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Effect of Decrease in the Protein Content of Cells on the Proteolytic Enzyme System

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In the previous works from this laboratory [1, 2] enormous changes were noted to take place in the enzymatic activity of *Escherichia coli* if the protein content of the cells was lowered by growing the bacteria in a nutrient solution containing plenty of sugar but little nitrogen. The nitrogen content of *E. coli* grown in a normal nutrient solution is about 13 % of the dry matter, but when *E. coli* is grown in a nitrogen-deficient medium, the nitrogen value can easily fall to 9—10 % and in some cases to half of the normal nitrogen content. The cells with different nitrogen content have very different enzymatic machineries; the activity of certain enzymes in low-nitrogen cells remains fairly similar to that in normal cells, while the activity of other enzymes is enormously lower. On the basis of the results it seems that the *indispensable enzymes, i. e.*, the enzymes without which the cell is unable to live whatever the composition of nutrient, retain their activity very well even though the nitrogen content is lowered. On the contrary, the *dispensable enzymes i. e.*, the enzymes which expand the living conditions of the cell, enabling it to utilize several nutrients but not being necessary in all nutritional conditions, lose most or all of their activity when the nitrogen content is decreased. For instance, the activity of saccharase lowered to about 10 % of the maximum when the nitrogen content of the cell dry matter fell from about 13 % to about 10 %, whereas the activity of catalase did not lessen. The enzymes which improve the living conditions of the cell are mostly adaptive, as for instance, lactase, maltase, and the major part of saccharase of our *E. coli* strain. Only a few per cent of the maximum saccharase activity is retained in the cells produced in saccharose-free nutrient solutions. The great dependence of the adaptive enzymes on the protein content of the cells has later been confirmed in regard to many

adaptive enzymes of *E. coli* (Virtanen and Winkler [3] in regard to lactase, De Ley [4] in regard to enzymes mentioned below).

The starting point for these investigations was the hypothesis advanced by Virtanen [5] that the proteins of the active young cells are practically entirely or at least for the major part enzyme proteins. If this be true, the lowering of the protein content of the cells brings about either a weakening of the activity of all the enzymes in proportion to the protein content of the cells *or* a strong decrease in certain enzymes while the others remain unchanged. The experimental results are in agreement with the latter concept.

De Ley [4, 6] has continued in Ghent the studies started in this laboratory on the correlation of the nitrogen content and respiration in *E. coli*. His thesis contains some very noteworthy results. The rate of respiration is relatively little lessened by the fall in the protein content of the cells. Only in cells with an extremely low protein content (abt. 4 % N) has the rate of respiration fallen to half or a little more. The fall of respiration is similar on different substrates. On the other hand, the anaerobic fermentation (acid formation) ceases entirely when the nitrogen content of the cells is lowered to below 8 %, and is already weak, abt. 10 % of the maximum, when the nitrogen content of the cells is still 10 %. Thus the lowering of the protein content of the cells makes the system of fermentation enzymes incomplete. In aerobic conditions the acid formation is only slightly lessened with the lowering of the protein content, and the curves representing the respiration and aerobic fermentation run parallel. New possibilities have thus been opened for elucidation of the correlation between the mechanism of respiration and anaerobic fermentation.

The adaptive formic hydrogenlyase, which catalyses the formation of H_2 and CO_2 from formate, disappears in N deficiency [4]. Cells with 10 % N of dry matter are already completely devoid of this enzyme. Decarboxylating enzymes disappear in low-nitrogen cells.

The results which so far have been obtained in regard to the dependence of the enzyme activity on the protein content of the cells open many new aspects. Fig. 1 illustrates these results graphically.

The present paper gives an account of our findings on the dependence of the proteolytic enzyme system on the nitrogen content of cells. The test organism used was the same strain of *E. coli* (K_3) as in the previous experiments of this laborbtory.

