INITIATION OF OVALBUMIN SYNTHESIS IN HEN'S OVIDUCT MINCES

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In previous communications, Narita (1962a, 1962b, 1965) presented a hypothesis that protein synthesis will initiate from an acetylated amino terminal group on a polysome and polypeptide chain will grow to the C-terminal direction. The terminal acetyl group of the completed protein molecule will be removed enzymatically. If such a deacetylase is absent in a protein synthesizing system, the acetylated protein is detached from a polysome and released into the soluble fraction. This hypothesis was derived from the fact that several proteins were terminated with an acetyl group at the amino end. Since the N-terminal acetyl group of the protein of tobacco mosaic virus was established by Narita (1958), the reports of the acetylated proteins have been accumulated and two dozen of different molecular species of the acetylated proteins have so far been reported.

In order to examine the above hypothesis, Narita et al. (1965) studied the activation of sixteen acetylamino acids with a cell free protein synthesizing system prepared from the rat liver. The results were unfortunately unsuccessful. Marcker and Sanger (1964) reported the isolation of N-formylmethionyl s-RNA from cell-free systems prepared from E. coli and yeast, and

suggested that N-formylmethionine may be an initiator for polypeptide synthesis in the microorganisms. Adams and Capecchi (1966) and Webster et al. (1966) clearly demonstrated that N-formylmethionine was the initiator of the protein synthesis in both RNA-phages R17 and f_2.

Many efforts to find the initiator of protein synthesis in higher organisms such as mammalian systems have not so far been successful. Since all of the reported acetylated proteins are synthesized by vertebrates and higher plants, there exists a possibility that the N-acetylamino acid functions as the initiator of peptide synthesis in higher organisms. We have studied the initiation of ovalbumin synthesis using hen's oviduct minces, because ovalbumin has been established to have acetyl-Gly-Ser- sequence at the amino end (Narita, 1961; Narita and Ishii, 1962). We have now obtained some indication for the above possibility as presented preliminarily in this communication.

Hen's oviduct minces (800 mg) were incubated with C^{14}-acetate (12.8 μc, 0.224 μmole) and an amino acid mixture (each 2 mM) in the Krebs-Ringer-Tris buffer (50 mM) at pH 7.4 containing glucose (10 mM) at 37°C for 60 minutes. The mixture (total 8 ml) was subjected to freezing and thawing for two or three times and then homogenized. To the supernatant of the homogenate after centrifugation at 10,000 r.p.m. for 15 minutes was added the carrier crystalline ovalbumin and the pH was adjusted to 4.8. The precipitate which was obtained by the addition of ammonium sulfate in the concentration between 40 and 60 % saturation was collected after 12 hours at 25°C. The precipitate was chromatographed on a CM-cellulose column (Rhodes et al., 1958). The peak of radioactivity was slightly behind that of absorbancy at 280 mμ and it seemed to correspond to ovalbumin A_3 containing no phosphate group (Sanger and Hocquard, 1962). The radioactivity of the ovalbumin did not decrease on the treatment with hydroxylamine, suggesting that the radioactive acetyl group was introduced to amino groups.

The radioactive protein fraction was digested with pronase after heat

denaturation. The digest was fractionated into the acidic peptide fraction containing acetylpeptides and the dipolar peptide fraction by the aid of a Dowex 50-X8 column, H^+ form, as described previously (Narita and Ishii, 1962). Paper chromatography of the acidic fraction revealed the radioactive acetyl-Gly-Ser-OH in addition to traces of acetylglycine as shown in Fig. 1. Hydrazinolysate of the extracted acetyl-Gly-Ser-OH at 100°C for 5 hours gave only radioactive spot of acetyl hydrazide on a paper chromatogram (Rf 0.69, pyridine-aniline-water, 9 : 1 : 4, v/v). Radioactive material that stayed at the origin in Fig. 1 behaved neutral on paper electrophoresis at pH 3.5 and traveled slightly toward the anode together with the standard oligosaccharide-peptide complex which had been isolated from the pronase digest of ovalbumin (Yamashina and Makino, 1962) and contained aspartic acid, leucine, glucosamine and mannose in molar ratio of 1 : 1 : 3 : 5, approximately. Therefore the above radioactive material that stayed at the origin in Fig. 1 seemed to be the oligosaccharide-peptide complex. The hydrazino-

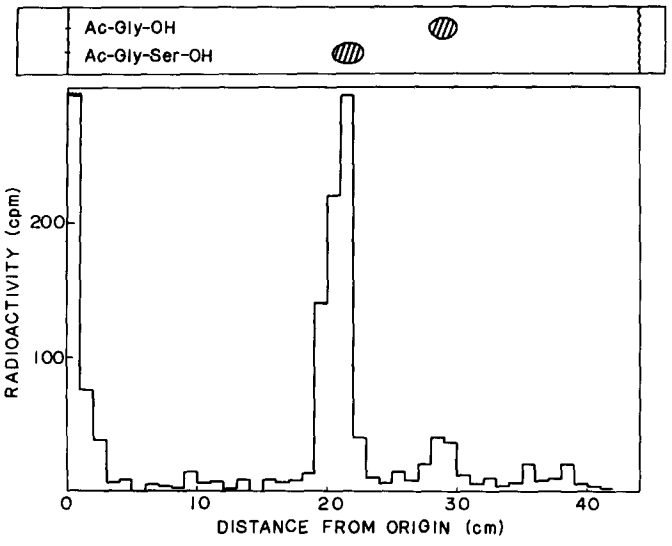

Fig. 1. Paper chromatography of the acidic fraction of the pronase digest of radioactive ovalbumin. Chromatography was carried out with n-butanol-acetic acid-water (4 : 1 : 1, v/v) with standard cold acetyl-Gly-Ser-OH and acetyl-glycine. Chromatogram was cut in 1 cm width and radioactivity of the cut paper was counted with a Nuclear Chicago gas flow counter.

