CHROMATOGRAPHIC ANALYSIS OF POLYPEPTIDES FROM POLYRIBONUCLEOTIDE STIMULATED ENZYMATIC SYNTHESIS

by Marvin A. Smith and Mark A. Stahmann

Department of Biochemistry, University of Wisconsin
Madison 6, Wisconsin

Received August 29, 1963

In this communication we report the use of co-chromatography for fractionating products from the enzymatic incorporation of lysine-C^{14} into polypeptides, and the identity of these products with known polypeptides obtained from a partial hydrolyzate of synthetic polylysine. Samples of the lysine-C^{14} polypeptides, enzymatically synthesized in the laboratories of Dr. S. Ochoa (Gardner et al., 1962; Kaziro et al., 1963) using a polyadenylic acid (poly-A) preparation and an enzyme system isolated from E. coli, were added to a partial acid hydrolyzate of polylysine and subjected to chromatography on carboxymethyl-cellulose columns. All the radioactivity in the enzymatically synthesized products was recovered in the first 14 polylysine peaks. The highest activity was recovered as dilysine, but significant activity was found in all lysine polypeptides up to the tetradecamer. Activities in larger peptides were not significantly greater than background.

In such biosynthetic systems, poly-A is considered to be the template for the incorporation of lysine residues into polypeptides. Assuming that the amino acid code requires a fixed number of nucleotides for the incorporation of each amino acid residue, one would expect a correlation

between the size of the poly-A and the size of the resulting polypeptide. Consequently, a determination of the size distribution of the poly-A used to stimulate the synthesis and of the polylysine synthesized should reveal the number of nucleotide residues required to code a single amino acid residue. In this communication we are concerned with the chromatographic determination of the size distribution of the polypeptides.

Materials and Methods. Aliquots of the cell-free E. coli reaction mixtures were kindly provided by Drs. Y. Kaziro, A. Grossman and S. Ochoa. These reaction mixtures were boiled after the incubation period to inactivate enzymes, and frozen until analyzed. Analysis was made by co-chromatography on carboxymethyl-cellulose using techniques already described (Stewart, J. W., et al., 1962) which allows resolution of the first 20 homologous lysine polypeptides. One ml of the reaction mixture was diluted with 10 ml of water containing 10 mg of carrier polylysine and added to carboxymethyl-cellulose columns. The carrier polylysine was prepared by partial acid hydrolysis of high molecular weight synthetic polylysine hydrobromide at 70°C in 6 N hydrochloric acid for 80 minutes. The resulting hydrolyzate contained a continuous distribution of polypeptides from lysine through polypeptides containing about 20 lysine residues. The effluent passed through a flow cell. The absorbancy at 220 mμ was measured and recorded. Aliquots were collected at 5-minute intervals for determination of C^{14} activity. This was measured with a Packard Tri-Carb Scintillation Spectrometer. The scintillation mixture was that described by Bray (1960). One ml aliquots from each of the fractions were added to 10 ml of scintillation mixture. When the activity was corrected for background, a quantitative recovery of the lysine-C^{14} added to the column was obtained. The operating efficiency of the instrument was about 47%.

Results and Discussion. The results are presented in Fig. 1. Figure 1(A) shows that no incorporation of lysine-C^{14} occurred in the absence of poly-A, and that chromatography allowed separation of the first 20 lysine

polypeptides in the carrier polylysine hydrolyzate with no interference from the incubation mixture. Over 99% of the radioactivity was eluted as lysine which did not interfere with separation of the higher peptides. Less than 1% passed through with the frontal peak as an acidic component. This may represent the maximum amount of lysine-C^{14} that was linked to carrier RNA. No incorporation into peptides occurred in the absence of poly-A indicating that peptide synthesis by routes other than poly-A initiation did not occur.