METHODS

E. coli which had been cultivated for 290—315 times in a saccharose nutrient solution containing 8 g $(NH_4)_2SO_4$ per 5 l, was grown for the experiment in a nutrient solution of the same composition as in the previous invest-

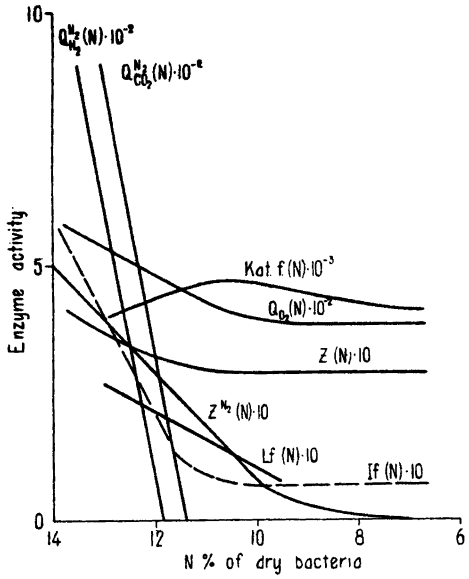

Fig. 1. Changes in the activity of different enzymes as affected by the N-content of E. coli. All activities calculated per N-content of cells.
Kat. f. (N) · 10^3 Catalase effect (Virtanen and De Ley); If (N) · 10 Saccharase effect (Virtanen and De Ley); Q_{O_2} (N) · 10^{-2} Respiration (De Ley); Z (N) · 10 Aerobic acid formation (De Ley); Z^{N_2}(N) · 10 Anaerobic acid formation (De Ley); $Q^{N_2}_{H_2}$(N) · 10^{-2H} H_2 formation from formic acid (De Ley); $Q^{N_2}_{CO_2}$ (N) · 10^{-2} CO_2 formation from formic acid (De Ley); Lf (N) · 10 Lactase effect (Virtanen and Winkler).

igations, *viz.*, 25 g saccharose, 25 g K_2HPO_4, 10 g NaCl, 100 mg $MgSO_4$, H_2O to 5 l. The amount of $(NH_4)_2SO_4$ ranged in different flasks from 8 to 0.1 g per 5 l. Since the yield of bacteria in higher N-concentrations was greater than in lower ones, only 1 litre cultures were used in ammonium sulphate concentrations of 8 to 1 g per 5 l, and 5 litre cultures in lower concentrations. In this way, sufficient bacteria were produced in all N-concentrations and an equal amount of bacterial dry matter could be used in each experimental series for determination of enzyme activity. In order to prevent losses in nitrogen, ammonium sulphate solutions were sterilized separately and added to the main solution just before inoculation. The pH was kept at about 6.5 by neutralizing with NaOH, the temperature of growth was 31° C, and duration 60 h.

Bacteria were separated by centrifuging, washed once, and suspended in 40 ml distilled water. Determinations of dry matter at 96° C and of nitrogen were made on the suspension. For each experiment a sample was taken containing 40 mg bacteria (calculated as dry bacteria). This was added to 120 ml of 2 % casein solution. First a 10 % casein (technical) solution was prepared with pH about 10. It was diluted to 2 %, and the pH lowered to 6.5. Toluene was added to all solutions.

Hydrolysis of casein was observed by determining amino-N with the Cu-method[7] (coeff. 0.28). In one experiment, besides, soluble N was determined as well as amino N according to van Slyke. Determination of soluble N was

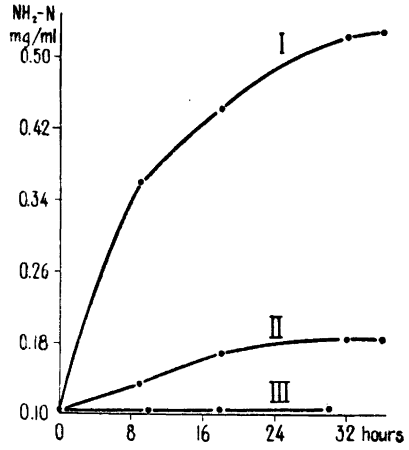

Fig. 2. Formation of amino-nitrogen from casein by E. coli.

