Catalytic Mechanism of Isoleucyl-tRNA Synthetase of *Escherichia coli* K10. Effect of pH and Chemical Modification†

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ABSTRACT: The pH dependence has been measured in the pH range 5–10 in the case of (1) aminoacylation catalyzed by isoleucyl-tRNA synthetase, (2) dissociation constants of binary complexes of the enzyme with L-isoleucine, L-isoleucinol, ATP, and adenosine, and (3) dissociation constants of ternary complexes between the enzyme, ATP, and L-isoleucinol. The effect of chemical modification at the highly reactive cysteine sulfhydryl group on ligand-binding properties has been studied. The catalytic rate constant follows the ionization of at least two types of amino acid side chains, which have been tentatively identified as histidine and cysteine. While several side chains of His are involved, only a single Cys is implicated. The pH dependence may be typical for aminoacyl-tRNA synthetases in general, if corrections for pH-dependent variation of the values for the Michaelis–Menten constants are introduced. The Michaelis–Menten constant for $tRNA^{Ile}$ has been interpreted in terms of the dissociation constant of binding to the enzyme·ATP·L-isoleucine complex. Its values are higher by more than one order of magnitude when compared with those of the dissociation constants. The observation is referred to anticooperativity between $tRNA^{Ile}$ and the small substrates. The pH dependence follows in a qualitative fashion the dependence of the catalytic rate constant and seems to involve as well His side chains in addition to others ionizing at higher pH. The stability of the enzyme·ATP complex decreases as pH is raised from 6.5 to 9, corresponding to a shift in pK from 6.9 in the free enzyme to 7.8 in the complex. The ionizing group may be a His side chain or a Lys moiety having low pK values because of special nonaqueous environments. Derivatization of the reactive Cys sulfhydryl group does not seriously affect the dissociation constant and its pH dependence. The value of the dissociation constant of the enzyme·L-isoleucine complex increases above pH 9. The pK of the free enzyme is 9.2. It could be a lysyl side chain, which interacts with the substrate carboxyl group. The complex of the enzyme with L-isoleucinol, which lacks the carboxylate, is pH independent. Derivatization of the Cys sulfhydryl group provokes destabilization of both L-isoleucine and L-isoleucinol complexes. Since the ionizing group in the case of L-isoleucine cannot be assigned to this Cys, the existence of another ionizing group has been postulated that counteracts ionization of Cys and eliminates pH dependence of the dissociation constant. Synergistically coupled binding to the enzyme of ATP and L-isoleucinol is pH dependent for the association of L-isoleucinol with the enzyme·ATP complex but not of ATP with the enzyme·L-isoleucinol complex. The pH dependence mirrors that of enzyme·ATP complex formation, leading to an increase in the strength of coupling at higher pH. Conversion of 5′-thio-2-nitrobenzoate–isoleucyl-tRNA synthetase into the *S*-cyano derivative does not lead to a recovery of the aminoacylation activity of the enzyme. This finding rules out that the observed inhibition by covalent attachment of sulfhydryl-specific reagents could be caused by the bulkiness or the electric charge of the modifying residue. The free Cys sulfhydryl may be required because of hydrogen-bond formation or mercaptide anion participation in the native enzyme. However, whereas His side chains appear to be essential for enzymatic activity, this group is only optimizing catalysis.

Our present knowledge about a participation of amino acid side chains of protein in the action of aminoacyl-tRNA synthetases is underdeveloped in comparison with that of other enzymes. Cysteine sulfhydryl and histidine imidazole groups have long been shown to be required intact to conserve maximum enzyme activity, but their function is obscure (Holler, 1978). Recently, the side chains of lysine (Fayat et al., 1978, 1979) and of a dicarboxylic acid (Kovaleva et al., 1978) have been found likely to be involved in the enzyme complex formation with ATP or tRNA and in the formation of an aminoacyl-enzyme, respectively.

As a rule, modification work alone is not sufficient to demonstrate the involvement of an amino acid side chain in catalysis but must be supported by results for an unmodified enzyme. One kind of such results is obtained from the effects of pH on the elementary steps of the catalysis revealing the ionization constants of amino acid side chains. Prior to modification work, such results can provide suggestions of the kind of side chain involved in a particular reaction.

In the present work, we have initiated the investigation of the pH dependence of the isoleucine specific system covering enzyme–substrate association and the overall aminoacylation of $tRNA^{Ile}$.

Materials and Methods

Materials. Isoleucyl-tRNA synthetase of *Escherichia coli* K10 was purified as described (Rainey et al., 1974). The number of active sites (0.6–1.0 mol of active sites/mol of enzyme) was routinely measured at time intervals of a few months according to the method of Rainey et al. (1977). Carboxymethylated isoleucyl-tRNA synthetase was obtained by treatment of the native enzyme with iodoacetate as follows. Enzyme was reactivated prior to carboxymethylation by 30-min incubation at 37 °C in 20 mM potassium phosphate buffer (pH 7.5) containing 0.1 mM EDTA and 20 mM dithioerythritol. The reduced enzyme was equilibrated with the buffer containing 0.2 mM dithioerythritol by desalting over a Sephadex G-75 column (1.3 × 13 cm). An amount of 9 μmol of enzyme was incubated with 0.015 M iodoacetate in the presence of 0.1 M Tris[1]-HCl (pH 8), 0.1 mM EDTA, and

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0.2 mM dithioerythritol in a total of 400 μL. After 1 h at 25 °C, the mixture was desalted over a Sephadex G-75 superfine column (1 × 18 cm) equilibrated with 20 mM potassium phosphate buffer (pH 7) containing 0.1 mM EDTA and 0.2 mM dithioerythritol. When [^{14}C]iodoacetate was used, it was found that 1 mol of acetate/mol of enzyme was incorporated while the aminoacylation activity was less than 5% of that of the untreated enzyme. Details of the filter method employed for radioactive labeling of the enzyme have been described (Rainey et al., 1976). The protein obtained by preferential attack of a single cysteine residue by a sulfhydryl specific reagent is known to be blocked at a unique cysteine moiety, which has been identified (Iaccarino & Berg, 1969; Rainey et al., 1977).

The enzyme was reacted with 5,5′-dithiobis(2-nitrobenzoic acid) following an established procedure (Ellman, 1959). Between 1 and 2 mol of 5-thio-2-nitrobenzoic acid residues/mol of enzyme are incorporated before inactivation becomes almost complete (Baldwin & Berg, 1966; Kuo & De Luca, 1969).

