MUTANTS OF ESCHERICHIA COLI DEFECTIVE IN THE B PROTEIN OF TRYPTOPHAN SYNTHETASE I. SELECTION AND CLASSIFICATION

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AN investigation of the genetic control of the structure of a specific protein is likely to be influenced by the method of selecting the mutants used for study. If the protein has enzymatic activity and use is made of the lack of enzymatic activity in mutants for identification and selection, there will doubtless be many alterations of the protein which pass unnoticed because their effect on the enzymatic activity is slight enough to permit them to evade the screen. This failure to find partially inactivated proteins will be most pronounced when the wild-type strain possesses a means of increasing the rate of synthesis to a level far above normal. A modest decrease in the enzymatic capability of a protein can then be compensated for by an increase in the amount of that protein synthesized. These considerations apply to most of the studies of gene-protein relationships in bacteria, no less those concerning the enzyme tryptophan synthetase (TSase) in *Escherichia coli*. Using the penicillin technique, the selection of auxotrophs unable to grow on tryptophan from wild-type *E. coli* has yielded strains with certain specific alterations in one or the other of the two protein components of this bipartite enzyme (YANOFSKY and CRAWFORD 1959). Since derepression can bring about a level of synthesis of these components 50 to 100 fold greater than in wild type, we began a search for a less discriminating screening method.

Prior investigations reviewed by YANOFSKY (1960) have demonstrated that the biosynthesis of tryptophan depends on the conversion of indole-3-glycerol phosphate (InGP) to L-tryptophan. In this process the glycerol phosphate side chain of InGP is cleaved and replaced by the alanine moiety of L-serine. Although indole does not appear as a free intermediate during this process (YANOFSKY and RACHMELER 1958; CRAWFORD and YANOFSKY 1958), it can be utilized by TSase for tryptophan formation in the presence of serine. Notwithstanding that the overall reaction, InGP to tryptophan, requires the presence of both TSase components, this latter reaction, indole to tryptophan, can be catalyzed by the TSase B-component acting alone. In ordinary buffers the activity of the B protein in this catalysis is markedly stimulated by component A; it can be estimated that the B component performs with only 3 to 10 percent maximal

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efficiency in this reaction in the absence of component A, (HATANAKA, WHITE, HORIBATA and CRAWFORD 1962). It is not known that this value accurately reflects the functioning of the protein within the cell, but it is known that mutants lacking component A but possessing normal component B when grown on indole derepress component B synthesis to the extent of forming approximately ten times the normal amount of protein (YANOFSKY and CRAWFORD 1959). Therefore cells of this type, lacking component A but able to utilize indole in place of tryptophan, were irradiated and the survivors screened by the penicillin selection method for mutants unable to grow on indole. It will be shown that this procedure has permitted the selection of mutants modified specifically in the B protein, and that many of these modifications are unlike the ones previously seen in mutants selected from the wild type.

MATERIALS AND METHODS

The auxotroph of *E. coli* K-12 used was isolated by YANOFSKY using the penicillin method following ultraviolet irradiation; it was first called T8 (YANOFSKY 1957), but was redesignated A2 when its mutational defect was localized to the A protein (YANOFSKY and CRAWFORD 1959). Cells in the exponential phase of growth in a complex medium (L-broth, LENNOX 1955) were centrifuged, resuspended in the minimal medium of VOGEL and BONNER (1956), irradiated and selected according to the ADELBERG and MEYERS (1953) modification of the penicillin technique. In all procedures subsequent to irradiation the minimal medium contained 2 mg/ml glucose and 20 μg/ml indole. After penicillinase treatment, tryptophan was added to a final concentration of 3 μg/ml. Colonies appearing during the ensuing nine days were transferred to complete medium then screened for their ability to grow on minimal medium plus indole (10 μg/ml) or tryptophan (10 μg/ml) agar plates. Isolates which grew on tryptophan but failed to grow on indole were retested from the growth on the tryptophan plate. Only those which again failed to show growth on indole were selected for additional testing. Mutants were maintained by bimonthly transfers on complete medium (L-agar, LENNOX 1955).

