reaction, as indicated by the observation that it is not replaceable by valinol, indicates that the carboxyl group participates either because valyladenylate is an intermediate in the interchange reaction or because it induces an essential conformational change in the enzyme upon binding.

The interchange might occur by a one-step mechanism, whereby the α–β bond cleavage and α–γ bond formation would occur simultaneously. As an alternative, a two-step sequence in which either a valyladenylate– or pyrophosphoryl–enzyme intermediate (Cooper & Kornberg, 1969) would be formed might be considered.

The finding that the valyl-tRNA synthetase can catalyze the readily observed β- to γ-P interchange of ATPβS raises the question of the physiological significance of this reaction, if any, with ATP as the substrate. One possible function is suggested by our observation that isoleucine can readily substitute for valine in the interchange reaction. This finding suggests that the interchange reaction may function as a mechanism for editing, i.e., a mechanism by which the "wrong" amino acid could interact with the synthetase but would not result in aminoacyl-tRNA formation. Although other mechanisms for editing have been proposed and investigated (Fersht & Dingwall, 1979; von der Haar & Cramer, 1976; Yamane & Hopfield, 1977), the interchange reaction reported in this paper, which would cycle the wrong amino acid in a nonproductive reaction resulting in no loss of ATP, may contribute yet an additional mechanism for ensuring the fidelity of aminoacylation. This possibility is presently being investigated.

Acknowledgments

We thank Dr. J. Bock for providing the valyl-tRNA synthetase used in the early stages of this work, Dr. E. K. Jaffe for providing advice in the preparation of the ATP thio analogues, and Dr. George Reed for many helpful discussions.

References

Bruton, C., Jakes, R., & Atkinson, T. (1975) *Eur. J. Biochem. 59*, 327.
Cooper, R. A., & Kornberg, H. L. (1969) *Biochim. Biophys. Acta 141*, 211.
Eckstein, F., & Goody, R. S. (1976) *Biochemistry 15*, 1685.
Fayat, G., Fromant, M., Kahn, D., & Blanquet, S. (1977) *Eur. J. Biochem. 78*, 333.
Fersht, A. R., & Dingwall, C. (1979) *Biochemistry 18*, 1238.
Jaffe, E. K., & Cohn, M. (1978) *Biochemistry 17*, 652.
Jaffe, E. K., & Cohn, M. (1979) *J. Biol. Chem.* (in press).
Loftfield, R. B. (1972) *Prog. Nucleic Acid Res. Mol. Biol. 12*, 87.
Mehler, A. M., & Mitra, S. K. (1967) *J. Biol. Chem. 242*, 5495.
Nogc, L. D., Jebeleanu, G., & Barzu, O. (1979) *FEBS Lett. 97*, 65.
Pimmer, J., Holler, E., & Eckstein, F. (1976) *Eur. J. Biochem. 67*, 171.
Posorske, L. H., Cohn, M., Yanagisawa, N., & Auld, D. S. (1979) *Biochim. Biophys. Acta 579*, 128.
Ravel, J. M., Wang, S.-F., Heinemeyer, C., & Shive, W. (1965) *J. Biol. Chem. 240*, 432.
Smith, L. T., Rossomando, E. F., & Bock, J. (1979) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 38*, 822.
von der Haar, F., & Cramer, F. (1976) *Biochemistry 15*, 4131.
von der Haar, F., Cramer, F., Eckstein, F., & Stahl, K.-W. (1977) *Eur. J. Biochem. 76*, 263.
Yamane, T., & Hopfield, J. J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 2246.
Yaniv, M., & Gros, F. (1969) *J. Mol. Biol. 44*, 1.

Affinity Labeling of a Reactive Sulfhydryl Residue at the Peptidyl Transferase P Site in *Drosophila* Ribosomes†

Steven Fabijanski and Maria Pellegrini*

ABSTRACT: An affinity label has been prepared that is specific for the P site of an eucaryotic peptidyl transferase, that of *Drosophila melanogaster*. It has the sequence C-A-C-C-A-(Ac[^{3}H]Leu) with a mercury atom added at the C-5 position of all three cytosine residues (referred to as the mercurated fragment). This label is an analogue of the 3′ terminus of *N*-acetylleucyl-tRNA. The mercurated fragment binds specifically to the P site of peptidyl transferase. It participates fully in peptide bond formation as judged by its ability to transfer *N*-acetylleucine to puromycin with at least the same efficiency as a nonmercurated fragment. Once bound to the P site, the mercurated fragment reacts covalently with a ribosomal protein(s). This affinity-labeling process can be effectively competed by nonmercurated fragment, which indicates a site-specific reaction. The covalent attachment of the affinity label to a ribosomal protein(s) occurs through the formation of a mercury–sulfur bond, as judged by its lability in the presence of thiol reducing agents. The major ribosomal protein labeled at the P site of *D. melanogaster* was found to be a small, basic protein. The electrophoretic behavior of this protein parallels that of major P site proteins found in *Escherichia coli* ribosomes and in other eucaryotes. These results suggest conservation of some of the overall properties of the P site proteins from these organisms.