The *S*-cyano derivative of isoleucyl-tRNA synthetase was obtained by an exchange of the covalently linked 5-thio-2-nitrobenzoate moiety against cyanide (Vanaman & Stark, 1970; Der Terrossian & Kassab, 1976). The enzyme derivative used contained 1.0 mol of 5-thio-2-nitrobenzoate/mol of enzyme and had a residual aminoacylation activity of 2.6% that of the native enzyme, which had been treated similarly but was not exposed to 5,5′-dithiobis(2-nitrobenzoic acid). For the replacement, the enzyme derivative was incubated for 24 h at room temperature with a mixture of 0.05 M [^{14}C]KCN (20 μCi/μmol) in 0.5 M potassium phosphate buffer (pH 8). The exchange could be followed by the appearance of colored 5-thio-2-nitrobenzoate. At the end, the mixture was desalted over a Sephadex G-50 (0.6 × 25 cm) column with 0.05 M Tris-HCl buffer (pH 7.5) as the eluant, including 0.1 mM EDTA and 0.1 M KCl. The incorporation of [^{14}C]cyanide was followed by using DEAE- or nitrocellulose filter disks, as has been described for the determination of enzyme·[^{14}C]-isoleucyl·AMP complexes (Rainey et al., 1977). The *S*-cyano enzyme had a final ratio of 1.0 mol of [^{14}C]CN incorporated/mol of enzyme. The aminoacylation activity was less than 1% of that of the native enzyme. In a control experiment, the ^{14}CN could be removed by incubation with a mixture of 25 mM Tris-HCl (pH 7.5), 50 mM 2-mercaptoethanol, and 50 mM KCl. After 48 h at room temperature, 16% of the ^{14}CN was removed from the protein, corresponding to a reactivation of 14% if compared with a 5-thio-2-nitrobenzoic acid treated enzyme that had been treated with the same reactivation mixture.

A derivative of isoleucyl-tRNA synthetase with isoleucyl bromomethyl ketone was prepared as described by Rainey et al. (1976, 1977). At a stoichiometry of 1.2–1.6 mol of isoleucyl bromomethyl ketone incorporated per mole of enzyme, a residual aminoacylation activity of 3–8% of that of native enzyme was measured.

tRNA^{Ile} (1200 pmol/A_{260} unit in water) was purified from unfractionated tRNA (Zubay, 1962) *E. coli* K10 by using benzoylated DEAE-cellulose (Gillam et al., 1967) and RPC-5 columns (Pearson et al., 1971). Unfractionated tRNA contained 15–50 pmol of tRNA^{Ile}/A_{260} unit in water, depending on bacterial growth and storage.

L-[^{14}C]Isoleucine (300–500 μCi/μmol), [^{3}H]iodoacetic acid (158 μCi/μmol), and potassium [^{14}C]cyanide (61 μCi/μmol) were purchased from Amersham Buchler (Braunschweig). ATP was obtained from Boehringer (Mannheim), potassium 2-(*p*-toluidinyl)naphthalene-6-sulfonate and dithioerythritol were from Serva (Heidelberg), L-isoleucinol hydrochloride was from Cyclo (Los Angeles), DEAE-cellulose filter disks (DE 81) were from Whatman, nitrocellulose filter disks (BA 85) were from Schleicher & Schüll (Dassel), and all other reagents of highest possible grade were from Merck (Darmstadt).

Methods. Kinetics of aminoacylation of tRNA^{Ile} were followed by the method of Kosakowski & Böck (1970). Tris-HCl (0.1 M) and 2-(*N*-morpholino)ethanesulfonate (0.1 M) were used as buffers. NaCl was added, maintaining a constant ionic strength of 0.1 M. Incubation times were 1–10 min, during which product formation was linear with time. If fixed, the concentrations of tRNA^{Ile}, ATP, or L-isoleucine were 4 μM, 20 mM, or 20 μM, respectively. Initial velocities were plotted according to Eadie (1942). Values of the catalytic rate constant, k_{cat}, were calculated from the maximum rates when [tRNA^{Ile}] was varied. Since L-isoleucine and MgATP were subsaturating with respect to their Michaelis–Menten constants, the k_{cat} values were corrected by the factor

$$\left(\frac{[\text{Ile}]_0}{K_m(\text{Ile}) + [\text{Ile}]_0}\right)\left(\frac{[\text{MgATP}]_0}{K_m(\text{MgATP}) + [\text{MgATP}]_0}\right)$$

In a few instances, L-isoleucine and MgATP were the varied substrates. The correction factors were in these cases

$$R\left(\frac{[\text{tRNA}^{Ile}]_0}{K_m(\text{tRNA}) + [\text{tRNA}^{Ile}]_0}\right)$$

where R stands for either $[\text{Ile}]_0/\{K_m(\text{Ile}) + [\text{Ile}]_0\}$ or $[\text{MgATP}]_0/\{K_m(\text{MgATP}) + [\text{MgATP}]_0\}$. If the present enzyme system is a bi-uni-uni-bi Ping-Pong ter-ter system (Moe & Piszkiewicz, 1979), the correction factors can be shown to be an excellent approach (Segel, 1975). The various values of the Michaelis–Menten constants needed for the correction were taken as averages from Figure 2.

The formation of enzyme·substrate complexes was followed by the fluorimetric method employing the reporter group 2-(*p*-toluidinyl)naphthalene-6-sulfonate, as has been described (Holler et al., 1971). At pH <7, potassium phosphate buffer was used, between pH 7 and 8.5 Tris-HCl buffer was used, and at pH >8.5, glycine/NaOH buffer was used. NaCl was added in order to maintain a constant ionic strength of 0.05 M. Controls indicated that the values of the dissociation constants were independent of the kind of buffer used. However, the dissociation constants exhibited an ionic strength dependence as is recognized in the case of ATP. In 20 mM Tris-HCl buffer (pH 7.5), the dissociation constants were 0.084 mM, 0.075 mM, 0.13 mM, and 0.50 mM (25 °C) in the presence of 0, 50, 100, and 200 mM KCl, respectively. All buffers contained 1 mM dithioerythritol, 0.1 mM EDTA, 0.5–0.8 μM isoleucyl-tRNA synthetase, and 20–40 μM TNS. Fluorescence was measured at 450-nm emission wavelength in a Perkin-Elmer Model MPF-2A fluorescence spectrophotometer. Excitation light was 366 (TNS) or 295 nm (enzyme). Excitation of the enzyme-intrinsic Trp fluorescence results in an energy transfer to the enzyme-bound TNS. This mode is slightly more sensitive, generating a larger decrease in fluorescence intensity during the binding of substrates. Corrections due to inner filter effects (in cases of ATP and adenosine as varied ligands) were performed by substituting tryptophan for the enzyme. The same correction was applied when the binding of tRNA^{Ile} was followed by observation of

[1] Abbreviations used: EDTA, (ethylenedinitrilo)tetraacetic acid; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; TNS, 2-(*p*-toluidinyl)-naphthalene-6-sulfonate; Ileol, L-isoleucinol (2-amino-3-methylpentanol); tRNA^{Ile}, tRNA specific for the acceptance of Ile.

