues in the protein. This was used to correct for any evaporation of the medium that may have occurred since the extent of degradation is equal to the ratio of free ^{14}C-leucine / total ^{14}C present (see the equation under Calculations). This ratio would be the same at a given point in the experiment whether there was evaporation or not.

Development of the method for quantitatively collecting $^{14}CO_2$ from the ^{14}C-leucine presented many difficult problems. The procedure that was finally used was an amalgamation of those used by Pine (52), Chang and Fenton (51), and Eisenberg and Dobrogosz (53).

Trial experiments, using a 0.1 N NaOH solution to collect $^{14}CO_2$, as Pine had done, gave very low results. For that reason it was decided to use ethanolamine as the CO_2 trap. After much trial and error it was determined that 0.1 mL was the best volume of ethanolamine to use. Eisenberg and Dobrogosz used 1 mL, apparently with good results. However, during the course of the present research, it was found that 1 mL and even a volume as small as 0.2 mL gave low and/or imprecise results. This may have something to do with the great quenching of ethanolamine. Perhaps using a different scintillation fluid or a method for quench correction that was better suited for larger volumes of ethanolamine would have solved the problem also.

The reaction of chloramine-T with an amino acid is as follows (50):



Chloramine-T was a very good decarboxylating agent. About 100% of the ^{14}C available for decarboxylation, that is, in the form of free ^{14}C-leucine, was collected and counted as $^{14}CO_2$ if the reaction was run for 2 hours at 30° or higher with swirling. The shorter 20 minute reaction time used by Chang and Fenton was tried and resulted in poor precision. Chloramine-T was not only much quicker than ninhydrin as a decarboxylating agent (51,60), it also gave more accurate and precise data. In trial experiments, where ninhydrin was used, only about 65% - 75% of the $^{14}CO_2$ that should have been

released from the labeled amino acid was actually trapped and counted.

The 1.0 mL of water, present in the Warburg flask along with the 1.00 mL aliquot of cell suspension, was needed in order to keep the viscosity of the reaction mixture low enough to allow complete decarboxylation of the ^{14}C-leucine. Under certain conditions, adequate swirling of the flask contents could not have been achieved without the additional one mL of water.

Results

The change in protein degradation as a function of time is shown in Figure 1 for the mesophile. The data used to calculate the percent degradation are contained in Table I.

For the mesophile, the degradation has been measured at five different temperatures. As can be seen, the extent of degradation and the overall degradation rate of the mesophilic proteins were greater as the temperature was raised from 30° through 55°. Chaloupka and Strnadova (6) found that the protein degradation rate in growing *B. megaterium* increased as the temperature was raised. Pine (52) showed that the degradation rate in growing *E. coli* also increased with temperature. This increase in degradation was expected since chemical reaction rates usually increase with temperature. In the case of enzyme reactions though, the rate increase with temperature is seen only up to a point due to the increasing significance of protein denaturation. The very low degradation observed at 65° is believed to be an example of this since protein degradation is, of course, an enzymatic reaction. On the other hand, the extent of degradation at 30° was probably so low because of low enzymatic activity at that temperature.

At the optimum growth temperature of the mesophile (37°), the degradation rate had a value comparable to that reported in the literature. Under the present experimental conditions, maximum growth was obtained between 6 and 8 hours at 37°. During this