Affinity-labeling techniques have been instrumental in the investigation of the 70S ribosome of *Escherichia coli*. Various structural components have been correlated with particular ribosomal functions using these specifically designed probes. Numerous affinity-labeling experiments, in addition to data from cross-linking, immune electron microscopy, and chemical modification experiments, have contributed to an understanding of the three-dimensional structure of the bacterial ribosome and of the molecular events which occur during protein synthesis.

† From the Molecular Biology Section, University of Southern California, Los Angeles, California 90007. *Received May 21, 1979; revised manuscript received September 14, 1979.* This work was supported by Grant GM-24572 from the National Institutes of Health and a grant from the Cottrell Research Corporation.

0006-2960/79/0418-5674$01.00/0

Eucaryotic protein synthesis systems have many features which distinguish them from those found in procaryotes. These include the increased number and size of ribosomal protein and RNA species in eucaryotes and differences in the number and type of accessory translational factors [see a review by Miller & Weissbach (1977)]. Subtle differences in responses to chemical modification and antibiotics also distinguish procaryotic and eucaryotic ribosomes (Pestka, 1977; Bermek et al., 1971). One chemical modification study has shown that eucaryotic peptidyl transferase activity can be stimulated by blocking ribosome sulfhydryl groups with low molecular weight thiol reagents (Carrasco & Vâsquez, 1975). No similar enhancement of peptide bond formation has been observed with procaryotic ribosomes.

The peptidyl transferase center can be probed directly with the "fragment assay" system (Monro, 1971). This system allows the study of the peptidyl transferase site in the absence of mRNA, translation factors, and energy sources. Changes in peptidyl transferase can, therefore, be ascribed to effects localized on the ribosome. Using the fragment assay system, we sought to further characterize the role of sulfhydryl residues in eucaryotic peptidyl transferase, specifically, to explore the proximity of sulfhydryl residues to the peptidyl transferase center. We have synthesized an affinity label that preferentially reacts with sulfhydryl groups. It is a mercurated analogue of the 3′ terminus of an *N*-acetylated aminoacyl-tRNA, a natural substrate for the P site of peptidyl transferase. This affinity label reacts specifically and covalently at the P site of *Drosophila melanogaster* ribosomes. A labeled ribosomal protein has been identified and reveals interesting parallels between proteins present at the procaryotic and eucaryotic peptidyl transferase centers.

Materials and Methods

Ribosome Isolation. Ribosomes were isolated from *D. melanogaster* mid-third instar larvae as follows. Routinely, 30 g of larvae were homogenized in 10 volumes of 10 mM Tris-HCl, pH 7.6, 3.3 mM $MgCl_2$, 50 mM NH_4Cl, 1 mM DTT,[1] 1% Triton X-100, and 250 mM sucrose. All operations were carried out at 4 °C. The homogenate was centrifuged at 12000*g* for 30 min. The supernatant was filtered through cheesecloth, to help to remove lipids, and was recentrifuged. This supernatant, in 8-mL aliquots, was layered over 30 mL of 30% sucrose, 10 mM Tris-HCl, pH 7.6, 3.3 mM $MgCl_2$, 50 mM NH_4Cl, and 1 mM DTT, and centrifuged for 15.5 h at 24 500 rpm in a SW27 rotor. Ribosomal pellets obtained in this fashion were essentially pure 80S monosomes as judged by sucrose gradient analysis. Ribosomes were stored as pellets at −90 °C for up to 6 months with no apparent loss of activity. For use in the fragment assay, ribosomes were resuspended in 10 mM Tris-HCl, pH 7.6, and 100 mM KCl at greater than 250 A_{260} units/mL.

Preparation of the 3′-Terminal Fragment of tRNA. C-A-C-C-A(Ac[^{3}H]Leu) (specific activity 2 Ci/mM) was prepared according to the procedure of Pestka (1971), with the following modification. Fractions containing the fragment when eluted from DEAE-Sephadex were pooled and lyophilized. To remove excess salt and further purify the fragment, we resuspended this material in a minimum volume of 10 mM sodium acetate, pH 5.0, and chromatographed on a 30 × 1 cm Sephadex G-25 column, equilibrated in the same buffer. Purified fragment was lyophilized and stored at −20 °C.

Fragment Assay. The fragment assay was performed essentially according to Monro (1971). The assay mixture contained 2.5 A_{260} units of 80S *D. melanogaster* ribosomes, 13 nM fragment, 1 mM puromycin, 50 mM Tris-HCl, pH 7.6, 400 mM KCl, and 15 mM $MgCl_2$ in 0.1 mL. The reactions were initiated by the addition of methanol to 30% (v/v). Incubations were carried out at 0 °C for 45 min. *N*-Acetyl[^{3}H]leucylpuromycin formation was measured according to Monro (1971).