Accumulation tests: Cultures grown 16 hours or more on a reciprocal shaker at 37°C in suitably supplemented minimal medium were centrifuged to remove the cells. Indole and indoleglycerol were detected in the supernatant fluids by qualitative tests previously described employing Ehrlich's reagent and a ferric chloride reagent respectively (YANOFSKY 1956). If examination of the culture under ultraviolet light disclosed a blue-fluorescent substance, it was identified as anthranilate or 1-(o-carboxyphenyl amino)-1-deoxyribuloside (anthanilic deoxyribuloside) by extraction from the acidified supernatant fluids into ethyl acetate and chromatography of the extract in the solvent of DOY and GIBSON (1959).

Extract preparation: Cells were grown 16–18 hours (to the stationary phase) in 4-liter flasks containing 1.5 liters of minimal medium plus 4 to 6 μg/ml tryptophan and 0.2 to 0.5 mg/ml acid-hydrolyzed casein (Difco). Cells were

harvested by centrifugation, washed once in 0.1 M tris buffer (pH 7.8), and resuspended in four times their weight of the same buffer. Succeeding steps were performed at 0° to 4°. After disruption for 20 minutes in a 10 kc sonic oscillator (Raytheon), cell debris was removed by centrifugation at 30,000 × g for 30 minutes. Extracts were stored at −15° until use.

Enzyme assays: The assay for the conversion of InGP to indole was performed as described by YANOFSKY and STADLER (1958), except for the inclusion of pyridoxal phosphate (10 μg/ml) instead of hydroxylamine. The assay for the conversion of indole to tryptophan by noting indole disappearance in the presence of pyridoxal phosphate and serine was also described by YANOFSKY and STADLER (1958). The buffer and ionic strength of the incubation mixture were varied as will be described. The conversion of InGP to tryptophan was quantitated by assaying the presence of tryptophan microbiologically (YANOFSKY 1954) in a 1 ml reaction mixture which had contained 0.4 μmole of InGP, pyridoxal phosphate, serine and a buffer to be described. The disappearance of InGP from this reaction mixture was occasionally quantitated by YANOFSKY's (1956) method.

A unit of activity in any reaction is the conversion of 0.1 μM of substrate to product in 20 minutes at 37°. Protein was determined by the Lowry method and specific activity is expressed as units of activity per mg protein.

Component A: Unless otherwise mentioned, all assays were done in a threefold or greater excess of normal A protein obtained from extracts of the mutant B8 (see HENNING, HELINSKI, CHAO and YANOFSKY 1961).

B-CRM assays: Some of the proteins immunologically related to component B (B-CRM's, i.e., cross-reacting materials) obtained in this study seemed to react slowly with antiserum to the normal component. A similar phenomenon has been noticed in some mutationally altered *Neurospora crassa* TSase proteins by SUSKIND, WICKHAM, and CARSIOTIS (1962). Consequently, the following procedure was adopted for assay of B-CRM. A suitable amount of a CRM-containing extract was incubated with 2.5 neutralizing units of anti-normal component-B rabbit serum in 0.4 ml of 0.025 M potassium phosphate buffer, pH 7.8, containing 125 μg/ml bovine serum albumin, 2.5 μg/ml pyridoxal phosphate, and 1.2×10^{-3} M β-mercaptoethanol. The mixture was allowed to stand for 16 hours at 5°, then 3.5 units of normal component B were added. After a pre-incubation of 10 minutes at 0°, the usual indole to tryptophan substrates were added along with excess component A. The titer of B-CRM was estimated by the decrease in neutralizing ability of the antiserum. Controls with normal rabbit serum, without B-CRM and without normal B were performed for each extract.

RESULTS

From a single mutant selection experiment 86 tryptophan requiring mutants of strain A2 were obtained. These were assigned the numbers A2B10 to A2B95 to accord with their supposed double-mutant nature. Following the preliminary classification tests, one of these strains (A2B17) was lost. Enough information had been obtained to permit classification, however, so this strain is included

in the discussion to follow, though information on the localization of its mutant site and the precise nature of its protein defect may not be forthcoming.

