Initiation and Translation in Vitro of mRNA for MOPC 315 Immunoglobulin Heavy Chain and Characterization of Translation Product*

Donna L. Bedard† and Ru Chih C. Huang

From the Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

An initiation study of mineral oil-induced plasmacytoma (MOPC) 315 heavy chain immunoglobulin (H₃₁₅) in vitro has been conducted using formyl-[¹⁴S]methionyl-tRNA₅₁₅ and a highly purified 18 S message from MOPC 315 solid tumor in a crude rabbit reticulocyte lysate system. The product was specifically precipitated by antibodies directed against MOPC 315 immunoglobulin and H₃₁₅.

The in vitro H₃₁₅ products terminally labeled with formyl-[¹⁴S]methionine or internally labeled with [³H]leucine were electrophoretically identical with in vivo H₃₁₅ on sodium dodecyl sulfate-polyacrylamide gels. All of the [¹⁴S]methionine was incorporated at the NH₂ terminus, not internally, since there is a near complete recovery of [¹⁴S]methionine following one cycle of Edman degradation.

The NH₂-terminal cyanogen bromide peptide, CN₁, of in vivo and in vitro H₃₁₅ co-migrated exactly on gel electrophoresis under conditions which completely resolved two proteins differing in size by only 14 amino acids. These data strongly suggest that there is no NH₂-terminal precursor of H₃₁₅ in this system.

Cyanogen bromide peptide profiles of in vivo and in vitro H₃₁₅ were chromatographically indistinguishable.

Three peptides, CN₁, CN₂, and CN₃, which represent approximately 85% of the total amino acids of H₃₁₅ were isolated and further characterized by electrophoresis and paper chromatography. All were very similar to the corresponding peptides of authentic H₃₁₅. We conclude that the fidelity of H₃₁₅ translation is preserved in vitro.

The cell-free translation of partially purified immunoglobulin heavy chain mRNA has been reported by several laboratories (1-4). The putative heavy chains synthesized in these systems were found to be antigenically related to authentic heavy chain (2-4); however, several central issues concerning the initiation, elongation, and termination of in vitro synthesis of heavy chain were not adequately resolved. It has been postulated that heavy chain immunoglobulin, like light chain immunoglobulin, is synthesized in vitro as a precursor molecule with additional amino acids at the NH₂ terminus (1, 3, 4). Until now this matter has not been investigated. Secondly, none of the studies so far reported have precisely determined the extent of chain elongation in vitro. Finally, although the tryptic peptides of the in vitro product were grossly similar to those of authentic heavy chain, unmatching peptides were also evident (1, 4).

The experiments reported here were designed to determine whether there is an NH₂-terminal precursor for mineral oil-induced plasmacytoma 315 heavy chain immunoglobulin and to judge the accuracy of translation and extent of elongation of H₃₁₅ mRNA in a crude rabbit reticulocyte system.

Eukaryotic protein synthesis can be initiated by methionyl-tRNA₅₁₅ (5, 6), but the NH₂-terminal methionine residue is often cleaved from the nascent protein. However, formyl-methionyl-tRNA₅₁₅ can also be used to initiate eukaryotic protein synthesis in vitro (7-10). Since the formyl group prevents the removal of the NH₂-terminal methionine residue (7) we have used this initiator in our studies.

We have characterized the translation product of MOPC 315 heavy chain (H₃₁₅) mRNA by specific antibody precipitation, gel electrophoresis, and gel filtration. Furthermore, we have isolated and analyzed three of the six cyanogen bromide peptides including the NH₂ terminus and an octapeptide near the COOH terminus.

Our data provide strong evidence for the faithful initiation and elongation of H₃₁₅ in crude rabbit reticulocyte lysate. No evidence was found for an NH₂-terminal precursor in this system.

