REVIEW ARTICLE

Biochemical Importance, Deficiency and Clinical Assay of Cyanocobalamin

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ABSTRACT
The present review describes the biochemical importance of cyanocobalamin (vitamin B₁₂) and its role in the biosynthesis of adenosylcobamide and methylcobamide co-enzymes which perform important functions in human metabolism. Cyanocobalamin deficiency is a significant public health problem particularly among the elderly. It has been reported that the prevalence of vitamin B₁₂ deficiency may be as high as 30-40% among the elderly due to food B₁₂ malabsorption. Various analytical methods have been used for the clinical assay of cyanocobalamin in biological samples. These include microbiological, radioisotope, spectrophotometric and chromatographic methods.

Keywords: Cyanocobalamin, deficiency, clinical analysis.

INTRODUCTION
Cyanocobalamin (vitamin B₁₂) is chemically 5, 6-dimethylbenzimidazolylycyanocobamide. It is a cobalt coordination complex in which the cobalt is trivalent and has a coordination number of six. The complex is neutral and is composed of two heterocyclic systems, a benzimidazole and a modified porphyrin nucleus. It is soluble in water and ethanol (90%). Cyanocobalamin exhibits absorption maxima at 278, 361 and 550 nm in aqueous solution. The infrared peaks occur at 1660, 1497, 1575, 1070, 1150 and 1220 cm⁻¹ in KBr disc. It is highly sensitive to light and is degraded to hydroxocobalamin (vitamin B₂₉) and further degradation products. The reaction is accelerated in the presence of nicotinamide.

BIOCHEMICAL IMPORTANCE
Cyanocobalamin occurs in nature as a co-factor in the form of adenosylcobalamin. It was originally isolated as cyanocobalamin and hydroxocobalamin from clinically active liver fractions. Minute amounts of the vitamin are highly effective in promoting the growth of Lactobacillus lactis. Single doses of the vitamin (3-6 mcg) have been found to produce positive hematological activity in patients with Addisonian pernicious anemia. The best dietary sources of cyanocobalamin include meat, egg and sea food, dairy and fermented products. The dietary vitamin is protein bound and its absorption results in its release in the stomach. The release is enhanced by gastric pH and pancreatic proteases.

In the biosynthesis of co-enzyme from vitamin B₁₂, cobalt is reduced from a trivalent to a monovalent state before the anionic ligands are attached to the molecule. The two types of cobamides that participate as co-enzymes in human metabolism are the adenosylcobamides and the methylcobamides. These co-enzymes perform important functions in methylmelonate-succinate isomerationization and in methylation of homocysteine to methionine. Methylcobalamin is the major form of the co-enzyme in the plasma; 5-deoxyadenosylcobalamin is the major form in the liver. The methylation of homocysteine to methionine is carried out by methylcobalamin. It is catalyzed by transmethylase in the presence of 5-methyltetrahydrofolic acid and reduce FAD.
In the cell cyanocobalamin has been found to participate as co-factor in two important metabolic reactions, i.e., mitochondrial and cytosolic reactions. In the mitochondrial reaction, the 5-deoxyadenosylcobalamin form of the vitamin is required for the enzyme methylmalonylCoA mutase that catalyses the conversion of methylmalonyl CoA to succinyl CoA. This is an intermediate step in the conversion of propionate to succinate during the oxidation of odd-chain fatty acids and the catabolism of ketogenic amino acids. In the cytosolic reaction the methylcobalamin form of the vitamin is required in the folate-dependent methylation of the sulphur amino acid, homocysteine, to form methionine, catalyzed by methionine synthetase\textsuperscript{11,12}.

**DEFICIENCY**

Cyanocobalamin deficiency is considered as a significant public health problem particularly among the elderly. Many workers have reported a high prevalence of cyanocobalamin deficiency on the basis of raised serum or urine methylmalonic acid or homocysteine levels with or without low serum B\textsubscript{12} concentrations\textsuperscript{13-15}. It has been suggested that the prevalence of B\textsubscript{12} deficiency may be as high as 30-40\% among the elderly due to food B\textsubscript{12} malabsorption caused by chronic gastritis, gastric atrophy and other causes\textsuperscript{16}. The clinical manifestations of cyanocobalamin deficiency, i.e., megaloblastic anemia, occur only in severely vitamin depleted individuals\textsuperscript{17}. Recent surveys of cayano cobalamin status in the elderly people indicate that the prevalence of the deficiency is much higher on the basis of serum or urine methylmalonic acid concentrations\textsuperscript{13}. It has been suggested that the usual cut-off values for serum B\textsubscript{12} (i.e., <300 pg/ml or <221 pmol/L for mild deficiency and <200 pg/ml or <148 pmol/L for severe deficiency) are too low, and that <350 pg/mL (258 pmol/L) is the cut-off value below which serum methylmalonic acid and homocysteine concentrations may become elevated.