Preparation of the Mercurated Fragment. $^{203}Hg(NO_3)_2$ (specific activity 38 mCi/M) at a 20-fold molar excess over the fragment present was incubated in 2 mL of 100 mM sodium acetate, pH 5.0, for 90 min at 65 °C. NaCl was added to a final concentration of 100 mM to stop the reaction, and the mixture was then passed over a Chelex 100 (Bio-Rad Laboratories) column equilibrated in 100 mM sodium acetate, pH 5.0. Chelex 100 has an extremely high affinity for free mercury atoms but does not readily absorb mercurated polynucleotides. The material which was not bound to Chelex was then desalted on a 15 × 1 cm Sephadex G-10 column equilibrated in 10 mM sodium acetate, pH 5.0. The mercurated fragment was then lyophilized and stored at −20 °C.

Under similar conditions, used by Dale et al. (1975), mercuration of pyrimidine residues in polynucleotides was found to be essentially quantitative. Comparison of the specific activity of the [^{203}Hg]- and the [^{3}H]leucine present in the purified mercurated fragment indicated quantitative addition of ^{203}Hg to all cytosine residues present in the fragment. It should be noted here that the ^{203}Hg used in these studies emits both β and γ radiation of 0.21 and 0.28 MeV, respectively. Although the mercuric salts and nucleotides worked with here are nonvolatile, experiments were carried out in a hood where possible. Chelating agents such as EDTA were used to help clean minor spills.

Isolation and Characterization of Ribosomal Components. Ribosomal proteins and ribosomal RNA were isolated by using the Mg^{2+}–acetic acid procedure of Sherton & Wool (1974). Separation of basic ribosomal proteins was carried out by both one-dimensional and two-dimensional gel electrophoresis. Overall dimensions of these gels are as described by Howard & Traut (1973). One-dimensional gels contained 4% (stacking gel) or 8% acrylamide (separation gel), 0.13% bis(acrylamide), 6 M urea, 1 mM Na_2EDTA, 26 mM boric acid, 20 mM Tris-HCl, pH 8.6, and 0.2% Temed (v/v). The running buffer was 0.06 M Tris-HCl, pH 8.6, 0.003 M Na_2EDTA, 0.078 M boric acid, and 6 M urea. Conditions of electrophoresis are described in the figure legends. For two-dimensional electrophoresis, one-dimensional gels were polymerized on top of a slab gel containing a 1-cm stacking gel. The stacking gel was 18% acrylamide, 0.5% bis(acrylamide), 6 M urea, 0.92 M acetic acid, 0.048 M KOH, and 0.58% Temed (v/v), pH 4.5. This stacking gel was polymerized by the addition of 1 μL of a solution of riboflavin (1 mg/mL) per mL of gel solution. The second-dimension separation gel was identical with the stacking gel except that polymerization was accomplished by the addition of ammonium persulfate to a final concentration of 0.15 mM. The second-dimension running buffer was 0.186 M glycine and 0.026 M acetic acid, pH 4.5. During electrophoresis and processing of labeled protein, reducing agents such as DTT and 2-mercaptoethanol were omitted.

Radioactivity was localized in the one-dimensional gels by slicing each gel into 3-mm sections which were placed in 1 mL of 0.5 M 2-mercaptoethanol and heated at 65 °C overnight.

[1] Abbreviations used: fragment, C-A-C-C-A(Ac[^{3}H]Leu); mercurated fragment, C(Hg)-A-C(Hg)-C(Hg)-A(Ac[^{3}H]Leu); DTT, dithiothreitol; PPO, 2,5-diphenyloxazole; NEM, *N*-ethylmaleimide; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); pCMB, *p*-(chloromercuri)benzoate.

Table I: Peptidyl Transferase Activity[a]

ribosome type	fmol of [^{3}H]Leu-puromycin produced
80S *D. melanogaster*, 2.50 A_{260}	101
70S *E. coli B*, 2.50 A_{260}	408

[a] Peptidyl transferase activity was measured by using the fragment assay as described under Material and Methods.

Table II: Stimulation of Peptidyl Transferase by Sulfhydryl Reagents[a]

reagent	concn (mM)	transfer of [^{3}H]Leu to puromycin, % of control
NEM	1.25	190
DTNB	1.25	280
pCMB	1.25	180

[a] Ribosomes were incubated with the indicated concentrations of reagents for 5 min at 0 °C prior to their addition to the fragment assay. Each reaction contained 2.5 A_{260} units of 80S ribosomes

This allows for elution of the ^{203}Hg mercurated fragment. After the samples were cooled, 10 mL of a Triton–toluene base fluor was added, and the samples were counted in a liquid scintillation counter. Two-dimensional gels, after destaining, were impregnated with PPO and fluorographed (Bonner & Laskey, 1976).

