Separation of hydroxocobalamin and cyanocobalamin by thin-layer chromatography

Recently hydroxocobalamin has been used extensively for therapeutic purposes. It has haematopoietic activity approximately equivalent to that of cyanocobalamin, but is a more desirable form of vitamin B_{12} for human use than cyanocobalamin because of its more effective retention by the body, its greater reactivity and high ability to form stronger bonds with proteins[1,2]. Hydroxocobalamin, isolated from microbial fermentations[3,4], or obtained from cyanocobalamin (by irradiation[5] or by catalytic hydrogenation[6,7]) is accompanied by other cobalamins, very often by cyanocobalamin.

There have been reports[8,9] on the separation of hydroxocobalamin from cyanocobalamin on silica thin layers, but the suggested methods are complicated and time consuming.

A new, very rapid and simple method for the separation of hydroxocobalamin and cyanocobalamin on thin layers of dry alumina is reported here. Advantage was taken of the property of hydroxocobalamin to form a compound of the cobalichrome group with NH_4OH[10]. Under these conditions cyanocobalamin does not change, so that both compounds can be easily separated, and the individual cobalamins can then be determined quantitatively by known techniques[11].

Experimental and results

The experiments were performed with aqueous solutions of cyanocobalamin, hydroxocobalamin and mixtures thereof whose pH had been adjusted to 8.5 with dilute NH_4OH.

Different mixtures of isobutanol, *n*-butanol, isopropanol and water with the addition of NaOH, NH_4OH and CH_3COOH were examined as solvent systems. The best separation, with good compact spots, was obtained with the mixture isobutanol–isopropanol–water, 1.5:1:1.25 (with addition of NH_4OH until the pH was 8.5). This is due probably to the fact that only under these conditions will hydroxocobalamin form a cobalichrome with NH_4OH, since with the same solvent system, but with the pH adjusted to 8.5 with dilute NaOH, the spots were elongated and not clearly separated.

Neutral alumina (second degree of acitivity according to Brockmann) was the most suitable as the adsorption medium. The R_F values in this case are: hydroxocobalamin, 0.30 and cyanocobalamin, 0.46.

When basic alumina was used, the spots were not well defined, and acid alumina did not give any separation.

Thin layers of dry alumina (about 1 mm thick) were prepared according to the usual method[12] on frosted glass plates, 30 × 8 cm.

The solutions of the cobalamins were applied 2–3 cm from the edge of the plate. After the spots had been dried, the plate was placed in an inclined position (20°) in an airtight chromatographic chamber, saturated previously with solvent system, 1–2 cm of the lower edge of the plate dipping into the solvent. The development of the chromatogram was effected for 4 h (temperature, 20 ± 0.5°) until the solvent had run about 24–26 cm.

The minimum amount of each compound detectable was 0.5 μg.

The method described for the separation of hydroxocobalamin and cyanocobalamin is quick and simple, can be easily carried out, and can be used for quantitative determination.

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1 G. B. Y. GLASS, H. R. SKEGGS, D. H. LEE, E. L. JONES AND W. W. HARDY, *Nature*, 189 (1961) 138.
2 J. I. TOOHEY AND H. A. BARKER, *J. Biol. Chem.*, 236 (1961) 560.
3 J. V. PIERCE, A. C. PAGE, E. L. R. STOKSTAD AND T. H. JUKES, *J. Am. Chem. Soc.*, 71 (1949) 2952.
4 W. G. JACKSON, G. B. WHITFIELD, W. H. DEVRIES, H. NELSON AND J. S. EVANS, *J. Am. Chem. Soc.*, 73 (1951) 337.
5 W. L. C. VEER, J. H. EDELHAUSEN, H. G. WIJMENGA AND J. LENS, *Biochim. Biophys. Acta*, 6 (1950) 225.
6 E. A. KACZKA, D. E. WOLF AND K. FOLKERS, *J. Am. Chem. Soc.*, 71 (1949) 1514.
7 J. A. BROCKMAN, J. V. PIERCE, E. L. R. STOKSTAD, H. P. BROQUIST AND T. H. JUKES, *J. Am. Chem. Soc.*, 72 (1950) 1042.
8 L. CIMA AND R. MANTOVAN, *Farmaco (Pavia), Ed. Prat.*, 17 (1962) 473.
9 O. TADAYOSHI, *Bitamin*, 30 (1964) 280; *C.A.*, 62 (1965) 1957a.
10 G. COOLEY, B. ELLIS, V. G. PETROV, G. H. BEAVEN, E. R. HOLIDAY AND E. A. JOHNSON, *J. Pharm. Pharmacol.*, 3 (1951) 271.
11 YA. POPOVA, CH. POPOV AND M. ILIEVA, *Prikl. Biokhim. Mikrobiol.*, 1 (1965) 693.
12 J. DAVÍDEK AND J. BLATTNÁ, *J. Chromatog.*, 7 (1962) 204.

Received March 11th, 1966

J. Chromatog., 24 (1966) 263–264

Ion exchange chromatography of some acidic and aromatic amino acids*

Available methods for separating phosphorylated and other acidic amino acids by ion-exchange column chromatography are time-consuming and do not provide good resolution. In view of the significance of glutamic acid and its metabolites in neural function and metabolism, and because the phosphoamino acids serve as the active sites of enzymes involved in phosphorus metabolism, it was desirable to develop an effective method for their separation.

Experimental

About 500 g of Dowex 1-X8 (AG 200–400 mesh BIO-RAD) was suspended in 2 l of water in a 3 l beaker and allowed to settle for five minutes. Approximately three-fourths of liquid containing the finer particles was decanted for subsequent use. After washing in the usual manner, the resin was converted to the acetate from the chloride form using 2 *M* sodium acetate.

* This research was supported by USPHS grant NB-05856.