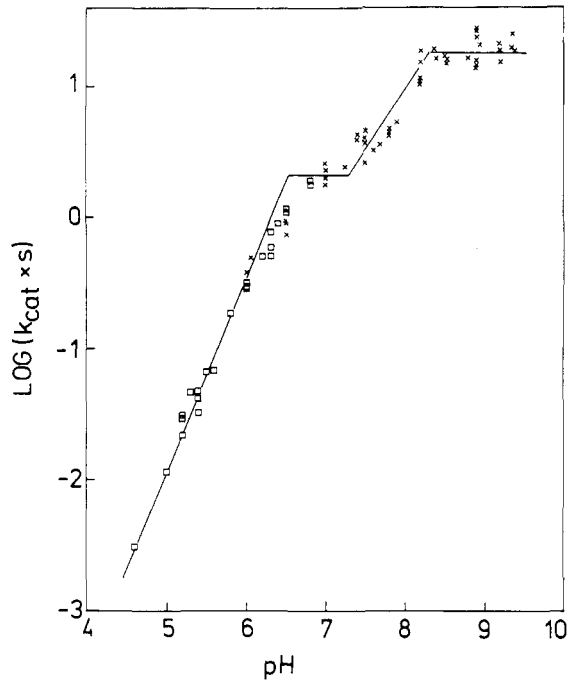


FIGURE 1: Effect of pH on the catalytic rate constant of aminoacylation. Rate constants k_{cat} were determined as described in the text. At pH below 5.5 it was verified by control experiments that K_m values for ATP and L-isoleucine were of the same order of magnitude as above pH 5.5. Conditions were (□) 0.1 M 2-(*N*-morpholino)ethanesulfonate or (×) 0.1 M Tris-HCl buffer, ionic strength 0.1 M adjusted with NaCl, 10 mM $MgCl_2$, 10 mM KCl, and 1 mM reduced glutathione at 28 °C. Total enzyme varied between 0.5 and 300 nM.

the protein intrinsic fluorescence at 340 nm (excitation wavelength, 294 nm). At a given concentration of $tRNA^{Ile}$, the corrected relative decrease in fluorescence intensity due to formation of an enzyme·$tRNA^{Ile}$ complex is given by the approximation $\Delta F_{E,tRNA} = (F_{Trp,tRNA} - F_{E,tRNA}) \times F_{Trp,0}/F_{Trp,tRNA}$. $F_{Trp,tRNA}$ and $F_{E,tRNA}$ refer to the fluorescence intensities of Trp and enzyme, respectively, in the presence of $tRNA^{Ile}$ or, in case of $F_{Trp,0}$, in the absence of tRNA. The fluorescence $F_{Trp,0}$ has to be the same as the fluorescence intensity of the enzyme in the absence of ligand.

Dissociation constants were calculated from linearized plots of the observed relative decrease in fluorescence intensity, as has been described (Holler et al., 1971; Bartmann et al., 1975). Part of the evaluation of the pH dependence of the dissociation constants was to determine limiting values of these constants at the alkaline and acid branches of the dissociation constant vs. pH dependence. The limiting values were obtained by extrapolation to zero concentration of $[H^+]$ and $[OH^-]$, respectively, by using plots of dissociation constant vs. $[H^+]$ and $[OH^-]$, respectively.

The intensity of light emitted by the enzyme·TNS complex as well as the decrease observed in the presence of the ligand was pH independent below pH 9 and decreased at pH values above 9 (Holler et al., 1973). For the carboxymethylated enzyme and for the 5-thio-2-nitrobenzoate enzyme, the intensity as well as the effect of ligand binding was the same as for the native enzyme.

Results

Aminoacylation. In Figure 1, the catalytic rate constant, k_{cat}, increases from pH 4.5 to 8.5 by 4 orders of magnitude. It remains constant between pH 8.5 and 9.5. Beyond pH 4.5 and 10, enzyme stability is decreased, rendering these pH ranges unsuitable for an investigation. We have no indication that the pH profile has an activity maximum if denaturation is excluded. The analysis in Figure 1 is based on the scheme shown in eq 1, which relates enzyme activity and association

$$\begin{array}{ccc} ES & \xrightarrow{k_1} & E + P \\ nH^+ \downarrow\uparrow K & & \\ H_nES & \xrightarrow{k_2} & H_nE + P \end{array} \qquad (1)$$

of *n* protons to amino acid side chains with identical ionization constants. ES and H_nES resemble the free and *n*-fold protonated enzyme·substrate complexes, which break down in the rate limiting step to give free or *n*-fold protonated enzyme and product, respectively. The protonation equilibrium is given by the apparent dissociation constant

$$K = \frac{[ES][H^+]^n}{[H_nES]} \qquad (2)$$

Product is formed at rates given by rate constants k_1 and k_2

$$\frac{d[P]}{dt} = k_1[ES] + k_2[H_nES] \qquad (3)$$

Including the conservation equation $[E]_0 = [ES] + [H_nES]$, the expression for the observed rate constant is

$$k_{cat} = \frac{\frac{k_1}{k_2}K + [H^+]^n}{\frac{1}{k_2}(K + [H^+]^n)} \qquad (4)$$

with an acid branch ($[H^+] \rightarrow \infty$) for $k_{cat} = k_2$ and with a base branch ($[H^+] \rightarrow 0$) for $k_{cat} = k_1$. The portion between the branches is given by a linear pH dependence, assuming $K \ll [H^+]^n \ll (k_1/k_2)K$ and using eq 4:

$$\log k_{cat} = \log k_1 - pK + npH \qquad (5)$$

The slope of the linear portion is equal to *n*. Upon extrapolation, the linear portion and the base branch intersect at pH equal to pK/n.

Inspection of Figure 1 reveals the acid branch to be absent ($k_2 = 0$ or undetectably low), the base branch to follow $k_1 = 20\ s^{-1}$, and the linear portion to be composed of at least two segments separated by a pH-independent interval. They are not well separated, but if an attempt is made, the segment on the acid side is found to follow $n = 1.5$ and to intersect at pH 6.5 ± 0.2 with the short pH-independent branch that follows $k_{cat} = 2\ s^{-1}$. Deprotonation of amino acid side chains that follow the $pK = 1.5 \times 6.5 = 9.75$ is essential for enzyme activity to occur. The other linear segment follows $n = 1$ and intersects the base branch at pH $= 8.3 \pm 0.2$. Its effect is to maximize the catalytic rate constant.

pH dependencies of the Michaelis–Menten constants for L-isoleucine, ATP, and $tRNA^{Ile}$ are given in Figure 2. In the range investigated, K_m of ATP remained almost constant while those of Ile and $tRNA^{Ile}$ show a definite dependence. In the case of Ile, an apparent discontinuity is seen at pH 9. A plot of log K_m vs. pH in the case of $tRNA^{Ile}$ reveals changes along the acidic side of the pH scale that are reminiscent of those found for the dependence of k_{cat} (Figure 1). An analysis will be attempted subsequently in analogy to that of dissociation constants of enzyme·substrate complexes.

