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A Chemical Approach to the Fine Structure of Biomolecular Complexes: The Amino Terminal Region of the 50S Ribosomal "A" Protein From *Bacillus stearothermophilus*†

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ABSTRACT: An experimental approach and methodology are described for determining the reactive properties and ionization constants of individual functional groups of proteins within biomolecular complexes. The ionization constants and reactivities of the methionyl-1 amino terminus and the lysyl-3 residue of the alanine rich 50S ribosomal "A" protein from *Bacillus stearothermophilus* have been determined by an extension of the competitive labeling technique used by H. Kaplan, K. J. Stevenson, and B. S. Hartley ((1971), *Biochem. J. 124*, 289–299). This approach employs [1-^{14}C]- and [^{3}H]acetic anhydride in a double-labeling procedure. In 0.1 M KCl–0.02 M Mg^{2+}–0.05 M Veronal at 10° the methionyl-1 amino terminus has a pK_a of 7.5 and is exposed on the surface of the ribosome. The lysyl-3 has a pK_a of 10 and is also exposed to solvent at the surface of the 50S subunit. Based on a linear free energy relationship (Bronsted plot) obtained with a series of standard amines the methionyl amino terminus has a substantially higher reactivity than expected from its ionization constant. The lysyl ε-amino group has the expected reactivity. The abnormally high reactivity of the methionyl amino terminus can only be accounted for by a specific interaction with other functional groups in the ribosome. These data support the proposal that the charged state of this residue is important in the structure and function of the "A" protein at the surface of the ribosome.

Many cellular functions are carried out in multiphasic molecular groupings, which at the highest level of complexity are defined as organelles. At this plane of organization, structure–function relationships are determined and/or mediated by highly specific interactions, orientations, and reactive properties of functional groups within the macrostructure. At present, no general method exists for determining the fine-structural properties of individual proteins and their functional groups within such multiprotein complexes. Our objective was to develop one such method using a competitive labeling technique which has already been successfully employed to determine the properties of individual ionizable groups with the native structures of monomeric proteins (Kaplan *et al.*, 1971; Cruickshank and Kaplan, 1972; Kaplan, 1972) and in assessing quantitatively the degree of exposure of proteins in ribosomes (Visentin *et al.*, 1973) and chromatin (Malchy and Kaplan, 1974). Such a study on individual functional groups of proteins in biomolecular complexes will provide useful topographical data concerning catalytic sites, their microenvironments, as well as a description of their dynamic properties within the structural matrix.

As a model system for such a study, the ribosome is a most appropriate choice. Indeed, as a catalytic unit its function in the translation of the genetic message is of fundamental importance to all other cellular structures and functions. Of the polypeptides within ribosome, the "A" proteins [the L7–L12 multimer in the *Escherichia coli* nomenclature (Kaltschmidt and Wittmann, 1971)] are most interesting as their complete sequence is known (Terhorst *et al.*, 1972, 1973; Möller *et al.*, 1972) and both forms have been implicated in the translocation of the growing peptide in association with the elongation factors G and T (Sander *et al.*, 1972; Brot *et al.*, 1972, 1973, 1974; Brot and Weissbach, 1972; Hamel *et al.*, 1972). Furthermore, it has been proposed that the acetylation of the amino terminus of L12 in *E. coli* attenuates the process of elongation itself (Sander *et al.*, 1972). Contradictory data have been presented by more recent results (Kung *et al.*, 1973). Given this information our objective in this study was to define precisely the properties of this amino terminal region of the "A" protein as it exists within the native 50S ribosomal subunit. In doing so we feel that this approach can serve as a focal point for further studies of fine structure and properties of other functional groups in the ribosome and other multiprotein mosaics, the complexity of which presently excludes their analysis by X-ray crystallography.

Experimental Section

Materials

Cells. Bacillus stearothermophilus cells were grown in

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the complex medium of Pace and Campbell (1967) in a 120-l. fermenter with vigorous aeration at 65° and harvested in early exponential-phase growth. The cells were carefully washed and either used immediately or stored as a pellet in liquid nitrogen.

