PYRROLID-2-ONE-5 CARBOXYLIC ACID INVOLVEMENT IN THE BIOSYNTHESIS OF RABBIT IMMUNOGLOBULIN

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Recent studies on the structure of rabbit immunoglobulin-G (IgG) established the presence of pyrrolid-2-one-5 carboxylic acid (PCA) as the N-terminal amino acid in 90% of the heavy chains (Wilkinson et al., 1966; Press et al., 1966).

The present communication reports that free PCA-C^{14} is not incorporated into IgG, and suggests that PCA is bound to transfer RNA in immunoglobulin-producing cells.

RESULTS AND DISCUSSION

PCA incorporation. L-PCA was synthesized from L-glutamic acid by heating a solution of L-glutamic acid (chromatographic grade, Calbiochem) in H_2O (pH 3.5) for 2 hours at 140° C in a sealed ampule. PCA was isolated by chromatography on a column of Dowex 50W-X8 H^+ form, 20 x 1 cm, and eluted with water. PCA, which has no free α-NH_2 group, was not retained by the cation exchange resin, while the glutamic acid was completely retained, and could be displaced by 2N ammonia. The yield of PCA was about 90% and the product did not react with ninhydrin.

Rabbit popliteal lymph node cells were prepared and suspended in Hanks' salt solution with 10% of normal rabbit serum. Addition of L-PCA for various times (1 minute to 60 minutes) did not affect the rate of protein synthesis in these cells, as measured by incorporation of C^{14}-labelled amino acids into hot trichloroacetic-insoluble material.

L-PCA-C^{14} was synthesized from L-glutamic-acid-C^{14} (uniformly labelled, 200 mC/mM from ICN), as described above. The isolated L-PCA-C^{14} was chromatographed on paper in n-butanol:acetic acid:water 2:1:1, and the radioactivity was located on the chromatogram with a Packard scanner. R_f values for L-PCA-C^{14} and L-glutamic were 75 and 45, respectively. Rabbit lymph node cells were incubated for various times (1 to 60 minutes) with 2μC of L-PCA-C^{14}, washed four times with cold Hanks' salt solution and fractionated. Ribosomes were obtained from the cytoplasmic extract by centrifugation for 4 hours at 10^5 g in 5 to 20% sucrose gradients. Both the ribosomes and the cytoplasmic proteins were precipitated (15', 90°C) and washed with 5% trichloroacetic acid. The level of radioactivity found in the two fractions was a few counts per minute above the background rate. We suggest that this low level of activity represents a non-specific contamination rather than incorporation, since there was no increase in radioactivity as a function of time.

Transfer-RNA analysis. The negative results in the experiments on incorporation of PCA-C^{14} rule out the possibility that free glutamic acid undergoes cyclization to form free PCA which is then incorporated into the N-terminal position of IgG. A possible site for PCA synthesis is the glutamic-tRNA complex, which would become, by cyclization, a PCA-tRNA. To test this hypothesis, we analyzed the bulk of charged tRNA in the immunoglobulin-producing cells for the presence of bound PCA.

The procedure was in general that described by Yegian et al. (1966) for E. coli-tRNA extraction and the stripping off of the bound amino acids. The tRNA was extracted with phenol from spleens, popliteal and mesenteric lymph nodes. The extract was precipitated, washed several times and finally incubated for 3 hours at 37°C in 0.17 M Tris buffer, pH 8.8, in order to release the acylated amino acids from the charged tRNA's.
The solution was adjusted to 0.1 M Na acetate, pH 5.0, and the nucleic acids were precipitated with 70% ethanol. The precipitate was washed with

a mixture of 75% ethanol and 25% 0.1 M Na acetate, pH 5.0. The supernatant and the wash fluid were combined and concentrated by evaporation in vacuum. The concentrated extract was applied to a Dowex-50W column, as described above. Amino acids that contained a free α-NH_2 group were retained, while the amino acids with blocked α-NH_2 group emerged directly from the column. The fractions of this unadsorbed material were immediately neutralized with NaOH. In order to distinguish between a free PCA and a peptide-bound PCA (an N-terminal peptide still bound to RNA), the Dowex-50W eluate was lyophilized, applied to a Sephadex G-10 column (110 x 2 cm) and eluated with water. Five-ml fractions were collected, lyophilized, and redissolved in 0.2 ml of water; 0.1 ml of each fraction was dried on a filter paper and sprayed with ninhydrin. No ninhydrin positive spots were detected. Treatment of the same fractions with 3N HCl for 30 minutes at 100°C caused the appearance of four ninhydrin-positive fractions. Using the same conditions in a control experiment, PCA was transformed to glutamic acid, as demonstrated by the apperance of ninhydrin-positive spots on a paper chromatogram (R_f 45). Analysis of PCA-C^{14} on the same Sephadex G-10 column showed the radioactivity at the same place where the ninhydrin-positive fractions were eluted. The acid-treated ninhydrin-positive fractions were pooled, desalted by chromatography on Dowex 50W and eluted with 2N ammonia. Two-dimensional ascending-paper chromatography of the desalted material revealed two ninhydrin-positive spots; one corresponding to glutamic acid and the other to glycine.

The results demonstrate that the source of PCA as N-terminal amino acid of immunoglobulins is not the free PCA, but possibly a PCA-tRNA complex. It is tempting to explain the synthesis of PCA by a comparison to the synthesis of N-formyl-methionyl-RNA as described by Marcker (1965). In both cases the N-terminal amino acid is involved and the α-NH_2 group is blocked. In the N-formyl-methionine case the formylation takes place on a specific methionyl-$tRNA_{F\text{-}met}$. By comparison, we can speculate that the cyclization of glutamic acid occurs on a specific glutamyl-$tRNA_{PCA}$. Incorporation of

PCA into the N-terminal position may be directed by a specific initiation codon. Experiments are in progress along these lines.

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