

Regulatory Mechanism of the Tryptophan Operon in Escherichia
coli: Possible Interaction between
trpR and trpS Gene Products

(Running head: Regulation of trp Operon)

KOREAKI ITO

Institute for Virus Research, Kyoto University,
Kyoto, Japan

Summary. The trpS5 mutation (a mutation in the structural gene for tryptophanyl-tRNA synthetase (TRSase) in E. coli), when present in the genetic background of strain KY913 (HfrH), results in the failure to grow at high temperature (42°C) in a complete medium. The rel (RC relaxed) marker present in this strain was found to be partly responsible for this temperature sensitivity. TRSase in such a strain was rapidly inactivated during growth at 42°C in rich media, but not in minimal media or in the presence of chloramphenicol. A partial derepression of anthranilate synthetase formation took place in the presence of excess tryptophan at growth-restricting temperatures. When some of the trpR mutations (including amber mutations) were combined with trpS5, the resulting double mutants (trpR trpS5) were temperature-insensitive, and TRSase was not inactivated at high temperature, in contrast to the trpR⁺ trpS5 strain. This effect of trpR mutations on temperature sensitivity was shown not to be a secondary consequence of the constitutive expression of the trp operon. These findings suggest that the trpR⁺ product interacts with the TRSase of the trpS5 mutant so as to bring about the growth-dependent inactivation of the enzyme. Furthermore, a special class of trpR mutants was obtained whose constitutivity with respect to the trp operon is manifested only in strains carrying trpS5 (but not trpS⁺) grown at high temperatures. It is proposed that TRSase participates in repression through direct interaction with the product of the trpR gene.

Introduction

Involvement of aminoacyl-tRNA synthetases in regulation of the amino acid biosynthetic operons has been suggested in several bacterial systems (Eidlic and Neidhardt, 1965; Roth and Ames, 1966; Nass, 1967; Kano, Matsushiro and Shimura, 1968; Ito, Hiraga and Yura, 1969 a,b; Iaccarino and Berg, 1971; Alexander, Calvo and Freundlich, 1971). However, the mode of their involvement and, hence, the mechanism of repression of these operons remains obscure at the present time. The tryptophan (trp) operon of Escherichia coli is under the control of at least one regulatory gene, trpR, which specifies the structure of a protein involved in repression (Cohen and Jacob, 1959; Ito et al., 1969b; Morse and Yanofsky, 1969). In addition, we and other workers have isolated and characterized tryptophanyl-tRNA synthetase (TRSase)¹ mutants (trpS) that require L-tryptophan for growth. Evidence has been presented that the trpS gene located between malA and str on the chromosome represents the structural gene for TRSase (Hiraga, Ito, Hamada and Yura, 1967; Doolittle and Yanofsky, 1968; Kano et al., 1968; Ito et al., 1969a) which somehow participates in repression of the trp operon (Kano et al., 1968; Ito et al., 1969 a,b).

We have briefly reported previously that one of the trpS mutations (trpS5) gives rise to temperature-sensitive growth when introduced into certain genetic backgrounds; trpS5 cells failed to grow at high temperature

Footnotes. ¹ Abbreviations: TRSase, tryptophanyl-tRNA synthetase; ASase, anthranilate synthetase.

even in a complete medium and became partially derepressed with respect to the expression of the trp operon (Ito et al., 1969b). These observations prompted us to investigate genetic and other factors affecting temperature sensitivity of such strains. It will be shown in this paper that mutations in the rel (or "RC") gene as well as the trpR gene can affect the temperature sensitivity of the trpS5 mutant. The results obtained further substantiate the involvement of TRSase in repression of the trp operon and provide some information on the mechanism by which TRSase participates in the actual process of regulation.

Materials and Methods

Bacterial and Phage Strains. The bacterial strains used are derivatives of Escherichia coli K-12 and are listed in Table 1. Strains KY4040, KY970 and KY4108 have been described previously (Ito et al., 1969 a,b). Other strains will be described in this paper. Phage $\phi 80$ -psuIII⁺ was obtained from H. Ozeki. Phage Pl, λ vir and T₁ were used for transduction, isolation of malA mutants, and for isolation of tonB-trp deletion mutants, respectively.

Media. Medium E (Vogel and Bonner, 1956) with 0.5% glucose was used as a minimal medium. Medium EA was medium E supplemented with 0.2% Difco Casamino Acids. Medium EAT contained 50 μ g of L-tryptophan per ml in addition to Casamino Acids. For growing tonB-trp deletion mutants, 10^{-4} M FeSO₄ was included in the medium (Wang and Newton, 1969). 5-Methyltryptophan medium was as described previously (Ito et al., 1969a). Peptone-glucose medium consisted of 20 g of polypeptone (Wako Drug Co.), 5 g of NaCl and 2 g of glucose per liter; pH was adjusted to 7.2.

Transduction of the trpS and the trpR Markers. Transduction with phage Pl was performed as described by Hiraga (1969). A desired allele of trpS was introduced into appropriate strains to obtain a pair of isogenic strains with difference only in the trpS region. A recipient strain carrying malA (isolated as a λ vir-resistant and maltose negative mutant) was infected with Pl grown on a donor strain and the malA⁺ transductants

that also received the trpS marker from the donor (10 to 20%) were purified. The presence of the trpS marker in the transductants was confirmed by examining TRSase activity in dialysed crude extracts (Ito et al., 1969a).

The trpR marker was introduced into threonine-requiring recipients by linked transduction, selecting for thr⁺. A trpR marker was checked by examinig either resistance to 5-methyltryptophan (when the recipient was trpS⁺) or the requirement for tryptophan for growth (when the recipient was trpS⁻) (Ito et al., 1969 a,b). It was usually confirmed by further transduction into other recipient strains and by measuring the repressed level of anthranilate synthetase (ASase) activity.

Test of Temperature Sensitivity on Agar Plates. Over-night cultures grown at 30°C in peptone-glucose medium were diluted 100 fold in saline and were streaked onto peptone-glucose agar. Plates were then incubated for 24 hr at 30 and 42°C.

Leucine Sensitivity of rel Strains. The rel marker was tested by sensitivity to leucine following shift down of a log-phase broth culture to a minimal medium, as described by Rogerson and Freundlich (1970). In the case of rel strains, the number of colonies formed on a minimal agar medium containing L-leucine (25 µg/ml) and other required amino acids (50 µg/ml) was 10⁻³ or less of that on a broth agar plate, in contrast to the rel⁺ strains that formed similar numbers of colonies on both media.

Preparation of Extract and Assay of Enzymes. Activity of ASase

was measured as described previously (Ito et al., 1969a). For assay of TRSase, extract was prepared as described previously except that the 105,000 x g centrifugation and dialysis were omitted and the buffer was supplemented with 10^{-5} M L-tryptophan and 10^{-3} M ATP to stabilize the enzyme of the trpS5 mutant (Ito et al., 1969a). The enzyme activity was determined in terms of tryptophanyl-tRNA formation as described previously (Ito et al., 1969a), but ^{14}C -L-tryptophan (The Radiochemical Centre) was substituted for ^3H -tryptophan and the reaction was carried out at 30°C. Protein was measured by the method of Lowry et al. (1951).

