The Non-linear Relationship Between the Enzyme Activity and Structural Protein Concentration of Thiogalactoside Transacetylase of *E. coli*

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Introduction

When the appropriate inducer is added to a growing culture of *E. coli*, the synthesis of the three structural proteins in the Lac operon is stimulated. These three proteins, β-galactosidase (G), β-galactoside permease (M) and thiogalactoside transacetylase (TA), have been widely reported to be synthesized coordinately. That is, the ratio of the activities of any pair (G to TA, for example) is a constant which does not depend on the degree to which the operon has been induced (1, 2, 3). While confirming these results for most levels of induction, experiments reported here show that at low levels of induction the appearance of G and TA enzyme activity is not coordinate. Rather, the formation of TA enzyme activity lags behind that of G. This non-coordinate appearance of TA activity with increasing inducer concentration is particularly noticeable in strains with y^- polar mutations which produce normal levels of G but low levels of TA. In these mutants significant amounts of G activity are formed at concentrations of inducer which give no TA activity. From the data presented in this paper, it is possible to show that the observed non-coordinate phenomena are consistent with the assumption that structural protein synthesis of TA and G is completely coordinate while only their maturation to active enzyme is discoordinate. Thus, it is not necessary to invoke non-coordinate transcription or translation.

Results

(1) The non-coordinate appearance of G and TA enzyme activity. The y gene

(specifying M) of the Lac operon maps between the z gene (specifying G) and the a gene (specifying TA). A polar mutant in y will have two effects. First, it will destroy M activity and thus prevent the accumulation of β-galactosides. Secondly, it will lower the amount of TA made, i.e., it is polar. The first of these effects allows us to change the degree of induction by simply varying the external level of inducer. The effect of increasing concentration of inducer on the steady state level of G and TA activity of two y^- polar mutants was studied. The mutants used are NG745, a UGA mutant whose maximal level of TA activity is about 5% of wild type and NG707, a UAG mutant with a maximal TA level of about 23% wild type. Both of these mutants produce wild type levels of G at saturating inducer concentrations. In Fig. 1, TA activity at each inducer concentration is plotted against G activity. Note that if the linear (i.e., coordinate) portions of the curves in Fig. 1 are extrapolated they reach a zero rate of TA synthesis while there is still significant G synthesis.

(2) The origin of the non-coordinate induction pattern. As we have seen above, inducer at low concentrations evokes the appearance of G activity more efficiently than TA activity. The possibility that the inducer (IPTG in this case) has some direct effect on the activity of the TA or G proteins themselves can be tested. If there were a direct inducer effect, then in a strain lacking functional repressor (i.e., i^-) there should be some systematic effect of inducer concentration on G or TA activity. When the experiment shown in Fig. 1 was repeated using the strain i^-NG707, increasing concentrations of inducer had no effect on the constitutive level of TA or G activity found. Thus we are left with two reasonable classes of alternative explanations for the observed non-coordinate phenomena.

I. The translation or transcription of the structural genes of the Lac operon is not completely coordinate.

II. The maturation of TA structural protein to active enzyme is concentration dependent.

What we would like to show here is that our data are consistent with II

if coordinate synthesis of TA and G are assumed.