Curve I: E. coli suspension plus casein
» *II: Casein*
» *III: E. coli suspension.*

made as follows. The pH of the solution was lowered to 4.6 with 1 % acetic acid, the flask was heated for 3 min in a boiling saturated solution of NaCl and let stand over-night. The contents were filtered through a filter Jena G 3, and nitrogen was determined on the clear filtrate.

Experiment 1. The casein-decomposing ability of normal nitrogen bacteria was examined 1. in casein solution *plus* bacteria, 2. in bacterial suspension without casein, and 3. in casein solution without bacteria. Only amino N was determined. The results appear from Fig. 2.

Experiment 2. Bacteria were grown in four different N-concentrations, and thus bacterial masses were obtained with the following N-contents.

	I	II	III	IV
$(NH_4)_2SO_4$, g/5 l	8	1	0.25	0.1
N % of bacteria dry matter	13.1	12.3	10.4	9.5

Decomposition of casein was observed with these bacterial masses by determining amino-N in the beginning of the experiment and after 5 and 20 days. The results are given in Table 1.

Experiment 3. Bacteria were grown in four different N-concentrations. The N-contents of the bacterial masses obtained were the following.

	I	II	III	IV
$(NH_4)_2SO_4$, g/5 l	8	1	0.25	0.1
N % of bacteria dry matter	13.2	12.6	10.4	10.4

Table 1. Decomposition of casein by E. coli with different N-content. NH_2-N determined by the Cu-method. In each experiment 40 mg bacteria (dry matter) in 120 ml 2 % casein solution, pH 6.5.

N of bacteria, % of dry matter	NH_2-N mg per ml		
	at start	after 5 days	after 20 days
13.1	0.100	0.284	0.478
12.3	0.101	0.277	0.464
10.4	0.100	0.271	0.441
9.5	0.100	0.270	0.413

The experiment comprised 8 test solutions each containing 40 mg bacteria dry matter in 120 ml 2 % casein solution. Four of these were examined after 6 days and four after 17 days. Decomposition of casein was observed with these bacterial masses by determining total N and amino N in the hydrolysate with the Cu-method. After that casein was precipitated and from the clear filtrate soluble N and amino N were determined both with the Cu and van Slyke methods. The results are seen from Table 2 and Fig. 3.

Table 2.

N of bacteria, % of dry matter	Total N of hydrolysate mg N/ml	NH_2-N of hydrolysate (Cu-method)		Soluble N % of total N	NH_2-N of the clear filtrate		
					(Cu-method)		(van Slyke)
		mg/ml	% of total N		mg/ml	% of sol. N	mg/ml
After 6 days							
13.2	2.53	0.33	13.0	64.2	0.28	17.3	
12.6	2.53	0.34	13.4	64.8	0.29	17.7	
10.4	2.40	0.31	12.9	61.7	0.28	18.9	
10.4	2.51	0.31	12.4	60.2	0.28	18.5	
Control *	2.50	0.11	—	8.3	0.04	—	
After 17 days							
13.2	2.50	0.50	20.0	78.8	0.42	21.3	0.42
12.6	2.51	0.51	20.3	78.1	0.42	21.2	0.45
10.4	2.39	0.45	18.8	79.9	0.38	20.0	0.39
10.4	2.50	0.46	18.4	77.6	0.39	20.1	0.40
Control *	2.50	0.15	—	17.8	0.06	—	0.06

* Without bacteria.

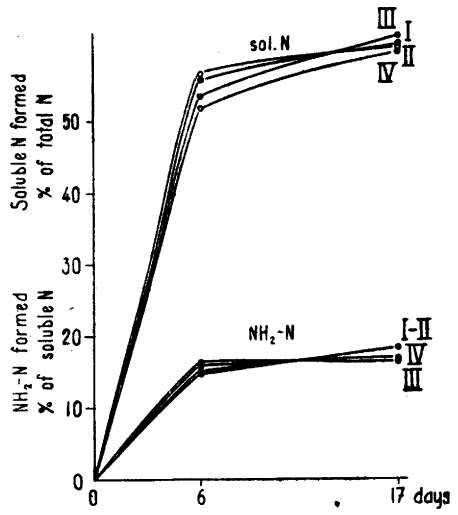

Fig. 3. Formation of soluble and amino nitrogen from casein by E. coli.

Curve I: 13.2 % N of dry bacteria
» *II: 12.6* » » » » »
» *III: 10.4* » » » » »
» *IV: 10.4* » » » » »

DISCUSSION

On the basis of the results obtained in the earlier works it is suggested that the decrease in the protein content of the cells is accompanied by a sharp weakening in the activity of the dispensable enzymes due to the fact that there will no longer be sufficient protein to build up the proteins of all the enzymes. A relatively slight lowering of the rate of respiration [4, 6] with a great decrease in the protein content of the cells is in agreement with this hypothesis since the indispensable enzyme system is then concerned. Good retention of the activity of catalase [1, 2] seems to imply that even this enzyme is indispensable for cells, though its role at the present is very unclear. The observation that the proteolytic enzymes maintain their activity fairly constant though the N-content of the cell varies from 13 to 9.5 % supports the idea that they are indispensable for the cell metabolism irrespective of the nature of nutrition. The proteolytic enzymes of *E. coli* are not adaptive.

The very distinct lessening of the activity of the disaccharide-hydrolyzing enzymes, saccharase [1, 2] and lactase [3], which takes place even when the nitrogen content of the cell lowers only 10—20 % of the optimum value, further confirms the idea that the dispensable enzymes are largely dependent on the protein content of the cells. The function of these enzymes is, according to the present knowledge, to enable the cells to use the particular disaccharides for their nutrition. If these enzymes disappear, the cells are no longer able to grow on the respective disaccharides though they are able to do so by means of other suitable carbon compounds, such as glucose. The said enzymes, arising

through adaptation, widen the living conditions of the bacteria but they are not indispensable for life. When the nitrogen nutrition is very poor the possibilities for the formation of adaptive enzymes seem to be lessened. So, for instance, our strain of *E. coli*, when transferred from lactose to lactose with only 0.1 g ammonium sulphate per 5 litres, starts to grow more slowly than when transferred from lactose to corresponding glucose nutrient solution. And yet the velocity of growth is almost the same in both solutions when the ammonium sulphate amount is high.

The enzyme decomposing formic acid disappears completely from our strain of *E. coli*, according to De Ley [4], when the N-content of the cell falls only 10 %. This enzyme is also adaptive and evidently not indispensable for life under all conditions. The rapid decrease in the anaerobic fermentation accompanying the drop in the N-content of the cell, and its total cessation when the N-content falls about 40 % is particularly noteworthy, as mentioned already at the beginning of this paper. According to our findings, the less the N-supply of the nutrient solution the more oxygen is needed by *E. coli* for its growth. These findings are in full accordance with those of De Ley on the disappearance of anaerobic fermentation.

The examination of the enzymatic activity of low-nitrogen bacteria and other organisms and the comparison with the respective normal nitrogen cells seems to open new lines for research in many directions.

SUMMARY

The proteolytic enzyme system of *E. coli* (strain K_3) retains its activity practically constant while the N-content of the cells falls from 13 % to 9.5 %. The dispensable enzymes, saccharase and lactase, decrease very sharply while the N-percentage lowers to 11.5 or more. The adaptive enzymes which are necessary only in definite nutritional conditions seem, as a rule, to decrease powerfully or to disappear entirely with the lowering of the N-content of the cells.

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