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Macromolecular Affinity Labeling Agents. Reaction of *N*-Bromoacetylisoleucyl Transfer Ribonucleic Acid with Isoleucyl Transfer Ribonucleic Acid Synthetase†

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ABSTRACT: *N*-Bromoacetyl-Ile-tRNA forms a covalent bond with isoleucyl-tRNA synthetase from *Escherichia coli* B. The complexes are readily isolable by gel filtration and are not disrupted by equilibration with $tRNA^{Ile}$ or treatment with 6 M urea in the presence of β-mercaptoethanol. When $tRNA^{Ile}$ is present during incubation, the enzyme is protected against alkylation by this reagent. Under conditions comparable to those used for the affinity labeling experiments, *N*-bromoacetyl-Ile does not alkylate the enzyme in the presence or absence of $tRNA^{Ile}$. The results demonstrate that *N*-bromoacetyl-Ile-tRNA binds to the enzyme by reversible interactions with the tRNA moiety, and then forms a covalent bond between the alkylating group and an amino acid residue of the protein.

Affinity labeling, or active-site directed irreversible inhibition, has provided much information regarding protein–small molecule interactions (Singer, 1967; Baker, 1967; Shaw, 1970). Although the utility of this technique in investigations of interactions of two or more macromolecules can readily be visualized, the formidable technical difficulties of attaching chemically reactive groups to specific noncontact points of macromolecules has precluded progress in this direction. One class of macromolecules which appears amenable to be used as affinity labeling agents is the aminoacyl-tRNAs. Techniques have been devised in which the α-amino group of the amino acid moiety can be selectively and quantitatively acylated (see Lapidot and de Groot, 1972); fortunately, since recognition of tRNA by its biological receptors likely involves a composite of numerous interactions, perturbations resulting from introduction of a single reactive group are unlikely to dramatically effect binding. The successful acylation of Met-tRNA synthetase with *p*-nitrophenylcarbamyl-Met-tRNA (Bruton and Hartley, 1970) provided the first indication that this approach was indeed feasible. In conjunction with ongoing studies of the aminoacyl-tRNA synthetases (Santi and Peña, 1973; Santi and Danenberg, 1971; Santi *et al.*, 1971a,b), we sought to prepare a chemically reactive derivative of aminoacyl-tRNA which would be capable of reacting with a wider variety of nucleophiles than the aforementioned reagent and which would yield a modified amino acid readily amenable to isolation and identification.

Bromoacetamides are capable of alkylating histidine, cysteine, methionine, and lysine residues of proteins (Shaw, 1970; Singer, 1967), and *N*-bromoacetyl-Phe-tRNA has recently been reported to be a labeling reagent for the 50S particle of *Escherichia coli* ribosomes (Pellegrini *et al.*, 1972). We describe here the preparation of *N*-bromoacetyl-Ile-tRNA, and provide evidence that this analog serves as an affinity labeling reagent of Ile-tRNA synthetase.[1]

Materials and Methods

[^{3}H]Ile (105 Ci/mmol) was a product of New England Nuclear and diluted to desired specific activity with unlabeled Ile (Nutritional Biochemicals Corp.). Bromoacetic acid (Matheson Coleman and Bell) was distilled before use; bp 98° (15 mm). Unfractionated tRNA (*E. coli* B) was obtained from Schwarz/Mann and had 16 pmol of $tRNA^{Ile}/A_{260}$. All other reagents were the highest purity available and used without further purification.

Ile-tRNA synthetase obtained by the method of Eldred and Schimmel (1972) moved as a single band on sodium dodecyl sulfate disc gel electrophoresis and showed 50% maximal activity using the ATP–PP_i exchange assay (Baldwin and Berg, 1966a). Purified Ile-tRNA was obtained by published methods (Gillam *et al.*, 1968; Yarus and Berg, 1969) and had an amino acid capacity of ~1.7 nmol of Ile-tRNA/A_{260}. Preparations of Ile-tRNA were performed essentially as described by Baldwin and Berg (1966b). Unless otherwise specified, the extent of acylation was monitored by acid precipitation of [^{3}H]Ile-tRNA and filtration through Whatman GF/C glass filters (Calendar and Berg, 1966). Isolation of [^{3}H]Ile-tRNA for subsequent reactions was accomplished by the addition of 1/5 vol of 2.5 M sodium acetate (pH 5.0) and precipitation with 2 vol of cold 95% ethanol. After centrifugation at 0°, the precipitate was washed in cold 95% ethanol, dissolved in 0.1 M sodium acetate (pH 4.5)–0.4 M sodium chloride, and reprecipitated. Radioactivity adsorbed on glass filters was counted under toluene containing 0.4% 2,5-diphenyloxazole

