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Isoleucyl Transfer Ribonucleic Acid Synthetase. Competitive Inhibition with Respect to Transfer Ribonucleic Acid by Blue Dextran†

John G. Moe‡ and Dennis Piszkiewicz*

ABSTRACT: The inhibitory effects of blue dextran and a small dye molecule derived from it (F3GA-OH) on the steady-state reaction catalyzed by *Escherichia coli* isoleucyl-tRNA synthetase have been studied. Blue dextran gave uncompetitive inhibition with respect to Mg·ATP, mixed inhibition with respect to L-isoleucine, and competitive inhibition with respect to tRNA. The small dye molecule (F3GA-OH) was also competitive with respect to tRNA. These inhibition patterns were not consistent with the bi-uni-uni-bi Ping Pong mechanism generally accepted for aminoacyl-tRNA synthetases. They were consistent with a mechanism in which a second L-isoleucine is bound after isoleucyl-AMP synthesis and before transfer of the isoleucyl moiety to tRNA. Enzyme-bound L-isoleucine lowered the affinity of the enzyme for blue dextran approximately fivefold, a value comparable to the ninefold lowering of the enzyme's affinity for tRNA upon binding L-isoleucine. The affinity of the synthetase for F3GA-OH (K_I = 1.0 × 10^{-7} M) is approximately fivefold higher than its affinity for blue dextran (K_I = 5.3 × 10^{-7} M). These results indicate that blue dextran and its derivatives may be useful for kinetic and physical studies of polynucleotide binding sites on proteins as well as NAD and ATP sites.

The mechanism of action and active-site topography of isoleucyl-tRNA synthetase of *Escherichia coli* (EC 6.1.1.5) have been probed through the use of many inhibitors of its catalytic reaction. These inhibitors have included analogues of L-isoleucine such as its methyl and ethyl esters, 2-methyl-1-butylamine and L-isoleucinol (Holler et al., 1973). Aminoalkyl adenylates, compounds in which the mixed anhydride of the aminoacyl adenylate has been replaced by an ester bond (Cassio et al., 1967), have been extensively studied in their interactions with isoleucyl-tRNA synthetase. Chemically modified tRNAs have been used as competitive inhibitors of isoleucyl-tRNA synthetase with respect to $tRNA^{Ile}$ (Baldwin & Berg, 1966; McNeil & Schimmel, 1972). Also, the trinucleotide U-A-G, a sequence derived from a part of the $tRNA^{Ile}$ structure which has been implicated in its binding to the synthetase (Schoemaker & Schimmel, 1977a), has been shown to be a competitive inhibitor of the synthetase with respect to $tRNA^{Ile}$ (Schoemaker & Schimmel, 1977b).

Previously, we have reported (Moe & Piszkiewicz, 1976) that isoleucyl-tRNA synthetase could be purified by affinity chromatography on blue dextran–Sepharose, presumably due to an attraction of the blue chromophore to a nucleotide binding site of the enzyme (Thompson et al., 1975). In this study we have used blue dextran and the dye molecule derived from it as dead-end inhibitors of the reaction catalyzed by isoleucyl-tRNA synthetase. The results we now report summarize our use of these inhibitors in analyzing the enzyme mechanism, including the finding that the chromophore of blue dextran is competitive for isoleucyl-tRNA synthetase with respect to tRNA.