Enzyme·Substrate Complexes. The dissociation constants of the enzyme·ligand complexes have been measured fluorimetrically in the presence of TNS as a reporter group. Figure 3A shows the results for ATP as a function of pH. The value of the dissociation constant increases by a factor of 10 when the pH varies from 6.5 to 9.5. The pH dependence for L-

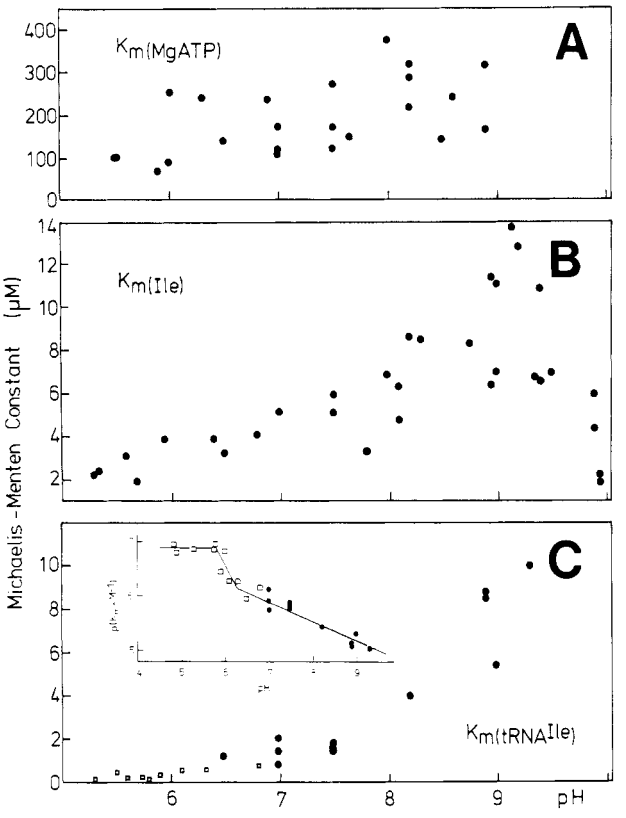


FIGURE 2: Effect of pH on the Michaelis–Menten constants of MgATP (A), L-isoleucine (B), and $tRNA^{Ile}$ (C) of the aminoacylation reaction. Results in the inset were plotted according to eq 6–15, assuming that the Michaelis–Menten constant can be referred to the dissociation constant. When not varied, concentrations were 2 mM MgATP, 20 μM L-isoleucine, and 4 μM $tRNA^{Ile}$. (□) 0.1 M 2-(*N*-morpholino)ethanesulfonate or (●) 0.1 M Tris-HCl buffer as in Figure 1.

isoleucine is seen in Figure 4A. The dissociation constant remains constant between pH 5.8 and 9 but rises sharply at higher pH. An investigation at even higher pH is limited by the beginning instability of the enzyme.

An evaluation of the pH dependence is based on the ionization scheme shown in eq 6. Free enzyme and enzyme-substrate complexes exist in free (E, ES) and protonated states (H_nE, H_nES). The number of strictly cooperatively binding protons is *n*. Apparent ionization constants are defined as

$$\begin{array}{ccc} & \overset{S}{\rightleftharpoons} & \\ E & \underset{K_{S,0}}{\rightleftharpoons} & ES \\ {\scriptstyle n}H^+ \;\; \downarrow\uparrow {\scriptstyle K_1} & & {\scriptstyle K_2}\;\downarrow\uparrow \;{\scriptstyle n}H^+ \\ H_nE & \underset{S}{\rightleftharpoons} & H_nES \end{array} \qquad (6)$$

$$K_1 = \frac{[E][H^+]^n}{[H_nE]}$$

$$K_{S,0} = \frac{[E][S]}{[ES]} \qquad (7)$$

$$K_2 = \frac{[ES][H^+]^n}{[H_nES]}$$

$$[E]_0 = [E] + [H_nE] + [ES] + [H_nES]$$

$$[S]_0 = [S] \gg [E]_0$$

The observed decrease in fluorescence intensity of TNS is pH independent below pH 9 and is considered to be proportional

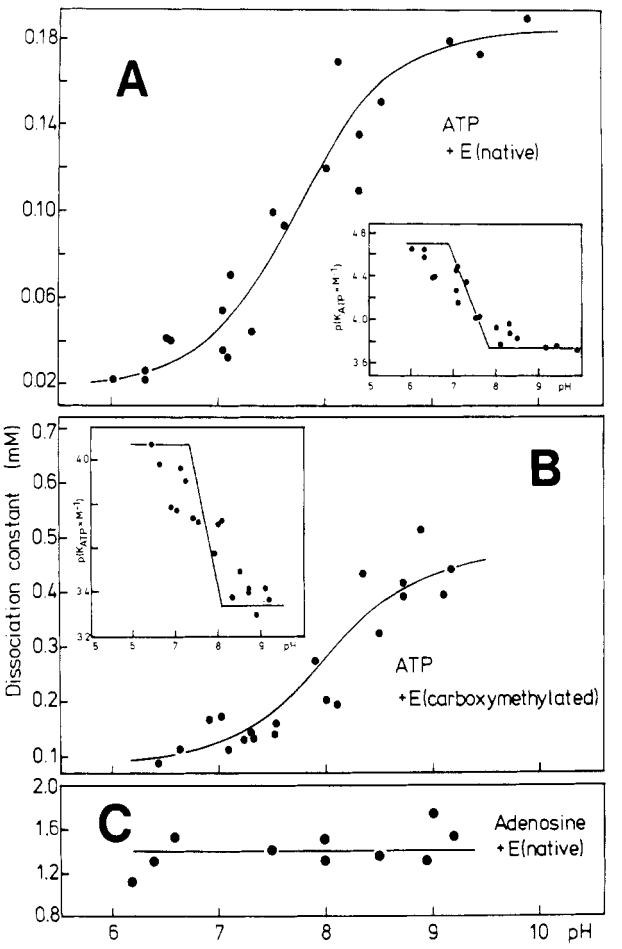


FIGURE 3: Effect of pH on the dissociation constants of Ile-tRNA synthetase with ATP (A) or adenosine (C) and of carboxymethyl-Ile-tRNA synthetase with ATP (B). Temperature was 25 ± 0.5 °C. Drawn curves are computed by standard procedures, using $pK_2 = 7.8$ (panel A) and $pK_2 = 8.1$ (panel B) of the respective enzyme·ATP complexes (Table I). A single proton was assumed. The insets were used for the determination of the p*K*'s of an ionizing group as described in the text, assuming the association of a single proton. The fit of the experimental points by the drawn curves in (A) and (B) supports the validity of the evaluation procedure.

to the concentration of ES plus H_nES formed. At higher pH, the fluorescence intensity of E·TNS as well as the ligand-induced decrease of the intensity becomes increasingly smaller. We assume that this dependence of the TNS fluorescence is not coupled to the same ionizing group(s) involved in substrate binding, one of the arguments being that the binding of TNS and substrates to the enzyme appears to be uncoupled (Holler et al., 1971). From eq 7, the expression for the concentration dependence of the decrease in fluorescence intensity, ΔF, is

$$\Delta F \sim [ES] + [H_nES] = \frac{[E]_0[S]_0}{K_{S,0}\frac{K_2}{K_1}\left(\frac{K_1 + [H^+]^n}{K_2 + [H^+]^n}\right) + [S]_0} \qquad (8)$$

The observed dissociation constant is

$$K_s = K_{S,0}\frac{K_2}{K_1}\left(\frac{K_1 + [H^+]^n}{K_2 + [H^+]^n}\right) \qquad (9)$$

or in logarithmic form

$$pK_s = pK_{S,0} - \log\frac{K_2}{K_1} - \log\frac{K_1 + [H^+]^n}{K_2 + [H^+]^n} \qquad (10)$$

Table I: pH Dependence of Dissociation Constants[a]

system	dissociation constants		ionization constants		
	$[H^+] \rightarrow \infty$ (mM)	$[H^+] \rightarrow 0$ (mM)	pK_1	pK_2	n^c
ATP + E	0.02 ± 0.005	0.18 ± 0.03	6.9 ± 0.2	7.8 ± 0.2	1
ATP + E(carboxymethylated)	0.09 ± 0.02	0.4 ± 0.1	7.3 ± 0.2	8.1 ± 0.2	1
adenosine + E	1.4 ± 0.2	same	none	none	
Ile + E	$(6.2 \pm 0.6) \times 10^{-3}$	nd[b]	9.2 ± 0.2	nd	1
Ileol + E	2.7 ± 0.7	same	none	none	
Ileol + E·ATP	$(1.3 \pm 0.3) \times 10^{-3}$	$(1.5 \pm 0.4) \times 10^{-4}$	7.8	6.9	1
ATP + E·Ileol	$(1.0 \pm 0.3) \times 10^{-5}$	same	none	none	

[a] Conditions: buffers and NaCl at constant ionic strength of 50 mM (see Materials and Methods), 0.1 mM EDTA, and 0.2 mM dithioerythritol at 25 °C. [b] nd = not determined. [c] n = number of H^+ ionizing.