**EXPERIMENTAL PROCEDURES**

Reagents-[-4,5-³H]Leucine, [-4,5-³H]isoleucine, [-U-¹⁴C]leucine, and [-U-¹⁴C]isoleucine were obtained from New England Nuclear. [¹⁴S]Methionine was purchased from Amersham/Searle. Oligo(dT)-cellulose, type T₂, was purchased from Collaborative Research, Inc. Cyanogen bromide was purchased from J. T. Baker, Inc.

Tumors-MOPC 315 OP plasmacytoma was obtained (through Bionetics, Inc.) from Dr. H. Eisen. The plasmacytoma was maintained in tumor form in BALB/c mice by serial subcutaneous passage of minced tumor fragments at 11- to 14-day intervals.

Isolation of MOPC 315 Protein and Separation into Component Heavy Chain (H₃₁₅) and Light Chain (L₃₁₅)—MOPC 315 protein was purified from a crude ascites preparation purchased from Bionetics, Inc., or from the pooled sera of tumor-bearing mice (11, 12). Total

* This research was supported by National Institutes of Health Grants R01CA13993 and 1TO1HD00826.
† Recipient of National Institutes of Health Postdoctoral Fellowship 1P22AM01408-02.
were pooled and precipitation curves used to determine the amount
was excised and minced in 90% Dulbecco's Modified Eagle's Medium,
for us by Dr. William Merrick using 1"Slmethionine (400 Ci/
trypsinized for 30 min at 37" in 10 ml of 0.2% trypsin and 10 ml of the
bine with any heavy chain synthesized, and 2.35 Kg of carrier IgABls

were homogenized in 5% SDS, 5 mm Tris HCl, pH 7.4 (2.2 ml/g of tumor), and
extracted twice with an equal volume of rediluted water-saturated
phenol. The RNA was ethanol precipitated, washed extensively, and
solved in 0.5% SDS, 0.1 M NaCl, 0.01 M EDTA (pH 7.2), 0.01 M Tris HCl (pH 7.4). RNA was centrifuged on 10 to 70% (w/v) sucrose gradients (16) in the same buffer as above, in an SW 27 rotor for 21/2 h (16.5 cm sedimentation). The RNA was
together in a 1 M sucrose, pH 7.4. The dialyzed sample was separated into component II15 and L15 by chromatography on Sephadex G-100 in 6 M guanidine HCl, 0.1 M sodium acetate, 1 M iodate, pH 5. Preparation of Antibody to Rabbit IgA15 - Rabbit antiserum to rabbit IgA15 was immunized by oral injection of 1 mg of IgA15 or 0.5 mg of totally reduced and alkylated H15 emulsified with Freund's complete adjuvant. Rabbits were bled every 2 or 3 days for 2 weeks beginning on Day 21. Sera were pooled and precipitation curves used to determine the amount of antiserum necessary to give maximum precipitation of the IgA15 or H15 in a given sample.

Isolation of mRNA - Messenger RNA for H15 was prepared from the
micosomal fraction of solid tumor (14, 15). Microsomes
in 0.525% agarose (27) in a running buffer of 0.1% SDS, 0.4
markers were applied adjacent to the radioactive samples and were
developed for 30 h.

Preparative Gel Filtration of Cyanogen Bromide Fragments - In vitro

cyanogen bromide fragments were treated separately when the final goal was analysis by electrophoresis or rechromatography. The gel was
solved in 0.05 M NH4OH and chromatographed on Sephadex G-50 (0.9 
220 cm). Small aliquots of each fraction were counted in a scintilla-
tion counter. Uncleaved H315 and L315 were identified by a peak at the void volume. CN, and CN, followed in a barely
discernible peak. Fractions containing CN, and CN, were lyophi-
ized and stored frozen. The fractions containing the larger fragments were
lyophilized and rechromatographed on Sephadex G-100 (0.9 
220 cm) in 5 M guanidine HCl, 0.1 M sodium acetate, pH 5. 30% of

High Voltage Paper Electrophoresis and Descending Chromatogra-
phy - Aliquots of CN, and CN, from in vitro and in vivo H315 were applied to Whatman No. 3MM paper and electrophoresed at 3.6 V in 0.5% pyridine, 5% acetic acid (29) at 2250 V for 1/2 h in a Savant
tank.