Materials and Methods

Nomenclature. Cibacron Blue F3GA and Derivatives. Cibacron Blue F3GA is a sulfonated, polyaromatic blue dye derived from 2,4,6-trichloro-*s*-triazine (Thompson et al., 1975). The commercially available dye is the monochlorotriazine derivative (Figure 1) which is used in the manufacture of blue dextran 2000. If this dye is incubated in 0.01 N NaOH at 60 °C, it is hydrolyzed. The expected product of this reaction would be the derivative of Cibacron Blue F3GA in which the chloride is replaced by either a hydroxyl group or its keto tautomer. In this report the Cibacron Blue F3GA and its hydrolysis product will be referred to as F3GA-Cl and F3GA-OH, respectively. The use of F3GA-OH to designate the hydrolysis product is not meant to imply that the molecule

† From the Department of Biological Chemistry, California College of Medicine, University of California, Irvine, Irvine, California 92717. *Received August 22, 1978.* This investigation was supported by Grant GM 19508 from the National Institutes of Health. This work was done by J.G.M. in partial fulfillment of the requirements for the Ph.D. Degree.
* Present address: Department of Chemistry, Duquesne University, Pittsburgh, PA 15219.
‡ Present address: Ph.D. → M.D. Program, University of Miami School of Medicine, Miami, FL 33152.

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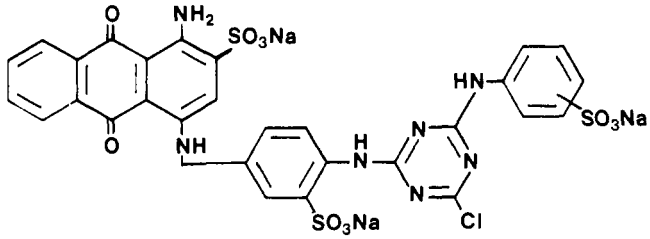


FIGURE 1: Chemical structure of Cibacron Blue F3GA.

exists predominantly as the hydroxyl tautomer. In fact, in many similar systems, the keto tautomer predominates in solution (Beak, 1977).

Purification of Cibacron Blue F3GA (F3GA-Cl). Cibacron Blue F3GA (Figure 1) was purchased from Polysciences, Inc. This commercial product was resolved into a major blue component and at least eight other colored components by thin-layer chromatography on activated silica gel (Eastman 6061) developed by tetrahydrofuran–water (48:7 v/v). The R_f of the major component was 0.57. The dye was purified before use by preparative column chromatography on silica gel (J. T. Baker Chemical Co.). In a typical experiment, 600 mg of the crude dye was dissolved in water and mixed with 3 g of silica gel. This mixture was evaporated to dryness at 40 °C in a heated vacuum desiccator. The dried mixture was suspended in ethyl acetate–tetrahydrofuran–water (48:48:4 v/v/v), applied to a column (2.0 × 44 cm) of silica gel equilibrated with the same solvent, and then washed with 1 L of this solvent. The eluting solvent was then changed to ethyl acetate–tetrahydrofuran–water (20:48:7 v/v/v). This mixture (2 L) was passed through the column while 20-mL fractions were collected. The fractions were assayed by thin-layer chromatography as described above. The fractions containing only the major blue component (R_f = 0.57) were pooled. The organic solvents were removed by rotary evaporation, and the aqueous dye solution was lyophilized. This procedure resulted in the isolation of 340 mg of pure F3GA-Cl.

Preparation of F3GA-OH. Pure F3GA-Cl was dissolved in 0.01 N NaOH and incubated at 60 °C for 115 h. One-tenth volume of 0.1 N NaOH was added every 24 h to compensate for formation of sodium carbonate. The course of the reaction was followed by thin-layer chromatography on silica gel. The intensity of the spot corresponding to F3GA-Cl (R_f = 0.57) decreased with time, and the intensity of the spot corresponding to the blue product, F3GA-OH (R_f = 0.28), increased with time. After 115 h of hydrolysis, the reaction mixture was cooled to room temperature and neutralized with 0.1 N HCl. The dye was then purified by column chromatography on silica gel as described above. The procedure for purification of this derivative was modified from that of the monochloride by substitution of ethyl acetate–tetrahydrofuran–water (10:48:7 v/v/v) for the eluting solvent used above.