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[1] Abbreviations used are: $tRNA^{Ile}$, tRNA specific for isoleucine acceptance; Ile-tRNA, $tRNA^{Ile}$ which has been esterified with Ile.

and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene in a Nuclear-Chicago Isocap 300 scintillation counter. Counting efficiencies were determined by the dual channel ratio method.

Bromoacetic Acid N-Hydroxysuccinimide Ester. The ester was prepared by a modification of the general method reported by Anderson *et al.* (1964). To a magnetically stirred solution of 139 mg (1.0 mmol) of bromoacetic acid and 115 mg (1.0 mmol) of *N*-hydroxysuccinimide in 5 ml of dioxane was added 206 mg (1.0 mmol) of *N,N'*-dicyclohexylcarbodiimide. After stirring at ambient temperature for 1 hr, the precipitated dicyclohexylurea was filtered and washed with several small portions of dioxane. The combined mother liquor and washings were evaporated *in vacuo* (25°) and the residue recrystallized from isopropyl alcohol: mp 115–117°; γ_{max}^{Nujol} 1790, 1820 cm^{-1}. The product gave a positive test for active halogen (Baker *et al.*, 1966; Epstein *et al.*, 1955). The ester was stored at −20° over calcium sulfate. *Anal.* Calcd for $C_6H_6BrNO_4$: C, 65.73; H, 8.27; N, 9.58. Found: C, 65.78; H, 8.51; N, 9.48.

N-Bromoacetyl-Ile. A solution of 131 mg (1 mmol) of Ile, 168 mg (2 mmol) of $NaHCO_3$, and 236 mg (1 mmol) of the *N*-hydroxysuccinimide ester of bromoacetic acid in 6 ml of 50% aqueous dioxane was allowed to stand for 1 hr at ambient temperature. After adjusting the pH to *ca.* 1 with concentrated HCl, the product was extracted into three 20-ml portions of $CHCl_3$, dried with $MgSO_4$, and evaporated *in vacuo*. The oily residue was crystallized from benzene to give 197 mg (78%) of white needles: mp 119–119.5; γ_{max}^{Nujol} 1720 (CO_2H), 1650 cm^{-1} (CONH). The product moved as a single spot (R_F 0.7; R_F^{Ile} 0.1) on tlc (silica gel) using 95% EtOH, and on Whatman No. 1 using *n*-BuOH–AcOH–H_2O (2:1:1) (R_F 0.95; R_F^{Ile} 0.77) or the upper phase of *i*-BuOH–H_2O (R_F 0.75; R_F^{Ile} 0.24); spots were detected by spraying with *p*-nitrobenzylpyridine (Baker *et al.*, 1966) and ninhydrin. *Anal.* Calcd for $C_8H_{14}BrNO_3$: C, 38.11; H, 5.60; N, 5.56. Found: C, 38.28; H, 5.48; N, 5.66.

N-Bromoacetyl-[^{3}H]Ile. The radioactive analog was prepared as above using 10 μl of [^{3}H]Ile (11 nmol; 1.25 Ci/mmol), 10 μl of 0.6 M sodium bicarbonate (6 μmol), and 20 μl of 0.15 M bromoacetic acid *N*-hydroxysuccinimide ester (3 μmol) in dioxane. After 30 min at ambient temperature the reaction mixture was chromatographed on Whatman No. 1 paper with *n*-butyl alcohol–acetic acid–water (2:1:1). The product (R_F 0.95) was eluted with 95% ethanol using the technique of Edstrom (1968) and was shown to co-chromatograph with the unlabeled material in the systems described above.