Initiation Assay - Formyl-[3H]methionyl tRNA, was prepared for us by Dr. William Merrick using [3H]methionyl (400 Ci/
Isolation of mRNA - Microsomal RNA was isolated from solid tumor and fractionated on sucrose gradients as described. Nine grams of tumor yielded approximately 22.2 mg of RNA of which 6.6 mg sedimented at 16 to 24 S. Oligo(dT)-cellulose bound 3 to 4% of this fraction on the first purification and 60 to 75% on the second purification, giving a total yield of 100 to 200 µg of mRNA.

Fig. 1 shows the purity of our mRNA as analyzed on SDS-polyacrylamide gels. After one purification on oligo(dT)-cellulose the major band migrated with 18 S ribosomal RNA, and five minor bands were visible in the region between the 18 and 28 S markers. A second purification on oligo(dT)-cellulose did not alter the banding pattern.

Cell-free Translation of IgA<sub>315</sub> mRNA - Microsomal RNA isolated from solid tumor was fractionated on a sucrose gradient and the RNA sedimenting at 16 to 24 S was translated in a crude reticulocyte lysate. As shown in Table I, there was a high level of protein synthesis in lysates without added RNA. This was due to globin synthesis directed by endogenous mes-.

We also found that the translation product of 16 to 24 S (dT)-retained RNA could be specifically precipitated by anti-IgA<sub>315</sub>. However, for reasons which are not readily apparent, precipitation with anti-H<sub>315</sub> was less efficient and was therefore not routinely used.

Polyacrylamide Gel Electrophoresis of Translation Product - H<sub>315</sub> labeled in vitro with [35S]methionine was compared with nonradioactive authentic H<sub>315</sub> by polyacrylamide gel electrophoresis (Fig. 2). Native H<sub>315</sub> moved as a diffuse band with a concentrated region at the front (Fig. 2E). The migration of totally reduced and carboxymethylated H<sub>315</sub> was retarded relative to native H<sub>315</sub> (Fig. 2F), presumably due to chemical modification. Since the in vitro translation product of H<sub>315</sub> was not reduced and carboxymethylated, it can only properly be compared with native H<sub>315</sub> (Fig. 2E).

The translation product of H<sub>315</sub> migrated in four sharp bands with identical \( R_f \) values for products labeled with either [3H]leucine or formyl-[35S]methionine. The primary band represented approximately 85% of the total cell-free products and migrated identically with authentic H<sub>315</sub> (\( R_f = 0.390 \) for cell-free product and 0.393 for native H<sub>315</sub>). The second band (approximately 10% of the total) moved slightly ahead of native H<sub>315</sub> (\( R_f = 0.408 \)). Two other products (representing less than 5% of the total radioactivity) migrated with \( R_f \) values of 0.502 and 0.605. In the [35S]methionine-labeled sample (Fig. 2D) a faint radioactive band was also present which migrated slightly behind L<sub>24</sub> (\( R_f = 0.798 \) for cell-free product and 0.858 for authentic L<sub>24</sub>). This band probably represents the synthesis of a small amount of light chain precursor.

Very faint radioactive bands were also detected in both the control (no RNA) and experimental (H<sub>315</sub> mRNA) samples at the dye front and at the rear boundary of authentic H<sub>315</sub>. These bands most likely represent nonspecific background radioactivity.

