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Threonine Deaminases from *Saccharomyces cerevisiae* Mutationally Altered in Regulatory Properties*

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ABSTRACT: Two mutants of *Saccharomyces cerevisiae* resistant to 5×10^{-3} M thiaisoleucine were found to have threonine deaminases 10- and 100-fold less sensitive than the wild type to L-isoleucine as inhibitor. The apparent affinity for L-threonine as substrate, L-valine as activator, and L-isoleucine as activator was unchanged. Reversal of the isoleucine inhibition required higher concentrations of valine in the mutants as compared to the wild type. The enzyme was shown to exhibit cooperativity with respect to substrate in the absence of effectors, and with respect to activators as well. The kinetics of activation by valine or by isoleucine may be described by a modification of the allosteric equation of Monod, Wyman, and Changeux (1965, *J. Mol. Biol. 12*, 88). A tentative model for the interactions of the enzyme with its substrate and effectors, with two sorts of sites able to bind isoleucine or valine, is proposed.

This model accounts for the activation as well as the inhibition by isoleucine.

Threonine deaminase (L-threonine hydro-lyase ((deaminating)), EC 4.2 2.16) from a variety of sources has been studied for its allosteric properties (Changeux, 1963; Maeba and Sanwal, 1966; Zarlengo *et al.*, 1968; de Robichon-Szulmajster and Magee, 1968; Brunner *et al.*, 1969; Hatfield and Umbarger, 1970a,b; Sharma and Mazumder, 1970). All

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the enzymes so far studied are inhibited by isoleucine, the inhibition being reversed by valine. Despite earlier reports to the contrary (Burns, 1966) threonine deaminase from *Salmonella typhimurium* shows Michaelis–Menten substrate saturation kinetics in the absence of added isoleucine (Burns and Zarlengo, 1968) as do the enzymes from *Bacillus subtilis* (Hatfield and Umbarger, 1970a) and spinach (Sharma and Mazumder, 1970). The enzyme from yeast, however, differs from these in that it exhibits sigmoidal substrate saturation curves in the absence of isoleucine, and it is activated (*via* "normalization" of the substrate saturation curve) by low levels of isoleucine or valine (de Robichon-Szulmajster and Magee, 1968). Since it has so far proved impossible to desensitize the yeast enzyme to isoleucine by treatment with heat or mercurials, a search was undertaken for mutants whose threonine deaminase has lost sensitivity to the inhibitor. Such mutants would greatly facilitate study of the interactions between the various sites. Two such mutants were obtained by use of the isoleucine analog thiaisoleucine (McCord *et al.*, 1965). This paper compares the kinetic properties of the enzymes from two of the mutants with the parental enzyme, and describes some experiments which indicate that there may be at least two kinds of sites for each effector on the enzyme molecule.

Materials and Methods

Yeast Strains. MD9 (*his*^{1}) and MD11 (*trp*^{2}) are segregants isolated from a diploid of M14XM2, strains obtained from Dr. D. Hawthorne. TIR-9 was derived from MD9, and MAR-33 from MD11 as described below.

Isolation of Thiaisoleucine-Resistant Mutants. A strain of haploid *Saccharomyces cerevisiae* was grown overnight in medium Y (Magee and Hereford, 1969). The cells were centrifuged, washed, and suspended in 0.1 M Tris-maleate buffer (pH 6.5) at a density of 10^8 cells/ml. A solution of *N*-methyl-*N*-nitro-*N'*-nitrosoguanidine was added to a final concentration of 0.005 mg/ml; the cells were incubated for 30 min at 30°. The cells were harvested, washed twice with distilled water, and incubated for 4 hr in Y at 30° to allow them to recover. They were then spread at a density of 10^8 cells/plate on medium M (Magee and Hereford, 1969), supplemented with histidine or tryptophan and 5×10^{-3} in thiaisoleucine (Reef Chemical Co., Lafayette, Ind.). After 5–10 days numerous colonies appeared. These were picked, restreaked, and tested for excretion of isoleucine by spotting on a plate of M medium seeded with a background of isoleucine-requiring *Escherichia coli* K12. About one in 50 resistant colonies showed excretion.

Preparation of Cell-Free Extracts. Cells grown overnight in Y medium to a density of 2×10^8 cells/ml were harvested by centrifugation, washed once with 0.1 M Tris-Cl buffer (pH 7.8), and resuspended at 1×10^{10} cells/ml in the Tris–glycerol–EDTA buffer used previously (Magee and Hereford, 1969) except that 1 mM dithiothreitol was added.