Results

Temperature Sensitivity of the trpS5 Mutant in the Genetic

Background of KY913

When the trpS5 mutation was introduced into strain KY913 (an HfrH strain) by P1 transduction, the resulting trpS5 transductant (KY4570) was found to be incapable of growth at the high temperature (42°C) either in EAT or peptone-glucose media. Figures 1A and B show the growth characteristics of several strains in medium EAT. Strain KY4570, unlike the parental trpS⁺ strain KY913, ceased to grow about 60 min after the temperature shift (30 to 42°C). Other trpS5 mutants in the background of Y-mel (KY4040) or W3350 (W3350-trpS5), however, continued to grow at 42°C, though at a reduced rate (Fig. 1A). The temperature sensitivity of KY4570 could also be shown clearly on agar plates provided that rich media are used. When minimal medium was employed, on the other hand, KY4570 grew normally at 42°C at least for two generations (Fig. 1C). The growth at high temperature of this strain was also influenced by cell density at the time of the temperature shift or by the amount of aeration; the most striking temperature sensitivity was observed at low cell density with good aeration. Under these conditions, no growth was observed for at least 120 min after shifting the temperature back to 30°C.

Effect of rel Mutations on the Temperature Sensitivity

In addition to KY913 (HfrH), a trpS5 HfrC strain manifested the temperature sensitivity, but trpS5 derivatives of strain Y-mel and W3350 did not. Since the latter two strains are rel⁺ (RC stringent) in contrast to the former strains carrying rel-1 (RC relaxed) (Edlin and Broda, 1968), it was thought that the rel locus might be responsible for the observed difference in temperature sensitivity. When trpS5 was introduced into a pair of isogenic rel⁺ (CP78) and rel (CP79) strains isolated by Fill and Freisen (1968), the latter (CP79-trpS5) but not the former (CP78-trpS5) was shown to be strikingly temperature-sensitive by streak tests. Thus, a transduction analysis was performed to confirm the effect of the rel mutation.

A thymine-requiring mutant (thyA) isolated from strain KY4570 was infected with phage P1 grown on CP78; thyA⁺ transductants were selected and were examined for their ability to grow at 42°C. Among 240 transductants tested, 11 could grow at 42°C. When tested for the rel marker by the sensitivity to leucine (Alföldi, Stent, Hoogs and Hill, 1963; Rogerson and Freundlich, 1970), the latter 11 transductants were found to be rel⁺ (one of which was designated as KY4635), whereas all the temperature-sensitive transductants tested remained rel. Thus, the rel mutation appears to be responsible for the temperature sensitivity of KY4570. However, growth experiments in liquid media revealed that KY4635 (a rel⁺ transductant of KY4570) does not grow as fast as KY4040 or W3350-trpS5 at 42°C, and could grow only after a long lag (about 6 hr, Fig. 1B). Thus, additional factors

beside rel may be responsible for the difference between KY4570 and KY4040 or W3350-trpS5.

Effect of Temperature on TRSase in vivo and in vitro

When cells of strain KY4570 that had been grown at 30°C were transferred to 42°C, specific activity of TRSase decreased to about 20% of the initial value within 15 min (Fig. 2). No striking decrease was observed with KY4040 under these conditions. The level of charged tRNA^{Trp} decreased when KY4570 was incubated at the high temperature (Table 2). As shown in Fig. 3, little or no inactivation of TRSase occurred in minimal medium with required amino acids, in contrast to the marked inactivation in EAT or peptone-glucose media. Chloramphenicol added to medium EAT prevented the inactivation completely. These results suggest that inactivation of TRSase in strain KY4570 involves growth or protein synthesis, a conclusion which is consistent with the growth characteristics of this strain as described above. On the other hand, the heat stability of the enzyme in vitro from KY4570 and KY4040 was about the same; enzymes from both strains were inactivated rapidly as compared to the enzyme of the trpS⁺ strain (Fig. 4).

Effect of Temperature on the Formation of ASase

It was suggested previously that TRSase is somehow involved in repression of the trp operon. This came from the examination of derepression kinetics and the levels of ASase activity under repression

condition with the trpS5 mutant (Ito et al., 1969 a,b). To establish further the regulatory role of TRSase, the formation of ASase was studied with KY4570 at growth-restricting temperatures. Table 3 shows specific activity of ASase in cultures grown at various temperatures in the presence of excess tryptophan. It was found that the ASase activity in this strain was higher the higher the growth temperature, attaining 4 to 5 fold the value obtained with isogenic trpS⁺ parent (KY913) at 37°C.

ASase formation in the presence of excess tryptophan was then followed after transfer of the culture from 30 to 42°C. As shown in Fig. 5, marked derepression of ASase formation occurs with KY4570 after the temperature shift, in addition to the higher basal level at 30°C. The differential rate of ASase formation at 42°C in KY4570 was found to be at least ten fold higher than in the trpS⁺ control, although still much lower than the maximum rate of derepression observed with a tryptophan auxotroph (trpA2) during tryptophan starvation (Ito et al., 1969a).

Effect of trpR Mutations on the Temperature Sensitivity

When trpS5 was introduced into strain KY970 (a trpR mutant derived from KY913), the recombinant obtained carrying both trpR970 and trpS5 (KY4571) was found to be temperature-insensitive, in contrast to KY4570 (trpR⁺ trpS5) (see Fig. 1B). That this difference in temperature sensitivity is due to the trpR970 allele was shown by further genetic analysis. A thr mutant (KY4200) was isolated from KY970 after 2-aminopurine mutagenesis. The trpS5 marker was transduced into this strain by phage P1 and

the resulting strain (KY4587) carrying thr, trpR970 and trpS5 was infected with P1 grown on KY913 (thr⁺ trpR⁺ trpS⁺). Transductants (thr⁺) were selected and were tested for tryptophan requirement and for temperature sensitivity. It was found that transductants that received trpR⁺ together with thr⁺ from the donor (122/150) exhibit a strict requirement for tryptophan and fail to grow at 42°C on peptone glucose medium, whereas the remaining ⁿ transductants (28/150) are not tryptophan requiring and can grow at 42°C. That the trpR mutations suppress the tryptophan requirement of the trpS5 mutant was previously reported (Ito *et al.*, 1969 a,b). It thus seems to be the trpR mutation itself that affects the temperature sensitivity of the present strain. It was also found that the temperature sensitivity of strain CP79-trpS5 was relieved by the trpR970 mutation.

That trpR mutation can affect the temperature sensitivity of the strain carrying trpS5 was further demonstrated by studying temperature-insensitive revertants isolated from KY4570 on peptone-glucose medium at 42°C either spontaneously or after 2-aminopurine treatment. These revertants were examined for their trpR genotype by P1 transduction. Among 19 revertants tested, 5 were shown to carry a trpR mutation. All these revertants carrying trpR exhibited the fully constitutive level of ASase activity in the presence of excess tryptophan.