Edman Degradation of in vitro H<sub>315</sub> Labeled with Formyl-
Faithful Initiation and Translation of Heavy Chain Immunoglobulin mRNA

TABLE II
Edman degradation of in vitro \(\text{H}^{31s}\)

In vitro \(\text{H}^{31s}\) labeled with formyl-\([3\text{S}]\)methionine was deformylated as described and subjected to two cycles of manual Edman degradation. An aliquot of the ethyl acetate phase, containing the released NH\(_2\)-terminal amino acid, was dissolved in Triton toluene 21 scintillation fluid (Yorktown Research). The HCl phase containing contaminating peptide was neutralized and dissolved, and the peptide remaining after two cycles of degradation was also dissolved in Triton toluene 21. All samples were counted on a Packard scintillation counter (model 2405).

| Cycle | Free NH\(_2\)-terminal (ethyl acetate phase) | Internal methionines (HCl phase) | Internal methionines (dried peptide) |
|-------|------------------------------------------|-------------------------------|----------------------------------|
| 1     | 2,580                                    | 190                           | 40                               |
| 2     | 190                                      | 235                           | 90                               |
| Peptide remaining after two cycles |                              |                               |                                  |

Fig. 2. SDS-polyacrylamide (10%) slab gel electrophoresis of antibody-precipitable cell-free translation products. A to D, fluorograms of labeled cell-free translation products; E and F, purified marker proteins stained with Coomassie blue. A and B are labeled with \([\text{PH}]\)leucine; A, no RNA added; B, \(\text{H}^{31s}\) mRNA added. C and D are labeled with formyl-\([3\text{S}]\)methionine; C, no RNA added; D, \(\text{H}^{31s}\) mRNA added. E, native \(\text{IgA}^{31s}\) protein; F, totally reduced and carboxymethylated \(\text{H}^{31s}\).

\([3\text{S}]\)methionine – After deformylation, an Edman degradation was carried out for two cycles on the in vitro \(\text{H}^{31s}\) to establish that the \([3\text{S}]\)methionine was indeed at the NH\(_2\) terminus. At each cycle the PTH-amino acid derivative was extracted into the ethyl acetate phase. Any contaminating peptide accidentally extracted with butyl chloride should remain in the HCl phase.

Table II shows that 82% of the radioactivity was recovered as PTH-amino acid derivative in the first cycle and 6% in the second cycle. Less than 3% of the radioactivity remained in the peptide after two cycles of the Edman procedure. These results confirm that the \([3\text{S}]\)methionine was incorporated only at the NH\(_2\) terminus.

Comparison of CNBr Fragments of in Vivo and in Vitro \(\text{H}^{31s}\) – To further establish the identity of our cell-free \(\text{H}^{31s}\) with authentic \(\text{H}^{31s}\), we prepared in vivo \(\text{[14C]}\)IgA\(^{31s}\) as described in the text except that \([\text{PH}]\)leucine and \([\text{PH}]\)isoleucine were used at specific activities of 60 and 80 Ci/mmol, respectively. The in vitro sample consisted of the antibody-precipitable material from 22 100-\(\mu\)l cell-free syntheses which were run at two different times with RNA prepared at two different times. Fraction size was 1 ml. Twenty-microliter aliquots were dissolved in Triton toluene 21 scintillation fluid (Yorktown Research) and counted for 4 min at counting efficiencies of 21% \((\text{C})\) and 59% \((\text{H})\). ○－－－－○, in vivo \(\text{[14C]}\)IgA\(^{31s}\); ●－－－－●, in vitro \(\text{[3H]}\)IgA\(^{31s}\).

The \(\text{H}^{31s}\) peak was recovered and cleaved with cyanogen bromide (28). Cyanogen bromide cleavage results in six fragments, five of which can be separated. These fragments have been characterized as follows (28). \(\text{CN}_1\) has 228 amino acids; \(\text{CN}_3\) has 156 amino acids and includes the NH\(_2\) terminus, the entire variable region, and part of the constant region. \(\text{CN}_{1a}\) and \(\text{CN}_{1b}\) are each approximately 30 amino acids long and both appear to contain carbohydrate. They are not separated under the conditions used. \(\text{CN}_1\) and \(\text{CN}_2\) are both octapeptides and \(\text{CN}_1\) includes the COOH terminus. Proposed alignment of the fragments is: \(\text{CN}_2\), \(\text{CN}_1\), \(\text{CN}_{1a}\), \(\text{CN}_{1b}\), \(\text{CN}_1\), \(\text{CN}_3\).