Comparison of the infrared spectra of F3GA-Cl and F3GA-OH showed that the absorbance band corresponding to aryl C–Cl stretching (Silverstein et al., 1974) was no longer present after hydrolysis. The relative reactivities of F3GA-Cl and F3GA-OH toward dextran were determined. In separate 30-mL centrifuge tubes, 20 mg of the dye and its hydrolysis product were dissolved with 100 mg of dextran in 7.5 ml of water. The mixtures were heated with stirring to 60 °C. NaCl (750 mg) was added and stirring continued for 30 min at 60 °C. The stirred mixture was then heated to 80 °C. Na_2CO_3 (100 mg) was added and stirring continued for 2 h. The mixtures were allowed to cool to room temperature. Isopropyl alcohol (2 volumes) was added to precipitate the dextran. After 30 min at −20 °C, the reaction mixtures were centrifuged at 35000*g* for 30 min at 0 °C. The supernatants were decanted, and the pellets were redissolved in 5 mL of water, reprecipitated by addition of 10 mL of 2-propanol, cooled at −20 °C for 30 min, and centrifuged as above. The pellets were washed in this manner 4 times. At the end of this washing, the pellet from the reaction mixture containing F3GA-Cl was dark blue. The pellet from the reaction mixture containing F3GA-OH was white. Thus, the hydrolysis product of Cibacron Blue F3GA does not react with dextran.

The blue dextran which had been synthesized by using F3GA-Cl was then incubated in 6 N HCl at 30 °C for 60 h. Blue dextran 2000 purchased from Pharmacia was also subjected to this treatment. After incubation, the samples were evaporated to dryness at 60 °C in a heated vacuum desiccator. Dimethylformamide (Mallinckrodt) was added to the samples to dissolve the dye. Aliquots were taken for thin-layer chromatography. The mobility of the dye released by acid hydrolysis of the blue dextran made by using F3GA-Cl was identical with that of the dye released from blue dextran 2000 in tetrahydrofuran–water (48:7 v/v) (R_f = 0.28).

Purification of Isoleucyl-tRNA Synthetase and Kinetic Analyses. The method of preparation of homogeneous isoleucyl-tRNA synthetase from *E. coli* has been described in the preceding paper (Moe & Piszkiewicz, 1979). Kinetic measurements of the aminoacylation of unfractionated tRNA by isoleucyl-tRNA synthetase were made at 20 °C in 5 mM sodium phosphate buffer at pH 6.40 with 10 mM 2-mercaptoethanol. Constant components in the reaction mixture were isoleucyl-tRNA synthetase (1.5 μg/mL), inorganic pyrophosphatase (1 unit/mL), and magnesium acetate (5 mM excess over ATP). The concentration of [^{3}H]-L-isoleucine (either 50 or 100 mCi/mmol; ICN) was 100 μM when Mg·ATP or tRNA was the varied substrate, and it was varied from 5 to 100 μM when it was the varied substrate. The concentration of the Mg·ATP stock solution was determined from the molar extinction coefficient of ATP: ϵ_{259} = 15 400 mM^{-1} cm^{-1} (Bock et al., 1956). Unfractionated tRNA from *E. coli* B (Schwarz/Mann) was determined to be 0.84% $tRNA^{Ile}$ by weight on the basis of its maximum level of esterification by [^{3}H]-L-isoleucine. Its concentration was 3.0 mg/mL (810 nM $tRNA^{Ile}$) when [^{3}H]-L-isoleucine and Mg·ATP were the varied substrates, and it was varied from 0.20 to 3.00 mg/mL (54 to 810 nM $tRNA^{Ile}$) when it was the varied substrate. Blue dextran 2000 and F3GA-OH solutions were prepared in water. The concentration of the blue chromophore was determined spectrophotometrically (ϵ_{610} = 13.6 mM^{-1} cm^{-1}) (Thompson & Stellwagen, 1976). For each set of conditions, the enzyme and other reactants were incubated separately at 20 °C for 5 min in the presence of the appropriate concentration of blue dextran or F3GA-OH. After the preincubation, the reaction was initiated by addition of the enzyme to the other reactants. At a minimum of six timed intervals, 200-μL aliquots of the reaction mixture were withdrawn and immediately dispensed into cold 5% trichloroacetic acid. After 30 min on ice, the samples were collected on glass fiber filters (Reeve Angel 934AH), rinsed extensively with 5% trichloroacetic acid, given a final rinse of 10 mL of 95% ethanol, dried, and counted by liquid scintillation in toluene–PPO–POPOP. Initial rates were calculated by utilizing the linear regression program of a Texas Instruments SR-51 calculator.