The cells were broken by shaking for 90 sec with an equal volume of 0.5-μ glass beads on a Bronwill shaker cooled with CO_2. The extracts were centrifuged at 20,000*g* at −5° for 15 min and the supernatant was passed through a 0.9×25 cm Bio-Gel P-10 column equilibrated with Tris–glycerol–EDTA–dithiothreitol buffer. The eluate was used for the kinetic experiments. For the gradient experiments, the extracts, prepared in Tris–KCl–*allo*-threonine buffer (Brunner *et al.*, 1969), were concentrated 10-fold. First, 3 mg of protamine sulfate/mg of protein was added to precipitate nucleic acids. Then the 40–55% ammonium sulfate precipitate was collected and resuspended in the same buffer.

Determination of Molecular Weights. The molecular weights of the enzymes were determined as described by Brunner *et al.* (1969). Pyruvate kinase and alcohol dehydrogenase were added as markers.

Assay of Threonine Deaminase. The assay mix was the same as described by Brunner *et al.* (1969), but pH 8.0 was used throughout. The reaction was terminated after 10–20 min (depending on the activity of the extract) by the addition of 0.5 ml of 0.3% (saturated) dinitrophenylhydrazine in 2 N HCl. After 15 min at room temperature or at 37°, 2 ml of 1 N NaOH was added and the mixture let stand for 15 min at 37°. The tubes were centrifuged at top speed in a desktop International centrifuge and the supernatant was read at 520 mμ in a Bausch & Lomb Spectronic 20 or on a Gilford spectrophotometer. One micromole of pyruvate gives an optical density of 1.280 for a 1-cm light path under these conditions. Between 0.3 and 0.5 mg of protein was used for each assay.

For the molecular weight determinations, the coupled assay described by de Robichon-Szulmajster and Magee (1968) was used.

TABLE I: Generation Times of Mutant and Wild-Type Yeast on Thiaisoleucine (TIL)-Containing Media.[a]

TIL Concn (mM):	0	5	50
Strain		Times (hr)	
MD9	5.2	14.9	
TIR9	4.6	4.0	>24
MD11	3.0	9.0	
MAR33	4.8	5.2	5.2

[a] The cells were diluted from minimal medium into minimal medium containing the appropriate amount of thiaisoleucine. Growth was followed in a Klett photoelectric colorimeter using a 660-mμ filter.

Results

Response of MAR33 and TIR-9 to Thiaisoleucine. Table I shows the growth rates of TIR-9, MAR33, MD11, and MD9 in minimal medium supplemented with various levels of thiaisoleucine. It is evident that TIR-9, while more resistant to the analog than the parent strains, is less so than MAR33. A preliminary genetic analysis, based on random spore data and a small number of tetrads, indicates that both mutations map in or are very closely linked to the gene for threonine deaminase, *isol*.

Interactions of Substrate and Inhibitor with the Mutant Enzymes. After a preliminary screening showed that the mutants were less sensitive to isoleucine inhibition of threonine deaminase than the parent strains, the substrate saturation curves of the enzymes were compared. Table II shows that the mutants and the parents were identical with respect to substrate concentration required for half-maximal activity and the Hill coefficient. The specific activities of the mutants in crude extracts are not radically different from the parent strains, although TIR-9 seems somewhat depressed.

Figure 1 shows the effect of increasing concentrations of L-isoleucine on the activity of the threonine deaminase in

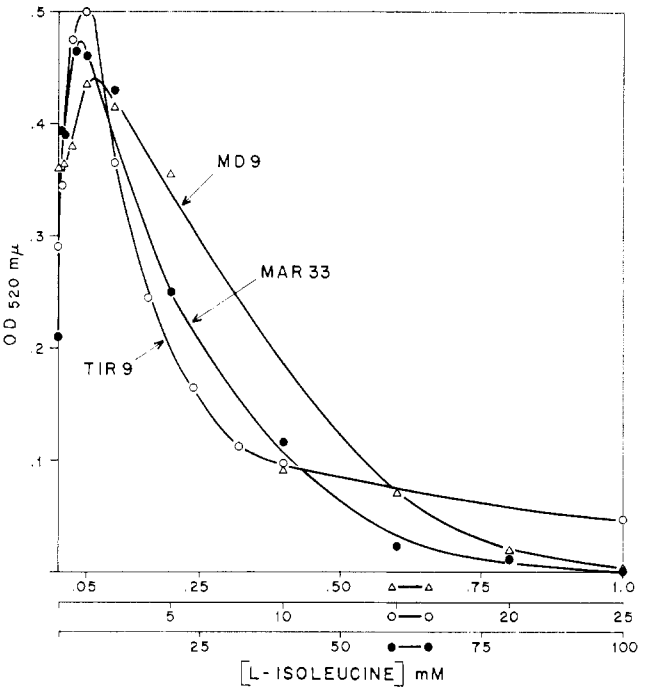

FIGURE 1: The effect of L-isoleucine on the activity of threonine deaminase from wild-type and mutant strains of *S. cerevisiae*. The extracts were prepared as described in Methods. L-Threonine concentration was 25 mM. Assays were for 15 min. (Δ—Δ) MD9, (○—○) TIR-9, and (●—●) MAR33.