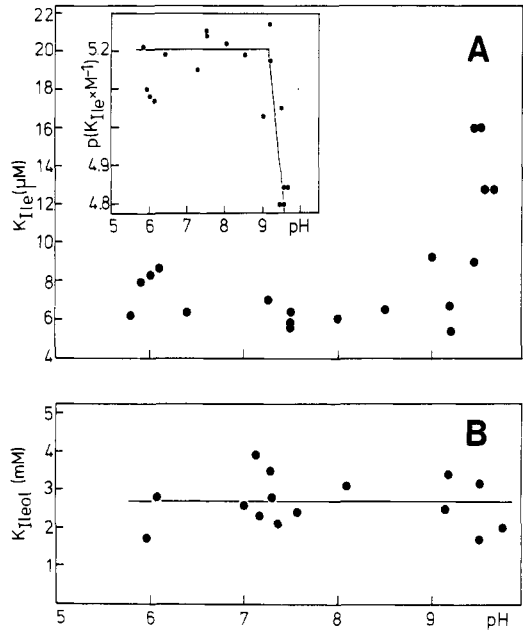


FIGURE 4: Effect of pH on the dissociation constant of Ile-tRNA synthetase complexes with L-isoleucine (A) and L-isoleucinol, respectively. The inset was used for the evaluation of the number of protons, n, and of the pK of an ionizing group in the free enzyme.

It has been shown (Dixon, 1953) that plots of pK_s vs. pH can be used to determine all parameters of eq 9. The following segments of the pH dependence (as shown in Figure 3) are then considered:

$[H^+] \rightarrow 0$ (the base branch)

$$pK_s = pK_{S,0} \tag{11}$$

$[H^+] \rightarrow \infty$ (the acid branch)

$$pK_s = \log (K_1/K_2) + pK_{S,0} \tag{12}$$

$K_2 \ll [H^+]^n \ll K_1$ (between the acid and the base branches)

$$pK_s = pK_2 - npH + pK_{S,0} \tag{13}$$

Equations 11–13 are linear in pK_s vs. pH. The following points of intersections exist:

$$\text{eq 11 with eq 13 at pH} = (1/n)pK_2 \tag{14}$$

$$\text{eq 12 with eq 13 at pH} = (1/n)pK_1 \tag{15}$$

The same equations, (8) through (14), are obtained if the substrate is the ionizing species (Dixon, 1953; Cleland, 1977). Similar calculations are valid in the case of the Michaelis–Menten constant as a function of pH, provided this parameter has the simple meaning of a dissociation constant.

The pH dependence for ATP is carried out as seen in Figure 3A, inset. The number of protons, n, cannot be obtained because pK_1 and pK_2 are not sufficiently apart. It is assumed that $n = 1$. The pK values are given in Table I. Figure 3C demonstrates that the ionizing group seen for ATP reflects the presence of the triphosphate group since adenosine exhibits no pH dependence. The pH dependence for L-isoleucine follows a pK_1 of 9.2 (Figure 4A) which is absent in the case of the amino alcohol, L-isoleucinol. This ionization clearly involves a single proton.

In the case of $tRNA^{Ile}$, the dissociation constant was determined only at a single pH of 7.5. Titration of the protein-intrinsic fluorescence was performed at 0.1 μM enzyme and 0.01–0.6 μM $tRNA^{Ile}$. Dissociation constants were (25 ± 5) nM in the absence and (13 ± 3) nM in the presence of 10 mM $MgCl_2$ in solutions otherwise containing 50 mM Tris-HCl, 0.2 mM EDTA, and 0.4 mM dithioerythritol. The intensity of the fluorescence emission decreased upon saturation with $tRNA^{Ile}$ by 15% of the intensity in the absence of tRNA. A single binding site per molecule of enzyme was indicated. Since ATP and L-isoleucine under conditions of tRNA aminoacylation were almost saturating with respect to enzyme, the Michaelis–Menten constant for $tRNA^{Ile}$ as a function of pH was assumed to resemble the pH dependence of the dissociation constant for $tRNA^{Ile}$ binding to the enzyme·Ile·ATP complex. This seems justified in the light of the established kinetic mechanism (Moe & Piszkiewicz, 1979). Accordingly, the log K_m vs. pH plot in Figure 2C is interpreted from the left to the right by (1) a pH-independent dissociation constant, $K_m = 0.13$ μM for $tRNA^{Ile}$ binding to the protonated enzyme, and (2) by a dissociation of more than one proton ($n = 1.5$) from the tRNA-free enzyme with a pK_1 of 5.8, and from the enzyme complex with tRNA with a pK_2 of $6.2 \times 1.5 = 9.3$. (3) Beyond pH 6.2, the value of the Michaelis–Menten constant is 0.8 μM and continues to rise with increasing pH. The value of $n = 0.3$ suggests a complex dependence on protonation of other ionizable groups. It is clear that the observed resolution of the pH dependence is limited because of relatively high experimental scatter.

Synergistic Binding of ATP and L-*Isoleucinol.* Synergistic binding is an enforcement of complex formation between the enzyme and either ATP or L-isoleucinol in the presence of both ligands (Holler et al., 1975). Binding of ATP to the enzyme·L-isoleucinol complex was found to follow a pH-independent dissociation constant, K_{ATP}^{Ileol} (Figure 5). Because of relations 16, 18, and K_{Ileol} (Figure 4B) as well as K_{ATP}^{Ileol} (Figure 5) being pH independent, it is concluded that binding of L-isoleucinol to the enzyme·ATP complex is pH dependent. The corresponding dissociation constant, K_{Ileol}^{ATP}, mirrors exactly the pH dependence for binding of ATP, thus canceling the pH dependence for K_{ATP}^{Ileol} (eq 16, 18). The strength of synergistic

Table II: Comparison of Dissociation Constants of Enzyme·Ligand Complexes for Native and Derivatized Isoleucyl-tRNA Synthetase[a]

ligand	dissociation constants (mM)					ratio of dissociation constants (modified:native)
		modifying residue				
	native	*S*-cyano	5-thio-2-nitrobenzoate	carboxy-methyl	isoleucyl methyl ketone	
ATP	0.08[c]	0.16	0.18	0.18	0.2[c]	~2.5
L-isoleucine	0.006[c]	0.08	0.09	0.12	0.08[c]	13–20
L-isoleucinol	2.7	nd[b]	nd	≳20	50[c]	~18

[a] 25 °C, 10 mM potassium phosphate buffer (pH 7.5), 0.2 mM EDTA. Experimental errors are of the order of 20%. [b] nd = not determined. [c] Rainey et al. (1977).