Results

Activity of D. melanogaster Ribosomes in the Fragment Assay for Peptidyl Transferase and Stimulation of This Activity by Sulfhydryl Reagents. The fragment assay is a method for determining the activity of peptidyl transferase. In this assay, a pentanucleotide fragment derived from the 3′ terminus of *N*-acetylleucyl-tRNA is bound specifically at the P site and the antibiotic, puromycin, is bound at the A site of peptidyl transferase. Ribosome-catalyzed transfer of *N*-acetylleucine to puromycin is used as a measure of the peptide bond forming activity of the enzymatic center. As judged by this assay, 80S ribosomes obtained from *D. melanogaster* larvae exhibit less peptidyl transferase activity than bacterial ribosomes. This is shown in Table I. However, the level of activity of *D. melanogaster* ribosomes is comparable to that of other higher eucaryotes (Stockmar et al., 1972). Peptidyl transferase activity is known to be localized on the large subunit of both procaryotic (50S) and eucaryotic (60S) ribosomes (Harris & Pestka, 1977). In our assay of *D. melanogaster* peptidyl transferase activity, we observe a strong dependence on the presence of the 40S as well as the 60S subunit. The nature of this dependence has not been explored further.

The enhancement of peptidyl transferase activity by reaction with sulfhydryl blocking reagents has been found with ribosomes isolated from higher eucaryotes, such as rabbit, but not in ribosomes isolated from yeast, a lower eucaryote (Carrasco & Vâsquez, 1975). *D. melanogaster* 80S ribosomes, like the mammalian ribosomes, show enhanced peptide bond forming activity following treatment with NEM, DTNB, and pCMB. As seen in Table II, there is nearly a threefold increase in activity after reaction with DTNB and a twofold increase after reaction with NEM and pCMB. Also, the level of stimulation of peptidyl transferase in *D. melanogaster* is directly comparable to that observed by using rabbit reticulocyte ribosomes (Carrasco & Vâsquez, 1975).

Table III: Peptidyl Transferase Activity of the Mercurated Fragment[a]

fragment concn (nM)	fmol of [^{3}H]Leu-puromycin produced
13, nonmercurated	101
13, mercurated	130

[a] Mercurated fragment was substituted for nonmercurated fragment under the conditions of the fragment assay described under Materials and Methods. Each reaction contained 2.5 A_{260} units of 80S ribosomes.

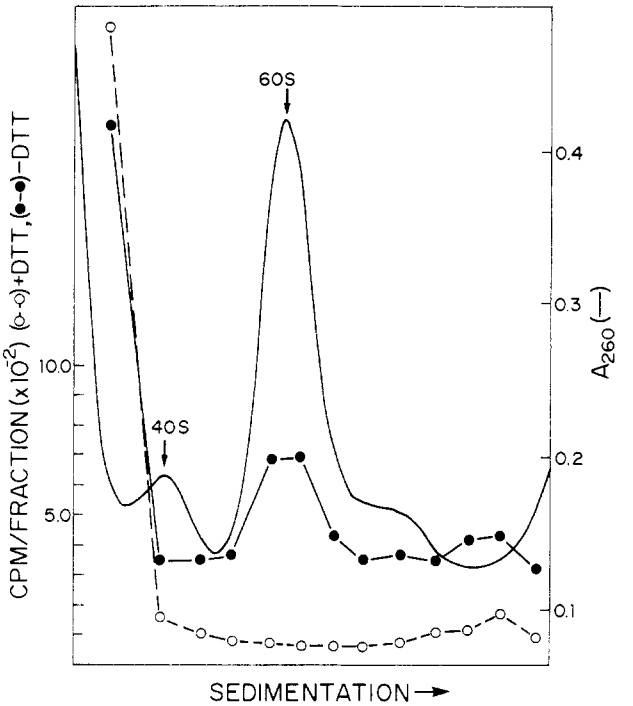

FIGURE 1: Sucrose gradient analysis of affinity-labeled ribosomes. Dissociated ribosomal subunits were separated on 10–30% linear sucrose gradients in 10 mM Tris-HCl, pH 7.6, and 100 mM KCl. Gradients were centrifuged at 40000 rpm for 6 h at 4 °C in an SW40 rotor. Gradients were monitored at 260 nm in a Beckman recording spectrophotometer (—). Fractions were collected and radioactivity was determined by liquid scintillation counting in a Triton–toluene base fluor. Gradient analysis was performed in the presence (O) or absence (●) of 3 mM dithiothreitol (DTT).

Activity of the Mercurated Fragment in the Peptidyl Transferase Reaction. The ability of the mercurated fragment to participate in peptidyl transferase parallels that of the nonmercurated fragment. The data presented in Table III clearly show that the mercurated fragment is as active a substrate for peptidyl transferase as the nonmercurated fragment. In fact, the slightly increased activity seen for the affinity label may suggest that the mercurated fragment functions as a better substrate for peptidyl transferase, perhaps as the result of the mercurated fragment having a high affinity for the P site.