Enzymes. α-Chymotrypsin and bovine pepsin were crystalline salt-free preparations purchased from Worthington Biochemical Corp., Freehold, N. J. Pronase and thermolysin were purchased from Calbiochem, Los Angeles, Calif.

Chemicals. [1-^{14}C]Acetic anhydride (specific activity 80 Ci/mol) and [^{3}H]acetic anhydride (specific activity 2.6 Ci/mmol) were obtained from Amersham/Searle Corp., Des Plaines, Ill. All other chemicals were high purity preparations obtained from commercial sources and were used without further purification.

Ribosomes and 50S Subunits. The *Bacillus stearothermophilus* ribosomal subunits were prepared as described previously (Chow *et al.*, 1972) except for a few minor changes. Separation and purification were achieved using isokinetic sucrose gradients (Zeijst and Bult, 1972) from 10 to 30% (w/v) in TMK buffer (10 mM Tris-HCl–50 mM KCl–0.3 mM Mg^{2+}–6 mM β-mercaptoethanol (pH 7.6). The 50S particles were concentrated by centrifugation and redissolved in VKM buffer (0.05 M Veronal–0.10 M KCl–0.02 M Mg^{2+} (pH 7.6), and stored in liquid nitrogen until ready for use. The purity of each preparation was checked on a Beckman Spinco Model E analytical ultracentrifuge.

The "A" Protein and the Acetylated Amino Terminal Peptide Marker. The "A" protein from the *Bacillus stearothermophilus* 50S particles were selectively extracted from 10 g of 50S subunit by the method of Hamel *et al.* (1972) and concentrated by precipitation with 2.25 volumes of −20° acetone. Separation, purification, and identification of the L7–L12 equivalents were then carried out as described previously (Visentin *et al.*, 1974). An amino terminal, ^{14}C-acetylated peptide, Ac-Met-Thr-Lys(Ac)Glu-Glu, derived from the thermophile "A" protein was prepared by adding 100 μCi of [^{14}C]acetic anhydride in acetonitrile to 10 mg of the protein at pH 9 followed by complete acetylation with 50 μl of unlabeled reagent. The acetylated protein was dialyzed and digested with pepsin (protein/pepsin = 10:1) in 10% formic acid and the acetylated amino terminal peptide was isolated by the selective amino terminal purification method of Kaplan (1974).

Methods

Competitive Labeling. Freshly prepared 50S subunits were trace labeled in a water-jacketed, nitrogen-flushed cell maintained at 10 ± 0.05°. A sample of subunit (0.091 μmol in 5.50 ml of solution containing VKM buffer and 0.526 μmol of L-phenylalanine) was equilibrated at 10°. The pH was adjusted with 5 M KOH or 5 M HCl to approximately desired value and then 100 μl of acetonitrile containing 0.96 μmol of [^{3}H]acetic anhydride (specific activity 2.6 Ci/mmol) was added with vigorous stirring. The reaction mixture was allowed to stand for 10 min, transferred to a centrifuge tube, and then spun at 35,000 rpm for 18 hr in a Beckman Spinco L265B ultracentrifuge. This procedure was carried out at the following pH's: 6.25, 6.83, 7.00, 8.01, 8.46, 9.00, 9.50, and 10.00.

Treatment of Ribosomal Proteins. The total 50S ribosomal protein from pellets obtained in the 18-hr centrifugation were prepared by the acetic acid extraction method (Hardy *et al.*, 1969), dialyzed, and freeze dried. The freeze-dried protein samples from each pH labeling were then combined with the corresponding supernatant from the 18-hr centrifugation step and solid urea was added to 8 M. The mixture was fully acetylated with [1-^{14}C]acetic anhydride (500 μCi diluted to 1 ml with neat acetic anhydride). The acetylphenylalanine was extracted from this mixture with ethyl acetate as previously described (Kaplan *et al.*, 1971). The fully acetylated mixture of 50S proteins were dialyzed against water and freeze dried.