Accumulation tests: When examined for accumulation products after growth in minimal medium plus glucose (2 mg/ml) and tryptophan (5 μg/ml), 85 of the 86 presumed double-mutant strains accumulated indoleglycerol as does the parent strain A2. Variable but usually small amounts of anthranilic deoxyribuloside and anthranilate were also occasionally detected. Unless the accumulation of these fluorescent compounds was marked, and these cases will be enumerated later, their presence was attributed to a "pile-up" of precursors before the main accumulation product. Traces of indole, attributable to tryptophanase action, were occasionally detected. (In support of this interpretation, it was observed that when the strains were grown in an excess of tryptophan, indole accumulation increased dramatically while indoleglycerol accumulation was drastically reduced). The one strain which failed to accumulate indoleglycerol was strain A2B27 which carries in addition to the ancestral A2 marker a "multisite mutation" or deletion covering a portion of the B gene and some of the adjacent chromosomal material concerned with enzymes prior to TSase in the tryptophan pathway, (CRAWFORD and JOHNSON, to be published). This strain accumulates anthranilate.

Four of the double mutants examined seemed to accumulate less than the usual amount of indoleglycerol and more than the usual amount of fluorescent material. These were A2B19 and A2B30, where the major fluorescent product was anthranilic deoxyribuloside, and A2B47 and A2B52, whose major fluorescent product was anthranilate.

Temperature sensitivity and leakiness: Cells from freshly grown slants of the 86 mutant stocks were suspended in sterile saline solution and inoculated onto duplicate minimal plus indole (5 μg/ml) and minimal plus tryptophan (5 μg/ml) agar plates. One set of plates was allowed to grow at room temperature (*ca.* 25°) while the other was incubated at 37°. All strains grew well on the tryptophan supplement at either temperature. Five strains showed slow but progressive growth on the indole-supplemented plates. These (A2B47, A2B52, A2B64, A2B67 and A2B68) were provisionally designated "leaky on indole." Only one, A2G67 seemed to grow better on indole at room temperature than at 37°.

Reversion studies: In classifying the double mutants considerable emphasis was placed on the occurrence and nature of spontaneous revertants. When grown overnight to a density of about 4×10^9 cells/ml in L-broth, all but 11 of the double mutants gave rise to revertant colonies when 0.1 ml of the culture was spread on the surface of minimal plus indole (5 μg/ml) or minimal agar plates. The reversion rates observed on indole-supplemented plates for the first nine numbered double mutants ranged from 0 revertants per 4×10^9 cells to one revertant per 3×10^7 cells. Several strains with a reversion rate estimated to be higher than 10^{-7} occurred among the remaining double mutants, but in no case was the rate high enough to preclude testing for leakiness as mentioned previously or to make frequent reselection of the double mutant necessary.

Before a mutant strain was scored as negative for spontaneous revertants in Table 1, about 10^{10} viable mutant cells were tested. Of the 76 mutants which *did* give indole-utilizing revertants, all but 11 also gave rise to prototrophic revertants. These findings gave immediate support to the thesis that the method of mutant selection used might provide novel mutant forms of the B protein. The most reasonable mechanism for the occurrence of prototrophic revertants is that reversion of the ancestral A2 mutation, by furnishing the cell with normal A protein, permits tryptophan biosynthesis. Apparently the modified form of the B protein obtained, though unable to form tryptophan from indole unassisted, can form it from InGP in the presence of normal component A. This explanation has received support from the investigation of cell-free extracts of several of the prototrophic revertants, which have contained normal component A as expected, and from the results of the accumulation studies which are also presented in Table 1.

Accumulation studies: When the revertants obtained on indole-supplemented or minimal media were tested for their accumulation products following growth in these media, sufficient data were on hand to provide a preliminary classification of the mutants. Classes I to III were reserved for the mutant types observed previously in conventional mutant selection experiments beginning with wild-type *E. coli* K-12 (YANOFSKY and CRAWFORD 1959). These are, respectively: I, the CRM-less mutant, containing no protein antigenically related to normal component B; II, the conventional CRM-forming mutant, containing a protein which is immunologically indistinguishable from normal B component, which lacks any activity in the tryptophan-forming reactions, even in the presence of normal component A, but is able to assist the A protein in forming indole from InGP; and III, the labile CRM-forming mutant, represented previously by a single mutant having a highly unstable form of component B which, though active in the indole to tryptophan reaction, becomes rapidly inactivated in the cell. In effect, a group-III mutant possessing normal component A should be able to grow on indole, but large amounts of indole will accumulate in the culture medium after growth has ceased. Auxotrophic revertants of one of the 13 mu-