Covalent Labeling of the Ribosome Occurs upon Binding of the Mercurated Fragment. Mercurated fragment (13 nM) was incubated with 5.0 A_{260} units of *D. melanogaster* 80S ribosomes and 1 mM puromycin under the fragment assay conditions (see Materials and Methods). As stated above, under these conditions the mercurated fragment participates fully in peptide bond formation. In order to obtain preliminary evidence as to whether the mercurated fragment could covalently attach to ribosomes, we dissociated the 80S particles from this reaction mixture into subunits by the addition of

Table IV: Recovery of ^{203}Hg following Ribosome Disassembly[a]

	cpm	% of total
ribosomal RNA	1472	4.9
ribosomal protein	28560	95.1

[a] Proteins were extracted from ethanol-precipitated ribosomes by the method of Sherton & Wool (1974). The ribosomal RNA pellet was washed 3 times, the washes were pooled, and the acetic acid soluble proteins were dialyzed overnight against 10% acetic acid at 4 °C and lyophilized. Ribosomal proteins were resuspended in 10 M urea, pH 8.6, for gel analysis. An aliquot of this solution was used for determination of radioactivity.

EDTA to a final concentration of 45 mM. The subunits were then separated on analytical sucrose gradients as shown in Figure 1. The ^{203}Hg radioactivity associated with the affinity label copurifies with the 60S subunits. The average yield of affinity-labeled 60S subunits in these and similar experiments was on the order of 1–2%. If covalent labeling occurs via a mercury–sulfur bond, this linkage, once formed, should be reversible by the reduction of this bond. Treatment of affinity-labeled ribosomes with DTT prior to dissociation and sucrose gradient analysis clearly eliminates labeling. As seen in Figure 1, in the presence of DTT, the mercurated fragment no longer copurifies with either subunit.

Even in the absence of puromycin, mercurated fragment is found to copurify with the 60S subunit. Under these conditions, nonmercurated fragment does not copurify with either subunit (data not shown). These data indicated that the affinity label covalently attaches to the 60S subunit. To provide more convincing evidence for the covalent attachment of the mercurated fragment to the ribosomes, we separated affinity-labeled 80S ribosomes into an RNA and a total protein fraction by treatment with Mg^{2+} and acetic acid (Sherton & Wool, 1974). Greater than 95% of the radioactivity originally associated with the affinity-labeled ribosomes is isolated in the protein fraction, as shown in Table IV. This radioactivity remains with the protein fraction even after extensive dialysis. These results provide further proof of the covalent attachment of the mercurated fragment to ribosomal components.

Site-Specific Labeling of the Ribosome with the Mercurated Fragment. To assure that the mercurated fragment has reacted specifically with the P site of peptidyl transferase, we performed the labeling reaction in the presence of increasing amounts of nonmercurated fragment. A constant amount of affinity label was mixed with a 5–400 molar excess of nonmercurated fragment. This mixture was reacted with ribosomes under the conditions of the fragment assay. Following the 45-minute incubation at 0 °C, ribosomes were collected by precipitation with 2 volumes of ethanol, and the protein fraction was extracted. The total ^{203}Hg radioactivity present in the protein fraction of each of these reactions is shown in Figure 2. Attachment of ^{203}Hg mercurated fragment to ribosomal proteins is dramatically reduced by the presence of nonmercurated fragment. It is clear that nonmercurated fragment effectively competes with the affinity-labeling reaction. These results indicate that the site at which covalent attachment of the mercurated fragment occurs and the binding site of the nonmercurated fragment are the same, i.e., the P site of peptidyl transferase. Further evidence for site-specific reaction is demonstrated by the ability of mercurated fragment, once bound to ribosomes, to transfer *N*-acetyl[^{3}H]leucine to puromycin, as discussed above.

Identification of Labeled Ribosomal Proteins. Results presented thus far suggest that the mercurated fragment binds specifically to the P site and reacts covalently with a ribosomal protein(s). In order to determine the identity of the affinity-labeled ribosomal species, we examined the basic ribosomal proteins by both one- and two-dimensional polyacrylamide gel electrophoresis. When affinity-labeled ribosomal protein was separated on 8% polyacrylamide gels, only one region of the gel was seen to contain substantial ^{203}Hg radioactivity. The position of this radioactivity corresponds to the position of a very basic protein. This is shown in Figure 3. Any mercurated fragment not attached to protein should not penetrate these gels. There is a visible aggregate of material which remains at the interface between the stacking and separation gels. Such an aggregate is also visible in the stacking gel of