Reaction of Ile-tRNA Synthetase and N-Bromoacetyl-[^{3}H]-Ile. A 2 mM potassium phosphate–1 mM magnesium chloride buffer (pH 7.5) (250 μl) containing 5 nmol (20 μM) of *N*-bromoacetyl-[^{3}H]Ile (0.75 Ci/mmol), 45 pmol (0.18 μM) of $tRNA^{Ile}$, and 45 pmol (0.18 μM) of the enzyme was incubated at 23°. An identical experiment was performed except tRNA was omitted. At time intervals 50-μl aliquots were withdrawn and passed through a Sephadex G-25 column (70 × 0.7 cm) at 23° using 10 mM sodium cacodylate–5 mM magnesium chloride (pH 7.5) as eluent. The flow rate was 0.5 ml/min and 0.3-ml fractions were taken and counted for radioactivity using 10 ml of toluene scintillation fluid containing 25% Triton X-100, 4% water, and 40 mg of Omnifluor. Radioactivity present in the void volume (7.6 ml) was considered alkylated enzyme.

Reaction of tRNA and N-Bromoacetyl-[^{3}H]Ile. A solution (350 μl) containing 0.1 M triethanolamine·HCl (pH 7.8), 0.01 M magnesium chloride, 0.6 mM unfractionated tRNA (106 A_{260}), and 5 mM *N*-bromoacetyl[^{3}H]Ile (1.7 mCi/mmol) in 30% dioxane was allowed to stand at ambient temperature. At time intervals, 100-μl aliquots were removed and added to 1 ml of cold 95% ethanol. After 20 min at −20°, the precipitate was centrifuged and washed with 1-ml portions of cold 95% ethanol. The tRNA was twice reprecipitated from 100 μl of 1 M NaCl and washed as above. The final wash contained less than twice background radioactivity. The precipitate was dissolved in 300 μl of 0.1 M potassium phosphate buffer (pH 6.8); a 10-μl aliquot was removed for A_{260} determination and the radioactivity determined from the remaining 290 μl in 10 ml of toluene containing 25% Triton X-100 and 40 mg of Omnifluor. The extent of reaction was calculated from disintegrations per minute per A_{260} unit and expressed as moles of alkylated tRNA per mole of total tRNA.

N-Bromoacetyl-[^{3}H]Ile-tRNA^{Ile}. A solution (100 μl) containing 0.6 μM purified [^{3}H]Ile-$tRNA^{Ile}$ (4.5 Ci/mmol), 10 mM magnesium chloride, 0.1 M triethanolamine·HCl (pH 7.8), and 5 mM bromoacetic acid *N*-hydroxysuccinimide ester in 30% aqueous dioxane was stirred at ambient temperature for 1 hr. Two volumes of cold 95% ethanol were added and the mixture was cooled at −20° for 20 min. The precipitate was centrifuged and redissolved in 0.4 M sodium chloride–0.1 M sodium acetate (pH 4.5) and reprecipitated with 2 vol of cold ethanol. Under these conditions 63% of the total radioactivity was recovered which was minimally 64% product according to cupric ion catalyzed hydrolysis of [^{3}H]Ile-tRNA (Schofield and Zamecnik, 1968).

N-Bromoacetyl-[^{3}H]Ile-tRNA^{Ile}-Ile-tRNA Synthetase Covalent Complex. A solution (100 μl) containing 10 mM potassium phosphate–5 mM magnesium chloride buffer (pH 7.5), 6 pmol (0.06 μM) of *N*-bromoacetyl-[^{3}H]Ile-$tRNA^{Ile}$ (4.5 Ci/mmol), and 10 pmol (0.1 μM) of the enzyme was incubated at 25° for 24 hr. An excess of unfractionated $tRNA^{Ile}$ (450 pmol; 27 A_{260} units of crude tRNA) was added and after 1 hr the reaction was filtered through a Sephadex G-100 (40–120 mesh) column (40 × 0.7 cm) at 5° using 10 mM sodium cacodylate–5 mM magnesium chloride buffer (pH 5.5) as eluent. The flow rate was 18 ml/hr and 0.5-ml fractions were collected. Radioactivity was determined in 10 ml of toluene containing 25% Triton X-100 and 40 mg of Omnifluor.