Fig. 4 shows a comparison of cyanogen bromide fragments from in vivo and in vitro synthesized heavy chain. Peptides \(\text{CN}_1\), \(\text{CN}_2\), and \(\text{CN}_3\) match very closely. Octapeptides \(\text{CN}_1\) and \(\text{CN}_2\) are octapeptides and \(\text{CN}_3\) includes the COOH terminus. Proposed alignment of the fragments is: \(\text{CN}_2\), \(\text{CN}_1\), \(\text{CN}_{1a}\), \(\text{CN}_{1b}\), \(\text{CN}_1\), \(\text{CN}_3\).

\(^a\) S. Francis and H. Eisen, personal communication.
Comparison of in Vivo and In Vitro CN, and Incompletely Cleaved H"-
-FIG. 5 shows a fluorogram of the CN, peak isolated from Sephadex chromatography and electrophoresed on 12% SDS-polyacrylamide gels. [14C]H" (214 amino acids) was used as a marker. There are three bands visible in the CN, fraction of both the in vivo and in vitro preparations. The weakest and slowest moving band, representing uncleaved H"., moved identically in in vivo and in vitro samples. The band migrating most rapidly represents CN, and the band following it probably represents a composite of CN, and another CNBr fragment. CN, moved as a diffuse band in both in vivo and in vitro samples, but particularly in the latter. CN, from in vitro H"., migrated slightly ahead of its in vivo counterpart. However, the more compact band of in vivo CN, moved within the outer boundaries of the in vitro CN, band. As discussed later, this slight difference in the migration of CN, probably reflects a difference in the way in vivo and in vitro CN, were handled. Electrophoresis of a mixture of in vivo and in vitro CN, (Fig. 5D) resulted in one broad band.

The composite fragment from in vitro H"., migrated as a sharp band and had a slightly higher mobility than the corresponding in vivo fragment. A mixture of the fragments from in vivo and in vitro samples (Fig. 5D) migrated as a single band with a sharp dense region at the front and a diffuse area behind.

Comparison of in Vivo and In Vitro CN, of H" by SDS-Acrylamide Gel Electrophoresis - A fluorogram of CN, the CN, were obscured by a spurious peak at Fraction 142 which probably represents salt. The shoulder on the left side of CN, most likely represents H31s which was incompletely cleaved.

NH2 terminus, isolated from in vivo and in vitro H" is shown in Fig. 6. The position of the band representing CN, from in vitro H" coincides exactly with that of CN, from authentic H". The absence of major contaminating composite fragments as seen in the CN, fraction can be largely explained by two facts. Since CN, is well within the separation range of Sephadex G-100 (M, = 16,000) the fragment could be isolated in purer form. Secondly, since CN, is the NH2 terminus, only one cleavage is necessary to produce CN, whereas two cleavages are necessary to free CN, an internal fragment.

Comparison of in Vivo and In Vitro CN, and CN5 - The octapeptides, CN, and CN, were purified on Sephadex G-50 as described under “Experimental Procedures,” and aliquots of the in vivo and in vitro samples were electrophoresed adjacent to each other in pyridyl acetate at pH 3.6. The radioactive profiles of in vivo and in vitro samples are identical (Fig. 7). Sharp peaks are evident at the origin and 16 to 17 cm away from the origin toward the cathode. A faint ninhydrin spot is visible at 16 to 17 cm and an even fainter spot at 6 to 8 cm. Based on the amino acid compositions of CN, and CN, we have tentatively identified the peptide at position 16 to 17 as CN5, since it contains a histidine which would be positively charged under these conditions. The ninhydrin spot at 6 to 7

CN, sequence: Val-Gly-His-Glx-Ala-Leu-Pro-Hsr (S. Francis and H. Eisen, personal communication).