Bio-Gel P-10 eluates of crude extracts from these strains. It is clear that the sensitivity of the enzyme to isoleucine is parallel to the analog sensitivity of the strain from which it came. It is interesting to note that the curves are quite similar in shape once the axes are adjusted. The isoleucine concentrations at which half-maximal inhibition (defined at one-half the rate in the absence of isoleucine) is reached are 0.3 mm for MD9, 7.0 mm for TIR-9, and 40 mm for MAR33. Thus there is a 100-fold variation in the sensitivity to the inhibitor. Isoleucine stimulates to a greater extent in the mutants, presumably due to greater saturation of the stimulatory site before inhibition becomes noticeable. The Hill numbers for isoleucine for the enzymes are quite similar, being between 2 and 3 in each case. Thus it seems likely that neither mutation has affected the homotropic interactions of isoleucine, only the affinity of the inhibitory site.

TABLE II: Characteristics of Threonine Saturation Curves of Mutant and Parental Enzymes.[a]

Strain	[L-Thr] at $^1/_2$ V_{max} (mM)	n^b	Sp Act. at 20 mM L-Thr (μmoles/20 min per mg of Protein)
MD9	30.7	1.9	3.75
TIR9	39.2	1.8	1.25
MD11	44.6	1.9[c]	2.11
MAR33	47.1	2.2[d]	2.56

[a] Assays as described in Methods. [b] Slope of log $(v/(V_{max} - v))$ *vs.* log (S). [c] Average of six determinations. [d] Average of four determinations.

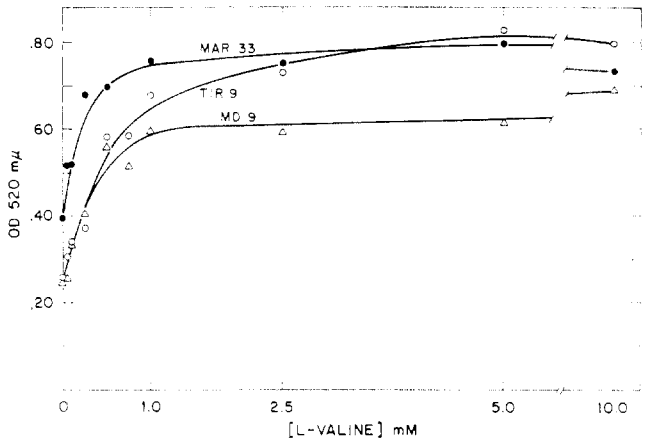

FIGURE 2: L-Valine activation of mutant and wild-type threonine deaminases. L-Threonine concentration was 25 mM. Assays were for 10 min. (Δ—Δ) MD9, (○—○) TIR9, and (●—●), MAR33.

Effects of Valine. Figure 2 shows the stimulation by valine of the enzymes from the three strains. The concentration of valine required for half-maximal stimulation at 25 mM threonine is virtually the same for all three strains, the values being 0.15 mM for TIR-9, 0.35 mM for MD9, and 0.37 mM for MAR-33 (values obtained by inspection). As previously shown (de Robichon-Szulmajster and Magee, 1968; Brunner and de Robichon-Szulmajster, 1969), valine stimulates by reducing or eliminating the cooperativity of the threonine saturation curve.

Valine, at considerably higher concentrations, partially reverses the inhibition by isoleucine of the wild-type enzyme. Figure 3 shows that valine also reverses the effect of isoleucine in the mutants, but at concentrations significantly higher than those required for the wild type. Furthermore, the lower the affinity for isoleucine, the greater the concentration of valine required for reversal. The quantity of L-valine required for half-maximal reversal for MD9 is 0.75 mM, for TIR-9, 16.7 mM, and for MAR33, 30 mM. In the wild type, valine concentrations above 10 mM begin to inhibit, while in the mutants stimulation continues up to 200 mM. The mutations thus seem