Results

The study of the properties of blue dextran as an inhibitor of isoleucyl-tRNA synthetase was carried out by determining

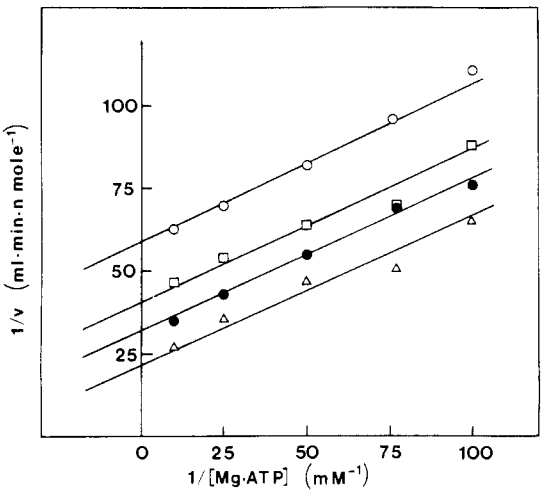


FIGURE 2: Kinetics of aminoacylation at 20 °C of unfractionated tRNA as a function of Mg·ATP and blue dextran concentrations. Mg·ATP was the varied substrate and blue dextran was present at changing fixed concentrations. The concentrations of blue dextran used were 0 (△), 4 (●), 8 (□), and 16 μM (○). Conditions and procedures used were those described under Materials and Methods.

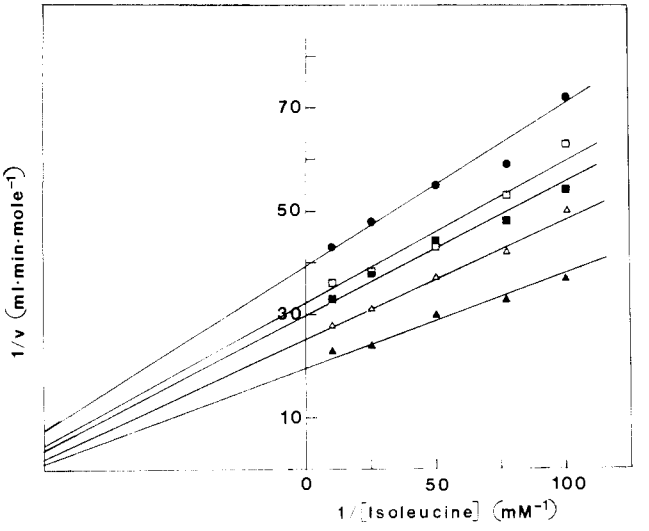


FIGURE 3: Kinetics of aminoacylation at 20 °C of unfractionated tRNA as a function of L-isoleucine and blue dextran concentrations. L-Isoleucine was the varied substrate and blue dextran was present at changing fixed concentrations. The concentrations of blue dextran used were 0 (▲), 4 (△), 8 (■), 12 (□), and 16 μM (●). Conditions and procedures used were those described under Materials and Methods.

the initial rate of the overall aminoacylation reaction as a function of varied substrate and blue dextran concentrations. When Mg·ATP was the varied substrate, the Lineweaver–Burk (1934) plot of the data generated in the presence of blue dextran (Figure 2) indicated that blue dextran acts as an uncompetitive inhibitor of isoleucyl-tRNA synthetase with respect to Mg·ATP. According to the rules of inhibition advanced by Cleland (1963b) this result implied that the binding of Mg·ATP and blue dextran to the enzyme was separated by an irreversible step (i.e., release of pyrophosphate) during the course of the aminoacylation reaction.