TABLE 1

Occurrence and accumulation products of spontaneous revertants of double mutants

Class	Reversion to:		Revertants' accumulation		Number
	Prototrophy	Indole auxotrophy	Prototroph	Auxotroph	
I–II	—	+	....	InG	11
III	—	+	....	Ind	1
IV	+	+	Ind	InG	11
V	+	+	InG	InG	38
VI	+	+	None	InG	15
VII	—	—	....	....	10
					86

tants failing to yield prototrophs accumulated indole; this strain (A2B65) was provisionally classified as having a type-III CRM.

Those double mutants giving rise to prototrophic revertants were placed in classes IV, V and VI, according to whether their prototrophs growing in minimal medium accumulated indole, indoleglycerol, or nothing detectable. In practice, occasional mutants gave revertants with discrepant results, i.e., some accumulating indoleglycerol and the remainder accumulating nothing. These were classified as group VI on the hypothesis, which requires further testing, that since the A2 marker may occasionally yield revertants or suppressed mutants with suboptimal levels of the A component, this may result from a "dosage effect" of the active A protein. Notwithstanding, it seems likely that all the 63 mutants in classes IV to VI represent novel forms of the B protein able to show significant activity in the conversion of InGP to tryptophan in the presence of component A.

Class VII was reserved for those double mutants where no revertants were observed. Of the ten members of the class, one (A2B27) is the large deletion involving portions of several genes. The other nine have proved to carry infrequently reverting mutations of the B gene calling for reclassification into other classes, as will be shown below.

Enzymatic studies: Double mutants falling into the I–II class by reversion and accumulation data were first chosen for enzymatic investigation. Cell-free extracts were prepared and examined for B-CRM activity in converting InGP to indole in the presence of normal component A. The results, summarized in Table 2, show that four mutants were CRM-less, four formed a conventional CRM, and one formed a novel type of CRM which can combine with antiserum for normal component B but which has no enzymatic activity, even in the presence of normal component A and various co-factors. Strains possessing conventional B-CRM will be labeled IIA, while those with the novel, enzymatically inert CRM will be called IIB. Certain results, to be reported in full later, suggest that CRM's of the IIA type form a normal complex with component A at neutral pH in 0.1 M potassium phosphate buffer, but that no such stable complex is formed by the type IIB CRM's. A2B38 and A2B62, originally placed in class IIA were later found to be misclassified (see below).

Table 3 shows the results of a similar investigation of the class-VII double mutants. All but one of these (A2B21, to be discussed later) proved to be mutations of classes I or II with a low rate of reversion of the new, B-gene mutation. A2B27, as previously mentioned, contains a large lesion behaving as a deletion. The other strains are point mutations as far as is known from preliminary recombination studies.

Summary of classification: On the basis of the classification tests performed to date, the 86 mutants obtained in this experiment can be grouped as follows: *Class I*—Nine strains lacking B component both enzymatically and by antiserum cross-reaction tests. This class includes one known "deletion." *Class IIA*—Seven strains possessing "conventional" B-CRM. These strains contain a protein capable of cross-reacting with antiserum for component B and of combining with

TABLE 2

*Specific enzymatic activity and immunological cross-reactivity with component B found in extracts of class I–II double mutants**

Strain	B-CRM units/mg	InGP → Ind† units/mg
A2B19	0	0
A2B41	0	0
A2B42	0	0
A2B43	0	0
A2B37	1.5	.028
A2B38‡	2.2	.044
A2B48	2.5	.055
A2B62‡	12.0	1.1
A2B72	2.0	.014
A2B87	4.0	.027
A2B59	0.7	0

* For comparison with the specific activities reported in this and the following table, the activity of the normal B protein in crude extracts of derepressed A2 cells has ranged in our hands from 20 to 30 indole-to-tryptophan units/mg (one B-CRM unit should be equivalent to one indole-to-tryptophan unit) and from 0.5 to 1.0 InGP-to-indole units/mg. Strain A2 grows well in minimal-glucose medium supplemented only with indole or tryptophan, however, while all the mutants reported here require a supplement of acid-hydrolyzed casein in addition to tryptophan for satisfactory growth. A detailed study of the effect of the acid-hydrolyzed casein supplement on enzyme levels has not been performed. Therefore, the activity levels reported in this study may not be representative of optimally derepressed cells. Values of 0 imply a level of activity less than one tenth that of the lowest activity reported.