lysate of the complex gave radioactive acetylhydrazide in addition to unknown radioactive components. The radioactive acetyl group appeared to attach to the amino group of the glucosamine residues.

In another experiment, equimolar amount of H^3-glycine to C^{14}-acetate was added to the incubation mixture and the acidic fraction was prepared from the pronase digest of the double-labeled ovalbumin. Molar ratio of acetyl group to glycine in the acetyl-Gly-Ser-OH isolated by paper chromatography was about 4.5 : 1 on the basis of their radioactive ratio of C^{14} to H^3. This result suggests that glycine pool in oviduct is greater than acetate pool. Very slight H^3-activity and high C^{14}-activity could be found in the oligosaccharide-peptide complex, suggesting that no glycine is contained in this fraction.

Puromycin inhibited the synthesis of radioactive ovalbumin and the yield of radioactive acetyl-Gly-Ser-OH from the ovalbumin preparation prepared in the presence of the inhibitor decreased dramatically, as shown in Fig. 2. In this experiment, C^{14}-acetate (10 μc, 0.189 μmole), H^3-glycine (100 μc, 0.172 μmole) and an amino acid mixture (minus glycine) were incubated with oviduct minces (1 g, total 10 ml) and puromycin (100 μg, 2.1 μmoles) was added at the point indicated in Fig. 2. These results suggest that the N-terminal acetylation is closely related with ovalbumin synthesis.

The ribosome-nascent ovalbumin complex which supposedly contained the incomplete protein, was isolated by the addition of sodium deoxycholate to the precipitate fraction of the homogenate of the incubation mixture with oviduct minces prepared as described previously, followed by the centrifugation at 40,000 r.p.m. for 2 hours. The radioactive acetyl-Gly-Ser-OH could also be identified in the pronase digest of the heat-treated ribosome-nascent ovalbumin complex. These results strongly suggest that ovalbumin synthesis will be initiated with an acetylglycine residue, although a possibility that the terminal amino group of the growing polypeptide chain on a polysome is acetylated cannot be excluded. The attempt to identify the re-

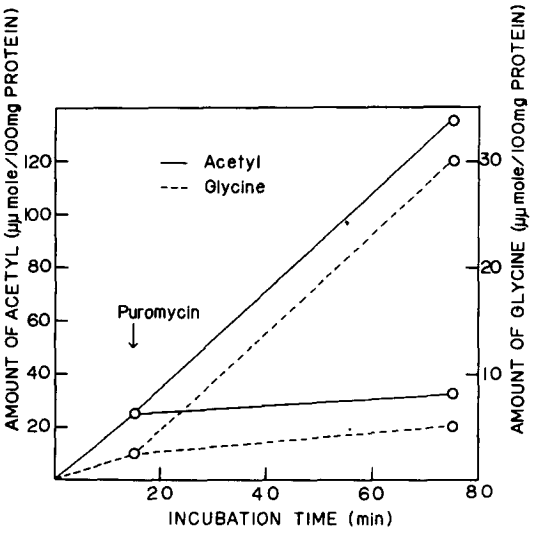

Fig. 2. Inhibition of ovalbumin synthesis by puromycin. Puromycin was added at 15 minutes to the incubation mixture containing C^{14}-acetate, H^3-glycine, amino acid mixture (minus glycine) and oviduct minces. Acetyl-glycylserine was isolated from the ovalbumin preparations as described in the text and radioactivities in the peptide were counted. Upper two lines in the figure are those obtained without addition of puromycin.

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lease of acetyl-glycyl-puromycin from the incubation mixture with puromycin have so far been unsuccessful and further experiments along this line are in progress.

Partial revision of the previous hypothesis for protein initiation (Narita, 1962a, 1962b, 1965) seems to be preferable on the basis of the present knowledge of the tertiary structures of several proteins. The tertiary structures deduced by the X-ray crystallographic analysis of several proteins have shown that their free N-terminal groups are located at or near the surface of their molecules. These results seem to indicate that the terminal acetyl group is cleaved enzymatically after the release of the completed protein molecule from a polysome. In the case of the terminal acetylated proteins, the acetyl terminal group is imbeded in the hydrophobic clusters formed inside of the protein molecule and thus deacetylase cannot

influence to the acetylated terminus. The X-ray crystallographic analysis by Dickerson et al. (1967) of horse heart cytochrome c, the terminal amino group of which is acetylated, will clarify this possibility.

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