When L-isoleucine was the varied substrate, the observed inhibition pattern in the presence of blue dextran (Figure 3) was mixed. According to the rules of Cleland (1963b), this indicated that L-isoleucine and blue dextran were simultaneously bound by the enzyme during the course of the aminoacylation reaction and that the binding of the substrate and inhibitor was not independent. The intersection of the experimentally generated lines below the 1/[L-isoleucine] axis indicated that the binding of blue dextran lowered the affinity of the enzyme for L-isoleucine and vice versa.

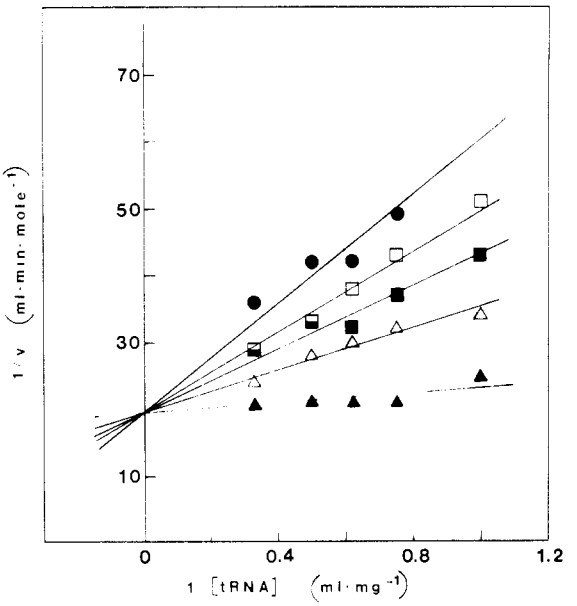
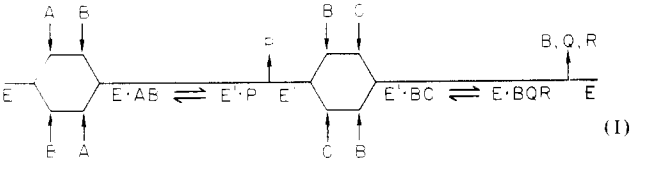


FIGURE 4: Kinetics of aminoacylation at 20 °C of unfractionated tRNA as a function of tRNA and blue dextran concentrations. tRNA was the varied substrate and blue dextran was present at changing fixed concentrations. The concentrations of blue dextran used were 0 (▲), 4 (△), 8 (■), 12 (□), and 16 μM (●). Conditions and procedures used were those described under Materials and Methods.

When tRNA was the varied substrate, the observed inhibition pattern in the presence of blue dextran was competitive (Figure 4). This indicated that the binding of tRNA and blue dextran to the enzyme during the course of the aminoacylation reaction is mutually exclusive and suggested that isoleucyl-tRNA synthetase interacted with blue dextran through a site normally used to bind tRNA.

We thought that it would be desirable to confirm the competitive inhibition of isoleucyl-tRNA synthetase with respect to tRNA using the dye molecule not conjugated with dextran. We decided against using the parent dye, Cibacron Blue F3GA (F3GA-Cl), because it is an inherently reactive compound (Weber et al., 1979). Any reaction of the inhibitor with the enzyme other than noncovalent complex formation would, of course, invalidate the kinetic analysis. The dye derived by hydrolysis of blue dextran and F3GA-Cl, F3GA-OH, was used in an additional experiment. When it was used as an inhibitor of the aminoacylation reaction with tRNA as the varied substrate, the observed inhibition pattern was once again competitive (figure not shown).