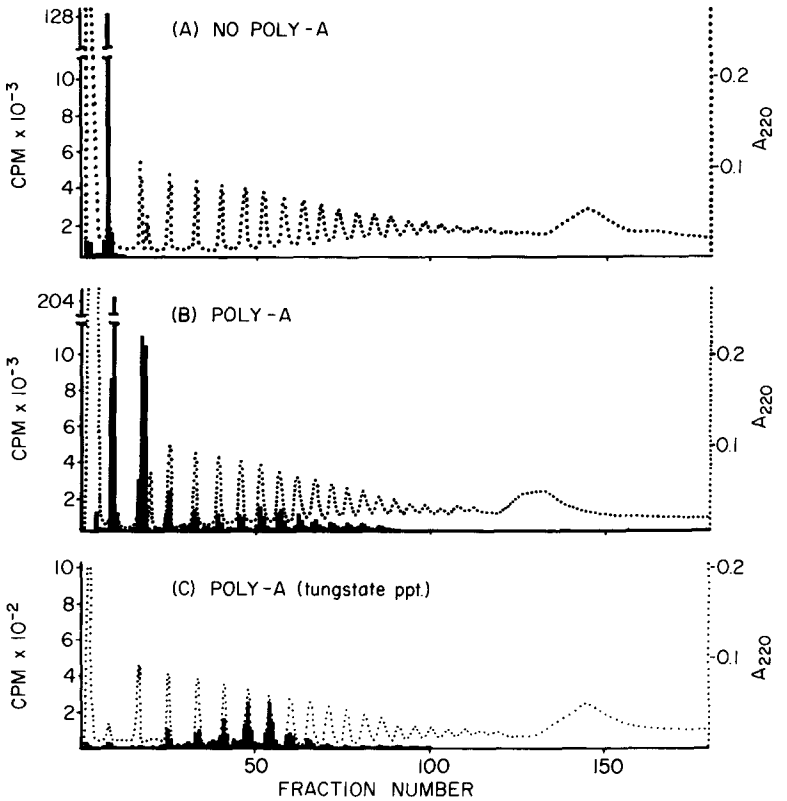

Figure 1. Co-chromatography of lysine-C^{14} incorporation products and known lysine polypeptides. (A) In the absence of poly-A stimulation. (B) With poly-A stimulation. (C) Tungstate precipitate of poly-A stimulated system. The bars represent radioactivity in CPM, and the dots absorbancy at 220 mμ. Lysine polypeptides were eluted from carboxymethyl-cellulose columns with an exponential sodium chloride gradient (Stewart et al., 1962). Peaks following the large frontal peak are lysine and consecutive oligomers, except the small peak following dimer in (A) and (B) which originates from the incubation mixture. The last large broad peak represents high molecular weight material eluted by increasing the gradient rate.

The results obtained when poly-A was added to this system are shown in Fig. 1(B). Radioactivity was recovered with lysine and all peptides containing from 2-14 lysine residues. Almost half was recovered as dilysine. This may arise from the cleavage of poly-A by ribonucleases to fragments that code for dilysine, or the cleavage of polylysine by tryptic-like enzymes. However, the distribution of the radioactivity was different from that obtained on tryptic hydrolysis of polylysine (Stewart, N. K., 1962). The position of the radioactivity coincided exactly with the position of peptides in the partial acid hydrolyzate. No significant radioactivity was detected in peptides containing more than 14 lysine residues. The increase in activity recovered in the hepta- and octa-lysine polypeptides, as compared with neighboring polypeptides, is significant and may be a reflection of the size distribution of the poly-A used to stimulate the biosynthesis.

The products from a similar experiment were precipitated with trichloroacetic acid-sodium tungstate (Gardner et al., 1962). This precipitate was extracted with 3% ammonium hydroxide and the soluble products analyzed after removing the excess ammonia. The results shown in Fig. 1(C) indicate that the tungstate does not precipitate dilysine and that precipitation of the lower lysine polypeptides in incomplete.

Conclusions. Since products from poly-A stimulated enzymatic biosynthesis co-chromatographed with known lysine polypeptides, we conclude that poly-A stimulation results in the biosynthesis of polylysine. Tryptic degradation of these products have been shown to form the same peptides as those from polylysine (Kaziro et al., 1963). Chromatography on carboxymethyl-cellulose was adequate to separate all the species formed in the biosynthetic reactions.

Polynucleic acids have been separated by chromatography on diethylaminoethyl-cellulose (Khorana et al., 1961). If there is a correlation between the size of the initiating nucleic acid and the size of the resulting polypeptide it would follow that chromatographic analysis

of the nucleic acid and the resulting polypeptide should reveal this relationship. However, such experiments are complicated by the cleavage of nucleic acids or the peptides during the experiment. Inasmuch as our results indicate that more than half of the radioactive lysine was found as dilysine, and as the distribution of the polypeptides suggests that this is not entirely due to tryptic hydrolysis, we suggest that the dilysine may arise from a biosynthesis stimulated by the most abundant active poly-A species in the reaction mixture. It would therefore seem important to determine the size distribution of the poly-A after incubation and to correlate this with the size distribution of the polylysine peptides produced. This may reveal the number of nucleotide residues required to code single amino acid residues.

Acknowledgments. The authors wish to express their gratitude to Drs. S. Ochoa, Y. Kaziro and A. Grossman for the E. coli incubation mixtures used in this work. The investigation was supported by grants E 101 and GPM-14, 672-R1 from the National Institutes of Health, U. S. Public Health Service.

References

Bray, G. A., Anal. Biochem., 1, 279 (1960).
Gardner, R. S., Wahba, A. J., Basilio, C., Miller, R. S., Lengyel, P., and Speyer, J. F., Proc. Nat. Acad. Sci., 48, 2087 (1962).
Kaziro, Y., Grossman, A., and Ochoa, S., Proc. Nat. Acad. Sci., 50, 54 (1963).
Khorana, H. G., and Vizsolyi, J. P., J. Am. Chem. Soc., 83, 675 (1961).
Stewart, J. W., and Stahmann, M. A., J. Chromatography, 9, 233 (1962); Polyamino Acids, Polypeptides and Proteins, Stahmann, M. A., ed., p. 95, University of Wisconsin Press, Madison, Wis., 1962.
Stewart, M. K., M.S. Thesis, University of Wisconsin, Madison, Wis., 1962.