Isolation of the "A" Protein Amino Terminal Peptides from a Total Mixture of 70S Particle Proteins. Each protein sample was dissolved in 10 ml of 10% formic acid and digested with 5 mg of bovine pepsin for 24 hr at 37° (protein/pepsin = 10:1). The protein digest was then freeze-dried. The freeze-dried peptic digest was then dissolved and spotted out along a 40-cm band on Whatman 3MM paper. The ^{14}C-marker amino terminal peptide was spotted at each end of the band. The following purification steps were carried out: (1) pH 6.5 paper electrophoresis for 40 min at 60 V/cm, (2) pH 2.1 paper electrophoresis for 60 min at 60 V/cm, (3) chromatography in butanol–acetic acid–water–pyridine (15:3:12:10, v/v.) (solvent BAWP) for 16 hr, and (4) pH 3.5 paper electrophoresis for 2 hr at 60 V/cm. In going from one purification step to the next, the ^{14}C-labeled spots were cut out along with the entire band and served as markers for the next step. After the final electrophoresis step the ^{14}C-labeled amino terminal peptide at either end of the strip was removed and the remainder of the band eluted with 20 mM NH_3 and evaporated to dryness.

Treatment of Isolated Peptides. The isolated peptides were dissolved in 100 μl of 1% (w/v) NH_4HCO_3 and digested with 0.01 mg of Pronase. The digest peptides were spotted out along 8 cm of Whatman 1 paper. The ^{14}C-marker peptides were treated in identical manner and spotted at each end of the bands and then subjected to pH 6.5 high-voltage electrophoresis for 1 hr at 60 V/cm. The radioactive peptides were located by radioautography, the markers removed, and the peptides eluted with 20 mM NH_3.

Amino acid analyses were carried out in a Durrum D-500 amino acid analyzer. Acetylated peptides and amino acids were hydrolyzed in 5.7 M HCl at 110° in sealed evacuated tubes.

Scintillation counting was carried out with a Beckman LA-250 liquid scintillation counter with Aquasol (New England Nuclear Corp.) as the scintillation medium.

Calculation of Reactivities. The pH dependent relative rate constants ($\alpha_x r$) were calculated from the $^{3}H/^{14}C$ ratios of the isolated acetylated peptides and the internal standard acetylphenylalanine by substituting in eq 1, where α_s is the

$$\alpha_x r = \frac{(^{3}H/14_C) \text{ of acetylated peptide}}{(^{3}H/14_C) \text{ of acetylphenylalanine}} \times \alpha_s \qquad (1)$$

degree of ionization of the amino group of phenylalanine and is calculated at each pH value using a pK_a of 9.5 (Kaplan, 1972). The two experimentally determined parameters for the particular amino group under study are α_x (the degree of ionization) and r (the ratio of the second-order velocity constants for the reaction of the unprotonated amino group under study with acetic anhydride to that of the unprotonated amino group of phenylalanine).

Results

Isolation of the Acetylated Amino Terminal Peptide of the "A" Protein from a Total Peptic Digest of 70S Ribosomal Protein. The acetylated amino terminal peptide of the "A" protein was located by the selective isolation proce-