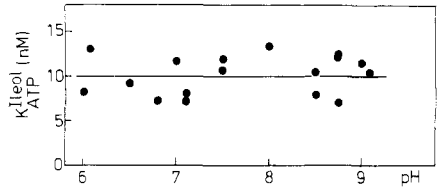


FIGURE 5: Effect of pH on the dissociation constant, K^{Ileol}_{ATP}, of binding ATP to the enzyme·L-isoleucinol complex. Titration experiments were performed at 25 °C in the presence of 40 μM TNS by adding ATP to mixtures containing 0.5 μM isoleucyl-tRNA synthetase, buffer (see Methods) and NaCl at an ionic strength of 50 mM, 0.1 mM EDTA, 0.2 mM dithioerythritol, and 1.75 mM L-isoleucinol. The apparent dissociation constant, K^{Ileol}_{ATP} (app), for ATP binding to the enzyme·L-isoleucinol complex was determined from the observed decrease in fluorescence intensity as described under Methods. The dissociation constants, K^{ATP}_{Ileol} and K^{Ileol}_{ATP}, defined in eq 16 were calculated on the basis of eq 17 and 18 by using the above values of isoleucinol and K^{Ileol}_{ATP} (app) and the experimental values of K_{ATP} and K_{Ileol} as a function of pH (Figures 3A and 4B). These equations have been derived previously (Kosakowski & Holler, 1973).

binding as defined by $s = K_{Ileol}/K^{ATP}_{Ileol} = K_{ATP}/K^{Ileol}_{ATP}$ varies from $s = 2000$ at low pH to $s = 18\,000$ at high pH.

Formation of Enzyme·Ligand Complexes after the Derivatization of Isoleucyl-tRNA Synthetase with Sulfhydryl-Specific Reagents. Dissociation constants of complexes of ATP, L-isoleucine, and L-isoleucinol with covalent derivatives of isoleucyl-tRNA synthetase are compared with those for the native enzyme (Table II). In all complexes, the observed fluorescence intensity of the enzyme-bound TNS, as well as the decrease in intensity upon formation of the complexes, was within 70–100% of that of the native enzyme. This rules out possible misinterpretations, which could arise from strong fluorescence properties of residual native enzyme and weak properties of modified enzyme.

Derivatizations (Table II) affect binding of ligands by decreasing the affinity for complex formation. In the case of ATP, the value of the dissociation constant increases 2.5-fold; in the case of both L-isoleucine and L-isoleucinol, 13–20-fold. Most interestingly, the effect does not depend on the size or electric charge of the derivating moiety; thus the effect of the CN moiety is the same as is that of the negatively charged 5-thio-2-nitrobenzoate or the (pH dependent) positively charged isoleucyl methyl ketone moiety. Furthermore, the carboxymethylation of the enzyme does not eliminate the pH dependence seen for ATP, though the ionizing group has slightly higher values of pK_1 and pK_2 (Figure 3B; Table I). This enzyme derivative has a pH dependence for the dissociation constant of the enzyme·Ile complex that is indistinguishable from that of the native complex (pH 6–10, not shown).

Discussion

Aminoacylation of tRNA. Michaelis–Menten constants of complex systems, as are aminoacyl-tRNA synthetases, are functions of kinetic and equilibrium constants and may not be meaningfully discussed in the context of pH dependencies (Segel, 1975). There are yet some observations which might be of value. (1) K_m (ATP) is independent of pH although this has a pronounced effect on the dissociation constant of the enzyme·ATP complex. The independence resembles that for K^{Ileol}_{ATP} obtained for ATP binding to the enzyme·L-isoleucinol complex. It might be speculated that under aminoacylation conditions, the other substrates, L-isoleucine, $tRNA^{Ile}$, and Mg^{2+}, could have a similar effect as that of L-isoleucinol. This is supported by the idea that synergistic binding of ATP and L-isoleucinol expresses a part of the catalytic mechanism (Holler et al., 1975). (2) K_m (ATP) in the range pH 8.3–9 and K_m(Ile) in the range pH 5.3–9 (Figure 2A,B) have values that seem to agree with those of the dissociation constants of the binary complexes (Figures 3A and 4A). At first glance, this suggests that the pH functions in these ranges are dominated by the particular dissociation constants. It turns out from the analysis below that the agreement is a coincidence. The dissociation constants in the presence of $tRNA^{Ile}$ are not the same as for the binary complexes. (3) The Michaelis–Menten constants of $tRNA^{Ile}$ (Figure 2C) are considerably higher than the corresponding dissociation constants [13 nM and 25 nM at pH 7.5; ≲10 nM at pH 5.5 at 17 °C; Lam & Schimmel (1975)]. The discrepancy can be referred to two observations. (a) Dissociation constants of enzyme·tRNA complexes are salt dependent, in the case of the yeast-specific system leading to an almost 10-fold increase in the value for the dissociation constant after addition of 0.1 M KCl (Krauss et al., 1976). (b) A modulation of the dissociation constant for $tRNA^{Ile}$ in the Ile-specific system (*E. coli*) by the presence of either ATP or Ile was observed as a 5-fold increase of its value (Hustedt & Kula, 1977). The effect is reciprocal as it leads to an increase in the values of the dissociation constants for ATP or L-isoleucine. We are concerned here mainly with observation b. It predicts, in the absence of other effects, that the K_m values for ATP, L-isoleucine, and, reciprocally, $tRNA^{Ile}$ be higher than those of the dissociation constants. In contrast, accumulation of the enzyme·Ile~AMP complex under steady-state charging condition tends to decrease the values for ATP and L-isoleucine similarly, as has been discussed (Fersht & Kaethner, 1976; Gutfreund & Sturtevant, 1956; Pimmer & Holler, 1979). Both effects, anticooperativity by $tRNA^{Ile}$ and binding in the form of the adenylate, seem to cancel out for ATP (pH range 8.3–9) and L-isoleucine (pH range 5.3–9). Since accumulation of the adenylate has no effect on the accessibility of the tRNA binding site, the anticooperativity between $tRNA^{Ile}$ and the small substrates can still be detected as an increase in the value of K_m for tRNA.

The value of the catalytic rate constant, k_{cat}, increases as a function of pH (Figure 1) from pH 4.5 to pH 8.2, where it arrives at a plateau value. This is in contrast to an activity optimum which has been reported for the ATP–PP_i exchange

(Bergmann et al., 1961). Figure 1 indicates that at least two kinds of amino acid side chains and several protons are involved. A similar pH dependence has been observed for phenylalanyl-tRNA synthetase of *E. coli* (E. Holler and M. Baltzinger, unpublished results). Essential for enzymatic activity to occur is the deprotonation observed below pH 7 (Figure 1). Interestingly, it is the same range exhibiting a decrease in the K_m value for tRNA (Figure 2C, inset), a decrease in the value of the dissociation constant of the enzyme·$tRNA^{Ile}$ complex (Lam & Schimmel, 1975), and a decrease in protein-intrinsic fluorescence intensity (Lam & Schimmel, 1975). Deprotonation in this pH range involves the removal of at least two protons from the enzyme, as indicated by $n = 1.5$ (Figures 1 and 2C, inset). The experimental $pK = 9.75$ cannot, in the absence of interaction factors (Segel, 1975; see Hill equation), be resolved into intrinsic pK's of individual amino acid side chains. From the order of magnitude it might be tentatively assumed that histidyl or carboxyl moieties are involved.