CN, sequence: Ser-Glx-Gly-Asx-Gly-Ile-Cys-Tyr (28).
Faithful Initiation and Translation of Heavy Chain Immunoglobulin mRNA

FIG. 8. Descending chromatography of in vivo and in vitro CN₄, CN₅ fractions. The CN₄, CN₅ fractions of in vitro and in vivo H₃₁₅ were applied adjacent to each other and chromatographed as described under "Experimental Procedures." CN₄, CN₅ fraction from in vivo [¹⁴C]H₃₁₅ (A), and from in vitro [³H]H₃₁₅ (B).

cm and the small radioactive peak of the in vitro sample in this area may represent CN₅.

Aliquots of in vitro and in vivo octapeptides were spotted separately on Whatman No. 3MM paper and subjected to descending paper chromatography. The results (Fig. 8) show a small peak at the origin and a large peak at 22 to 26 cm in both samples. A single ninhydrin spot marked the location of the peptide from nonradioactive carrier H₃₁₅ at the front edge of the radioactive peak (data not shown). The Rᵥ of the in vitro peptide (0.657) was virtually identical with that of the in vivo [¹⁴C]H₃₁₅ (A), and from in vitro [³H]H₃₁₅ (B).

DISCUSSION

The major band of RNA which has been twice purified on oligo(dT)-cellulose and which is active in the synthesis of H₃₁₅ migrates with the 18 S marker on SDS-acrylamide-agarose gels (Fig. 1). This is consistent with reports that the mRNAs for MOPC 21 heavy chain and a MOPC 21 deletion mutant have sizes of 17 S and 16 S, respectively (1, 31), and with reports that H₃₁₅ mRNA sediments at 16 to 19 S (3, 4) or at 17 S (32, 33) on sucrose gradients.

Our in vitro translation assay was highly reproducible. In a total of 15 experiments, the amount of H₃₁₅ synthesized ranged from 0.55 to 1.95% (mean = 1.04%) of total protein synthesized. Nonspecific precipitation (background) ranged from 0.04 to 0.21% (mean = 0.12%) of total protein synthesized. Since H₃₁₅ represented such a small fraction of total protein, it was imperative that nonspecific precipitation be minimal. We found this assay to be very sensitive and reproducible with various preparations of RNA and of crude reticulocyte lysate.

The protein synthesized in our cell-free system was shown to be related to authentic H₃₁₅ by several criteria. Our cell-free product had antigenicity for both anti-IgA₃₁₅ and anti-H₃₁₅. The size of our primary translation product was identical with that of authentic H₃₁₅ as judged by SDS-polyacrylamide gel electrophoresis and gel filtration under denaturing conditions. CN₁ from native and in vitro H₃₁₅ co-chromatographed on gel filtration and moved closely on gel electrophoresis. CN₄ and CN₅ moved identically with their authentic counterparts on both electrophoresis and chromatography. CN₅ moved identically on Sephadex chromatography but was not analyzed further.

CN₅ and an unidentified composite fragment from in vivo and in vitro samples differed slightly in mobility on electrophoresis. It is probable that this reflects a slight difference in the treatment of in vivo and in vitro samples. In vivo H₃₁₅ was isolated from IgA₃₁₅ which had been totally reduced and carboxymethylated. In vitro H₃₁₅ was not subjected to this treatment. CN₄ contains 8 cysteine residues which were carboxymethylated and could account for slower migration on SDS-acrylamide gels. Indeed we have found that after total reduction and carboxymethylation H₃₁₅ and L₃₁₅ migrate more slowly on SDS-polyacrylamide gels than H₃₁₅ and L₃₁₅ of native IgA₃₁₅ which has not been so harshly treated. Fig. 2 shows that native H₃₁₅ has an Rᵥ of 0.393 while totally reduced and carboxymethylated H₃₁₅ has an Rᵥ of 0.362. Native and totally reduced and carboxymethylated L₃₁₅ migrate with Rᵥ values of 0.858 and 0.772, respectively (data not shown).