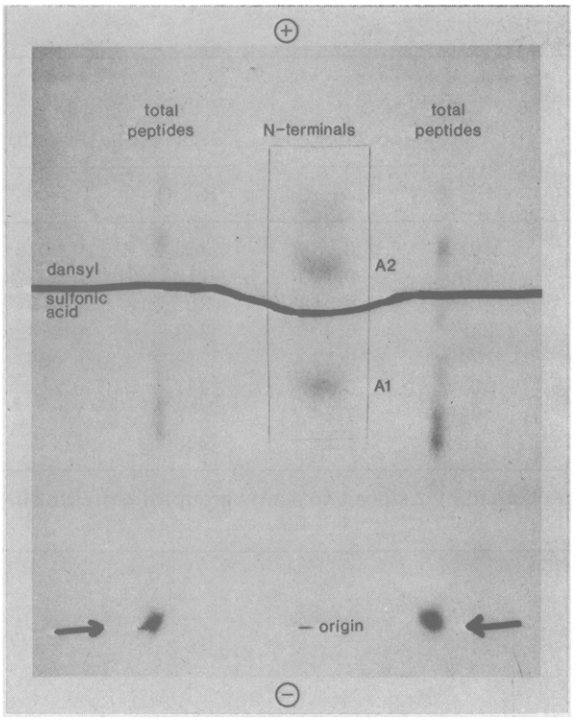

FIGURE 1: Radioautogram of ^{14}C peptides derived from a peptic digest of the "A" protein following pH 6.5 high-voltage electrophoresis.

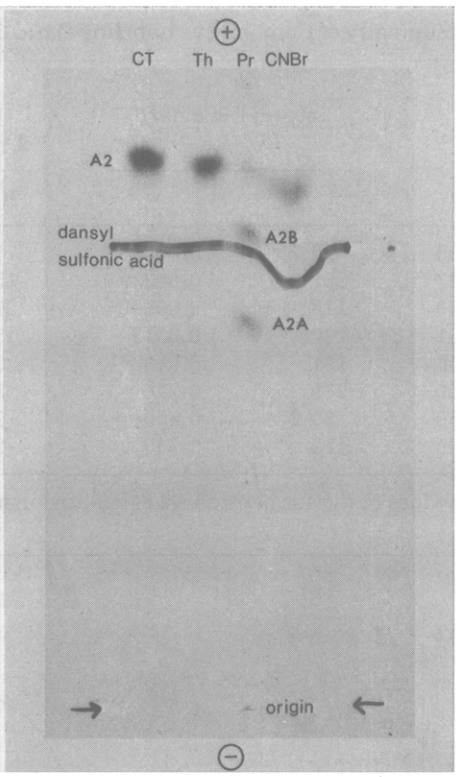

FIGURE 2: Radioautogram of peptide A2 after treatment with chymotrypsin (CT); thermolysin (Th); Pronase (Pr), and cyanogen bromide (CNBr) followed by pH 6.5 electrophoresis.

dure for amino terminal peptides of Kaplan (1974). Figure 1 shows a radioautogram of total ^{14}C-labeled peptides from a peptic digest of the "A" protein following pH 6.5 electrophoresis. The peptide bands in the center are the amino terminal peptides derived from this digest by the amino terminal procedure of Kaplan (1974). There appears to be two major amino terminal peptides, Al(α_{Asp} = 0.54) and A2(α_{Asp} = 0.81). A2 was present in much larger yield and therefore was used as a marker to locate the peptide after the procedure of Kaplan *et al.* (1971) from the digest of the total 70S ribosomal proteins. After employing the purification procedure described in methods the peptide derived from the total 70S mixture had the following amino acid composition:

acetyl-methionine-threonine-lysine(Ac)-glutamine-glutamic
0.63 0.84 1.0 1.1 1.1

This compostion corresponds to the known amino terminal sequence of the "A" protein from *B. stearothermophilus* (Visentin *et al.*, 1974). Treatment of this peptide with Pronase yielded two peptides A2A(μ_{Asp} = 0.58) and A2B(α_{Asp} = 0.73) as shown in the radioautogram after pH 6.5 electrophoresis (Figure 2). These peptides gave the following amino acid compositions:

A2A – lysine(Ac)-glutamine-glutamic
0.95 1.0 1.0

and

A2B – acetyl-methionine-threonine
0.60 1.0

The methionine in peptides A2 and A2B has been oxidized to either the sulfoxide or sulfone during the purification as evidenced by the lower yield of methionine on amino acid analysis and the failure of methionine to be cleaved by treatment with cyanogen bromide (Figure 2).