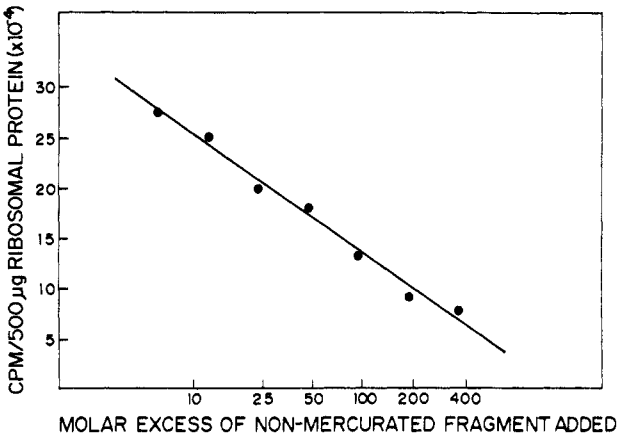

FIGURE 2: Competition of the affinity-labeling reaction with nonmercurated fragment. A total of 2.2 A_{260} units of 80S *D. melanogaster* ribosomes was incubated with 10 nM mercurated fragment and the indicated concentrations of nonmercurated fragment under the conditions of the fragment assay as described under Materials and Methods. Following incubation at 0 °C for 45 min, ribosomes were precipitated with 2 volumes of ethanol. A total of 20 A_{260} units of 80S ribosomes was added as carrier, and the total ribosomal protein fraction was extracted (see Materials and Methods) and dialyzed overnight against 10% acetic acid at 4 °C. The protein content of each reaction was measured by the method of Lowry et al. (1951). Radioactivity was determined as described in Figure 1.

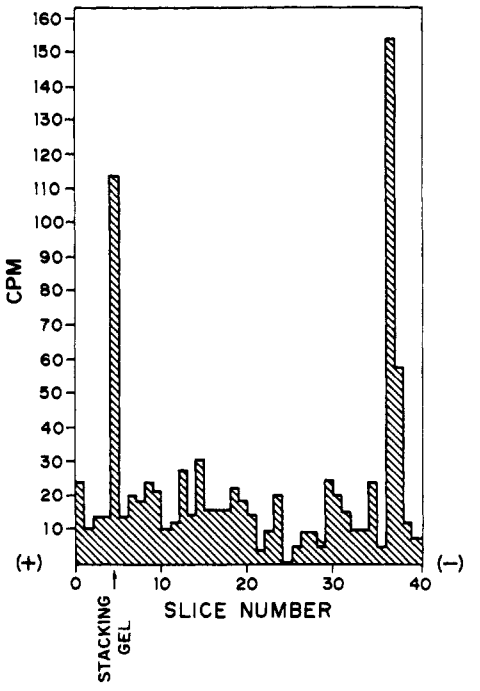

FIGURE 3: One-dimensional gel electrophoresis of labeled ribosomal proteins. Labeled ribosomal proteins were electrophoresed for 13 h at 1.25 mA/tube. Following electrophoresis, the gel was sliced in 3-mm sections, and the radioactivity in each was determined as described in the text.

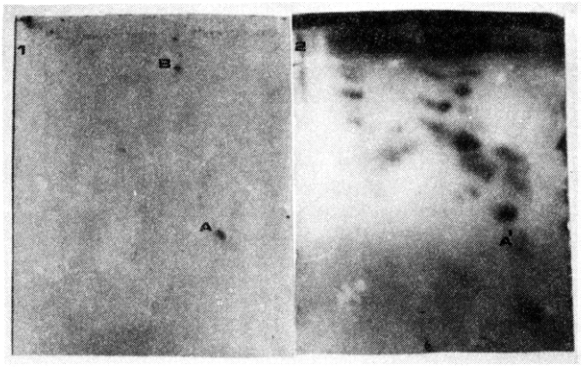

FIGURE 4: Fluorography of labeled ribosomal proteins following two-dimensional gel electrophoresis. Labeled ribosomal protein was subjected to two-dimensional gel electrophoresis as described under Materials and Methods. Electrophoresis was carried out for 13 h at 1.25 mA in the first dimension and at 200 V for 21 h in the second dimension. Following this separation, gels were stained with 0.2% Coomasie Blue and photographed. Fluorography was then used to determine areas of radioactivity present in the gel. Panel 2 shows a photograph of the stained gel and panel 1 is the corresponding fluorogram. A and B designate the major areas of radioactivity visible on the fluorogram. A′ denotes the stained protein thought to correspond to radioactive spot A. Details are discussed in the text.

the first dimension of two-dimensional gels. This is due to the fact that these gels are run in the absence of thiol reducing reagents such as DTT or 2-mercaptoethanol, since these reagents will readily cleave the protein–affinity label linkage. As a result, the numerous sulfhydryl groups present in ribosomal proteins cannot be totally prevented from forming interprotein disulfide cross-links. In affinity-labeled ribosome samples, such aggregates of ribosomal proteins are not capable of penetrating the separation gel. This is seen by the ^{203}Hg radioactivity remaining at the stacking gel–separation gel interface (Figure 3). Ribosomal proteins from ribosomes not treated with the affinity label also show such aggregation, i.e., many of them do not penetrate the separation gel when thiol reducing agents are absent. Nevertheless, the results of one-dimensional electrophoresis indicated that labeled ribosomal protein(s) could be identified by gel electrophoresis. In order to determine more precisely which ribosomal proteins were labeled, we performed two-dimensional electrophoresis, since it can provide resolution of individual ribosomal proteins. Following separation of labeled ribosomal proteins, the two-dimensional gels were stained and photographed, and areas of radioactivity were identified by fluorography (Bonner & Laskey, 1974). As shown in Figure 4, two areas of radioactivity were detected. One of these spots (A) can be correlated to a stained protein spot (A′). The actual spot on the fluorogram corresponds to a position shifted slightly up and to the left of the protein species indicated on the gel plate. This migration shift of the labeled species of protein relative to the unlabeled protein species is predicted due to the addition of the negative charges contributed by the phosphate groups of the affinity label and the addition of the molecular weight of the label. The net result of these two factors would be the appearance of the affinity-labeled protein species to the upper left of the same, nonlabeled species. Further proof of this migration shift could be obtained by isolation of the labeled ribosomal protein, removal of the affinity label, and subsequent two-dimensional electrophoresis of the free protein. Unfortunately, we were not able to obtain sufficient quantities of the labeled ribosomal protein to do this. The radioactivity visible in the upper center of the fluorogram (B) does not correlate well with any stained protein spot on the two-dimensional gel. Because of its position in the gel, we believe it may represent a protein aggregate.