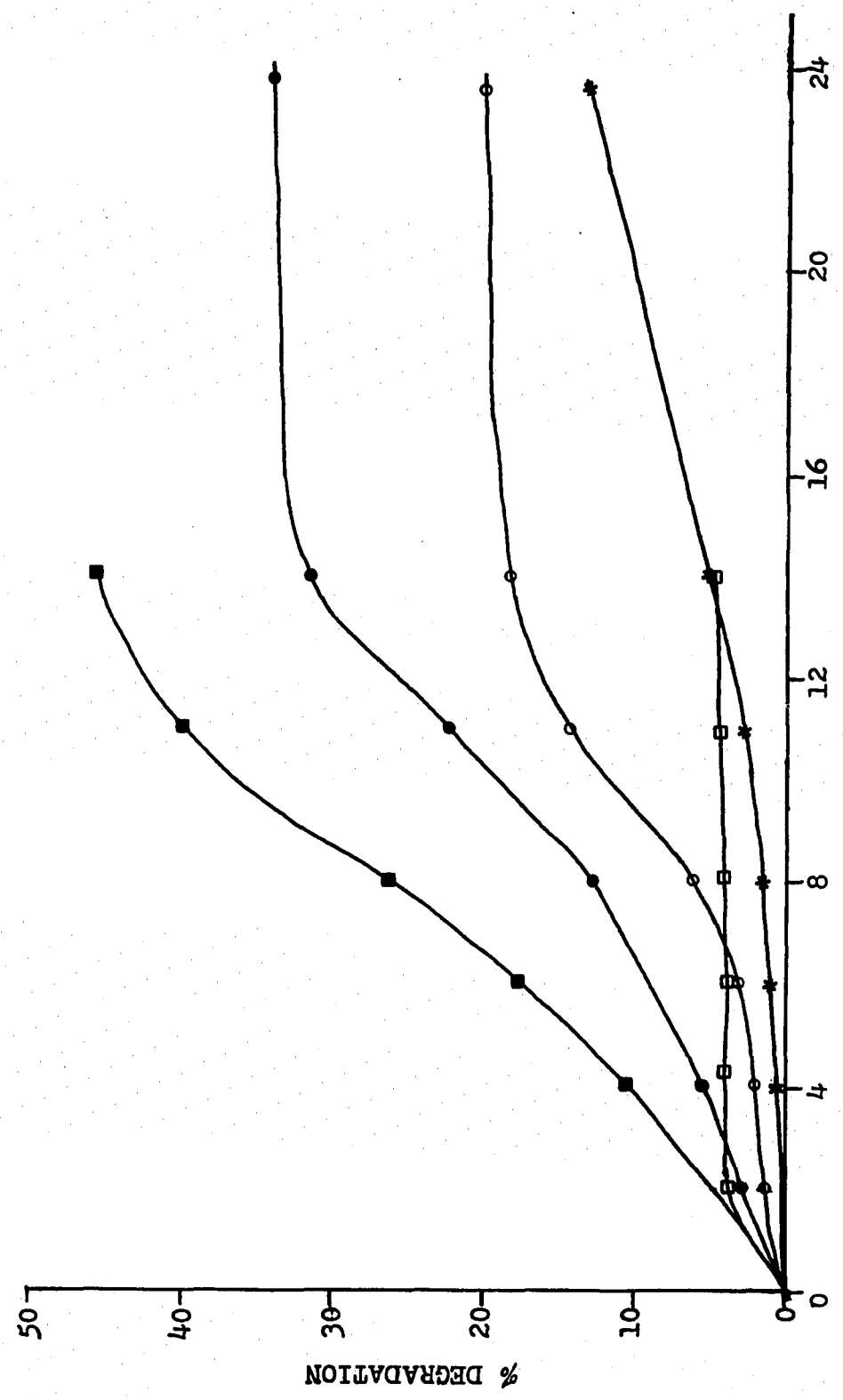


Figure I. Effect of Temperature on Protein Degradation in the Mesophile *Bacillus lichenifomis*. Symbols: *, 30°C; o, 37°C; ●, 45°C; ■, 55°C; □, 65°C.

Table 1

Values of $^{14}CO_2$ and Total ^{14}C Used to Calculate the Extent of Protein Degradation in the Mesophile+

Experiment Time (hours)	30 C		37 C		45 C		55 C	
	$^{14}CO_2$	Total	$^{14}CO_2$	Total	$^{14}CO_2$	Total	$^{14}CO_2$	Total
0*	106.0	–	67.9	–	94.7	–	108.0	–
2	–	–	83.8	309.9	173.8	589.6	–	–
	–	–	101.3	"	185.6	"	–	–
4	123.9	763.5	101.3	301.7	235.2	573.6	608.9	1027.5
	142.3	"	95.3	"	249.5	"	651.9	"
6	151.1	746.8	110.8	298.4	–	–	909.1	949.2
	149.5	"	115.8	"	–	–	936.8	"
8	176.7	718.4	138.2	287.4	422.0	559.2	1280.7	893.2
	151.1	"	151.2	"	438.6	"	1192.2	"
11	224.5	687.3	229.4	253.1	653.3	523.4	1679.7	803.8
	202.8	"	222.6	"	629.8	"	1593.0	"
14	265.7	664.6	239.5	238.0	658.2	392.9	1905.2	842.2
	274.5	"	261.4	"	650.8	"	1963.4	"
23.6	491.2	617.3	234.2	209.7	587.1	327.2	–	–
	491.2	"	250.6	"	591.8	"	–	–