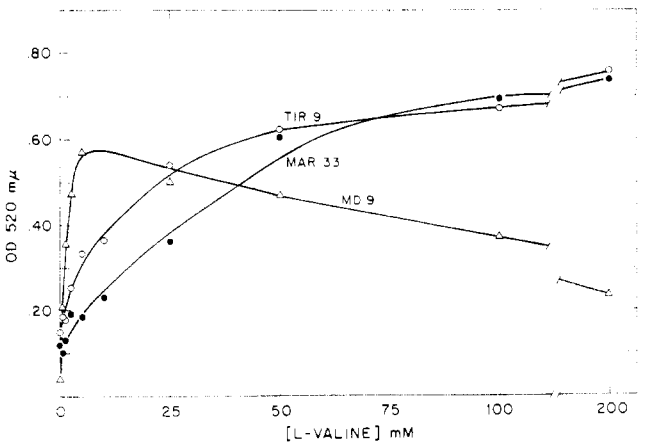

FIGURE 3: Reversal by L-valine of L-isoleucine inhibition. L-Threonine was 25 mM. L-Isoleucine was 0.5 mM for MD-9, 6.0 mM for TIR9, and 40 mM for MAR33. Assays were for 20 min. (Δ—Δ) MD9, (○—○) TIR9, and (●—●) MAR33.

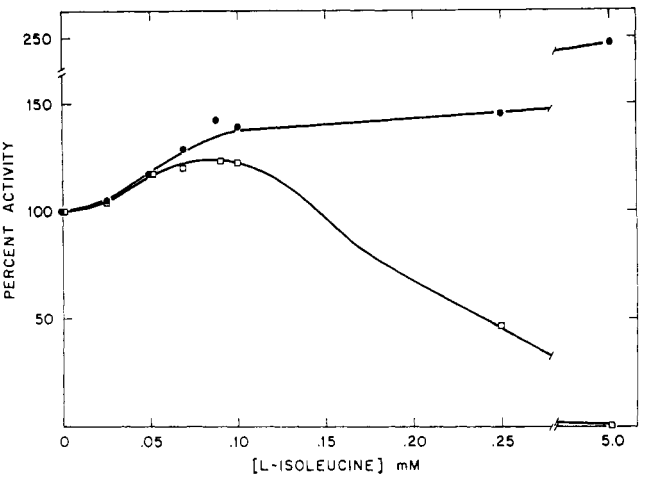

FIGURE 4: Stimulation by L-isoleucine. L-Threonine was 25 mM. Assays were for 20 min. (□—□) MD11 and (●—●) MAR33.

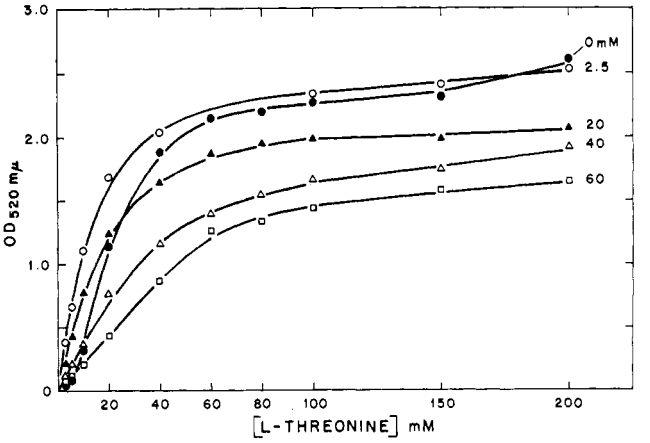

FIGURE 5: The effect of increasing concentrations of L-isoleucine on the threonine saturation curve of MAR33. Assays were for 20 min. L-Isoleucine concentrations: (●—●) 0, (○—○) 2.5 mM, (▲—▲) 20 mM, (Δ—Δ) 40 mM, and (□—□) 60 mM.

to have affected the site where valine binds to reverse the inhibition by isoleucine, but they have apparently not affected the valine stimulation site. Interestingly enough, the mutants show a complex homotropic effect of valine in the reversal. The parental strain may also do so, but it is very difficult to distinguish from eventual inhibition, since both give the same upwardly concave reciprocal plots.

Evidence of Two Kinds of Isoleucine Sites on the Enzyme. Figure 4 shows the effect of low concentrations of isoleucine on MAR33 and MD11 threonine deaminase. It seems likely that the stimulatory site in MAR33 has not been altered by the mutation to isoleucine insensitivity, since the curves are very similar in shape up to 0.07 mM L-isoleucine. At the latter concentration inhibition becomes apparent in the wild-type extracts and the curves diverge. The homotropic effect of isoleucine stimulation is also apparent in the shape of the curves in Figure 4. It seems likely, therefore, that there are two sorts of sites for isoleucine on the enzyme, one which causes stimulation at low substrate concentrations, and one which inhibits, and that the stimulating one has an affinity about 5-fold higher (in the wild type) than the inhibiting one. In MAR33 the amount of isoleucine required for half-maximal stimulation is about 0.50 mM.