Results

Since affinity labeling must proceed unimolecularly within a specific reversible complex, it is important to demonstrate that an analogous chemically reactive function which does not form a reversible complex will not alkylate the protein under similar conditions. Some indication that a bimolecular reaction between α-halocarbonyl alkylating agents and Ile-tRNA synthetase would not occur under the conditions employed here could be derived from the data of Iaccarino and Berg (1969). These workers reported the bimolecular rate constant for the reaction of the enzyme and iodoacetamide to be 7.9×10^{-2} M^{-1} min^{-1} at 17°. Using this value and concentrations of *ca.* 10^{-7} M for Ile-tRNA synthetase and *N*-bromoacetyl-[^{3}H]Ile-tRNA as in a typical affinity labeling experiment, we calculated that a negligible amount of enzyme would have reacted over the 24-hr duration of the experiment. To verify this, we performed an experiment in which *N*-bromoacetyl-[^{3}H]Ile and the enzyme were incubated at concentrations (2×10^{-5} and 2×10^{-7} M, respectively) which exceeded those used in the affinity labeling experiment. After 24 hr, the enzyme was separated from the unreacted alkylated agent by filtration through Sephadex G-25 and was shown not to contain radio-

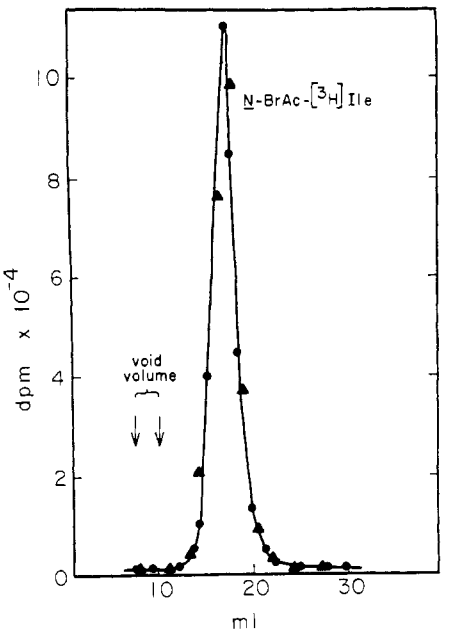

FIGURE 1: Sephadex G-25 filtration after incubation of *N*-bromoacetyl-[^{3}H]Ile (*N*-BrAc-[^{3}H]Ile) with Ile-tRNA synthetase with (●) and without (▲) tRNA for 24 hr as described under Materials and Methods. The void volume (7.6–9.8 ml) was determined with 50 μl of a 2-mg/ml blue dextran solution.

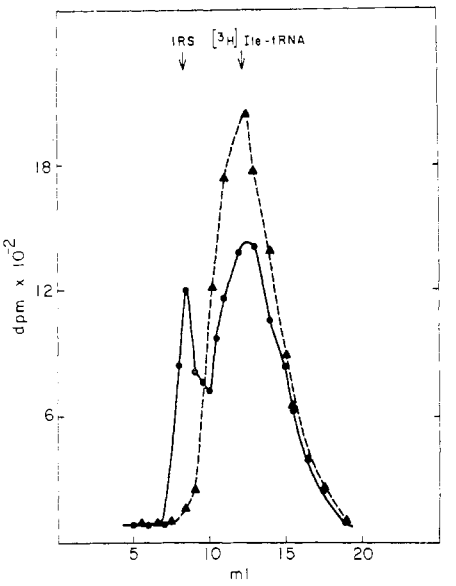

FIGURE 2: Separation of enzyme–*N*-acetyl-Ile-tRNA complex from *N*-acetyl-Ile-tRNA on Sephadex G-100; direct filtration (●) and addition of 16-fold excess tRNA before filtration (▲). Unfractionated tRNA containing 25 pmol of *N*-acetyl-[^{3}H]Ile-tRNA was incubated with 40 pmol of enzyme in 100 μl of 10 mM potassium phosphate–5 mM magnesium chloride buffer (pH 7.5) for 1 hr. The column was preequilibrated and eluted with 10 mM sodium cacodylate–5 mM magnesium chloride buffer (pH 5.5). The column was calibrated with the indicated markers.

activity (Figure 1). An identical result was obtained if tRNA^{Ile} was included in the reaction mixture.