We were unable to precisely locate radioactive CN₄, the COOH terminus of H₃₁₅, in either the in vivo or in vitro sample. Since CN₄ was labeled only at the third position from the COOH terminus, it is possible that the label was cleaved after synthesis by contaminating carboxypeptidase. Alternatively, it is possible that the specific activity was too low to be detected in the present study. In any case, CN₄, CN₅, and CN₆ represent 392 amino acids of H₃₁₅, thus providing excellent evidence for the synthesis of at least 85% of H₃₁₅.
The H\textsuperscript{35S} synthesized in our cell-free system migrated in four discrete bands. The major band, approximately 85% of the total product, migrated identically with cold marker H\textsuperscript{35S} while the second band moved slightly faster. The third and fourth bands represent less than 5% of the total translation product and migrate considerably ahead of H\textsuperscript{35S}. Cowan and Milstein noted that the y chain of MOPC 21 synthesized in vitro migrated as two discrete bands analogous to the first two bands in our system. These results could indicate different sites of initiation or termination. Schechter and co-workers have provided strong evidence for the latter possibility. They have identified cell-free translation products of MOPC 41 light chain immunoglobulin mRNA ranging from a molecular weight of 29,000 to 19,000 in the wheat germ cell-free system and migrate considerably ahead of H\textsuperscript{35S}. These data strongly suggest that there is no NH\textsubscript{2}-terminal cleavage in this system.

Two lines of evidence confirm that no NH\textsubscript{2}-terminal cleavage occurs in this system. Edman degradation showed that the \textsuperscript{35S}methionine was completely NH\textsubscript{2}-terminal. Since the H\textsuperscript{35S} protein was still labeled after isolation, the NH\textsubscript{2} terminus could not have been cleaved. Secondly, H\textsuperscript{35S} labeled with formyl-\textsuperscript{35S}methionine or \textsuperscript{3H}leucine migrated identically on SDS-acrylamide gels.

We have also shown that CN\textsubscript{r}, the NH\textsubscript{2} terminus, from in vitro H\textsuperscript{35S} migrates identically with CN\textsubscript{r} from authentic H\textsuperscript{35S} on SDS-acrylamide gels (Fig. 6). It should be pointed out that under the same electrophoretic conditions CN\textsubscript{r} (225 amino acids) and L\textsuperscript{35S} (214 amino acids) which differ by only 14 amino acids were completely resolved (Fig. 5). Finally, all of the CN\textsubscript{Br} fragments of in vitro H\textsuperscript{35S} corresponded to those of in vivo H\textsuperscript{35S}. These data strongly suggest that there is no NH\textsubscript{2}-terminal precursor for H\textsuperscript{35S} in this system.

Acknowledgments — We wish to thank Drs. Sharron Francis and Herman Eisen for providing us with the unpublished sequence of CN\textsubscript{r} and the proposed alignment of the cytochrome b6 fragments. We owe special thanks to Dr. William Merrick who synthesized formyl-\textsuperscript{35S}methionyl-tRNA\textsubscript{Met} for us. Finally, we wish to thank Dr. John Cebra and Ms. Nancy Weigel for expert advice and Mrs. Sue Auyang and Mr. S. L. Hu for excellent technical assistance.