That stimulation is a real effect and not due to a stabilization of the enzyme or abolition of a lag period is shown by the fact that valine and isoleucine act by changing the shape of the substrate saturation curve rather than the maximal velocity.[1] Figure 5 shows threonine dependence curves for the MAR33 enzyme in the presence of concentrations of L-isoleucine ranging from 2.5 to 60 mM. It is interesting to note that in MAR33 isoleucine, even at a concentration which gives 70% inhibition at 25 mM threonine, stimulates at low concentrations. Furthermore, at high threonine 2.5 mM isoleucine shows a very slight, but reproducible, inhibition, indicating that the inhibitory site has some affinity for isoleucine at this concentration.

The simplest explanation for isoleucine and valine stimulation is to suppose a single sort of stimulatory site with affinity for either. If this were true, the kinetics of stimulation by either effector ought to be described by a single equation. Accordingly, we used the enzyme from MAR33 and varied threonine in the presence of isoleucine or valine concentrations ranging from 0 to 2.5 mM. The data obtained were used to derive parameters to fit the equation for activation of an allosteric enzyme derived by Monod *et al.* (1965), using the transformation which allows use of kinetic rather than binding data (Frieden, 1967)

$$v = \frac{V_{max}\alpha(1 + \alpha)^{n-1}}{[L/(1 + \gamma)^n] + (1 + \alpha)^n} \quad (1)$$

where α = [L-threonine]/K_R, L is the allosteric equilibrium constant [T]/[R], γ = [activator]/K_A, [T] is the form of the enzyme with low affinity for the substrate, [R] is the form with high affinity, and n is the number of protomers. Equation 1 makes one assumption in addition to those listed by Frieden (1967), *viz.*, that K_T is very large and $K_R/K_T \simeq 0$.

The Marquardt algarithim (1963), run on the Yale Computer Center 7014–7040 system, was used to determine the best fits for these parameters, with the assumptions being made that n = 2 or 4. Separate runs were made for isoleucine and for valine as activators and the parameters derived were close enough so that it seemed worthwhile to make a joint run. The results are shown in Table III. The joint run gave quite good fits. The values for n were chosen as 2 since that is the slope of the Hill plot for threonine and 4 since that is the Hill number for isoleucine. Two gave the best fits to the data; 4 gives too low a velocity at low substrate and too rapid a rise in the velocity at intermediate substrate concentrations. Thus, the activation by either effector may be described by eq 1, and n = 2 gives the best fit. Figure 6 shows the data for three concentrations of effector compared to the theoretical line drawn using the parameters for the joint run in Table III, with n = 2. The sigmoid shape of the threonine saturation curve in the absence of effector is evident, as is the fit of the theoretical curve. Intermediate concentrations of effectors decrease the sigmoid nature of the curve, with valine being much more effective than isoleucine. The fit for valine is not perfect at low substrate. At high effector (about six times K_A for isoleucine) the curves and the points are almost identical for the two

[1] In contrast to the experiments cited by Hatfield and Umbarger (1970b), we find that the lag period of yeast threonine deaminase, observed using the coupled enzyme system, can be abolished by increasing lactic dehydrogenase concentration as previously reported (de Robichon-Szulmajster and Magee, 1968). Their reported inability to repeat this result may be due to the different strains they used.

TABLE III: Statistical Fits of Kinetic Parameters to Substrate Saturation and Activation Data.[a]

Strain	K_R (mM)	V_{max} ($OD_{520\ m\mu}$/ 15 min)	K_A (mM) L-Isoleucine	K_A (mM) L-Valine	L	d^2
			$n = 2$			
MAR33						
Isoleucine	13.1 ± 1.5	2.31 ± 0.04	0.428 ± 0.047		23.4 ± 4.8	0.215
Valine	14.2 ± 1.6	2.22 ± 0.05		0.119 ± 0.027	14.8 ± 3.2	0.553
Isoleucine + valine	13.7 ± 1.1	2.25 ± 0.03	0.389 ± 0.057	0.116 ± 0.022	19.3 ± 3.1	0.810
MD11						
No effector	11.9 ± 0.7	2.12 ± 0.06			19.3[b]	0.028
			$n = 4$			
MAR33						
Isoleucine + valine	19.3 ± 1.5	2.27 ± 0.04	0.422 ± 0.085	0.225 ± 0.056	101 ± 30	1.310 (95)
MD11						
No effector	16.7 ± 0.075	1.96 ± 0.04			101[b]	0.0309 (11)

[a] The values are fitted to threonine saturation curves run in the presence of 0, 0.05, 0.25, 1.0, and 2.5 mM L-isoleucine or L-valine for MAR33. For isoleucine, four separate extracts were assayed in duplicate; for valine, two duplicate determinations were made. For MD11, four separate extracts were assayed in duplicate, but only in the absence of effectors. The different extracts were normalized to a value of $v = 1.905$ for 200 mM L-threonine. For $n = 2$, the data for MAR33 were run with each effector separately (lines 1, 2) and then with the two effectors jointly (line 3). For $n = 4$, only a joint run was made. [b] To minimize the number of iterations required for convergence, the value of L derived from the joint run of MAR33 was fixed in the run with MD11.

activators. Also included in Table III are results of a similar run made on MD11, the parent of MAR33. In agreement with the hypothesis that the threonine site is unaffected by the MAR33 mutation, the computed value of K_R is very similar to that for MAR33.