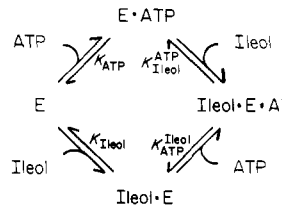
Besides the deprotonation which is essential, another deprotonation at higher pH is indicated (Figure 1) that exerts an enhancement of enzymatic activity. The slope, $n = 1$, indicates a single proton involved. The ionizing group in the enzyme·substrate complex has a $pK = 8.3$. In the same pH range, the strength of synergistic binding increases from $s = 2000$ (pH 6.5) to $s = 18\,000$ (pH 8.5), which mirrors the pH dependence of the dissociation constant for the enzyme·ATP complex. A correlation between enzyme activity and strength of synergism has been suggested by the idea that synergism promotes the formation of the transition state of enzymatic synthesis of aminoacyladenylate (Blanquet et al., 1975; Holler, 1978).

Enzyme·Substrate Complexes. All of the complexes between isoleucyl-tRNA synthetase and the substrates exhibit a pH dependence. For ATP, the highest stability is observed below pH 7, for L-isoleucine below pH 9, and for $tRNA^{Ile}$ below pH 6 (Figures 3A, 4A, and 2C).

Deprotonation of the enzyme·ATP complex leads to reduced stability (Figure 3A, Table I). Reciprocally, binding of ATP causes the pK of an ionizing group to shift from $pK = 6.9$ (free enzyme) to $pK = 7.8$ (enzyme·ATP complex; eq 6 and Table I). A single proton is assumed to be involved. the $pK = 6.9$ has been attributed to free enzyme but could as well be related to ionization of free ATP ($HATP^{3-} \rightleftharpoons H^+ + ATP^{4-}$, $pK = 6.95$; Alberty, 1969). Adenosine, which has been shown to compete with ATP for enzyme complex formation (Holler et al., 1973), does not exhibit such an ionizing group (Figure 3C) in support of the alternative explanation. However, the pH independence of binding ATP to the enzyme·L-isoleucinol complex (K_{ATP}^{Ileol}, Figure 5), while there is no evidence for an interaction between ATP and L-isoleucinol in the absence of enzyme [reference is made to formation of complexes between ATP and biogenic amines; Weder & Wiegand (1973)], seems to rule out ATP as the ionizing species. The upward pK shift implies that a proton is taken up during enzyme·ATP formation. Allowing small environmental effects, candidates for the ionizing amino acid side chains are histidine ($pK = 6.7$–7.1; Tanford & Hauenstein, 1956) and cysteine ($pK = 8.8$–9.1). If the group serves as an electrostatic anchorage, a histidine is likely. Results of affinity labeling with 2′,3′-ribose oxidized ATP of several aminoacyl-tRNA synthetases suggested a lysine amino side chain to be proximate to the ATP binding site (Fayat et al., 1978). The observed $pK = 6.9$ can be ascribed to an ϵ-amino group of lysine only if an unusual environment like positive charge(s) or nonpolar groups is assumed.

Stability of the enzyme·L-isoleucine complex decreases at higher pH 9 after the removal of a single proton from a group in the complex with a pK which is unknown but which is $pK = 9.2$ in the free enzyme. Again, it is assumed that the ionizing group belongs to the protein and not to substrate L-isoleucine (the pK of the amino group is 9.68: Fasman, 1974). The argument is that L-isoleucinol, having a similar pK value, exhibits pH-independent binding (Figure 4). The ionizing group on the enzyme could be a lysine side chain ($pK = 9.3$–9.5; Tanford & Hauenstein, 1956), which interacts electrostatically with the substrate carboxylate.

Synergistic binding of ATP and L-isoleucinol has been shown to follow the reaction scheme in eq 16 (Kosakowski & Holler,



(16)

$$K_{ATP}^{Ileol}(app) = K_{ATP}\left(\frac{K_{Ileol}^{ATP}}{K_{Ileol}^{ATP} + [Ileol]}\right)\left(\frac{K_{Ileol} + [Ileol]}{K_{Ileol}}\right) \quad (17)$$

$$K_{ATP}K_{Ileol}^{ATP} = K_{Ileol}K_{ATP}^{Ileol} \quad (18)$$

1973). The results of the present pH work indicate that the ternary enzyme·ATP·L-isoleucinol complex forms pH independently. The elements responsible for the synergism have been found to be a positive charge (L-isoleucinol) and negative charges (ATP). Positive and negative charges are also present in the substrate amino acid and, consequently, we have called their cooperative function in that case "intramolecular" synergism (Holler et al., 1975). The similarity of synergism between ATP/L-isoleucinol and intramolecular synergism is expressed by a common range of pH independence (pH 6–9).

Michaelis–Menten constants for $tRNA^{Ile}$ have been interpreted in terms of dissociation constants for binding $tRNA^{Ile}$ to the enzyme·ATP·Ile complex. The pH dependence indicates several protons are involved (Figure 2C). As the net negative charge of the enzyme increases by repeated deprotonation, the strength of interaction between the enzyme and the negatively charged tRNA decreases, as is expected. Tight binding correlates with less enzyme activity (Figure 1). This observation with isoleucyl-tRNA synthetase is typical for most other aminoacyl-tRNA synthetases. In the case of the *E. coli* Phe-specific system, tight binding of tRNA has been demonstrated below pH 7 (Bartmann et al., 1975). With most aminoacyl-tRNA synthetases, the nitrocellulose filter assay of Yarus & Berg (1970) is applicable only below pH 7 where complex stability is highest. Generally, synthetase activities fall off below pH 7. In contrast to our findings, most synthetases have been shown to have activity optima between pH 7 and 9. These results may be erroneous because fixed assay concentrations have been used. As the K_m value for tRNA increases with increasing pH (Figure 2C), a point might be reached where activity falls off because of subsaturation of the enzyme with tRNA. Similar explanations include the other substrates. The fact that tRNA binding and enzyme activity follow pH dependences in a qualitatively similar yet reciprocal fashion is in agreement with transfer of the aminoacyl moiety to tRNA as the rate-determining step of catalysis. Similar or identical ionizing moieties might be involved in binding and transfer (bond formation, group positioning, or