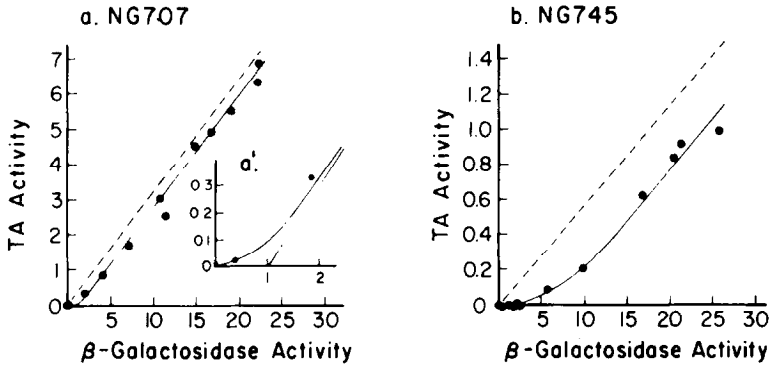

Figure 1: Relationship between TA activity and G activity at varying IPTG concentration. The E. coli K12 strains used are NG707 and NG745 (4). Both are mutants in the β-galactoside permease gene of the Lac operon. To insure that secondary mutations do not affect the results, both mutants were transferred into the same background, M0 sm^r, an F^- phototroph. To confirm the absence of differences between the TA genes of the strains used, several independent revertants to $lactose^+$ and $melibiose^+$ were selected for NG707 and NG745. These were tested to eliminate revertants due to suppression. Revertants of both strains synthesized the same level of TA activity. Carrier protein extracts or cell suspensions were made using $F^-514Lw\ sm^r$ (4). All cultures were grown in the following medium: 1% casamino acids, 1 mM $MgSO_4$, in M9, pH 7.3. For the growth of 514 Lw, 2.5 ml of 2 mg/ml tryptophan were added per 100 ml of medium. IPTG* was used as inducer, and was present in varying concentration in the above casamino acids medium throughout the growth period. Each culture was innoculated with 1/20 of the final culture volume of fresh, overnight, standing Penn broth culture. The cultures were then shaken at 37°C for 4 hours at which time they were growing exponentially with a doubling time of about 55 to 60 minutes. Cell density was determined using a Klett densitometer. β-galactosidase was assayed according to the procedure described by Zipser (1963). The whole cells were prepared for assay by adding two drops of chloroform and 0.025 ml of a 0.25% solution of SDS to 1 ml of cell culture. This was then shaken for 15 minutes at 37°C before assay. Transacetylase Assay - This procedure represents a modification of the assay according to Alpers, Appel, and Tomkins (3). The exponentially growing culture was centrifuged at 12,000 rpm for 10 minutes, and resuspended in 1/10 volume of 0.05 M Tris, 0.01 M EDTA at pH 7.9. The suspension was sonicated at 50 watts of power in a Biosonic Sonicator for 30 seconds, and then heated at 70°C for 5 minutes. Agglutinated protein was removed from the extract by centrifugation. This crude extract, if necessary, was diluted with a crude extract of a Lac deletion prepared in an identical fashion, so as to give an activity of about 5% of wild type. Dilution is linear. A 50λ aliquot of the diluted crude extract was mixed with 50λ of an assay medium containing 5 mg/ml acetyl coenzyme A and 250 mg/ml IPTG in 0.05 M Tris, 0.01 M EDTA, pH 7.9. The assay mixture was incubated at 25°C for one hour. The reaction is linear during the one hour of assay. The assay was stopped by the addition of 3 ml of a solution of 25 mg/100 ml DTNB in 0.05 M Tris, pH 7.9. Color development was determined at 412 mμ in an Hitachi Spectrophotometer. The different crude extracts were normalized by determining the absorbance at

* The abbreviations used are: IPTG (isopropyl-1-thio-β-D-galactoside), SDS (sodium dodecyl sulfate), DTNB (5,5'-dithiobis-2-nitrobenzoic acid).

260 mμ. One unit of TA = OD_{412}/OD_{260}/hour. Daily results were normalized to a standard value of NG707. Each point represents the enzyme activity of NG707 (a) and (a') or NG745 (b) when grown at a specific IPTG concentration. The insert, a', is an enlargement of the initial portion of a. The dashed lines give the expected concentrations of TA protein assuming true coordinate synthesis.