While stimulation is explained most simply by a single type of stimulatory site with affinity for both effectors, an alternative possibility is the existence of two types of site, one for isoleucine and one for valine. In the first case the equation for stimulation is

$$v = \frac{V_{max}\alpha(1 + \alpha)^{n-1}}{L/(1 + \delta + \gamma)^n + (1 + \alpha)^n} \quad (2)$$

when $\gamma = [\text{effector}_1]/K_{A_1}$ and $\delta = [\text{effector}_2]/K_{A_2}$.

For two sites the equation is

$$v = \frac{V_{max}\alpha(1 + \alpha)^{n-1}}{L/[(1 + \gamma)^n(1 + \delta)^n] + (1 + \alpha)^n} \quad (3)$$

The difference between these two is most apparent at low substrate and effector, where the points are least reliable. Data from assays in the presence of both effectors were not able to distinguish between the two possibilities.

Molecular Weights of Mutant and Parental Enzymes. Figure 7 shows the results obtained by centrifuging in glycerol gradients mixtures of the mutant and wild-type enzymes. It is evident that the major peak of activity coincides with the peak of isoleucine-insensitive activity, thus indicating that the mutant enzymes are very close in molecular weight to the wild type. The molecular weights calculated from the marker enzymes are 1.70×10^5 in both cases. This agrees with the results reported by Brunner *et al.* (1969) and is somewhat higher than the value of 1.4×10^5 originally reported by de Robichon-Szulmajster and Magee (1968).

Discussion

The present study of the kinetic properties of these desensitized mutants of yeast threonine deaminase helps to clarify our understanding of the interactions between the various effectors. For the first time a partial separation between catalytic and regulatory properties of the yeast enzyme has been achieved, with the catalytic properties remaining unchanged in the mutant. This is in contrast to the studies on mutant M6 by Brunner *et al.* (1969) who found a simultaneous increase in isoleucine sensitivity and a decrease in affinity for threonine. The results presented here provide additional evidence of the independence of the inhibitory and catalytic sites and demonstrate that a drastic alteration of the former need not affect the latter.

In addition, we have been able to study carefully the role of isoleucine as activator. Strong evidence that the activation takes place at a site different from inhibition is provided by the similarity of the early part of the curves for activation of MD11 and MAR33 in Figure 4. The mutation which changed the affinity at the inhibitory site has not affected stimulation by isoleucine nor by valine. The fact that n in eq 1 can be assumed to be 2 with a good fit to the data also distinguishes the stimulation from the inhibition, where the Hill coefficient is usually between three and four (de Robichon-Szulmajster and Magee, 1968; Brunner *et al.*, 1969).

Drawing together the data presented here and those in earlier publications (de Robichon-Szulmajster and Magee, 1968; Brunner *et al.*, 1969) we can form a tentative model which explains most of the observations on mutant and wild-