Discussion

We have presented evidence that the mercurated fragment labels a ribosomal protein at the P site of peptidyl transferase in *D. melanogaster* ribosomes. Several experiments support the site-specific nature of the labeling process. The ability of the mercurated fragment to transfer *N*-acetylleucine to puromycin in the fragment assay as efficiently as the nonmercurated fragment argues that both fragments bind to the P site of peptidyl transferase in a similar manner. This is further evidenced by the fact that both mercurated and nonmercurated fragment compete for the same site. When the mercurated fragment is present at the P site, the major species labeled is a single protein as determined by two-dimensional gel electrophoresis. This is additional evidence for a specific affinity-labeling process.

We also present evidence that the reaction of the affinity label with a ribosomal protein occurs via the formation of a covalent mercury–sulfur bond. Once the affinity-labeling process occurs, the mercurated fragment is found to copurify with a ribosomal protein throughout isolation of the total protein fraction and analysis of this fraction by two-dimensional gel electrophoresis. This strongly suggests covalent attachment of the affinity label. Linkage of the mercurated fragment to a ribosomal protein can be cleaved by thiol reducing agents. This is precisely what is expected if the linkage is a mercury–sulfur bond. The affinity label used in these experiments is novel in that it is designed to label via a specific protein residue, namely, sulfhydryl groups, rather than being reactive toward a number of different residues. This additional feature of design allows for the affinity label not only to identify active-site proteins but also to identify active-site proximal sulfhydryl groups.

The results of these affinity-labeling experiments provide new information concerning the nature of a eucaryotic peptidyl transferase. It is clear that sulfhydryl groups are present at or near the binding site for the 3′ terminus of peptidyl tRNA. Reaction of these sulfhydryl groups may directly or allosterically affect peptidyl transferase function. It is interesting that enhanced peptidyl transferase activity is seen for the mercurated fragment. Similarly, small thiol blocking reagents can stimulate peptidyl transferase (Carrasco & Vásquez, 1975). Perhaps these phenomena are both the result of alterations of P site proximal sulfhydryls.

The protein labeled with the mercurated fragment is a small, basic ribosomal protein. This was demonstrated by both one- and two-dimensional gel electrophoresis. This is noteworthy in that Reyes et al. (1977) have identified at least five small, basic proteins as being involved in the peptidyl transferase center of rat liver ribosomes. These investigators also presented evidence that the sulfhydryl stimulation of peptidyl transferase may be dependent on one or more of these proteins. There have been two recent reports (Gosálbez et al., 1978; Czernilofsky et al., 1977) in which proteins involved in eucaryotic peptidyl transferases have been identified by affinity labeling techniques. Both of these groups have identified a number of small, basic proteins as present at or near the peptidyl transferase center of rat liver and yeast ribosomes, respectively. Unfortunately, direct comparisons of proteins implicated in the peptidyl transferase centers of these organisms and the protein we have identified at the P site of peptidyl transferase of *D. melanogaster* are difficult to make. However, these results suggest similarities between the peptidyl transferase center of ribosomes isolated from *Drosophila* and those derived from other eucaryotes. Similarities between P site proteins

of eucaryotes and procaryotes are also seen. Using a peptidyl-tRNA affinity-label analogue to covalently label 70S *E. coli* ribosomes, Pellegrini et al. (1974) found the major reactive species to be a small, basic protein of the large subunit (L27). This reagent requires nucleophilic attack for the labeling to occur. The most likely residue for alkylation is the cysteine sulfhydryl. L27 is known to contain a reactive cysteine group in the intact particle (Moore, 1971; Bakardjieva & Crichton, 1974). These results suggest the presence of a sulfhydryl group at the P site of both eucaryotic and procaryotic peptidyl transferases. The labeling of a small, basic protein in *Drosophila* ribosomes suggests conservation of at least size and electrophoretic behavior and, possibly, some specific amino acid residues within the P site of both procaryotic and eucaryotic ribosomes. In collaboration with P. Butler and R. Traut, we are undertaking studies using this mercurated reagent and *E. coli* ribosomes to further explore these possibilities.

