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Note

Separation of adenosine and riboflavin nucleotides on Dowex AG 50W-X4 cation exchanger

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The cation exchanger Dowex AG 50W-X4, after charge reduction with formate or citrate buffer, has been used to separate different nucleic acid components[1–4]. In this study, attempts were made to separate different adenosine and riboflavin

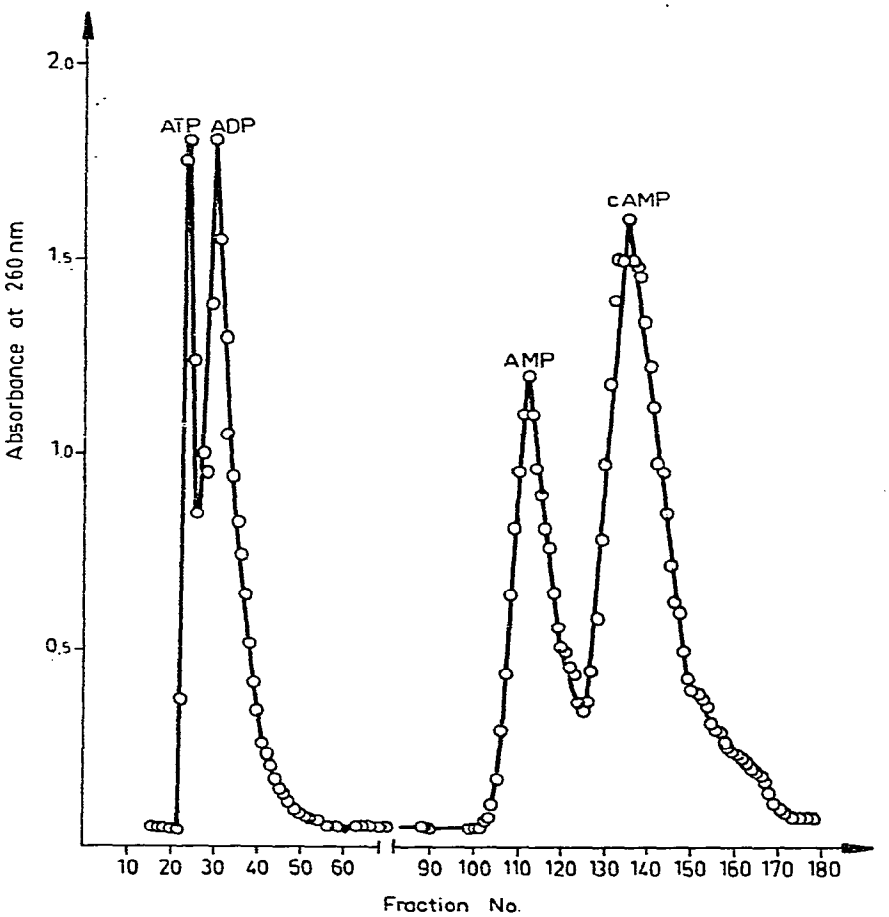

Fig. 1. Chromatography of adenosine ribonucleotides. Column dimensions: 185 × 21 mm. Each fraction contained 1.2 ml.

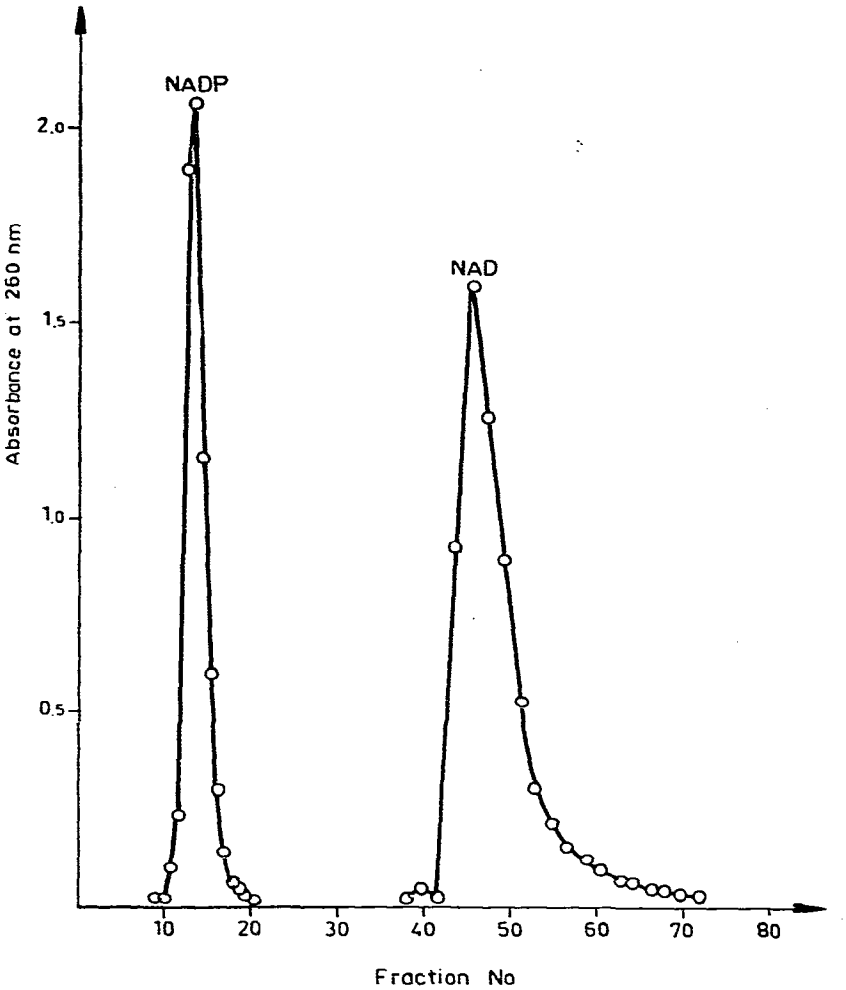

Fig. 2. Separation of NADP from NAD. Column dimensions: 370 × 10 mm. Each fraction contained 1 ml.

nucleotides (ATP, ADP, AMP, cyclic 3'5'-AMP, NADP, NAD, FMN and FAD) by this method. The advantage of the separation system is the rapidity of the chromatographic procedure and the use of a volatile buffer as eluent.