After isolation of peptide A2 from each pH sample, one-tenth was removed for scintillation counting and the remainder was digested with Pronase. The new peptides from each pH sample were located using [^{14}C]A2A and [^{14}C]A2B as radioactive markers. These peptides were eluted and one-fifth was taken for scintillation counting. The ($^3H/^{14}C$) ratios obtained for these peptides and the acetylphenylalanine (internal standard amine) were substituted in eq 1 to give the reactivities of the methionyl amino terminus and the lysyl ϵ-amino group at position 3. These data are summarized in Table I.

Reactivity of the Methionyl Amino Terminus. Figure 3 shows a plot of $\alpha_x r$ against pH for the amino terminus (peptide A2B) of the "A" protein in the native 50S subunit (open circles). The solid line is a theoretical titration curve for r = 0.58 and a pK_a = 7.5. Above pH 9 there is a discontinuity in the pH–reactivity profile indicating some minor conformational change.

Reactivity of the ϵ-Amino Group of Lysine-3. Figure 3 also shows a plot of $\alpha_x r$ against pH for the ϵ-amino group of lysine-3 (peptide A2A) of the "A" protein. The solid line is a theoretical titration curve for r = 2.3 and pK_a = 10. The data at the lower end of the titration curve give an excellent fit to the theoretical curve. However, above pH 9 there is a discontinuity which occurs at the same pH as that observed with the amino terminus.

Comparison of Reactivities with a Brønsted Plot for Primary Amines. Figure 4 shows where the methionyl amino terminus and the ϵ-amino group of lysine-3 lie in relation to a Brønsted plot for primar amines (Kaplan *et al.*, 1971). The reactivity of the ϵ-amino group of lysine-3 is close to that expected from the Brønsted plot and is therefore a fully exposed group on the surface of the ribosome with normal properties. The methionyl amino terminus, on the other hand, is approximately an order of magnitude more reactive

Table I: Summary of Competitive Labeling Data.

pH	Internal Standard (Ac-Phe)		Peptide A2 (Ac-Met-Thr-Lys(Ac)-Glu-Gln)		Peptide A2B (Ac-Met-Thr)		Peptide A2A (Lys-(Ac)-Glu-Gln)	
	$^3H/^{14}C$	α_s	$^3H/^{14}C$	$\alpha_x r^a$	$^3H/^{14}C$	$\alpha_x r$	$^3H/^{14}C$	$\alpha_x r$
6.25	32.2	0.000562	1510	0.05	3040	0.053	103	0.0018
6.83	38.9	0.00239	1570	0.19	3090	0.190	113	0.0070
7.50	118	0.00990	2500	0.42	4040	0.342	202	0.019
8.01	222	0.0313	1540	0.43	2820	0.40	243	0.034
8.46	191	0.0783	942	0.77	1990	0.82	214	0.088
9.00	276	0.240	522	0.91	877	0.76	241	0.21
9.50	89.2	0.500	246	2.8	238	1.3	279	1.6
10.00	112	0.760	209	2.8	166	1.1	266	1.8

[a] The values of $\alpha_x r$ for peptide A2 calculated using eq 1 must be multiplied by 2 since two acetyl groups are present in this peptide.

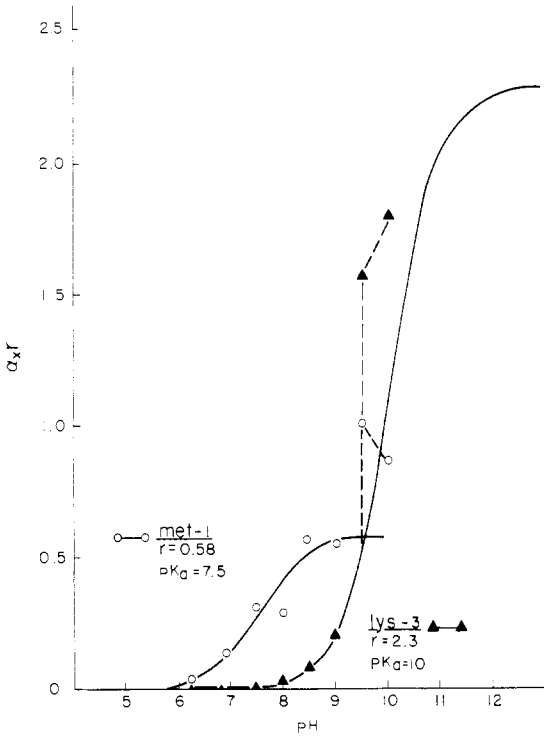

FIGURE 3: Plot of $\epsilon_x r$ against pH for the amino terminus (O) and the ϵ-amino group of lysine-3 (●) of the "A" protein.