The results so far presented might suggest that the observed effect of trpR mutations on temperature sensitivity is due to the constitutive expression of the trp operon, possibly through increased synthesis of endogenous tryptophan. This seems to be unlikely, however, since several

deletion mutants of KY4537 (thr trpR970 trpS5) lacking a part or the whole of the trp structural genes could grow at 42°C on peptone-glucose medium like the parental strain carrying the intact trp operon. (The deletion mutants used were selected for their resistance to phage T₁ and were characterized by testing accumulation of the intermediates of the tryptophan biosynthetic pathway.) Furthermore, when thr⁺ was transduced from KY913 into one of the deletion mutants (KY4595), most of the transductants obtained (18/20) were temperature-sensitive and carried trpR⁺. The growth curves of these presumably isogenic trpR⁺ and trpR⁻ transductants carrying a trp deletion are presented in Fig. 6. It should be noted that the trpR970 mutation results in almost full recovery of growth rate at 42°C, whether or not a complete set of the trp structural genes is present (Fig. 1, 6). Thus, relief of temperature sensitivity by trpR mutations does not seem to be a secondary consequence of the constitutive expression of the trp operon, suggesting the possibility of more direct interaction between the trpR and the trpS gene products.

Activity of TRSase was then examined with KY4571 carrying trpR970 and trpS5 upon temperature shift from 30 to 42°C. As shown in Fig. 2B, the enzyme was not inactivated appreciably, in contrast to the rapid inactivation observed with KY4570 (trpR⁺ trpS5). This agrees with the growth properties of these strains. Again, heat stability of the enzyme in vitro did not differ appreciably between these strains under a variety of conditions employed (see Fig. 4).

Effect of trpR Amber Mutations on the Temperature sensitivity

The effect of trpR mutations on the temperature sensitivity of the trpS5 mutant described hitherto can be explained in at least two different ways. (1) Altered and inactive trpR products of certain trpR mutant alleles but not of trpR⁺ somehow stabilize the TRSase. (2) The product of trpR⁺ but not of some trpR mutant alleles causes the growth-dependent inactivation of the enzyme. To discriminate between these alternatives, the effect of trpR amber mutations was examined. Two mutations trpR_{am6} and trpR_{am27} were chosen because they have been mapped at opposite extremities of the trpR mutational sites examined and could recombine with each other at an appreciable frequency (Morse and Yanofsky, 1969). When they were introduced into KY4587 (thr trpR970 trpS5) by transduction, the transductants that received trpR_{am} were all shown to grow at 42°C, indicating that the trpR_{am} mutations as well as trpR970 can relieve the temperature sensitivity of the trpS5 mutant. The trpR amber mutation in these transductants was checked and confirmed by its inability to grow at 42°C when lysogenized with $\phi 80\text{psuIII}^+$. These results seem to favour the second alternative described above. In agreement with this conclusion, preliminary experiments showed that trpR⁺ is dominant over trpR970 with respect to the effect on the temperature sensitivity of the trpS5 mutant.

Interaction between trpR and trpS with Respect to Repression of the trp Operon

In contrast to the trpR mutations described above, the trpR4108

mutation (partially constitutive, see Table 4) was unable to relieve the temperature sensitivity of the trpS5 mutant. To gain more insight into the problem of trpR-trpS interaction, repressible "revertants" were isolated from the fully constitutive trpR970 mutant. Previous studies (Ito et al., 1969 a,b) have shown that the trpS5-trpR double mutants can grow in the absence of tryptophan while the trpS5-trpR⁺ strains cannot. Thus, a strain carrying trpS5, trpR970 and an F'trp⁺ episome was treated with nitrosoguanidine followed by penicillin screening in the absence of tryptophan to select tryptophan auxotrophs. The partial diploid with respect to the trp operon was used to minimize the chance of isolating mutants in the trp structural genes. Among 6 tryptophan auxotrophs thus obtained, 2 exhibited considerable repressibility of the trp operon. The trpR locus of these strains (designated as trpR970-6 and trpR970-7) was transduced into the background of KY913 carrying the trpS⁺ (KY4625 and KY4627) or the trpS5 allele (KY4626 and KY4628). Examination of these strains revealed that the trpR970-6 and trpR970-7 alleles, like the parental trpR970, can relieve the temperature sensitivity of the trpS5 mutant.

When tested for the repressed level of ASase activity, these trpR revertants in the background of trpS⁺ (KY4625 and KY4627) exhibit^{ed} marked repression, though not as strong as with the trpR⁺ wild type, suggesting that they are partial revertants (Table 4). In the background of trpS5 (KY4626 and KY4628), however, they exhibited only slight repression when grown at 42°C (Table 4). Thus, these trpR revertants are peculiar in that

they are highly constitutive only when combined with trpS5 and grown at the high temperature. These results provide further evidence for the involvement of the trpS gene in repression and also suggest the possibility of a direct interaction between trpS and trpR gene products in the regulatory mechanism of the trp operon.

Table 5 summarizes the temperature sensitivity of various strains described in this paper.

Discussion

Interaction between *rel* and *trpS5*. The present study has shown that the *trpS5* mutation causes temperature-sensitive growth in the genetic background of *rel* strains. The observed effect of the *rel* mutation may be explained in view of the following considerations. It has been reported that the relaxed mutant (*rel*), in contrast to the stringent wild type (*rel*⁺), can not synthesize active proteins under the conditions of amino acid limitation (Edlin and Broda, 1968; Callant, Erlich, Hall and Lafeler, 1970; Hall and Callant, 1971). The stringent control system in *rel*⁺ bacteria thus provide the cells with better adaptability to the conditions that cause limitation of aminoacyl-tRNAs (Edlin and Broda, 1968; Sokawa, Sokawa and Kaziro, 1971). Thus, in the *trpS5* mutant, the *rel*⁺ strains may respond to the limitation of tryptophanyl-tRNA at 42°C and may continue to synthesize proteins (possibly those fitted to the new environmental conditions) and hence manage to recover its growth, while the *rel* strains may not^(be) able to do so. It should be noted, however, that some additional factors other than the *rel* locus might also be responsible for the difference observed between KY913 and Y-mel or W3350 (Fig. 1).

The temperature sensitivity was more striking in a rich medium than in a poor medium, in agreement with ^{the} growth dependence of the inactivation of TRSase. It was observed further that strains carrying *trpS5* exhibit reduced growth rate even at 30°C in rich media such as nutrient broth or

penassay broth. The growth rates in these media showed a striking correlation with temperature sensitivity exhibited in peptone-glucose or EAT media (Ito, unpublished results). Such growth properties have also been reported with other aminoacyl-tRNA synthetase mutants (Doolittle and Yanofsky, 1968; Schlesinger and Nester, 1969; Iaccarino and Berg, 1971). Anderson and Neidhardt (personal communication) have isolated a new class of valS mutants whose valyl-tRNA synthetase is inactivated in vivo at high temperature under growing conditions.

Interaction between trpR and trpS Affecting Temperature Sensitivity.