+ The value used for background dpm ("B" in the equation under Calculations) was 36.2 for all experiments.

* The values reported at zero experiment time are the average values of each pair of zero time aliquots.

time period in the growth of the cells, the proteins had a degradation rate of about 1.3 % per hour (Figure 1). Typically, literature values for protein degradation rates are 1-2 % per hour in growing bacterial cells (8).

It can also be seen from Figure 1 that the degradation rates at 37°, 45° and 55° had maximum values somewhere between 6 and 12 hours and then leveled off at about the 14 hour point. The maximum degradation rates at the above three temperatures

occurred at approximately the same time that the cell growth was beginning to slow down. The leveling off of the degradation rates around 14 hours requires an explanation. The leveling off occurred at roughly the same time that the cells entered the stationary phase of growth, which is a result of nutrient exhaustion. One might expect that the rate of protein degradation would *increase* beyond this point since, as mentioned in the Introduction, most bacterial studies show an increase in degradation as nutrients are depleted (7). In the present experiment, however, the organism is a spore-former. Sporulation begins when nutrients are exhausted (61), so that, for this organism, the rate of degradation does not increase but levels off.

Figure 2 shows the change in protein degradation with time, for the thermophile, at three different temperatures. The data are contained in Table 2.

No significant degradation took place in the first 30 hours. This may seem surprising, however Kenkel and Trela (62) found no measurable breakdown when protein degradation was followed over a two hour period in growing cultures of the extreme thermophile *Thermus aquaticus*. Likewise, Epstein and Grossowicz (56) found only negligible degradation in a growing unclassified thermophilic bacillus when measured over a five hour period. How long the negligible protein degradation of these two thermophiles would have continued is a matter of conjecture. It can be seen from Figure 2 that, after 30 hours, the experiment run at the optimum growth temperature of the thermophile (55^{o}) resulted in the greatest degradation rate and that the experiments run above and below 55^{o} showed significantly lower rates. Epstein and Grossowicz likewise found that, for the thermophilic bacillus in the stationary phase, maximum protein degradation occurred around 45^{o} and 55^{o} (the optimum growth temperature) and was considerably lower at temperatures above and below these. Why the degradation rates in the present study remained unmeasurable until about 30 hours had passed is tentatively attributed to the formation of spores, followed by breakage of dormancy (spore activation) upon prolonged