In several studies it has been reported that an apparently high prevalence of low B\textsubscript{12} status and varying degrees of B\textsubscript{12} deficiency occur in both children and adults in locations such as Mexico, India and Israel\textsuperscript{18-22}. The causes of B\textsubscript{12} deficiency in these populations may involve a combination of low intake and malabsorption. The above mentioned studies have shown that the most common causes of clinically evident B\textsubscript{12} deficiency is malabsorption and inadequate dietary intake\textsuperscript{12}. Chemical inactivation of B\textsubscript{12} by prolonged inhalation of the anesthetic gas nitrous oxide (NO\textsubscript{2}) can also cause or substantially contribute to B\textsubscript{12} deficiency\textsuperscript{23}.

**CLINICAL ASSAY**

Various analytical methods have been used for the assay of cyanocobalamin in biological samples. These methods have been reviewed by Hashmi\textsuperscript{24}, Ball\textsuperscript{25}, Lee and Griffiths\textsuperscript{26}, Kumar et al., 2010\textsuperscript{27} and Karmi, 2010\textsuperscript{28}. The details of these methods are described in the following sections:

**Microbiological Methods**

This method is among the oldest methods used for the assay of vitamin B\textsubscript{12}. Davis et al.\textsuperscript{29} described an automated method for the microbiological assay of vitamin B\textsubscript{12} using *L. leichmannii* as a test organism. This method gives the results in 24 hrs. Low serum vitamin B\textsubscript{12} levels can be measured with considerable precision by microbiological assay with the *Euglena gracilis* and its deficiency in humans can be recognized with the high level of consistency by either the Euglena or *L. leichmannii* assays and is ideally suited for the assay of large number of biological specimens. The *L. leichmannii* technique requires preliminary extraction of protein from the samples prior to assay\textsuperscript{30}. The microbiological methods for B\textsubscript{12} assay are tedious, time consuming and give low precision\textsuperscript{29}. These methods have now been replaced by other analytical techniques.

**Radioisotope Methods**

The radioisotope dilution assay of B\textsubscript{12} is an ideal method for determining the concentration of the vitamin in moderate number of samples. This method
is simple and can be carried out in any laboratory with suitable counting equipment. The technique is capable of differentiating B₁₂ deficiency from control subjects. The sub-normal serum levels found in pernicious anemia with this technique indicates severe reduction of liver B₁₂ level. If the fall in serum B₁₂ level is associated with folate or iron deficiency, the tissue B₁₂ levels are usually reduced but not to the low levels found in B₁₂ deficiency states. The evaluation of commercial radioisotope methods for the simultaneous determination of vitamin B₁₂ and folate in biological samples has been reported. The methods showed good correlation with the microbiological methods.

Spectrophotometric Methods
The various spectrophotometric methods for the assay of cyanocobalamin have been reviewed by Ahmad et al. These authors have developed multicomponent spectrophotometric methods for the simultaneous assay of cyanocobalamin and hydroxocobalamin, and cyanocobalamin, hydroxocobalamin and riboflavin. Elzanfaly et al. developed a spectrophotometric multivariate calibration method for the simultaneous determination of cyanocobalamin along with thiamine hydrochloride and pyridoxine hydrochloride. The method depends on the use of spectrophotometric data coupled to PLS and PCR multivariate calibration methods for the simultaneous determination of these vitamins.

Chromatographic Methods
High performance liquid chromatography (HPLC) methods have been used to separate and identify the various forms of cobalamines. A reversed-phase HPLC method has been developed for the determination of vitamin B₁₂ and other vitamins including B₁, B₃, B₆ and folic acid in mixtures using a UV-detector. The relative standard deviation (RSD) of the method ranged from 0.5 to 4.1%. Luo et al. have reported a HPLC-ESI-MS method for the analysis of vitamin B₁₂ in food products and biological samples.

A new, simple and accurate high-performance thin-layer chromatographic (HPTLC) method has been developed for the separation and simultaneous determination of B₁, B₂, B₆ and B₁₂ using pre-coated aluminium-backed silica gel G 60 F₂₅₄ HPTLC plates. Ethanol-chloroform-acetonitrile-toluene-amonia-water (7:4:4.5:0.5:1:1, v/v) mixture was used as a mobile phase and UV-detection was performed densitometrically at 254 nm. A TLC densitometric method has been reported for the simultaneous determination of vitamin B₁, B₂, B₆ and B₁₂ using chloroform-ethanol-water-acetic acid (2:8:2:0.5, V/V) as solvent system. The separated spot of vitamin B₁₂ was scanned at 361 nm for quantitative purpose.

DISCUSSION
Cyanocobalamin (vitamin B₁₂) is an important component of vitamin B-complex and is extensively used in various dosage forms for the vitamin deficiency. It has great biochemical importance and in the cell cyanocobalamin participates as a co-factor in metabolic process, i.e., mitochondrial and cytoplasmic reactions. Cyanocobalamin deficiency, particularly among the elderly, is largely due to low intake and malabsorption of food B₁₂ content. The clinical manifestations of B₁₂ deficiency (mechaloblastic anemia) have been found to occur in severely B₁₂ depleted persons. Several methods have been developed for the assay of cyanocobalamin in biological materials. These methods are based on different principles involving microbiological, radioisotope, spectrophotometric and chromatographic techniques.

REFERENCES