In the preparation and storage of *N*-bromoacetyl-[^{3}H]Ile-tRNA, the nucleic acid is continuously exposed to the alkylating agent. If reaction occurs, the alkylating potency will be abolished and the nucleic acid modified. Although unfractionated tRNA has been alkylated with halides of comparable reactivity (Smith and Yamane, 1967; Hara *et al.*, 1970; Grachev *et al.*, 1972), quantitative data were unavailable which would allow prediction as to whether such reactions would occur under the conditions used here. There was some indication that tRNA^{Ile} would not be susceptible to alkylation under the conditions used, since it does not contain the exposed 4-thiouridine residue (Yarus and Barrell, 1971) believed to react with α-bromoacetamides (Hara *et al.*, 1970). To ensure this, unfractionated tRNA was treated with a radioactive bromoacetamide under conditions more extreme than those employed in the preparation and storage of *N*-bromoacetyl-[^{3}H]Ile-tRNA. During incubation under N-acylating conditions (except for a 10^3-fold increase in tRNA) aliquots of the solution were taken at various intervals and repeatedly precipitated with ethanol to remove the alkylating agent. The extent of acylation was determined from the radioactivity associated with the tRNA. After the normal 1-hr incubation and after 24 hr, the extent of alkylation was determined to be $^1/_{1100}$th and $^1/_{140}$th mol of alkylating agent per mol of tRNA, respectively. It must be emphasized that unfractionated tRNA was used in this experiment as well as a large excess of alkylating agent; we conclude that alkylation of tRNA^{Ile} by the reactive chemical group is not significant enough to warrant concern.

A number of workers have demonstrated that aminoacyl-tRNAs form tight complexes with their cognate synthetases which may be isolated by gel filtration (Lagerkvist *et al.*, 1966; Myers *et al.*, 1971; Eldred and Schimmel, 1973) or adsorption on nitrocellulose membranes (Yarus and Berg, 1969). The rate of dissociation of the complex is relatively slow ($t_{1/2}$ for dissociation is 90 sec at 17°; Yarus and Berg, 1969) and under conditions of dilution with excess tRNA, bound aminoacyl-tRNA may be exchanged with the tRNA of the medium. The complex with *N*-acetyl-[^{3}H]Ile-tRNA and Ile-tRNA synthetase may be isolated in the excluded volume by Sephadex G-100 filtration (Figure 2), whereas *N*-acetyl-[^{3}H]-Ile-tRNA was eluted after the void volume. When the preformed complex was incubated with excess tRNA prior to filtration, a radioactive complex was not obtained.

When *N*-bromoacetyl-[^{3}H]Ile-tRNA prepared from highly purified tRNA^{Ile} was incubated with Ile-tRNA synthetase under the same conditions, Sephadex G-100 chromatography (Figure 3a) gave radioactive peaks corresponding to the enzyme–*N*-bromoacetyl-[^{3}H]Ile-tRNA complex and *N*-bromoacetyl-[^{3}H]Ile, apparently resulting from hydrolysis during the incubation period; the amount of free *N*-bromoacetyl-[^{3}H]Ile-tRNA was negligible. When the preformed complex was incubated with an excess of tRNA prior to filtration under conditions in which the Ile-tRNA synthetase–*N*-acetyl-[^{3}H]Ile-tRNA complex dissociated, there was little loss in the isolable radioactivity associated with the enzyme (Figure 3b). However, when the incubation of *N*-bromoacetyl-[^{3}H]Ile-tRNA and the synthetase was performed in the presence of excess tRNA, little or no radioactivity was associated with the enzyme (Figure 3b). Pooled fractions of the Ile-tRNA synthetase–*N*-bromoacetyl-[^{3}H]Ile-tRNA complex could be rechromatographed with little loss in radioactivity (Figure 4a), and served to remove contaminants of unbound *N*-bromoacetyl-[^{3}H]Ile-tRNA. An almost identical elution pattern (Figure 4b) was obtained when the complex was treated with 6 M urea and 1 mM β-mercaptoethanol for 1 hr prior to filtration; under these conditions the urea should denature the protein and disrupt any noncovalent complexes and the mercaptan should have reacted with any reactive halogen still remaining.