Table III: Summary of Ionizing Amino Acid Side Chains of Isoleucyl-tRNA Synthetase and Their Possible Functions

pH range	pK (enzyme·substrate complex)	no. of protons	catalytic functioning	derivatization: type of	derivatization: effect	proposed amino acid side chain
4.5–7	9.75[a]	≥2	κ_{cat} (aminoacylation)	carbethoxylation	inactivation[b]	His (I)[g]
5.5–7		≥2	intrinsic enzyme fluorescence[c]			His (I)
5.8–6.2	9.3[a]	≥2	K_m(tRNA^{Ile}), binding of tRNA^{Ile}[c]			His (I)
6.2–9.5		≥1	K_m(tRNA^{Ile}) binding of tRNA	Schiff base[d]	site blocked	Lys (I)
6.5–8.5	7.8	1	binding of ATP	alkylation of Cys-SH	almost no effect	His (II)
				Schiff base[e]	site blocked	Lys (II)
6.5–8.5	7.8	1	synergistic binding of ATP and Ileol			see binding of ATP
7–8.2	8.3	1	k_{cat} (aminoacylation)	alkylation of Cys-SH	33-fold reduction of k_{cat}[f]	Cys
9	9.2 free enzyme	1	binding of Ile	alkylation of Cys-SH	20-fold increased $K_{diss.}$	Cys ? Lys (III)

[a] Values for $n \geq 1$ resemble complex functions of intrinsic pKs and interaction factors (Segel, 1975). [b] E. Wimmer and E. Holler, unpublished results. [c] Lam & Schimmel, 1975. [d] Fayat et al. (1979) in the case of methionyl-tRNA synthetase, Baltzinger et al. (1979) in the case of yeast phenylalanyl-tRNA synthetase. [e] Fayat et al. (1978). [f] Rainey et al. (1977). [g] Numbers denote different side chains.

conformational changes). The position of pKs suggests below pH 7 several His moieties and above pH 7 one or more Lys side chains. Reaction of His side chains with diethyl pyrocarbonate has been reported in all cases investigated to lead to inactivation. Loss or reduction of tRNA binding has been found in the Trp-specific synthetase (Favorova et al., 1978) but not with synthetases specific for Phe of *E. coli* (Hennecke & Böck, 1974), of yeast (Raffin & Remy, 1978), or Ile (E. Wimmer and E. Holler, unpublished results). Participation of lysine as the ionizing group is indicated by the results of affinity labeling with periodate-treated tRNA (Fayat et al., 1979; Baltzinger et al., 1979).

Enzyme Derivatization. Alkylation of a single cysteine sulfhydryl group by L-isoleucyl bromomethyl ketone provokes a 33-fold decrease in enzyme activity (Rainey et al., 1976, 1977). Since a cysteine sulfhydryl group ionizes with a pK of 9.1 (Tanford & Roxby, 1972), it could well be a group reflected by the pH dependence of k_{cat} (Figure 1).

We have employed derivatization of the same sulfhydryl group that reacts with L-isoleucyl bromomethyl ketone in order to examine the nature of inhibition. Effects on dissociation constants for ATP and Ile are listed in Table II. Conclusions drawn from these and other results are briefly summarized. (1) Observed effects do not result from size or charge of the attached moiety at the sulfur atom. For maximum catalytic activity, the sulfhydryl group must then be either ionized or hydrogen bonded. (2) Although the value of the dissociation constant for L-isoleucine is elevated ~20-fold, the maximum rate of adenylate synthesis is unchanged, indicating that this sulfhydryl group does not participate in the amino acid activation reaction (Rainey et al., 1977). (3) Despite of the effect on the dissociation constant, it appears unlikely that the sulfhydryl group is the ionizing moiety seen in the pH dependence for L-isoleucine (Figure 4A). The reason is that L-isoleucinol does not have a pH dependence, although its dissociation constant is affected in the same way (Table II). The absence of a pH dependence for L-isoleucine in face of the observed increase of the value of the dissociation constant is intriguing. A possible explanation is that an effect of ionization of Cys in the native enzyme is compensated by another event, for instance, by the ionization of a group which, in the deprotonated state, exerts the destabilization while deprotonation of Cys would render the enzyme·Ile complex more stable. Cys and the putative group could form an ion pair as discussed (Holler et al., 1975). Further work is required to clarify this point. (4) Alkylation of the Cys has a small effect on the dissociation constant of ATP (Table II) and its pH dependence (Figure 3). We conclude that the Cys is not important in ATP binding.

Conclusion

The results in this and other investigations on the participation of ionizing side chains have been summarized in Table III. Two moieties (His and Lys) and a single moiety (Cys) have been made responsible either directly or, as yet unknown, via conformational changes. Of these side chains, His appears essential for enzymatic activity. The other moieties are involved in optimization of enzymatic properties. In this investigation, carboxyl groups and Tyr side chains have not been considered.

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Nonenzymic Translocation and Spontaneous Release of Noncognate Peptidyl Transfer Ribonucleic Acid from *Escherichia coli* Ribosomes†

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ABSTRACT: Poly(uridylic acid)-programmed ribosomes have been used to synthesize the noncognate peptidyl-tRNA AcPhe-Tyr-tRNA^{Tyr} and its cognate counterpart Ac(Phe)$_2$-tRNA^{Phe}. After synthesis, Ac(Phe)$_2$-tRNA^{Phe} remains, as expected, in the ribosomal acceptor (A) site, but the noncognate AcPhe-Tyr-tRNA^{Tyr} does not; part of it spontaneously falls off the ribosome and the rest translocates, without elongation factor (EF) G, to the ribosomal donor site. The inhibitor of translocation viomycin prevents both the spontaneous release and the nonenzymatic translocation by confining the noncognate peptidyl-tRNA to the A site. Under these conditions, the interaction of AcPhe-Tyr-tRNA^{Tyr} with the A site appears to be similar to that of Ac(Phe)$_2$-tRNA^{Phe} without the antibiotic, and EF-G promotes the translocation and subsequent elongation of both peptidyl-tRNAs to comparable extents. The results indicate that, without viomycin, the noncognate peptidyl-tRNA is weakly held in the ribosomal A site and support the proposal that the release of peptidyl-tRNA occurring during protein synthesis in vivo is related to a ribosomal editing mechanism which discards mistranslated nascent proteins [Menninger, J. R. (1977) *Mech. Ageing Dev. 6*, 131].

Translocation is possibly the most complex step in the polypeptide chain elongation cycle. It comprises the coordinated movement of peptidyl-tRNA from the ribosomal acceptor (A)[1] site to the donor (P) site, the advance of mRNA in the length of three nucleotides (one codon), and the ejection of deacylated tRNA from the P site. Translocation requires the participation of elongation factor (EF) G and the hydrolysis of GTP [Modolell & Vázquez, 1975 (review)]. Little is known of the detailed molecular interactions and rearrangements of peptidyl-tRNA, mRNA, and ribosomal subunits that take place during translocation. One of the many unresolved problems is how the fidelity of base pairing between the mRNA and peptidyl-tRNA affects translocation. That base-pairing errors or distorted codon–anticodon interactions may alter the num-

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[1] Abbreviations used: A site, ribosomal acceptor site; P site, ribosomal donor site; EF-G, elongation factor G; EF-Tu, elongation factor Tu; EF-T, a mixture of EF-Tu and elongation factor Ts; AcPhe-tRNA^{Phe}, phenylalanine-specific *N*-acetylphenylalanyl transfer ribonucleic acid; AcPhe-Tyr-tRNA^{Tyr}, tyrosine-specific *N*-acetylphenylalanyltyrosyl transfer ribonucleic acid.

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