A rate law has been derived to describe the initial rate of the overall animoacylation reaction in the presence of inhibitor (Moe, 1978). This reaction requires the binding of a second L-isoleucine after isoleucyl-AMP formation and prior to transfer of isoleucine to tRNA (Fersht & Kaethner, 1976; Moe & Piszkiewicz, 1979; see also Discussion). This reaction is represented (Cleland, 1963a) by eq 1, where E is the enzyme,



(I)

E′ is the isoleucyl-AMP·enzyme complex, A is Mg·ATP, B is L-isoleucine, C is tRNA, P is inorganic pyrophosphate

Table I: Dissociation Constants for Isoleucyl-tRNA Synthetase, Blue Dextran, and F3GA-OH

For Enzyme·Isoleucyl Adenylate Complex
K_I(blue dextran) = 5.3×10^{-7} M
K_I(F3GA-OH) = 1.0×10^{-7} M[a]
For Enzyme·Isoleucyl Adenylate·Isoleucine Complex
K'_I(blue dextran) = 2.6×10^{-6} M
K'_I(F3GA-OH) = 4.9×10^{-7} M[a]

[a] Calculated by assuming that K'_I/K_I for F3GA-OH is equal to K'_I/K_I for blue dextran.

(magnesium salt), Q is AMP, and R is isoleucyl-tRNA. The rate law including inhibition of this reaction by an inhibitor competitive with respect to tRNA is given by eq 2, where K_I

$$\frac{v}{V_{max}} = ([A][B][C])/\left[\beta K'_{ib}K'_c[A] \times \left[1 + \frac{[I]}{K_I} + \frac{[B]}{K'_{ib}}\left(1 + \frac{[I]}{K'_I}\right) + \frac{[C]}{K'_c} + \frac{[B][C]}{\beta K'_{ib}K'_c}\right]\right] + \frac{k_3}{k_1}\alpha K_A K_B[C]\left(1 + \frac{[A]}{K_A}\frac{[B]}{K_B} + \frac{[A][B]}{\alpha K_A K_B}\right) \quad (2)$$

$= [E'][I]/[E'\cdot I]$ (i.e., the dissociation constant of the inhibitor·enzyme·isoleucyl adenylate complex) and $K'_I = [E'\cdot B][I]/[E'\cdot B\cdot I]$ (i.e., the dissociation constant of the inhibitor·enzyme·isoleucyl adenylate·isoleucine complex).

When this equation is written in the form of Lineweaver & Burk (1934) for B (L-isoleucine) as the varied substrate (eq 3), it may be noted that the slope and the intercept of the line

$$\frac{V_{max}}{v} = \frac{1}{[B]}\left[\frac{\beta K'_{ib}K'_c}{[C]}\left(1 + \frac{[I]}{K_I}\right) + \beta K'_{ib} + \frac{k_3}{k_1}\alpha K_B\left(\frac{K_A}{[A]} + 1\right)\right] + \frac{\beta K'_c}{[C]}\left(1 + \frac{[I]}{K'_I}\right) + \frac{k_3}{k_1}\left(\frac{\alpha K_A}{[A]} + 1\right) + 1 \quad (3)$$

described by this equation are functions of the inhibitor concentration, [I] (Moe, 1978).

Also, the slope is a function of K_I (Moe, 1978), as shown in eq 4, while the intercept is a function of K'_I (Moe, 1978),

$$\text{slope} = \frac{1}{V_{max}}\left[\frac{\beta K'_{ib}K'_c}{[C]}\left(1 + \frac{[I]}{K_I}\right) + \beta K'_{ib} + \frac{k_3}{k_1}\alpha K_B\left(\frac{K_A}{[A]} + 1\right)\right] \quad (4)$$

as shown in eq 5. Thus, two secondary plots of the data

$$\text{intercept} = \frac{1}{V_{max}}\left[\frac{k_3}{k_1}\left(\frac{\alpha K_A}{[A]} + 1\right) + \frac{\beta K'_c}{[C]}\left(1 + \frac{[I]}{K'_I}\right) + 1\right] \quad (5)$$

presented in Figure 3 are necessary to calculate K'_I for the interaction of blue dextran with isoleucyl-tRNA synthetase. From a plot of slope vs. [I] (figure not shown), K_I may be calculated with the use of the equilibrium and kinetic constants presented in the preceding paper (Moe & Piszkiewicz, 1979). Similarly, K'_I may be calculated from a plot of intercept vs. [I] (figure not shown). These values are presented in Table I.