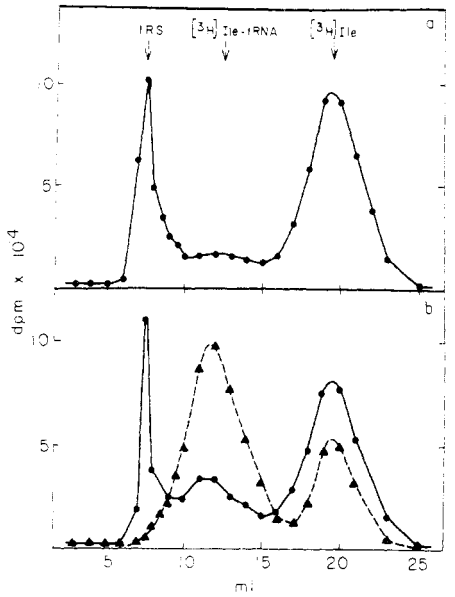

FIGURE 3: Sephadex G-100 chromatography after incubation of *N*-bromoacetyl-[^{3}H]Ile-tRNA with enzyme for 24 hr at 25°: (a) no excess tRNA added prior to filtration; (b) excess tRNA competitor added before (▲) and after (●) incubation period.

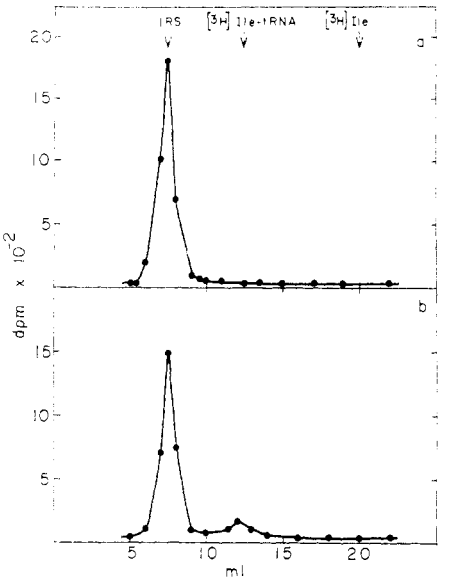

FIGURE 4: Sephadex G-100 chromatography of the purified enzyme–*N*-bromoacetyl-Ile-tRNA complex before (a) and after (b) treatment with 6 M urea–1 mM β-mercaptoethanol.

Discussion

N-Acetyl- and *N*-bromoacetyl-Ile-tRNA form complexes with Ile-tRNA synthetase which may be isolated by Sephadex G-100 gel filtration. However, the enzyme–*N*-acetyl-Ile-tRNA complex is disrupted upon equilibration with tRNA whereas the complex formed with the *N*-bromoacetyl derivative is stable. In fact, it may be rechromatographed or treated with 6 M urea and β-mercaptoethanol without apparent dissociation. These results clearly demonstrate that a covalent bond is formed between Ile-tRNA synthetase and *N*-bromoacetyl-Ile-tRNA (*viz.* enzyme–CH_2CO-Ile-tRNA), undoubtedly as a result of displacement of the reactive halogen by a nucleophilic group of the enzyme.

As with any irreversible inhibitor, the paramount question to be answered is whether the covalent bond forms *via* bimolecular reaction or from within a reversible complex. In the present case commonly used kinetic criteria (Baker, 1967) for affinity labeling agents are difficult to apply since the reversible dissociation of tRNA from Ile-tRNA synthetase is known to be slow (Eldred and Schimmel, 1973; Yarus and Berg, 1969); if formation of the covalent bond is faster than this process, the reaction would show bimolecular kinetics regardless of the mechanism and the distinguishing "rate saturation" phenomenon (Baker, 1967) would not be observed. In addition, the relative instability of ester linkage of *N*-acyl-Ile-tRNAs over the time periods used for the labeling experiments described precludes precise kinetic evaluation of the mechanisms of covalent bond formation. Nevertheless, several direct lines of evidence provide strong support that the reaction of Ile-tRNA synthetase with *N*-bromoacetyl-Ile-tRNA proceeds by the affinity labeling mechanism rather than bimolecular alkylation.