EXPERIMENTAL

The Bio-Rad resin Dowex AG 50W-X4 (—400 mesh) was pre-treated as described by Blattner and Erickson[1]. The columns used had dimensions of 370 × 10 and 185 × 21 mm, and were packed with a slurry of the resin and washed with the elution buffer for a few hours before use. Volumes of 1 ml of sample mixture in buffer were added at the top of the columns and the substances were eluted at room temperature with 0.1 *M* ammonium formate buffer, pH 3.2 (adjusted with formic acid). The flow-rate during packing, washing and elution was 1 ml/min, controlled using a peristaltic pump.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of the 5'-nucleotides ATP, ADP and AMP and also cyclic 3'5'-AMP. The peaks of ATP and ADP are incompletely separated. Adenosine could not be chromatographed on the resin under the conditions used, as it was so strongly absorbed that it could not be eluted with the buffer solution.

The cation exchanger could also be used to separate NADP from NAD (Fig. 2).

On chromatography of a mixture of FMN, FAD and riboflavin, FMN and FAD were separated. Riboflavin could easily be removed from FMN and FAD, as it was strongly retained by the resin (Fig. 3).

Some possible applications of this system are as follows:

(1) the metabolism of adenosine and adenosine derivatives may be followed;

(2) simple systems for separation of NADP from NAD are of importance in the determination of the NADP and NAD contents in cells using bacterial luciferase for detection of the compounds[5];

(3) in the determination of the FMN content in cell extracts using enzymatic assays (with bacterial luciferase) it is essential before the assay to remove FAD and riboflavin[6].

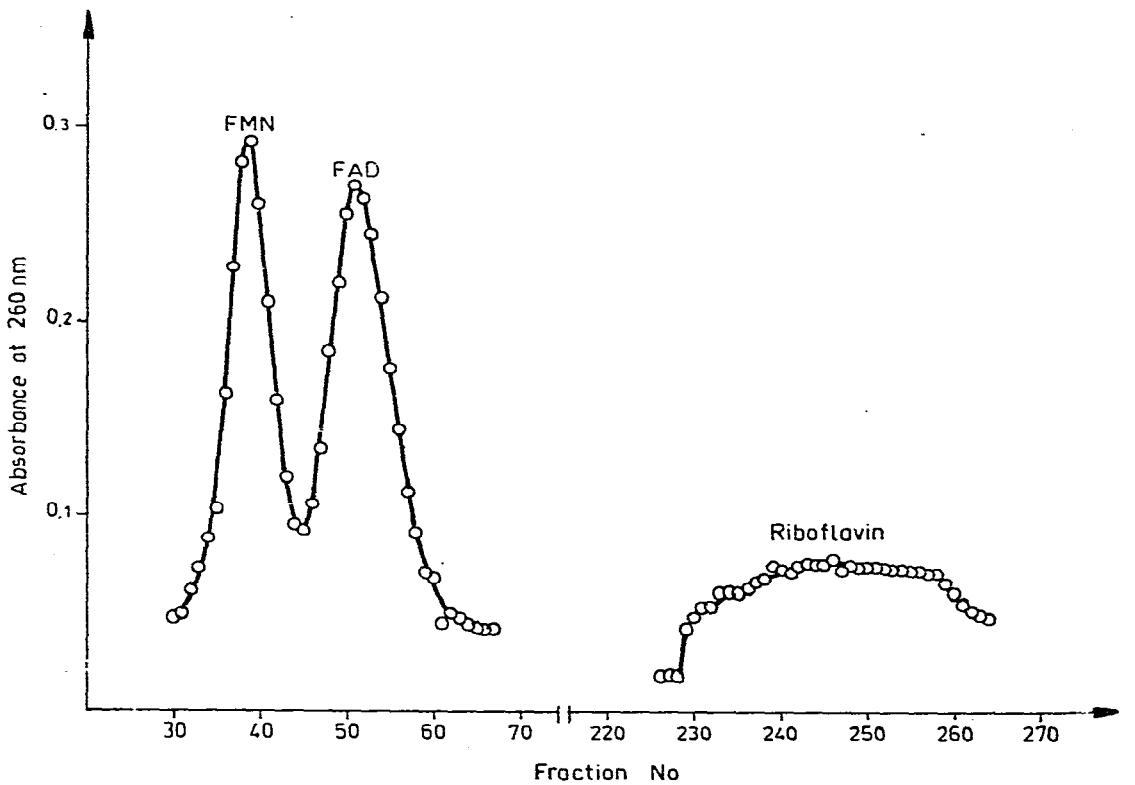

Fig. 3. Separation of a mixture of FMN, FAD and riboflavin. Column dimensions: 185 × 21 mm. Each fraction contained 1.2 ml.

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