REFERENCES

1. Cowan, N. J., and Milstein, C. (1973) Eur. J. Biochem. 36, 1-7
2. Schmeckpeper, J. J., Cory, S., and Adams, J. M. (1974) Mol. Biol. Rep. 1, 295-298
3. Green, M., Graves, P. N., Zehavi-Willner, T., McInnes, J., and Presthi, S. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 204-208
4. Green, M., Zehavi-Willner, T., Graves, P. N., McInnes, J., and Presthi, S. (1976) Arch. Biochem. Biophys. 172, 74-89
5. Smith, A. E., and Marcker, K. A. (1970) Nature 226, 607-610
6. Brown, J. C., and Smith, A. E. (1970) Nature 226, 610-612
7. Housman, J. D., Jacobs-Lorena, M., RajBhandary, U. L., and Lodish, H. F. (1970) Nature 227, 913-918
8. Oberg, B. F., and Shuktin, A. J. (1979) Proc. Natl. Acad. Sci. U. S. A. 69, 3589-3593
9. Jones, G., and Mach, B. (1973) Biochim. Biophys. Acta 312, 399-402
10. Jacobs-Lorena, M., and Baglioni, C. (1973) Mol. Biol. Rep. 1, 113-117
11. Eisen, H. N., Simms, E. E., and Potter, M. (1968) Biochemistry 7, 4126-4134
12. Underdown, B. J., Simms, E. E., and Eisen, H. N. (1971) Biochemistry 10, 4320-4330
13. Fish, W. W., Mann, K. G., and Tanford, C. (1969) J. Biol. Chem. 244, 4989-4994
14. Stavnezer, J., and Huang, R. C. C. (1971) Nature New Biol. 229, 172-176
15. Stavnezer, J., Huang, R. C. C., Stavnezer, E., and Bishop, J. M. (1974) J. Mol. Biol. 88, 63-63
16. Neal, M. W., and Florini, J. R. (1972) Anal. Biochem. 45, 271-278
17. Swan, D., Aviv, H., and Leder, P. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 1967-1971
18. Lockard, R. E., and Lindel, J. B. (1969) Biochem. Biophys. Res. Commun. 37, 294-212
19. Holmes, D. E., McKeown, S. G., and Schimke, R. T. (1973) J. Biol. Chem. 248, 2803-2809
20. Strous, G. J. A. M., Bollen, T., and Bloemendal, H. (1974) Mol. Biol. Rep. 1, 471-475
21. Bhaduri, S., Chatterjee, N. K., Bose, K. K., and Gupta, N. K. (1970) Biochem. Biophys. Res. Commun. 40, 402-407
22. Eison, N. A., Brewer, H. B., and Anderson, W. F. (1974) J. Biol. Chem. 249, 5227-5235
23. Gray, W. R. (1967) Methods Enzymol. 11, 469-475
24. Laemmli, U. K. (1970) Nature 227, 689-695
25. Bonner, W. M., and Laskey, R. A. (1974) Eur. J. Biochem. 46, 83-90
26. Laskey, R. A., and Mills, A. D. (1972) Eur. J. Biochem. 36, 353-361
27. Peacock, A. C., and Dingman, C. W. (1968) Biochemistry 7, 668-674
28. Francis, S. H., Leslie, R. G. Q., Hood, L., and Eisen, H. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 1123-1127
29. Kyle, A. F., Sanger, F., Smith, L. F., and Kitai, R. (1966) Biochem. J. 60, 541-556
30. Light, A. S., and Smith, E. L. (1962) J. Biol. Chem. 237, 2537-2546
31. Cowan, N. J., Secher, D. S., and Milstein, C. (1976) Eur. J. Biochem. 61, 355-368
32. Premkumar, E., Shoyab, M., and Williamson, A. R. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 99-103
33. Williamson, A. R., Premkumar, E., and Shoyab, M. (1975) Fed. Proc. 34, 28-32
34. Schechter, I., and Burstein, Y. (1976) Biochem. Biophys. Res. Commun. 68, 489-496
35. Schechter, I., McKeen, D. J., Guyer, R., and Terry, W. (1975) Science 188, 105-106
Initiation and translation in vitro of mRNA for MOPC 315 immunoglobulin heavy chain and characterization of translation product.

D L Bedard and R C Huang

J. Biol. Chem. 1977, 252:2592-2598.

Access the most updated version of this article at http://www.jbc.org/content/252/8/2592

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/8/2592.full.html#ref-list-1