It was shown that the temperature sensitivity of the trpS5 mutant can be relieved by some of the trpR mutations. Such effects of trpR appear to be independent of the expression of the trp operon, since similar effects were also observed with the trp deletion strains. Operator constitutive mutations (Hiraga, 1969) tested were not effective in relieving the temperature sensitivity (Ito, unpublished results). Thus, the observed effect of trpR mutations on the temperature sensitivity of the trpS5 mutant is distinct from the previous observations that amino acid requirement of aminoacyl-tRNA synthetase mutants is often suppressed by regulatory mutations, a phenomenon that can be ascribed to overproduction of the amino acid (Ito et al., 1969 a,b; Schlesinger and Nester, 1969; Chater, Lawrence, Rowbury and Gross, 1970; Iaccarino and Berg, 1971).

The effect of trpR mutations on the temperature sensitivity could be due to an increased uptake of exogenous tryptophan (I owe to S. Hiraga

for pointing out this possibility). This would be the case if the tryptophan permeation system were under the control of the trpR gene. However, measurement of activity of the aromatic and the tryptophan-specific permeases with glucose-starved cells of various strains showed no specific correlation with the temperature sensitivity they exhibited. Furthermore, preliminary experiment showed that the pool size of free tryptophan does not differ significantly between KY4596 (trpR⁺ trpS5 trp-del) and KY4597 (trpR970 trpS5 trp-del) (Ito, unpublished results). Thus, involvement of exogenous tryptophan seems to be unlikely at the present time.

Synthesis of aminoacyl-tRNA synthetases in E. coli has been shown not to be constitutive but to be subject to some sort of regulation (Williams and Neidhardt, 1969). If the trpR gene exerts its regulatory effect on the formation of TRSase, then it might be conceivable that the observed effect of trpR mutations is due to increased formation rather than to increased stability of the enzyme. This is unlikely, however, in view of the following experiments (Ito, unpublished results). (1) The specific activity of TRSase was not significantly different between trpR⁺ and trpR strains. (2) As reported for other aminoacyl-tRNA synthetases (Williams and Neidhardt, 1969), a leaky trp mutant isolated in this laboratory showed 1.5 to 2 fold higher activity of TRSase when grown in the absence of tryptophan than its presence. This derepression of the enzyme formation was also observed with a trpR970 derivative of this strain, indicating that the regulatory mechanism for TRSase formation remains intact in the trpR

mutant. Thus, the effect of trpR mutations on the temperature sensitivity described here suggests the possibility of a direct interaction between trpR and trpS gene products.

Interaction between trpR and trpS in Repression. The results presented in this paper add two further observations suggesting the involvement of TRSase in the regulation of the trp operon. First, the trpS5 mutant in certain genetic backgrounds (e. g. KY4570) exhibits partial derepression of ASase formation at high temperatures. Second, some of the trpR reversions such as trpR970-6 and trpR970-7 are conditionally constitutive; they are constitutive only when present in the trpS5 background and grown at 42°C (Table 4). The data in Table 4 show that the repressed levels of ASase are affected by the trpS5 mutation to different degrees that depend on the particular allelic state of the trpR gene, suggesting some interaction between these two genes with respect to repression of the trp operon.

Direct Involvement of TRSase in Repression. Although the mechanism of involvement of TRSase in repression is not clear at present, it seems unlikely that TRSase participates in repression through charging tRNA^{Trp}, which in turn acts as corepressor (Doolittle and Yanofsky, 1968; Ito et al., 1969a; Mosteller and Yanofsky, 1971). Thus an alternative model that TRSase is involved more directly by constituting a part of the repressor molecule may be suggested by the results presented above.

The effect of the trpR amber mutations on the temperature sensitivity suggested that the wild-type trpR⁺ product would interact with the mutant

TRSase so as to bring about the growth-dependent inactivation of the enzyme at high temperature. Furthermore, this kind of interaction may also occur between the trpR⁺ and the wild-type trpS⁺ products as an essential step for the normal process of repression, though in this case it does not give rise to the temperature-sensitive growth. The repressibility of the trp operon and temperature sensitivity of the growth of the various strains described in Tables 4 and 5 seem to be reasonably explained by this hypothesis as may be seen in Table 6: i. e. (1) The trpR⁺ product interacts with TRSase of either trpS⁺ or trpS5; (2) The trpR970 and trpR_{am} mutations lead to the complete failure in interaction; (3) The products of the trpR 970-6 and trpR970-7 mutant alleles can interact (though less effectively) with the trpS⁺ product at both low and high temperatures but not with the trpS5 product at high temperature; (4) The product of trpR4108 is able to interact with TRSase but carries a lesion at a different site from that involved in this interaction; (5) Even when combined with trpR⁺, the trpS5 mutation itself causes slight escape from normal repression (Tables 3 and 4, Fig. 5).

The model proposed here adds a new aspect to the regulatory mechanism for amino acid biosynthetic operons, though a direct demonstration of the trpR-trpS interaction awaits future studies at the molecular level.

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Table 1. Bacterial strains

Strain	Genetic characters				Origin ^a
	<u>trpR</u>	<u>trpS</u>	<u>rel</u>	Other markers	
KY4040	+	<u>5</u>	+	F ⁻ (λ)	Y-mel
KY913	+	+	<u>1</u>	HfrH <u>met</u> <u>str</u> (λ)	T. Nagata
KY970	<u>970</u>	+	<u>1</u>	same as in KY913	KY913
KY4108	<u>4108</u>	+	<u>1</u>	same as in KY913	KY913
KY4200	<u>970</u>	+	<u>1</u>	<u>thr</u> others same as in KY913	KY970
KY4570	+	<u>5</u>	<u>1</u>	same as in KY913	KY913
KY4571	<u>970</u>	<u>5</u>	<u>1</u>	same as in KY913	KY970
KY4572	<u>4108</u>	<u>5</u>	<u>1</u>	same as in KY913	KY4108
KY4587	<u>970</u>	<u>5</u>	<u>1</u>	<u>thr</u> others same as in KY913	KY4200
KY4595	<u>970</u>	<u>5</u>	<u>1</u>	<u>tonB-trp-del</u> ^b <u>thr</u> others same as in KY913	KY4587
KY4596	+	<u>5</u>	<u>1</u>	<u>tonB-trp-del</u> others same as in KY913	KY4595
KY4597	<u>970</u>	<u>5</u>	<u>1</u>	<u>tonB-trp-del</u> others same as in KY913	KY4595
KY4625	<u>970-6</u>	+	<u>1</u>	same as in KY913	KY4200
KY4626	<u>970-6</u>	<u>5</u>	<u>1</u>	same as in KY913	KY4587
KY4627	<u>970-7</u>	+	<u>1</u>	same as in KY913	KY4200
KY4628	<u>970-7</u>	<u>5</u>	<u>1</u>	same as in KY913	KY4587
KY4635	+	<u>5</u>	+	same as in KY913	KY4570
CP78	+	+	+	F ⁻ <u>thr</u> <u>leu</u> <u>his</u> <u>arg</u> <u>thi</u> <u>malA</u>	Y. Kaziro
CP79	+	+	-	same as in CP78	Y. Kaziro
W3350	+	+	+	F ⁻ <u>gal</u>	H. Ozeki
W3110#203	<u>am27</u>	+	+	F ⁻ <u>cysB</u>	C. Yanofsky
W3110#273	<u>am6</u>	+	+	F ⁻ <u>cysB</u> <u>leu</u> _{am227}	C. Yanofsky

^a Origin as indicated does not necessarily represent an immediate origin.