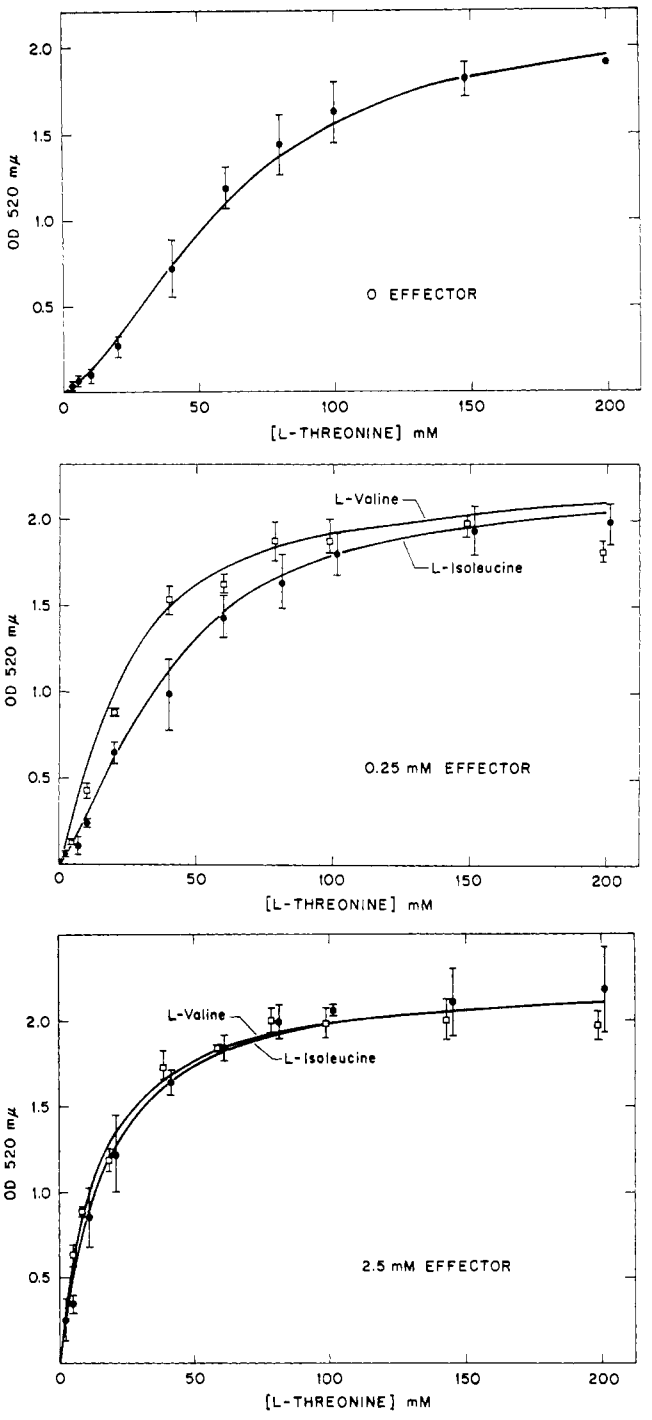

FIGURE 6: Theoretical curves and observed values for effector stimulation of MAR33 threonine deaminase. The points are the average of four (isoleucine) or two (valine) determinations. The bars indicate the standard error (isoleucine) or range (valine) of the determinations. The curves were computed from using the parameters from line 3 (joint run, $n = 2$) in Table III.

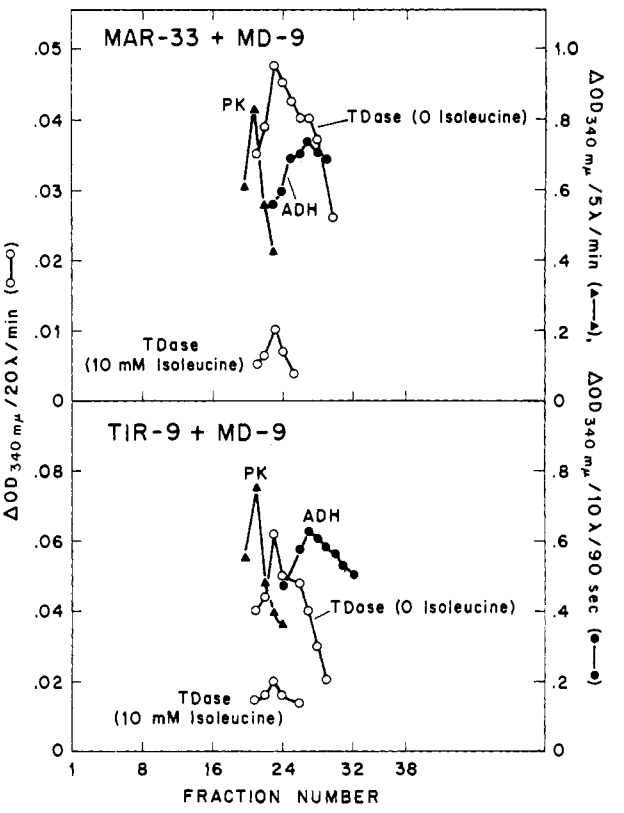

FIGURE 7: Glycerol gradient profiles of mutant and wild-type threonine deaminases. The gradient was 5–30% glycerol prepared in Tris–KCl *allo*-threonine buffer spun in a Spinco Model L for 10 hr at 40,000 rpm (SW50 rotor). The fractions were assayed for threonine deaminase in the presence and absence of 10 mM L-isoleucine, a quantity sufficient to inhibit completely the MD9 enzyme, and to inhibit 70% of the TIR9 enzyme. Approximately 2.5 mg of TIR9 extract was added, with a specific activity of about 2.0; 1.5 mg of MAR33 was added, with a specific activity of 2.0; and 2.5 mg of MD9, with a specific activity of 5.0.

type threonine deaminase from *S. cerevisiae*. Since it is based upon kinetic observations in crude extracts and partially purified preparations, the model naturally must await purification of the enzyme for its final test, but it is useful to consider it here, since the enzyme has so many complex interactions of effectors.