First, in the presence or absence of $tRNA^{Ile}$, *N*-bromoacetyl-Ile does not alkylate the enzyme; in this case, the alkylating agent is of identical chemical reactivity as the affinity labeling agent, but does not reversibly bind to the enzyme. Second, incubation of the affinity labeling agent with Ile-tRNA synthetase in the presence of a competitor for the nucleic acid binding site (*i.e.*, $tRNA^{Ile}$) decreases the extent of covalent bond formation. Together these data suggest that *N*-bromoacetyl-Ile-tRNA reversibly binds to Ile-tRNA synthetase *via* recognition of the tRNA moiety, and then undergoes a unimolecular reaction in which the chemically reactive α-bromoacetamide moiety forms a covalent bond with the enzyme. Since these experiments were performed with limiting concentrations of *N*-bromoacetyl-tRNA, they do not ascertain the stoichiometry of the reaction. However, since Ile-tRNA synthetase is a monomer of 114,000 daltons (Arndt and Berg, 1970; Baldwin and Berg, 1966a) and possesses a single binding site for Ile-tRNA (Yarus and Berg, 1967), it is reasonable to assume that the enzyme reacts with only 1 equiv of the affinity labeling agent.

Although we have not yet identified the target amino acid of Ile-tRNA synthetase which is alkylated, it is interesting to note that Iaccarino and Berg (1969) have reported that a cysteine residue exists at or near the acetive site of the enzyme which rapidly reacts with alkylating agents. Experiments are in progress which should identify the amino acid residue which reacts with *N*-bromoacetyl-Ile-tRNA. In addition, selective degradation of the affinity labeled enzyme should provide modified derivatives which will be useful in unraveling mechanistic features of the catalytic process.

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Induction of Stable Linkage between the Deoxyribonucleic Acid Dependent Ribonucleic Acid Polymerase and $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ by Ultraviolet Light†

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ABSTRACT: *Escherichia coli* RNA polymerase bound to the synthetic polymer $d(A\text{-}T)_n \cdot d(A\text{-}T)_n$ can be induced with ultraviolet (uv) light to form stable binary complexes. The stability of the complex most likely is due to covalent linkage(s) between the enzyme and polymer as shown by resistance of the complex to high ionic strength solutions and stability to alkali or heat treatment. Formation of stable complexes was found to be a function of the incident uv dose, the input molar ratio of enzyme–polymer, and the components present in the irradiation solution. Ultraviolet light induced enzyme–polymer complexes were detected by retention on Millipore filters after washing with high ionic strength solutions (2 M) which dissociate nonlinked enzyme–polymer complexes. Analysis of uv-induced stable complexes on cesium chloride sedimentation equilibrium gradients resulted in formation of an enzyme-dependent peak which essentially floated on top of the gradient. For a particular dose of uv light, the per cent total polymer present in this floating peak compared favorably with the per cent total polymer retained on Millipore filters after a high salt wash for several molar ratios of the enzyme–polymer tested. From density distribution profiles of detergent-treated, uv-induced enzyme–polymer complexes in cesium chloride equilibrium gradients, a significant fraction of the denatured enzyme–polymer complex banded at a density of 1.58 g/cm^3. This fraction most likely contains only one linked polypeptide subunit of the multisubunit enzyme, and this is possibly the α or σ subunit.

The first step in ribonucleic acid (RNA) synthesis is association of the deoxyribonucleic acid dependent ribonucleic acid polymerase (RNA polymerase) with the deoxyribonucleic acid (DNA) template; this initial process is designated as the binding step. Upon mixing purified RNA polymerase with a nucleic acid polymer *in vitro*, a binary complex is formed in the absence of RNA synthesis (Fox *et al.*, 1965). The *Escherichia coli* RNA polymerase is a complex multisubunit enzyme; the holoenzyme is composed of the polypeptide subunits $\alpha_2\beta\beta'\sigma(\omega)$ (Zillig *et al.*, 1970; Burgess and Travers, 1971). Recent studies using partially dissociated fragments of the RNA polymerase have provided evidence as to the function(s) of the various subunits of the enzyme in the process of RNA synthesis (Zillig *et al.*, 1970; Ishihama, 1972). However, in order to understand better the molecular mechanisms of transcription, it is necessary to know the spatial relationship of the various subunits of the enzyme in the RNA polymerase–DNA complex. Several attempts have been made with the electron microscope to determine the fine structure of RNA polymerase and RNA polymerase bound to DNA (Fuchs *et al.*, 1964; Slayter and Hall, 1966; Colvill *et al.*,

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