^b del, deletion.

Table 2. Effect of temperature on level of charged tRNA^{Trp}^a

Strain	Growth temperature	% of charged tRNA ^{Trp}
KY913	30	70
	42	66
KY4570	30	52
	42	14

^a Cultures were grown at 30°C in medium EAT to a turbidity of 100 Klett-units (No. 54 filter), divided into two parts, one part was transferred to 42°C and the other kept at 30°C. Cells were harvested after 60 min incubation at these temperatures. The level of charged tRNA^{Trp} was determined as described previously (Ito et al., 1969a).

Table 3. Effect of temperature on ASase formation
under repression condition^a

Strain	Growth temperature (°C)	Doubling time ^b (min)	ASase activity (units/mg)
KY913	30	73	0.005
	34	57	0.005
	37	48	0.005
	39	46	0.004
KY4570 ^c	30	59	0.012
	34	53	0.018
	37	72	0.023
	39	-	-

^a Bacteria were grown in medium EAT at the temperatures indicated for about 3 generations and were used for assay of ASase activity.

^b Doubling time was determined by measuring turbidity of the culture by a Klett-Summerson colorimeter with a filter No. 54.

^c This strain cannot grow at 39°C.

Table 4. Repressed levels of ASase activity

Strain	Relevant genotype		Growth at 42°C ^a	ASase activity (units/mg) ^b		
	<u>trpR</u>	<u>trpS</u>		30°C	37°C	42°C
KY913	+	+	+		0.002	
KY4570	+	<u>5</u>	-		0.018	
KY970	<u>970</u>	+	+		0.941	
KY4571	<u>970</u>	<u>5</u>	+		1.278	
KY4625	<u>970-6</u>	+	+	0.026	0.036	0.049
KY4626	<u>970-6</u>	<u>5</u>	+	0.034	0.072	0.572
KY4627	<u>970-7</u>	+	+	0.023	0.034	0.051
KY4628	<u>970-7</u>	<u>5</u>	+	0.028	0.058	0.516
KY4108	<u>4108</u>	+	+		0.105	
KY4572	<u>4108</u>	<u>5</u>	-		0.403	

^a + and - represent growth and no growth, respectively.

^b Bacteria were grown in medium EAT at the temperatures indicated for about 3 generations, harvested and extracts were assayed for ASase activity.

Table 5. Temperature sensitivity of various strains employed^a

Genetic background	Relevant genotype				Growth at 42°C ^b
	<u>trpR</u>	<u>trp</u>	<u>trp operon</u>	<u>rel</u>	
Y-mel or W3350	+	+	+	+	+
	+	<u>5</u>	+	+	+
KY913	+	+	+	<u>1</u>	+
	+	<u>5</u>	+	<u>1</u>	-
	+	<u>5</u>	+	+	±
	<u>970</u>	<u>5</u>	+	<u>1</u>	+
	+	<u>5</u>	del ¹	<u>1</u>	-
	<u>970</u>	<u>5</u>	del	<u>1</u>	+
	<u>970-6</u> or <u>970-7</u>	<u>5</u>	+	<u>1</u>	+
	<u>am27</u> or <u>am6</u>	<u>5</u>	+	<u>1</u>	+
	<u>4108</u>	<u>5</u>	+	<u>1</u>	-
CP78	+	<u>5</u>	+	+	±
CP79	+	<u>5</u>	+	-	-
	<u>970</u>	<u>5</u>	+	-	+

^a Isogenic strains in each genetic background with difference only in the genetic region indicated were constructed by P1 transduction as described in the text, and were compared as to their temperature sensitivity.

^b Growth was examined by streaking cell suspensions on peptone-glucose agar plates; +, ± and - represent growth, slow growth, and no growth, respectively.

Table 6. An interpretation of the trpR-trpS interaction

Allelic state		<u>trpR-trpS</u> inter- action	Repression of <u>trp</u> ^a operon	Inactivation of TRSase at 42°C	
<u>trpR</u>	<u>trpS</u>				
+	+	+	++		
+	<u>5</u>	+	+	+	
<u>970, am</u>	<u>5</u>	-	-	-	
<u>970-6, 970-7</u>	+	+	+		
<u>970-6, 970-7</u>	<u>5</u>	+	+		(at 30°C)
		-	-	-	(at 42°C)
<u>4108</u>	<u>5</u>	+	-	+	

^a ++, +, and - represent complete repression, partial repression (see Tables 3 and 4), and no repression (some repressibility still remains in the trpR4108 mutant, see Table 4), respectively.

Figure Legends

Fig. 1A-C. Effect of temperature shift on growth of bacteria. Cells were grown at 30°C with shaking and cultures were transferred to 42°C at a turbidity of 40 as measured ^{with} ~~by~~ a Klett-Summerson colorimeter, [^] ~~with~~ filter No. 54. Medium EAT was used in Experiments (A) and (B), whereas minimal medium supplemented with 50 µg/ml of L-methionine and L-tryptophan was employed in Experiment (C). Experiment A---(O) W3350, (Δ) W3350-trpS5, (□) KY4040. Experiment B---(O) KY913, (Δ) KY4570, (⊙) KY4571, (Δ) KY4635. Experiment C---(O) KY913, (Δ) KY4570.

Fig. 2A, B. Heat stability of TRSase in vivo. Cells were grown in medium EAT to a turbidity of 100 Klett-units (filter No. 54) and then transferred to 42°C. Aliquots of the culture were harvested at intervals, extracts were prepared and assayed for TRSase activity. Experiment A---Initial activities (pico moles of L-tryptophan incorporated in to tRNA per min per mg protein) were 66 and 33 for strains KY4040 (O) and KY4570 (Δ), respectively. Experiment B---Initial activities were 33 and 40 for strain KY4570 (Δ) and KY4571 (O), respectively.

