Synthesis of Glycosylated Triiodothyronine in Vitro

MUNETADA OIMOMI, YUKIO YOSHIMURA, TOMIYASU KAWASAKI, SHINZO KUBOTA, GENYO TANKE AND SHIGEAKI BABA

The Second Department of Internal Medicine, Kobe University School of Medicine, Ikuta-ku, