Adaptive Enzymes

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Preface

After working three months on adaptive enzymes of E. Coli and making very little headway, it seems worthwhile to summarize the reasons for the study and the progress made. This summary may then provide a basis for deciding what experiments, if any, should be done when it is possible to resume work in biophysics.

Reasons for program

Adaptive enzymes appeared to be an attractive point of attack on the more general problem of protein synthesis because in the adaptive process the cell could be directed to synthesize one particular protein as contrasted to the duplication of all cellular components that occurs during growth. Furthermore, adaptation is generally possible even in a nitrogen deficient medium where normal growth stops. For this reason it appeared that perhaps the process of adaptive enzyme formation involved only certain phases of protein synthesis and these particular steps might be more understandable than the complete process.

It is known that the formation of adaptive enzymes requires (with certain exceptions) the presence of the appropriate gene (Spiegelman, Monod, Lwoff) and the substrate. However, once initiated, the synthesis can continue in the absence of the gene (Spiegelman) or in the absence of the substrate (Hinshelwood). Furthermore, most enzymes show some adaptive behaviour as they are synthesized in greater quantity in the presence of their substrate. Consequently, adaptive enzymes are not unusual types of proteins and any insight into their formation should apply quite generally.

As adaptive enzyme formation proceeds without external nitrogen or sulfur sources, the origin of the nitrogen and sulfur was of interest. In the case of yeast, Spiegelman found that one adaptive enzyme was formed as another diminished indicating perhaps that one particular enzyme was transformed into another. If,

in this process, the enzyme was broken down into amino acids and those were then resynthesized, an exchange of labelled amino acids might be observed.

Under various conditions the concentration of RNA and DNA in the cell varies. If these are involved in the adaptation process, then some correlation might be observed between their concentration in the cell and the rate of adaptation.

Finally, the intensive study of any particular process using all the available techniques (particularly P^x and S^x) usually yields some new information. On this basis the study of adaptive enzymes was started.

Choice of Cell

E. Coli was used because of our previous experience and because adaptation is much more rapid than in yeast.

Choice of Enzyme

The sugars available in the laboratory were tested as substrates using glucose adapted cells and measuring oxygen uptake in the warburg. The results Table I show that lactose and arabinose are the most suitable as there is a very low initial rate, a rapid adaptation and a high final rate.

Table I

	Initial Rate	Final Rate	Adapting Time
Glucose	100	100	-
Mannose	100	100	-
Galactose	50-100	100	30 minutes
Arabinose	10	100	40 minutes
Ribose	30	30	3 hours +
Xylose	12	75	2 hours
Sucrose	10	3	-
Lactose	5	100	40 minutes
Maltose	100	100	-

Effect of Medium

Effect of NH_4 on glucose metabolism

The oxygen uptake with glucose as substrate is roughly doubled by the addition of NH_4 to the medium. This has been ascribed to the increased energy requirements due to growth which affects glucose metabolism by using up ATP and making ADP available as a phosphate acceptor.

This theory is not acceptable because the effect of NH_4 is present with 1) cells in a lag phase that are not growing. 2) cells of such high density that there is no growth. 3) Lysine deficient cells which cannot grow because of the absence of lysine.

There are probably some synthetic processes occurring in the presence of NH_4 which account for the increased uptake of oxygen (and a comparable increase in uptake of PO_4). One such process could be the synthesis of amino acids which then diffused out into the medium. In one experiment we looked for radioactive organic sulfur in the supernatent of lysine deficient cells incubated with glucose, NH_4 and S^*O_4 but without lysine. After two hours incubation a small quantity of non-sulfate sulfur was found, some of which would precipitate with an excess of cystine. These experiments should be followed up.

Effect of NH_4 on adaptation.

The same effect of NH_4 is found with lactose or arabinose as substrates, i. e., the final rate is about double in the presence of NH_4 but the time required for adaptation is the same. NH_4 does not appear to be involved in the formation of the adaptive enzyme.

Other ingredients of the medium

The adaptation time was not affected by the addition of methionine using normal cells or by the addition of lysine with lysine deficient cells. The effect of K, Mg, and SO_4 and PO_4 on adaptation should be tested. However,

we have not found any requirements for the adaptation process similar to the comparable requirement for growth. It appears that the cell contains the material for the new protein within itself and only the substrate and energy (derived from the substrate or from cellular reserves) are required to initiate the transformation.

State of the Cell

Growing cells, cells grown on low glucose and cells grown on full glucose show increasing times for adaptation similar to the increasing time required for the initiation of growth. This would be expected if one of the rate limiting factors in growth is a process similar to adaptation.

Low K and Mg Cells

Cells were grown in a low K low Mg medium, then washed and put in media containing Mg only K only and a control containing K and Mg for further growth. This was clearly limited in both cases, linear growth being observed in the K deficient medium and a levelling off in the Mg deficient medium. After the density had doubled the cells were washed and tested for adaptation to lactose - NH_4^+ in a medium with adequate K and Mg. The adaptation time was doubled by this pretreatment in deficient media. Growth on glucose in a complete medium was also delayed. These delays were probably not due to the time required to take up the lacking K or Mg as the oxygen uptake (with glucose) returned to normal as soon as the K ∩ Mg was restored. The main object of this experiment was to test adaptation of cells with different DNA/RNA ratios. However, this failed as measurements of RNA and DNA (by P) showed that both the DNA and RNA were normal.

Nitrogen deficient cells

If cells contain protein precursors (peptides?) these might be the source of nitrogen for the adaptive enzymes. This idea was tested by incubating the

cells with glucose in a N-free medium before adaptation. No effect was found on the rate of adaptation. As a more stringent test cells were adapted to arabinose in N-free medium and then adapted to lactose. This pre-adaptation did not affect the rate of the second adaptation. Consequently, there is no evidence that the nitrogen of adaptive enzymes is formed from any limited supply of precursors.

Loss of adaptive enzymes

Unlike Spiegelman we have not been able to reverse the adaptation in any of the treatments tried. These include incubation up to 24 hours with glucose, galactose, arabinose, $\pm$ NH_4 and in buffer only. The lactose adaptation remains after 5 generations of growth on glucose. We have not attempted the prolonged reversion methods of Hinshelwood. In a few cases a slight loss (20 %) has been found but this has not been repeatable.

Isolation of the lactose enzyme

It was found that supernatents from lactose cells and extracts of lactose grown cells speeded up the adaptation. This was traced to the presence of the lactase enzyme. As a quick test 2 ml of glucose grown cells (old) 1/2 ml of lactose and 1 drop of methylene blue were used. With glucose instead of lactose the M. B. was decolorized in 4 minutes instead of 40. A strong enzyme solution added would also decolorize in 4 minutes. This test was used to follow the lactose activity of various preparations. Roughly 50 % of the enzyme activity could be extracted from the cells or an acetone power of the cells by shaking at 60 cycles with glass beads. The enzyme could be precipitated with $(NH_4)_2SO_4$ or 75 % ethyl alcohol, it was stable in solution, could be dialyzed, and was destroyed by boiling. No activity was found in similar extracts of glucose grown cells. At this point Lederberg's article appeared which confirmed what we knew but did not help in further purification.

Cells were grown with P^x and S^x and these tracers used together were helpful in following purification. The methods used were not satisfactory but some little progress was made.

1. Very little can be extracted from acetone powder with PO_4 buffer H 4.5-8.
2. About 50 is extracted on shaking with glass beads (cells or acetone powder).
3. A second extraction does no good.
4. The extract is cloudy and can be cleared by repeated centrifugating without loss of activity.
5. Precipitation by alcohol and NH_4 SO_4 depends on concentration and previous purification and is not reliable.
6. Precipitation by NH_4SO_4 23 saturated does improve the S/P ratio but causes a loss of activity.
7. No improvement was achieved in the enzyme /S ratio.
8. The spectrum of the active extract looks like pure nucleic acid and no way was found to remove the NA and leave the enzyme.

Work on purification was interrupted. As a pure enzyme is highly desirable (see below) further trials may be attempted later, particularly if Lederberg has any further information.

Uptake of radioactivity during adaptation

Cells incubated with glucose and lactose one hour in N free medium with radioactive cystine.

	Glucose	Lactose
Total	37.	22
TCA Sol	24	11
TCA ppt	5.8	5.3

This uptake corresponds to .03 mg of sulfur per ml of cells (in the TCA ppt) or

1/100 of the total sulfur content. As there is no difference between glucose and lactose no conclusions can be drawn. Furthermore, the losses from both groups of cells on further incubation were negligible whether the second incubation was carried out in glucose or lactose. This is not surprising as the lactose activity is not removed.

Uptake of SxO_4

Cells incubated 1 hour $\pm$ NH_4 with glucose and lactose

	Glucose		Lactose	
	$+NH_4$	$-NH_4$	$+NH_4$	$-NH_4$
Total	5.6	.10	1.5	.12
Protein	2.1	.037	.47	.045

In presence of NH_4 there is much more SO_4 uptake with glucose due to more growth, etc. Without NH_4 there is slight excess with lactose possibly due to formation of adaptive enzyme.

As the effects are small it will be possible to detect incorporation of tracers into the adaptive enzymes (which may be 1/10% of the total protein) only by:

a) Preferentially removing the enzyme.

b) Purifying the enzyme.

The enzymes studied so far cannot be removed. Catalase (studied by Abelson) does decrease and might offer possibilities. Purification seems difficult.

Discussion

Most all the indications obtained so far are negative. The most consistent picture is that some cellular protein (probably an enzyme) is slightly altered by contact with lactose to form the lactase enzyme.

$$E \longrightarrow E^{*}$$

Once E is converted to E^*, E^* not E is synthesized by the cell even in the absence of lactose. As E is a slightly more stable configuration, reversion E^* → E may occur after many generations (Hinshelwood). The gene may not be involved in this transformation at all. In the absence of the gene an alternate form of E may occur which cannot transform to E^*. According to this picture there would be no uptake of material from the medium and no exchange of amino acids. The only hope of proof would be in obtaining highly purified lactase enzyme and demonstrating similarity to a protein extracted by the same process from glucose adapted cells which did not have lactase activity.

This transformation might conceivably be induced in vitro.

Further work requires either a good method for purification or a fresh approach.

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