F3GA-OH was studied as an inhibitor of isoleucyl-tRNA synthetase only with respect to tRNA as the varied substrate. Since competitive inhibition was observed (figure not shown), the $1/v$ intercept was independent of [I], K_I, and K'_I. The slope of the Lineweaver–Burk lines was a function of [I], K_I, and K'_I (eq 6) (Moe, 1978). From a plot of slope vs. [I], it is not

$$\text{slope} = \frac{1}{V_{max}}\left[\frac{\beta K'_{ib}K'_c}{[C]}\left(1 + \frac{[I]}{K_I}\right) + \beta K'_c\left(1 + \frac{[I]}{K'_I}\right)\right] \quad (6)$$

possible to directly calculate K_I and K'_I for F3GA-OH. However, if the assumption is made that K_I/K'_I is identical for both blue dextran and F3GA-OH, then values for K_I and K'_I may be calculated for F3GA-OH. These values are presented in Table I.

Discussion

This study of the inhibition of isoleucyl-tRNA synthetase of *E. coli* by blue dextran and the dye derived from it, F3GA-OH, was initiated as a result of our observations (Moe & Piszkiewicz, 1976) that this enzyme could be purified by affinity chromatography on blue dextran–Sepharose. It seemed likely that the blue dye interacted specifically with a nucleotide binding site of the enzyme since a low concentration of ATP would elute the enzyme and since blue dextran is known to interact with NAD and ATP binding sites of numerous enzymes (Thompson et al., 1975; Stellwagen, 1977). Somewhat surprisingly, however, this kinetic study demonstrated that blue dextran and the dye derived from it, F3GA-OH, were competitive not with ATP but with tRNA. While some caution is appropriate in interpreting this result (i.e., binding could be at an effector site which prevents tRNA binding), the simplest explanation is that the blue dye binds to the tRNA binding site of isoleucyl-tRNA synthetase.

The inhibition patterns of isoleucyl-tRNA synthetase by blue dextran gave useful information about the order of substrate addition and product release from this enzyme. The inhibition pattern found in Figure 3 indicated that L-isoleucine and blue dextran simultaneously bound to the enzyme during the course of the aminoacylation reaction. Since blue dextran was competitive with tRNA (Figure 4), one may infer that L-isoleucine and tRNA bind simultaneously during the overall reaction. This conclusion is inconsistent with the bi-uni-uni-bi Ping Pong mechanism which is generally accepted for the aminoacyl-tRNA synthetases [Söll & Schimmel, 1974; Kisselev & Favorova, 1974; see also the preceding paper by Moe & Piszkiewicz (1979)]. However, it is consistent with the mechanism which has a second L-isoleucine binding after isoleucyl-AMP formation and prior to transfer of isoleucine to tRNA (eq 1) (Fersht & Kaethner, 1976; Moe & Piszkiewicz, 1979). Furthermore, the results obtained in the kinetic study show that bound L-isoleucine lowers the affinity of the enzyme for blue dextran approximately fivefold (Table I). This is comparable to the ninefold lowering of the enzyme's affinity for tRNA upon binding L-isoleucine (Moe & Piszkiewicz, 1979).