Fig. 3. Effect of culture medium on heat stability of TRSase in vivo. Cultures of KY4570 grown at 30°C to a turbidity of 100 Klett-units in medium E supplemented with 50 µg/ml of L-methionine and L-tryptophan

Figure Legend 1

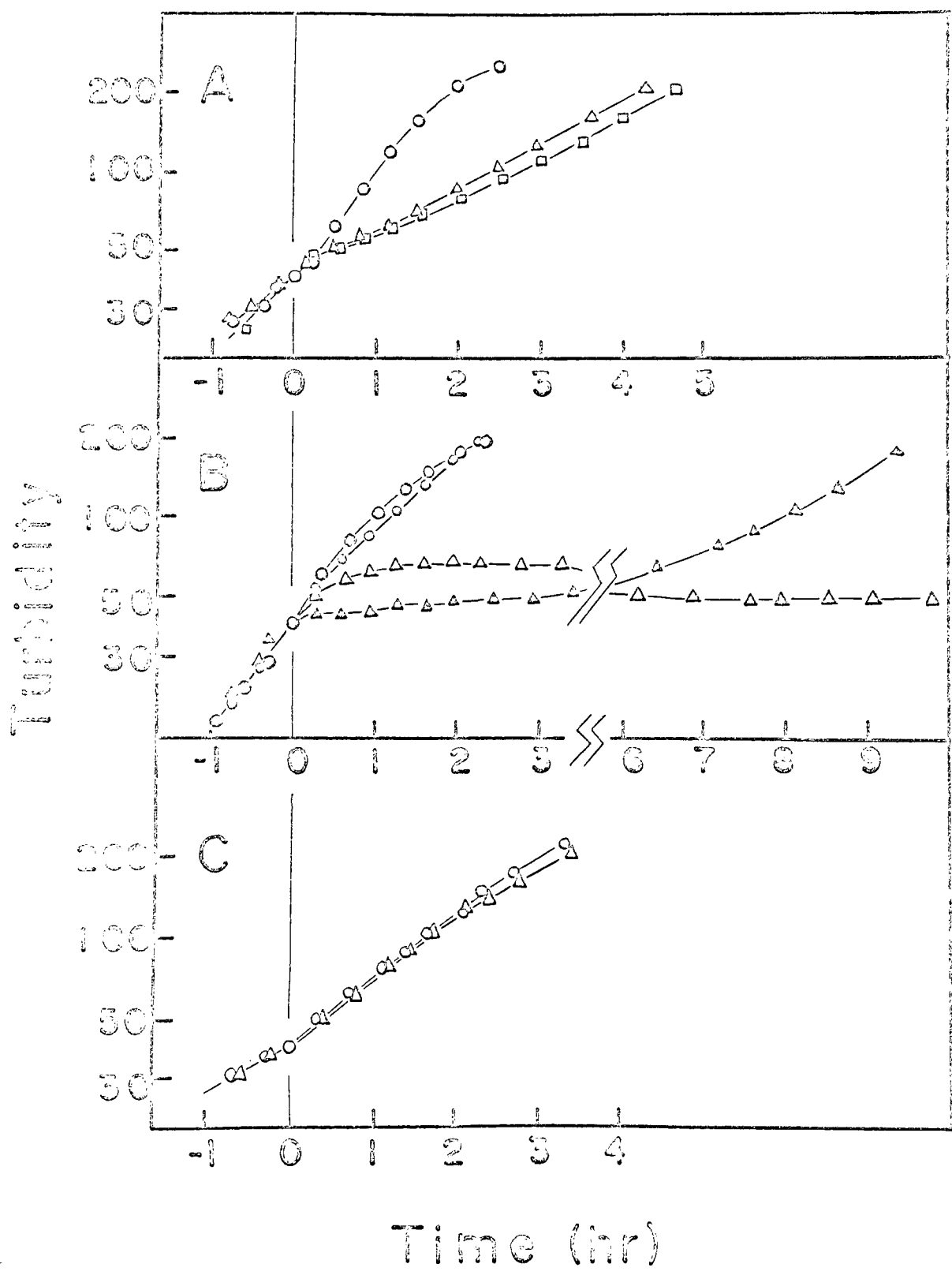
were centrifuged in the cold, cells were collected, washed and resuspended in the medium as indicated below, and were incubated at 42°C with shaking. Aliquots were removed at intervals and TRSase activity was determined. (○) medium E with L-methionine and L-tryptophan (50 µg/ml), (○) the same medium without glucose, (Δ) medium EAT, (□) peptone-glucose medium, (Δ) medium EAT containing 100 µg/ml of chloramphenicol.

Fig. 4. Heat stability of TRSase in vitro. Extracts were prepared as described in Materials and Methods from late log-phase cultures grown at 30°C and were diluted to 1 mg protein/ml for KY913 and 6 mg protein/ml for other strains. Samples (0.1 ml) were then heated at 42°C, chilled in ice-water, and assayed for residual TRSase activity. Activities (see legends to Fig. 2) of unheated extracts were 534, 67, 54, and 73 for KY913 (○), KY4040 (Δ), KY4570 (□), and KY4571 (○), respectively.

Fig. 5A, B. Formation of ASase under repression condition after temperature shift. Cultures were grown at 30°C in medium EAT to a turbidity of 100 Klett-units and were transferred to 42°C. Aliquots of the culture were removed at intervals, harvested and extracts were prepared and assayed for ASase activity. A portion of the culture was also treated with perchloric acid to measure protein content as described previously (Ito et al., 1969a). Arrows indicate the points of temperature shift from 30 to 42°C. (A) Amounts of ASase as a function of total protein

content of the culture. (E) Time course of increase in total protein of the culture after temperature shift. (O) KY913 at 30°C, (◐) KY913 after shift to 42°C, (Δ) KY4570 at 30°C, (◒) KY4570 after shift to 42°C.

Fig. 6. Temperature sensitivity of strains carrying a trp deletion. Growth was followed in medium EAT containing 10^{-4} M FeSO_4 as described in Fig. 1. (O) KY4596, (Δ) KY4597.



