CHROMATOGRAPHY OF VITAMIN B₁₂-BINDING SUBSTANCES FROM RAT STOMACH ON CARBOXYMETHYL-SEPHADEX

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(Received for publication October 27, 1964)

The purification of Intrinsic Factor from human gastric juice and hog stomach has been extensively studied and reports on partially purified preparations published¹⁻²⁾. Except for Okuda³⁾ there are few studies on purification of IF from rat stomach.

It is not known whether IF of these sources are identical or not. This knowledge would be of great biological interest.

To further purify IF from rat stomach, the author chromatographed the V.B₁₂-binding substances from rat stomach as the first step, since the IF has V.B₁₂-binding capacity.

The present paper describes the results of chromatography on Carboxymethyl-Sephadex.

MATERIALS AND METHODS

Rat stomachs were stored frozen and 7.5 g of scraped stomach mucosa was extracted by 2 % saline solution, and after centrifugation, the supernatant was mixed with 216 m μ of ⁵⁷Co-cyanocobalamin. After dialysis for 48 hr, the extract was stirred with 5 g of CM-Sephadex in 0.05 M potassium phosphate buffer of pH 5.7. The Sephadex was separated by centrifugation and washed twice with the same buffer. The eluate from Sephadex with 0.15 M potassium phosphate buffer of pH 7.3 was dialyzed and freeze-dried and the yield was 400 mg.

Column Chromatography

CM-sephadex (medium) was activated as usual and equilibrated with 0.05 M potassium phosphate buffer of pH 5.7. The material (60 mg) in the same buffer was placed on a column of CM-Sephadex (1.5 $\times$ 25 cm) in the same buffer at 0°C.

Elution was started with the same buffer, collecting 10 ml-fractions. At tube 18 gradient elution was started by introducing 0.3 M potassium phosphate buffer of pH 8.05 from a reservoir flask by syphon into the magnetically stirred mixing chamber which was previously filled with 0.05 M potassium phosphate buffer of pH 5.7 and connected to the column. The absorbancy at 280 m μ and radioactivity of the fractions were determined.

RESULTS AND DISCUSSION

Fig. 1 shows the results obtained and suggestion of the presence of two binding

Fig. 1 Elution profile from CM-Sephadex column chromatography.
Details are given in the text.

●—●, absorbance at 280mμ; ○—○, radioactivity

fractions. The initial fraction seems to be eluted below the ionic strength of 0.05 M phosphate buffer because it was eluted at the beginning.

In contrast, the second fraction seems to have a strong affinity with CM-Sephadex. CM-Sephadex has not been used as often as DEAE-Sephadex, but this suggests that the use of CM-Sephadex is advantageous in some cases for the isolation of single protein.

Holdsworth has reported the presence of two V.B₁₂-binding substances from hog stomach, one of which had potent IF activity¹⁾. Gräsbeck et al. also has shown similar results from human gastric juice²⁾. It is interesting that my result is consistent with theirs.

Properties and rechromatography of these fractions will be reported subsequently.

SUMMARY

The chromatography of V.B₁₂-binding substances with CM-Sephadex from rat stomach suggested the presence of at least two components.

Acknowledgments- The author wishes to thank Prof. Okuda for his advice and careful review of manuscript.

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