The inhibition constants (K_I) obtained for blue dextran and F3GA-OH are 5.3×10^{-7} and 1.0×10^{-7} M, respectively. These are only 1–2 orders of magnitude higher than the dissociation constant for tRNA (1.6×10^{-8} M) and approximately 4 orders of magnitude lower than the dissociation constant for ATP (2.6×10^{-3} M). Thus, the dye is bound with a much higher affinity than the mononucleotide cofactor and with an affinity approaching that of tRNA. The observed dissociation constant for tRNA represents the sum of all of the energies of interaction at the multiple points of contact with the enzyme. The fact that the dye is bound with an

affinity comparable to the whole tRNA molecule may indicate that the interaction of the tRNA with the enzyme occurs through high- and low-affinity sites of attachment and that the tight binding of one part of the tRNA molecule may include the interaction at the site which binds blue dextran. Apparently the lower affinity interactions of tRNA with the synthetase are essential for prevention of misacylation, since relaxing of the tRNA structure (Yarus, 1972) leads to tighter binding of tRNA to synthetases with an increased frequency of misacylation (Kern et al., 1972).

The affinity of the enzyme for F3GA-OH is approximately fivefold higher than its affinity for blue dextran. This same relationship has been seen for other enzymes (Wilson, 1976) and may reflect steric hinderance by the polysaccharide when the enzyme interacts with blue dextran.

We are aware of only one other small molecule which is a competitive, dead-end inhibitor of an aminoacyl-tRNA synthetase with respect to tRNA. The trinucleotide U-A-G, which is the sequence at the 8-9-10 position of $tRNA^{Ile}$, also inhibits isoleucyl-tRNA synthetase (Schoemaker & Schimmel, 1977b). The K_I values we obtained for blue dextran (Table I) compare very favorably with the K_I value reported for this trinucleotide ($K_I = 1.10 \times 10^{-4}$ M). The high affinity of the enzyme for blue dextran and F3GA-OH makes these substances excellent kinetic and physical probes of this and possibly other aminoacyl-tRNA synthetases.

The kinetic results provide an explanation for the observation that ATP elutes isoleucyl-tRNA synthetase from blue dextran–Sepharose (Moe & Piszkiewicz, 1976). Uncompetitive inhibition is observed for blue dextran with respect to ATP; this reflects the binding of blue dextran and ATP to different forms of the enzyme. Thus, the effect of ATP in eluting isoleucyl-tRNA synthetase from blue dextran–Sepharose is not exerted by direct competition for the site on the enzyme which binds the dye. The form of the enzyme which binds ATP is simply not capable of binding blue dextran. Thus, when ATP is present, the equilibrium between free enzyme and enzyme bound to blue dextran is shifted toward free enzyme.

The effect of added L-isoleucine on ATP elution of isoleucyl-tRNA synthetase from blue dextran–Sepharose (Moe & Piszkiewicz, 1976) can also be explained on the basis of the kinetic results. The presence of L-isoleucine causes the enzyme to elute at a lower concentration of ATP. This either could be due to direct interaction between the L-isoleucine and the blue dextran binding sites (Table I) or could reflect the enhanced affinity of the enzyme for ATP upon binding L-isoleucine (Holler et al., 1975).

Evidence is now mounting that blue dextran may interact with polynucleotide binding sites of enzymes as well as NAD and ATP sites recognized previously (Thompson et al., 1975; Stellwagen, 1977). Drocourt et al. (1978) have reported that *E. coli* tryptophanyl-tRNA synthetase could be retained on a blue dextran–Sepharose column and it could be eluted by ATP and its cognate tRNA while other tRNAs had no effect. They also presented evidence that indicated that *E. coli* polynucleotide phosphorylase bound to blue dextran–Sepharose at its polynucleotide binding site. Interferon bound to blue dextran–Sepharose (Jankowski et al., 1976; Thang et al., 1977) and could be eluted by polynucleotides but not mono- or dinucleotides. Clearly, blue dextran and its dye can be of use in kinetic and physical studies of proteins that bind polynucleotides as well as those that bind NAD and ATP.

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