† Component A was added. Assays were performed in the presence of 0.1 M hydroxylamine, pyridoxal phosphate (10 μg/ml), and pyridoxal phosphate (10 μg/ml) + .04 M L-serine. The activity obtained under the best of these conditions is recorded.

‡ Later found to have additional enzymatic activities.

TABLE 3

Specific activity and immunological cross-reactivity with component B found in extracts of class-VII double mutants

Strain	B-CRM units/mg	InGP → Ind* units/mg
A2B10	0	0
A2B23	0	0
A2B27	0	0
A2B34	0	0
A2B83	0	0
A2B12	3.6	.020
A2B21†	7.2	.12
A2B30	1.1	.015
A2B44	8.6	.11
A2B76	2.5	0

* Component A was added. Assays were performed in the presence of 0.1 M hydroxylamine, pyridoxal phosphate (10 μg/ml) and pyridoxal phosphate plus .04 M L-serine. The activity obtained under the best of these conditions is recorded.

† Later found to have additional enzymatic activities.

normal component A to assist in the conversion of InGP to indole. (Three strains provisionally thought to be of this type were later reclassified as type IV on the basis of enzymatic tests.) *Class IIB*—Two strains possessing "inert" B-CRM. These strains contain a protein capable of cross-reacting with antiserum for component B, but unable to combine stably with component A and therefore

inert in the InGP to indole reaction. *Class III*—One strain possessing a "labile" B-CRM. This strain seems to contain a B protein which has some activity in the conversion of indole to tryptophan in the presence of normal component A, but this protein is apparently quite labile, even within the cell. *Class IV*—14 strains possessing "indole-accumulating, active" B-CRM. These strains have a B protein able to form tryptophan from InGP in the presence of normal component A but unable to perform the indole to tryptophan reaction alone. Enzymatic studies bearing on the accumulation of indole by prototrophic revertants of these strains are reported below. *Class V*—38 strains possessing "inefficient but active" B-CRM. The B protein in these strains is, of course, unable to convert indole to tryptophan unassisted. In revertants possessing component A, however, a limited formation of tryptophan from InGP occurs with the accumulation of indoleglycerol but without the accumulation of significant amounts of indole. *Class VI*—15 strains possessing "efficient, active" B-CRM, The B protein in these strains, though unable to convert indole to tryptophan unassisted, is sufficiently active in converting InGP to tryptophan in the presence of component A that prototrophic revertants do not accumulate any known precursor of tryptophan.

Enzymatic studies with class-IV double mutants: When a cell-free extract of A2B15, a typical class-IV strain, was examined, it proved to contain 67 units of B-CRM per ml, readily demonstrable in the usual antiserum neutralization assay. In the presence of normal component A this extract had 7.7 units of activity per ml in the InGP to indole assay. When assayed in 0.1 M potassium phosphate buffer, pH 7.8, in the presence of normal component A, pyridoxal phosphate and L-serine, this extract caused the disappearance of InGP and its conversion to both indole and tryptophan in a ratio of about 2:1. This behavior adequately explains the most striking property of the prototrophic revertants of class-IV mutants, their accumulation of large amounts of indole. When an extract prepared from such an indole-accumulating prototroph was examined, it was found to contain both normal A and the peculiar B-CRM of its parent strain.

