Note

Application of Immobilized Antibody for Analysis and Purification of Prochymosin-specific mRNA from Calf Tissue

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Calf prochymosin (prorennin) is a precursor of a milk-clotting enzyme, chymosin (rennin), which is used for cheese manufacturing. Prochymosin with $M_r$ of about 41,000 (365 amino acids) is secreted from the mucosal tissue of the forth stomach of newborn calves and successive autocatalytic cleaving of the N-terminal 42 amino acids under acidic conditions produces active chymosin. We reported the first successful isolation of prochymosin-specific mRNA from calf tissue and cloned its cDNA which is expressed effectively in Escherichia coli. During these studies, procedures for isolation and purification of the specific mRNA have been improved, especially by the use of immobilized prochymosin-antibody for mRNA assays as described here.

Fresh mucosal tissue ($c=200g$) from two calf abomasa frozen at $-80^\circ C$ was pulverized with dry ice and mixed with 1 liter of 0.1 M Tris-HCl (pH 9.0) containing 25 mM EDTA, 0.1 M NaCl, 1% SDS, and 25 ml of bentonite solution. RNA was extracted with 500 ml portions of phenol–chloroform–isoamylalcohol (50:50:1) four times and was precipitated from the extract with 2 M LiCl twice and with ethanol five times. The final RNA precipitates, dissolved in 5 ml of 0.1 x SSC, were applied onto a poly(U) sepharose affinity column (7 ml bed vol., 1 x 2.3 cm) and the poly(A)-tailed RNA fraction eluted from the column was finally fractionated by sucrose density gradient (5~20%) centrifugation at 63,000 x $g$ for 16 hr (Fig. 1).

In order to detect prochymosin-encoding activity among the final centrifugal fractions, solid phase immunoadsorption analysis using immobilized prochymosin antibody was developed as follows. Anti-prochymosin antibody from rabbits was purified by affinity chromatography with immobilized prochymosin. The purified antibody (10 mg) was immobilized onto 1.5 g of CNBr-activated sepharose 4B. Selective absorption of $^3$H-prochymosin onto the immobilized antibody was confirmed by comparing with $^3$H-bovine serum albumin (BSA) as a control. After both proteins ($c=2~5ng$) labeled with Bolton-Hunter reagent were mixed separately with 25 mg (dry weight) of the immobilized

![Fig. 1. Sedimentation Profile of mRNA at Sucrose Gradient Centrifugation.](image)

Poly(A)-containing RNA (1.3 mg) was applied on 30 ml of a linear 5~20% sucrose density gradient. After centrifugation at 25,000 rpm for 16 hr at 15°C, RNA in each fraction (1.2 ml) was precipitated with ethanol and assayed for translation activity. (——) absorbance at 254 nm; (■) total translation activity per 1 μg RNA; (■) prochymosin specific translation activity per 1 μg RNA; (——) specific/total mRNA ratio.

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antibody, the radioactivity adsorbed was eluted with 1% SDS and counted. Radioactivity of prochymosin was adsorbed quantitatively while less than 2.5% of the radioactivity of BSA was recovered.

Each centrifugal fraction of mRNA (c = 1 µg) was introduced into 25 µl of a reticulocyte lysate system (the New England Nuclear Co.) with 300 µCi/ml 3H-leucine (c = 5000 Ci/mol) or 2 mCi/ml of 35S-methionine (c = 1000 Ci/mmoll) and incubated for 1 hr at 37°C. Half of the reaction mixture was mixed with 4 µl of 20 mM Na·phosphate (pH 7.0) containing 14 mM EDTA and 14% Triton X-100, and 50 µl of 150 mM NaCl containing 10 mM leucine, 25 mg/ml BSA, and 1% Na·deoxychorate. The mixture and 25 mg (dry weight) of the immobilized antibody in 100 µl of PBS (20 mM Na·phosphate, pH 7.0, and 150 mM NaCl) were mixed. After incubation at room temperature for 1 hr followed by gentle shaking at 4°C overnight, the adsorbent was collected by centrifugation, washed three times with 800 µl of PBS containing 10 mM leucine, 1% Triton X-100, and 10 mM EDTA, and then packed into a small glass capillary column, and the adsorbed materials were eluted with 1 ml of 1% SDS. Radioactivity in the eluate was counted as a measure of prochymosin-specific mRNA. On the other hand, the other half of the translation reaction mixture was added to a final 5% concentration of trichloroacetic acid to measure radioactivity incorporated into the acid-insoluble materials. As shown in Fig. 1, prochymosin-specific mRNA was distributed with a distinct peak at fraction 12, while total translational activity showed a peak at around fraction 9. The ratio of the specific/total mRNA was distinctly high at fraction 12, which suggested considerable enrichment of the prochymosin mRNA in this fraction. Its sedimentation constant of about 155 corresponds roughly to the size of 1500 bases which is reasonable for prochymosin mRNA containing 1095 bases of coding sequence with 5’ and 3’ extensions.

When the translational products encoded by each mRNA fraction were analyzed by SDS polyacrylamide gel electrophoresis and its fluorography, a single major product comigrating with authentic prochymosin was detected with fractions 12–14 (Fig. 2). These results indicate effectiveness of the solid phase immunoadsorption method to detect prochymosin mRNA. The method gives reproducible results with simple procedures compared with the usual liquid phase immunoprecipitation method.

REFERENCES