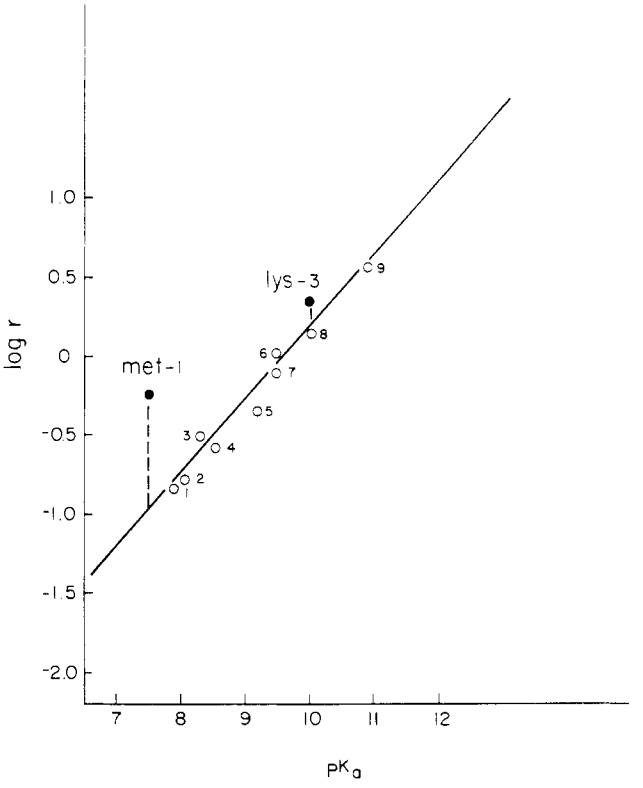

FIGURE 4: Bronsted plot showing the position of methionyl amino terminus and the ϵ-amino group of lysine-3 of the "A" protein in relation to a series of standard primary amines: (1) Gln-Gly; (2) Phe-Gly; (3) Leu-Gly-Gly; (4) Ala-Gly; (5) Asn; (6) Phe; (7) Gln; (8) Ala; (9) Bz-Gly-Lys.

than one would expect for an amino group with a pK_a of 7.5. This functional group therefore is exposed but as a result of some feature of its microenvironment is rendered super-reactive. The ionization constants, absolute reactivities, and the relative reactivities [reactivities relative to that expected on the basis of a Brønsted plot (Kaplan *et al.*, 1971)] are summarized in Table II.

Discussion

The technique of competitive labeling employs the functional groups of proteins as built-in probes whose chemical properties will reflect the nature of their environment as well as the dynamic properties of the protein molecule. This technique has two features which make it a particularly advantageous approach for studying structure and function relationships in proteins. Firstly, the properties determined are for the functional groups in the native protein. Secondly, the parameters, *viz.*, ionization constant and reactivity, can be unequivocally assigned to individual residues. The first feature is achieved by adding a sufficiently small amount of radioactive reagent to the reaction mixture so that the reagent must react predominantly with the unmodified protein. It therefore does not matter whether the protein is present as a monomer or as a constituent of a multiprotein complex. The reactivity of its functional groups will always reflect the properties of the native structure. The second feature is realized by the isolation of labeled peptides containing only the functional group under study. In

previous applications of competitive labeling, peptides containing the labeled functional groups were isolated and quantitated by amino acid analysis. However, such a procedure for multiprotein complexes presents some practical as well as technical problems. In the case of biomolecular complexes such as the ribosome with molecular weights of the order of 10^6, the amount of complex needed to contain enough of a particular protein ($\simeq 1$ μmol) to ensure a yield of peptide suitable for accurate amino acid analysis is operationally unrealistic, especially when one considers that this amount is required for each pH value at which labeling is carried out. Another difficulty is that if it is necessary to separate a particular protein from the other constituents of the complex, this operation would, in most cases, make the burden of carrying out a large number of parallel purifications prohibitive.