As would be expected from the accumulation of indole just described, mixtures of normal component A and A2B15 B-CRM showed very poor activity in converting indole to tryptophan in normal buffers. Representative results are shown in Table 4 where the activity of a partially purified preparation of A2B15 B-CRM was compared with a purified preparation of normal component B. Both preparations had been dialyzed against 0.1 M tris-acetate, pH 7.8, (supplemented with 10 μg/ml pyridoxal phosphate and 2×10^{-3} M β-mercaptoethanol) for six hours. The activity of the A2B15 B-CRM was very low in potassium phosphate and tris-NaCl buffered reaction mixtures where the normal B component performed best. In the presence of 1.4 N ammonium citrate, however a greater than ten-fold stimulation of this activity occurred. This level of activity was dependent on the presence of component A, though an A-CRM (from strain A3, YANOFSKY and CRAWFORD 1959) would substitute for the normal component. Higher or lower levels of ammonium ion were less effective. HATANAKA *et al.* (1962) noted that the activity of normal component B acting alone in the indole to tryptophan reaction was enormously stimuated by ammonium ions in

TABLE 4

The activity of wild-type and A2B15 components B in the formation of tryptophan from indole in several buffers

B component	Indole → tryptophan activity*		
	Tris-NaCl†	KPO_4‡	Tris-NH_4-citrate§
	pH 7.8	pH 7.0	pH 7.8
Wild type	55	36	22
A2B15	0.19	0.26	3.6

* The numbers recorded represent a ratio of the activity observed in the indole to tryptophan reaction under the given conditions to the activity of the same amount of B component in the standard InGP to indole assay. Only the ionic environment was varied in the three conditions recorded.
† Optimal conditions with normal components, 0.09 M tris-chloride plus 0.2 M NaCl.
‡ Optimal conditions for the InGP to indole reaction, 0.1 M potassium phosphate buffer.
§ Best conditions so far found for the A2B15 B-CRM in this reaction, 0.1 M tris-citrate buffer plus 1.4 N ammonium citrate.

high concentration. The interesting variant B protein present in the class-IV mutants apparently requires *both* an A protein and a high concentration of ammonium ions to perform the indole to tryptophan reaction adequately.

Resurvey of class-II mutants for leakiness: In the light of the ammonium ion effect just noted, it seemed of interest to examine the class-II mutants for *their* ability to participate in the indole to tryptophan reaction in the presence of component A and 1.4 N ammonium ion. In this manner, three mutants were found to have been misclassified, for not only could their B-CRM form tryptophan from indole under the conditions noted, but it was also capable of forming small amounts of tryptophan from InGP in the standard assay for this reaction. These activities are compared with those of an A2B15 (class IV) extract in Table 5. It seems reasonable to conclude that these three strains would have given rise to indole-accumulating prototrophic revertants if a suitable reversion of the A2 locus had occurred. Several additional attempts to select such revertants occurring spontaneously or following ultraviolet irradiation have not met with success. Nevertheless, we will provisionally assign these three mutants to class IV.

TABLE 5

Specific enzymatic activity in the three tryptophan synthetase reactions exhibited by several mutationally modified components B

Strain	B-CRM units/mg	InGP → Ind* units/mg	Ind → T† units/mg	InGP → T‡ units/mg	InGP → T‡ Ind/T
A2B15	5.5	0.66	1.7	0.38	1.6
A2B21	7.2	0.12	0.11	0.25	6.8
A2B38	2.2	0.044	0.30	0.035	1.0
A2B62	12.0	1.1	3.1	0.054	0.44

* In 0.1 M potassium phosphate buffer, pH 7.0, plus pyridoxal phosphate (10 μg/ml) and an excess of component A. Activities were not increased in this reaction by the addition of ammonium ions.
† In 0.1 M tris-citrate buffer pH 7.8 plus 1.4 N ammonium citrate. An excess of component A, pyridoxal phosphate and L-serine was present.
‡ In 0.05 M potassium phosphate buffer, pH 7.8 with an excess of component A, pyridoxal phosphate and L-serine present. Both indole and tryptophan were assayed in the reaction mixture. The addition of ammonium ions did not increase the rate of InGP utilization, though it influenced the ratio of products by decreasing the amount of indole found.