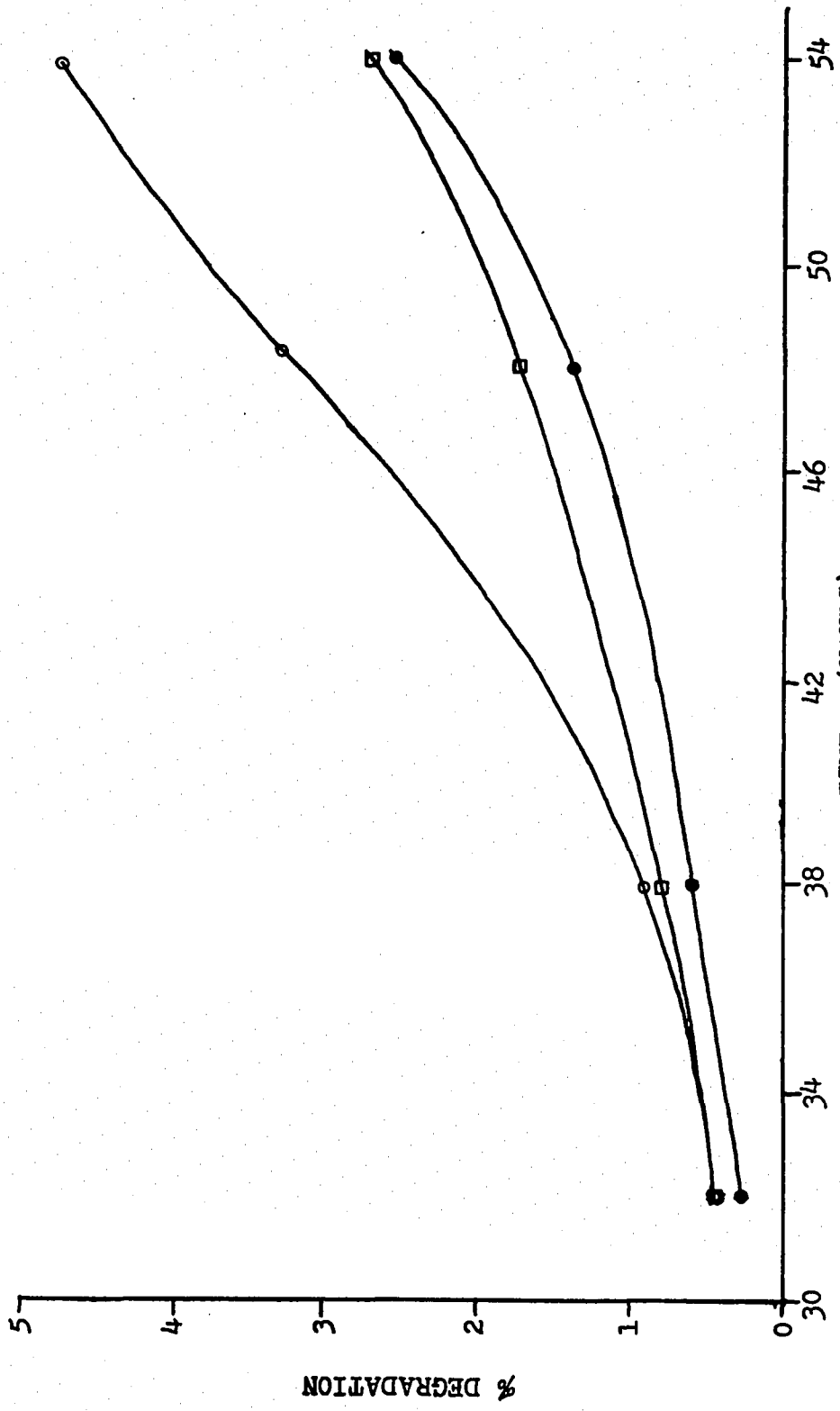


Figure 2. Effect of Temperature on Protein Degradation in the Thermophile *Bacillus stearothermophilus*. Symbols: ●, 45°C; ○, 55°C; □, 65°C.

Table 2

Values of $^{14}CO_2$ and Total ^{14}C Used to Calculate the Extent of Protein Degradation in the Thermophile+

Experiment Time (hours)	45 C		55 C		65 C	
	$^{14}CO_2$	Total	$^{14}CO_2$	Total	$^{14}CO_2$	Total
0*	44.9	–	46.1	–	34.0	–
32	57.3	987.4	63.5	845.4	47.6	674.6
	59.3	"	66.7	"	50.7	"
38	92.5	913.2	82.6	854.0	63.5	712.9
	72.2	"	85.2	"	61.0	"
48	116.4	1058.3	186.2	897.6	100.3	783.9
	115.9	"	188.2	"	97.6	"
54	166.4	969.4	255.8	932.9	154.8	956.0
	159.6	"	261.0	"	159.5	"

+ The value used for background dpm ("B" in the equation under Calculations) was 36.2 for all experiments.

* The values reported at zero experiment time are the average values of each pair of zero time aliquots.

exposure to heat.

Whatever the reason for the slow degradation rate, the data on the thermophile are in agreement with the now generally accepted conclusion that rapid protein turnover is not a factor in thermophily. In fact, they show that unusually slow turnover may be an important characteristic of thermophily.

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