A consequence of II is that below some minimal concentration of structural protein, active TA enzyme is not efficiently matured. This threshold concentration should be a property of the TA structural protein itself. In particular, the threshold should not be affected by inducer concentration or the presence of y^- polar mutations. From this we would predict that if II were correct then the plot of TA structural protein concentration vs. TA enzyme activity should be the same for all polar mutants. It is not yet practical to measure inactive TA structural protein directly. However, it is possible to determine the concentration of TA structural protein to be expected assuming possibility I to be incorrect, i.e., assuming that all the structural proteins of the operon are synthesized coordinately. This is done by constructing, for each mutant, a line on the TA vs. G activity plot (Fig. 1 dashed line)

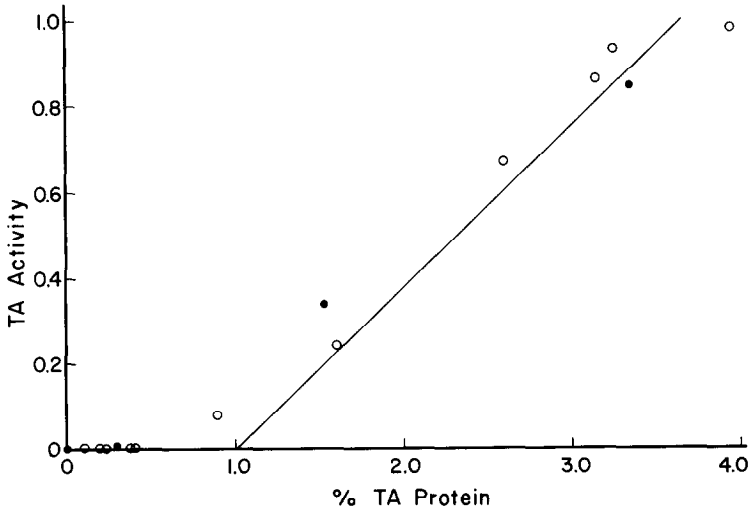

Figure 2: Relationship between TA activity and TA protein concentration. The results of plotting expected TA protein concentration, expressed as % saturated wild type level, against observed enzyme activity units are shown for NG707 (closed circles) and NG745 (open circles). The solid line has the slope and intercept of the linear portion of the solid line in Fig. 1a.

which has the same slope as the linear portion of the observed curve but is displaced so that it passes through the origin. This new straight line represents a plot of TA structural protein vs. G activity, assuming that both are synthesized coordinately. Now we can test the consistency of II with the observed data by plotting TA activity vs. expected TA structural protein for both polar mutants used in these experiments. Fig. 2 is such a plot and as can be seen, the points from both mutants fall on the same curve. From this we conclude:

a) Our data re consistent with explanation II.

b) That if II is correct, a pool of about 0.5% of the wild type saturated level of TA structural protein must accumulate before enzyme activity begins to appear. The full rate of enzyme maturation is not reached until the pool reaches about 1.0%.

Discussion

While effects on the levels of transcription and translation have not been ruled out, they seem quite unlikely considering our current knowledge of Lac operon control. We feel that the most reasonable hypothesis explaining these results is that a dimer or some higher multimer of TA is the enzymatically active form, and that the rate limiting step in the formation of active TA is a concentration dependent monomer to multimer reaction. From the data, it is not possible to determine how many monomeric units compose the active TA enzyme, but, based on results reported by Zabin (7), the dimeric form appears most likely. It is interesting to note that the formation of the multimer does not seem to be an equilibrium reaction since dilution of the TA enzyme to zero concentration is linear.

The results presented here must be considered in evaluating studies of the kinetics of induction of the lactose operon, or of the polarity of highly polar z or y mutants in which measurements of very low concentrations of TA protein are made. The magnitude of the time lag between the appearance of G activity and TA activity after induction found by Alpers and Tomkins (8) and Leive and Kollin (9) is greater than expected based on an independent estimate of the

rate of growth of the polypeptide chain. This discrepancy may be accounted for, in part, by the concentration dependent formation of active TA enzyme. By assuming a linear relationship between TA activity and TA protein concentration, instead of the one presented here, significant errors in estimating TA protein concentration will be introduced at low TA activities.

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