The procedure described in the present study requires only a single purification of the protein which contains the particular functional groups of interest and therefore the problem of studying the properties of an individual protein within a multiprotein complex is reduced to essentially the same as that of studying a monomeric protein. The isolated protein is derivatized with ^{14}C-labeling reagent (acetic anhydride in the present study) and the isolation procedures for the peptides containing the particular functional groups of interest are determined. These ^{14}C-labeled peptides can then be used as markers to locate the desired peptide from a digest of the total protein of the complex. Although in the present study only the 50S subunit was used, we have shown that an amino terminal peptide from the "A" protein could be easily purified from a digest of the total 70S proteins by placing the ^{14}C-marker peptide at the edges of the peptide bands prepared for high-voltage paper electrophoresis. Radioautography was used to locate the position after each purification step and the marker peptide was simply removed after the last purification step. Thus, the need to carry out a large number of parallel protein purifications is eliminated.

The problem of quantitating the labeled peptides was solved by using a double-labeling procedure. Instead of reacting the ^3H-trace-labeled protein with an excess of unlabeled reagent as in previous studies, the ^3H-trace-labeled protein was reacted with an excess of ^{14}C-labeled reagent. It has been shown (Kaplan *et al.*, 1971) that at a given pH the relative, pH-dependent, second-order velocity constant ($\alpha_x r$) for the reaction of the functional group with the radioactive label under trace-labeling conditions is equal to the degree of ionization of the internal standard (α_s) times the ratio of ^3H-specific radioactivity of the peptide, containing the derivatized functional group, to that of the derivatized internal standard. In the case of the double-labeling procedure the ratio of specific radioactivities will be equal to the ratio of $^3H/^{14}C$ ratios (eq 1 under Methods). Although the absolute amount of each peptide could be determined from the number of ^{14}C counts, there is no need to do so since the only experimental information required is the $^3H/^{14}C$ ratio. An obvious advantage of this double-labeling procedure is the relatively small amount of starting materials and isolated peptides required. The amount of peptides A2A and A2B (Figure 2) isolated were of the order of 500 pmol, an amount insufficient for accurate amino acid analysis, yet the ($^3H/^{14}C$) ratio could be easily determined. As it turns out, the double-labeling technique is the only approach by which the reactivity of the amino terminus could be determined. A portion of the amino terminus of the "A" protein is derivatized (L. P. Visentin, unpublished data) and therefore the ^3H-specific radioactivity of any acetylated peptide derived from the amino terminus would be lowered by the presence of an endogenous acetylated peptide. The ($^3H/^{14}C$) ratio, on the other hand, will not be affected by the endogenous acetylated peptide and the double-labeling approach therefore gives the chemical properties of the unacetylated portion. The properties of lysyl-3 ϵ-amino group, however, will be an average for the two forms of the protein.

$$\alpha_x r = \alpha_s X \frac{\text{specific radioactivity of peptide}}{\text{specific radioactivity of internal standard}}$$

Table II: Reactivity of the Amino Groups in the Amino Terminal Region of the "A" Protein of *B. stearothermophilus*.