We suppose, then, that the enzyme would have two sites for substrate, two activator sites, and four inhibitor sites. It would be a typical allosteric protein, with the equilibrium in favor of the T form, of the sort described by Monod *et al.* (1965). The activator sites could bind either isoleucine or valine, with valine being preferred. Such binding would shift the equilibrium toward the R form. The inhibitor sites would bind either isoleucine or valine, with isoleucine, the preferred ligand, binding with greater affinity to the T form than to the R form. Valine would bind to the inhibitory site of either the R form or the T form; when it displaced isoleucine, the equilibrium would shift to give more R form, reversing the inhibition.

It is essential to postulate four sites for inhibition, although this is not in accord with the assumption of Monod *et al.* (1965) that each protomer has one and only one type of site. Inspection of eq 4

$$v = \frac{V_{max}\alpha(1 + \alpha)^{n-1}}{L\frac{(1 + \beta)^n}{(1 + \gamma)^n} + (1 + \alpha)^n} \quad (4)$$

(where β = [isoleucine]/K_I and γ = [isoleucine]/K_A) shows that either inhibition or stimulation, but *not* both, will be observed, depending on the ratio K_A/K_I. However, if the numerator in eq 4 takes the form $L[(1 + \beta)^m/(1 + \gamma)^n] + (1 + \alpha)^n$, $m > n$, then if $K_A < K_I$, activation will occur at low isoleucine and inhibition at high isoleucine concentrations.

Finally, the same sort of argument which indicates separate sites for inhibition and stimulation by isoleucine would say that reversal of inhibition by valine is due to binding at the inhibitory site. The decrease in affinity for valine as a reverser of inhibition is not quantitatively identical with the decrease in affinity for isoleucine as inhibitor, but they change in the same direction in both cases, as shown in Figure 3. This argument is strengthened by the fact that substrate interactions and stimulation are *not* affected in either mutant. Unfortunately, the homotropic interactions of valine as inhibitor in MD9 are too complex to be fitted by a simple Hill plot, so no estimate of n can be obtained.

This model is in accord with all the previous observations on the enzyme with the exception of the effect of the competitive inhibitor *allo*-threonine, which shows a lack of cooperativity, and does not affect the homotropic interactions of threonine. These data were interpreted as meaning it binds to only one of the threonine sites (Brunner *et al.*, 1969). A much more likely explanation is that it binds equally well to both the R and the T forms, not affecting the equilibrium between them, but competing with threonine for all the active sites. In this case the equation for the velocity as a function of *allo*-threonine and threonine concentration becomes

$$v = \frac{V_{max}\alpha(1 + \alpha + 2\phi)}{(1 + \alpha + \phi)^2 + L(1 + \phi)^2}$$

where ϕ = [*allo*-threonine]/K_R(allo) and c' = K_R(allo)/K_T(allo) = 1 (again $c \simeq 0$). Using 0.85 mM as the value for K_R(allo) and holding [threonine] at 20 mM (Brunner *et al.*, 1969) this equation yields a straight line when $1/v$ is plotted as a function of *allo*-threonine concentrations above 2 mM. At lower *allo*-threonine concentrations the curve deviates upward from linearity, as do Brunner's points. All results in the mutant M6 can most simply be explained on the basis of an alteration in the allosteric equilibrium, so that L becomes greater. Then, without a change in the microscopic dissociation constants, the enzyme will show apparent decreased substrate affinity and increased inhibitor affinity. Of course, it is also possible that the mutation in M6 has so altered the structure of the enzyme that several of the microscopic dissociation constants are altered.

This model, while not strikingly different from those previously proposed (de Robichon-Szulmajster and Magee, 1968; Brunner *et al.*, 1969), has some appealing aspects. First, it dispenses with the idea of several sorts of threonine sites, requiring binding only at the catalytic site. Secondly, it explains isoleucine activation as a fortuitous by-product of valine activation, without having to invoke a separate activating site. Thirdly, it accounts for valine reversal of isoleucine inhibition in a simple manner. Finally, it explains the alteration in M6 on the basis of a change of a single parameter, rather than a disruption of several sites. Like all models based on kinetics, of course, it awaits purification of the enzyme for a final test.

It seems clear now that yeast threonine deaminase is different from all others so far reported in that it does show cooperative kinetics under conditions designed to exclude effectors. This property, and the fact that a number of mutants altered in different regulatory properties are available, indicate that if the enzyme is purified it will be an exceedingly useful tool for the study of the effect of primary structure on allosteric properties.

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