F_{12}

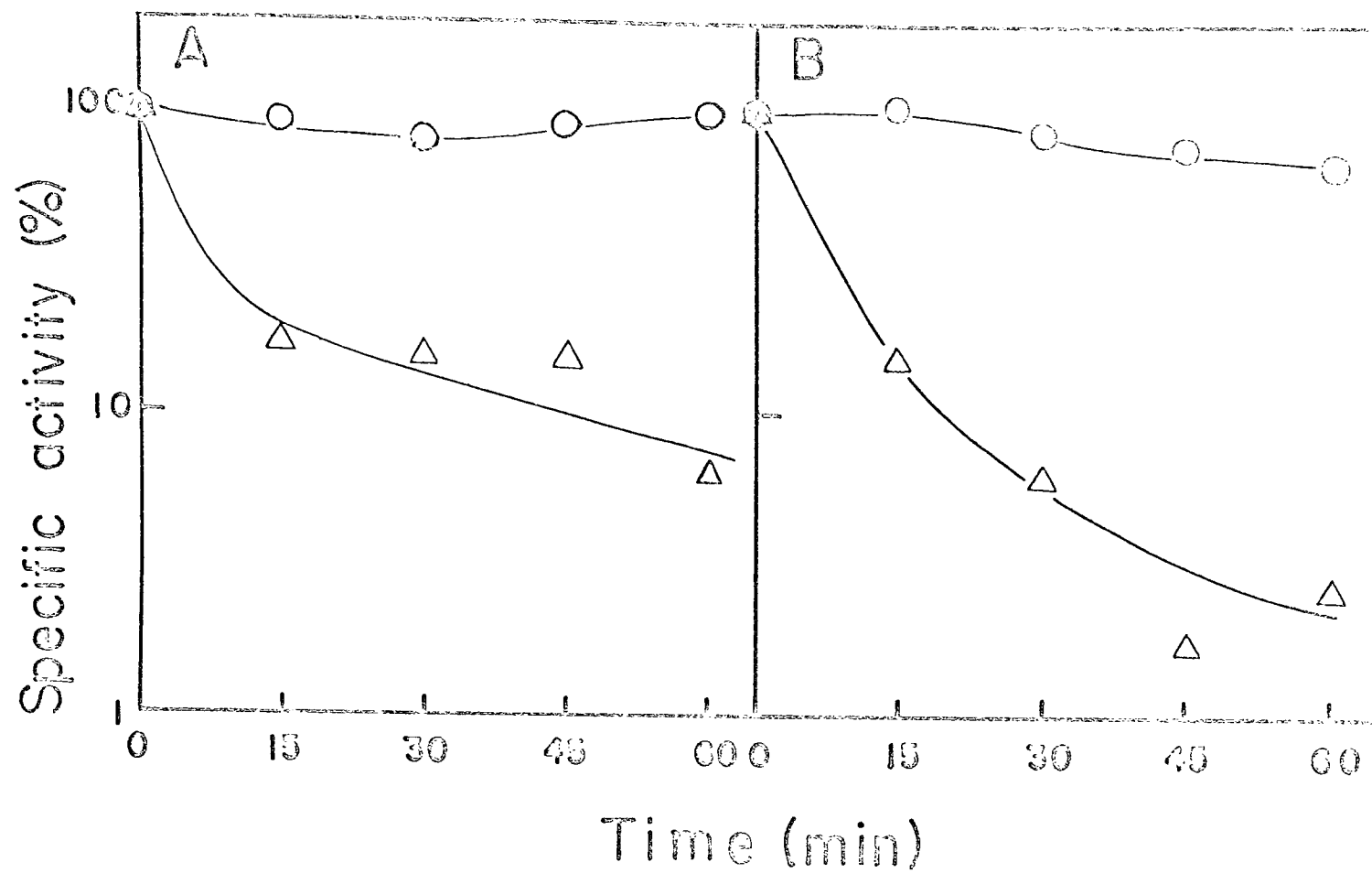


Fig 2

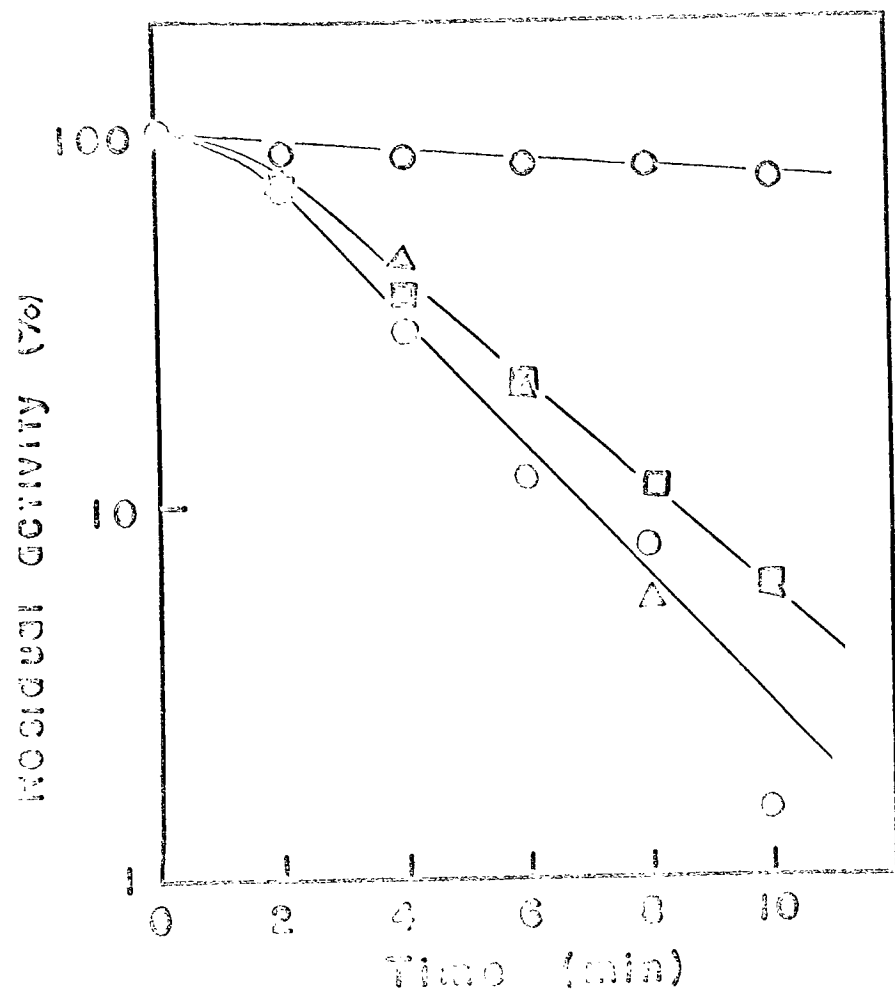


Fig 4

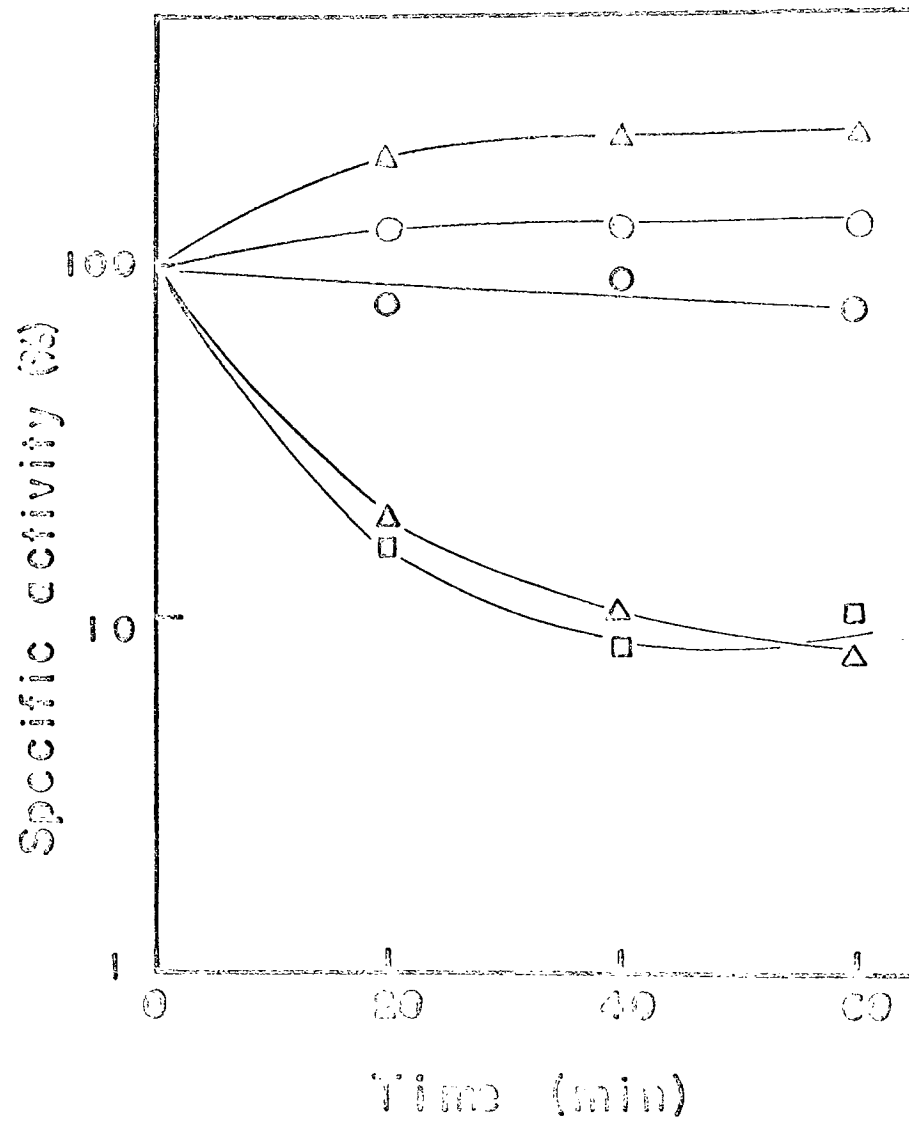