References

Bakardjieva, A., & Crichton, R. (1974) *Biochem. J. 143*, 599–606.
Bermek, E., Mönkemeyer, H., & Berg, R. (1971) *Biochem. Biophys. Res. Commun. 45*, 1294–1299.
Bonner, W. M., & Laskey, R. A. (1974) *Eur. J. Biochem. 46*, 83–91.
Carrasco, L., & Vâsquez, D. (1975) *Eur. J. Biochem. 50*, 317–323.
Czernilofsky, P. A., Collatz, E., Gressner, A. M., & Wool, I. G. (1977) *Mol. Gen. Genet. 153*, 231–235.
Dale, R. M. K., Martin, E., Livingston, D. C., & Ward, D. C. (1975) *Biochemistry 14*, 2447–2457.
Gosâlbez, M. P., Vâsquez, D., & Ballesta, J. P. G. (1978) *Mol. Gen. Genet. 163*, 29–34.
Harris, R. J., & Pestka, S. (1977) in *Molecular Mechanisms of Protein Biosynthesis* (Weissbach, H., & Pestka, S., Eds.) p 413, Academic Press, New York.
Howard, G. A., & Traut, R. R. (1973) *FEBS Lett. 29*, 177–180.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Miller, D. L., & Weissbach, H. (1977) in *Molecular Mechanisms of Protein Biosynthesis* (Weissbach, H., & Pestka, S., Eds.) p 324, Academic Press, New York.
Monro, R. E. (1971) *Methods Enzymol. 20*, 472–481.
Moore, P. B. (1971) *J. Mol. Biol. 60*, 169–184.
Pellegrini, M., Oen, H., Eilat, D., & Cantor, C. (1974) *J. Mol. Biol. 88*, 809–829.
Pestka, S. (1971) *Methods Enzymol. 20*, 502.
Pestka, S. (1977) in *Molecular Mechanisms of Protein Biosynthesis* (Weissbach, H., & Pestka, S., Eds.) p 468, Academic Press, New York.
Reyes, R., Vâsquez, D., & Ballesta, J. P. G. (1977) *Eur. J. Biochem. 73*, 25–31.
Sherton, C. C., & Wool, I. G. (1974) *Mol. Gen. Genet. 135*, 97–112.
Stockmar, R., Ringer, D., & McAllister, H. (1972) *FEBS Lett. 23*, 51–55.

Sequence of Histone 2B of *Drosophila melanogaster*†

Sarah C. R. Elgin,* James Schilling, and Leroy E. Hood

ABSTRACT: The complete sequence of histone 2B of *Drosophila* has been determined by using an improved Beckman sequenator. Comparing these data with those previously published by other investigators on the histone 2B of calf [Iwai, K., Hayashi, H., & Ishikawa, K. (1972) *J. Biochem. (Tokyo) 72*, 357–367], trout [Koostra, A., & Bailey, G. S. (1978) *Biochemistry 17*, 2504–2510], and *Patella* (a limpet) [van Helden, P. D., Strickland, W. N., Brandt, W. F., & von Holt, C. (1979) *Eur. J. Biochem. 93*, 71–78], it is possible to assess the evolutionary stability of this protein. There is little conservation of sequence in the N-terminal portion of the molecule (residues 1–26 numbering according to calf H2B), while the remainder of the protein, which we designate the C-terminal portion, is highly conserved. In the region of 27–125 residues, there are 9 substitutions in the composite data among the 98 positions, 8 of them conservative. These data indicate that very different selective pressures operate on the two different portions of the H2B molecule, implying the existence of two well-defined regions. Studies on the structure of the nucleosome by others have suggested that the C-terminal portion of H2B is involved in histone–histone interactions while the N-terminal portion is a relatively free "tail" binding to DNA. The sequence data indicate that the function of the C-terminal region of H2B requires considerable sequence specificity while that of the N-terminal region does not.

During the last few years considerable evidence has been obtained which has led to and supported the nucleosome or ν body model of chromatin structure. The chromatin fiber is visualized as a string of beads, each bead made up of eight molecules of the smaller histones, 2A, 2B, 3 and 4, around which the DNA is wrapped. There are ~200 base pairs of DNA associated with each unit. Histone 1 and the nonhistone chromosomal proteins are apparently associated with the DNA on the outside of the core structure. For reviews of the evidence leading to this model and a more detailed discussion, see Elgin & Weintraub (1975), Kornberg (1977), and Felsenfeld (1978).

The pioneering studies of Fambrough, DeLange, and their colleagues (Fambrough & Bonner, 1968; DeLange et al.,

† From the Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138 (S.C.R.E.), and the Division of Biology, California Institute of Technology, Pasadena, California 91125 (J.S. and L.E.H.). *Received April 30, 1979; revised manuscript received September 27, 1979.* Supported by Grant PCM 75-15259 from the National Science Foundation to S.C.R.E. and Grant GM 06945 from the National Institutes of Health to L.E.H. S.C.R.E. is the recipient of a Research Career Development Award from the National Institutes of Health.

0006-2960/79/0418-5679$01.00/0