Amino Group	pK_a	r	k (M^{-1} min^{-1})	Relative Reactivity
Methionine-1	7.5	0.58	26,000	6.5
Lysine-3	10	2.3	100,000	1.8

X-Ray crystallographic studies (Blow and Steitz, 1970) have shown that amino groups are usually exposed on the surface of proteins and are found to be interacting with other groups only when they have some functional role. It is expected therefore that the exposure of amino groups will reflect the exposure of the segment of polypeptide chain on which they are located. The results of the present study show the amino terminal region of the "A" protein of *B. stearothermophilus* is fully exposed on the surface of the 50S ribosomal subunit. The abnormally high reactivity of the amino terminus indicates that it probably has a specific interaction with a neighboring group. It has been demonstrated that hydrogen bonding of the imidazole moiety to the carbonyl function of the polypeptide chain or to a carboxylate anion renders this group super-reactive (Cruickshank and Kaplan, 1972; Kaplan, 1974) and it is possible that hydrogen bonding of this type is responsible for the enhanced reactivity observed for the amino terminus of the "A" protein. On the other hand, the lysyl ϵ-amino group which is nearby appears to be interacting only with solvent.

In view of the fact that the "A" protein is known to be involved in the translocation step of protein synthesis, and that acetylation of its amino terminus has been implicated in the modulation of its activity, the results of this investigation suggest that the amino terminal region is a functional site. The amino groups of this region could be involved in the binding of GTP or in the association of subunits, or could be directly involved in a catalytic process. With the extension of the technique of competitive labeling described in this communication, these various possibilities are amenable to experimental verification and are presently being investigated in our laboratories.

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Methylation of the Ribosomal Proteins in *Escherichia coli.* Nature and Stoichiometry of the Methylated Amino Acids in 50S Ribosomal Proteins†

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ABSTRACT: Methylated ribosomal proteins from *Escherichia coli* 50S subunit are localized by growing cells in a medium containing [1-^{14}C]methionine and [^{3}H-*methyl*]-methionine and comparing the ^{3}H/^{14}C ratio for each of the 50S ribosomal proteins. The following proteins are methylated: L11, L1, L3, L5,L7, L8, L9, L12, L18, and L33. The nature and stoichiometry of the methylated amino acid(s) in each of the methylated proteins are determined. Protein L11 is the most heavily methylated of all the 50S subunit proteins. This protein has previously been implicated in the peptidyl transferase reaction during protein synthesis (K. H. Nierhaus and V. Montejo (1973), *Proc. Nat. Acad. Sci. U. S. 47,* 1588–1602). Three proteins (L1, L3, and L5) have intermediate levels of methylation and contain about 0.4–0.6 methyl groups each per molecule of protein. Five other proteins (L7, L8, L9, L12, and L18) are also methylated to a slight extent (~0.1 methyl group/molecule of protein). One unknown methylated neutral amino acid was detected in protein L11 and at least one and possibly two other unidentified methylated amino acids appeared to be present in protein L33.

Methylated amino acids have been reported to be present in ribosomal proteins (Comb *et al.*, 1966; Terhorst *et al.*, 1972, 1973; Chang *et al.*, 1974; Alix and Hayes, 1974). Terhorst *et al.* (1972, 1973) showed that 50% of one lysine residue from two closely related ribosomal proteins, L7 and L12, was methylated to ϵ-*N*-monomethyllysine in *Escherichia coli* MRE600. Recently we have detected the presence of approximately 0.8–0.9 molecule of ϵ-*N*-trimethyllysine in protein L11 (Chang *et al.*, 1974). Similar observations have been reported by Alix and Hayes (1974). A rapid method for the localization of the methylated ribosomal proteins in *E. coli* has been described (Chang *et al.*, 1974; Chang and Chang, 1974). Using this method we have shown that methylation of ribosomal proteins occurs predominately in 50S subunit proteins.

Methyl-deficient 50S ribosomal particles have been obtained from an *E. coli rel*$^{-}$ *met*$^{-}$ strain after starvation for methionine. Such particles can be methylated *in vitro* using enzymes derived from the 0.5 M KCl wash of wild type ribosomes (Chang and Chang, 1974). Protein L11 and, to a much lesser extent, proteins L1, L3, and L5 were found to be methylated *in vitro*. The methylated amino acids in protein L11 have been characterized and shown to be predominately ϵ-*N*-trimethyllysine (Chang and Chang, 1974). Since most of the methylated 50S ribosomal proteins (except protein L11 and possibly protein L33) contain only small amounts of methylated amino acids, a sensitive procedure

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