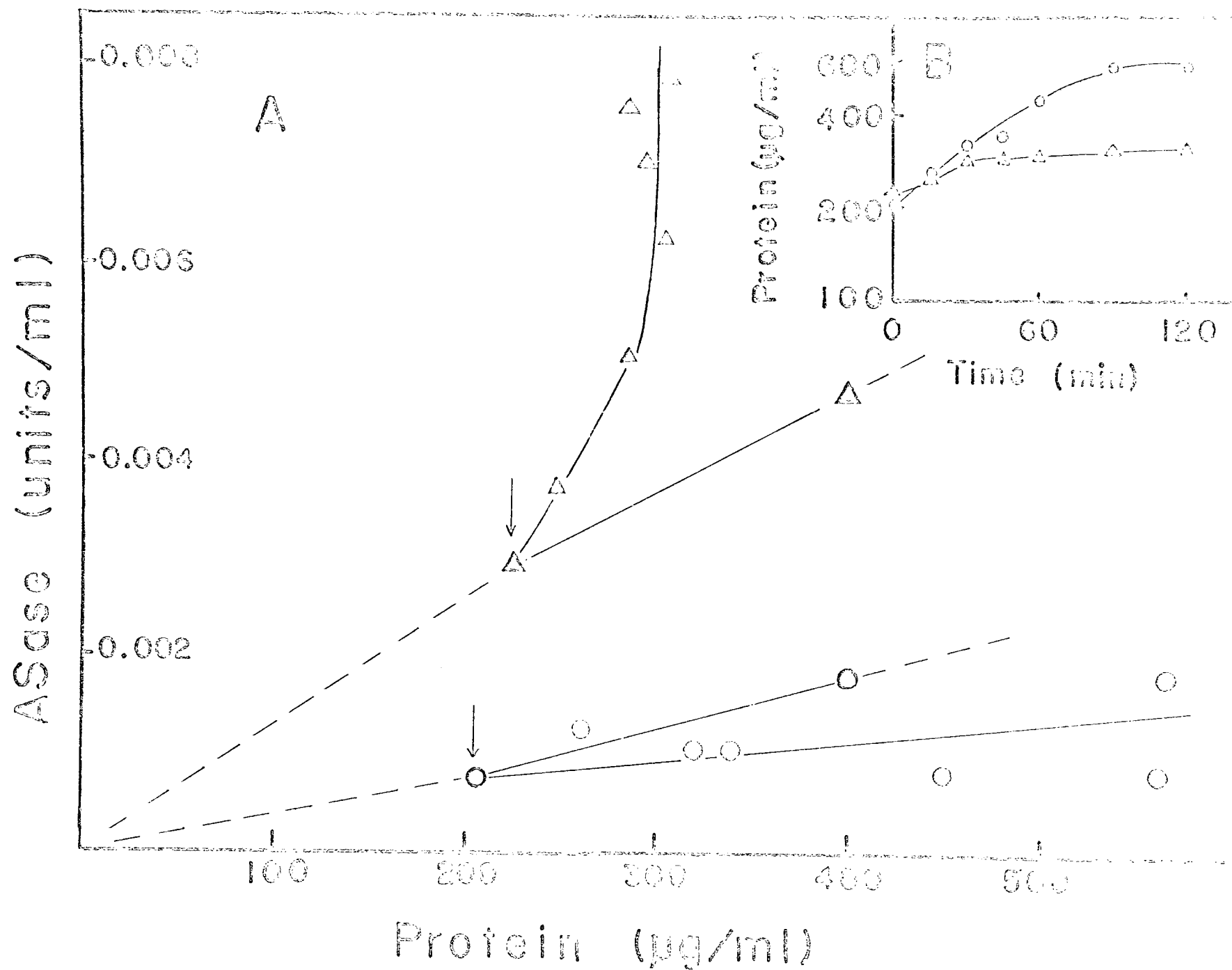
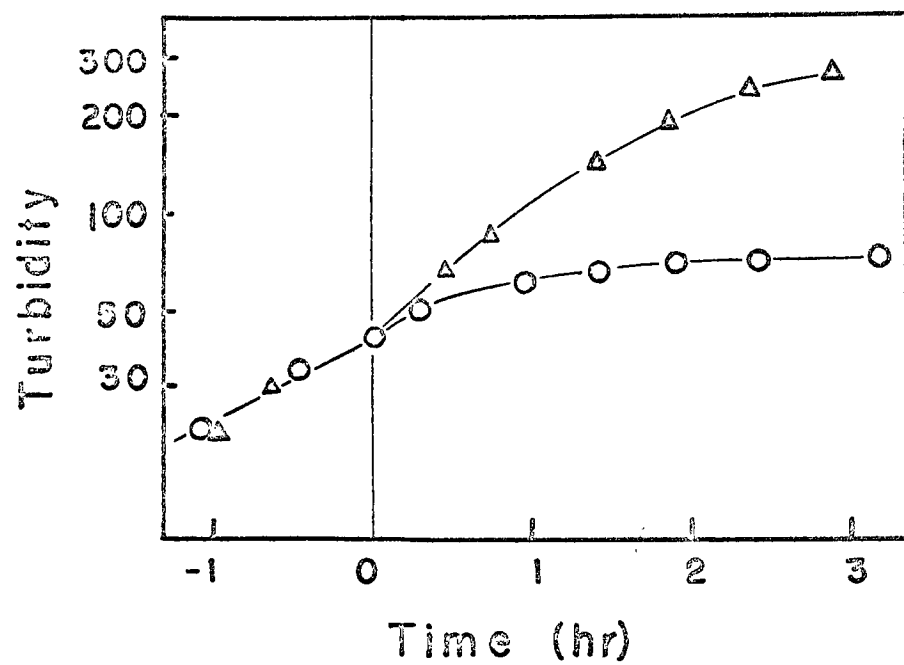


Fig 3



Figs



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