DISCUSSION

By permitting the selection of any strain defective in an "unusual" reaction involving the B component of tryptophan synthetase, the method described in this paper has furnished several new variants of this protein. Most of the variants when combined with the A protein still possess some activity in the physiologically important function, the conversion of InGP to tryptophan. In addition, certain altered B proteins lacking activity in all reactions either alone or in the presence of the A component were recovered. There is no obvious reason why mutants of this latter type were not seen among strains selected in the conventional way (YANOFSKY and CRAWFORD 1959) unless insufficient numbers were screened. The present method differs from the methods of "direct selection" of double mutants described by DAVIS (1952) in the shikimic acid pathway, by BOYER, ENGLESBERG and WEINBERG (1962) in the arabinose pathway, and by NIKAIDO (1961) in the galactose pathway, partly in that the singly mutant parental strain is not self-destructive. In the present experiment the lethal agent, penicillin, was added to the cell's environment by the experimenter; more importantly, however, the enzymatic activity through which the singly mutant parental cell destroys itself is not the primary or physiologically crucial attribute of the protein. Thus, though the method is probably inapplicable to most other systems, it proves to be the method of choice for obtaining a wide variety of mutationally altered B proteins.

As has consistently been the case, there are parallels between the present findings with the *E. coli* two-component TSase and those obtained with the corresponding *N. crassa* enzyme which has a single component. Among the earlier protein alterations observed in *N. crassa* were CRM's lacking activity in any reaction (see DEMOSS and BONNER 1959). At least one type of lesion producing this sort of enzyme in *E. coli* has now been localized to the *E. coli* B protein. It is interesting that although this alteration of the *E. coli* B component is drastic enough so that there is no demonstrable combination with the A protein, antiserum to normal component B combines with the mutant protein.

Mutants of classes IV, V and VI, the majority types recovered in the experiment, would have been classified as "leaky" mutants or would not have appeared to be mutant at all had they appeared in the conventional mutant-selection experiment beginning with wild-type *E. coli*. Only five of these permitted any growth at all on indole in cells lacking the A protein, however, and this was extremely slight. The enzymatic capabilities of the remainder can be expressed only in the presence of the A component. It seems interesting that enzymes of class IV show activity in the indole to tryptophan reaction only when *both* component A and a high concentration of ammonium ions are present. Either one of these conditions is sufficient to normal component B for satisfactory activity in this reaction. It has been hypothesized that the effect on the normal B component of ammonium ions in these high concentrations is to alter the tertiary structure of the protein and perhaps promote its aggregation. It seems likely that a similar effect is being observed with the altered enzyme.

It is improbable that ammonium ions are influencing only the combination of A and B components in this case, since activity in the InGP to tryptophan reaction, which also depends on the formation of an A-B complex, is quite good with the class-IV mutant enzymes.

It has been noted that alterations of *N. crassa* tryptophan synthetase which retain the InGP to indole reaction (corresponding to the class IIA mutants of *E. coli* reported here) may require pyridoxal phosphate or pyridoxal phosphate plus L-serine for this reaction (DEMOSS and BONNER 1959). Most of the mutants of class IIA in this study performed this activity much better in the presence than in the absence of pyridoxal phosphate, in contrast to the normal enzyme whose activity is increased somewhat by the presence of hydroxylamine. (Hydroxylamine is a potent inactivator of pyridoxal phosphate.) No mutant enzymes requiring *both* pyridoxal phosphate and L-serine for indole-forming activity were found in this experiment. A mutant of this type appearing in *E. coli* strain 518 has been studied by GIBSON, GIBSON and YANOFSKY (1961), however.

In *N. crassa* it has been reported that mutational sites giving rise to similar enzymatically deficient proteins cluster in the same locality within the gene (BONNER, SUYAMA and DEMOSS 1960). An investigation of the intragenic location of the mutations giving rise to the mutants reported in the paper is in progress and will be reported in a subsequent communication.

SUMMARY

A wide variety of mutationally altered versions of the B protein of *E. coli* tryptophan synthetase was obtained by a novel selection procedure. The cells were forced to rely on an unusual catalytic activity of the B protein, the ability to convert indole to tryptophan in the absence of the A-protein portion of this enzyme complex. Penicillin selection of variants deficient in the unusual activity resulted in the collection of a biochemically varied group of proteins many of which could perform the usual physiologically critical reaction, the conversion of InGP to tryptophan, when the A protein was provided. A tentative classification of 86 mutants with affected B proteins, utilizing chiefly reversion and biochemical criteria, is presented.

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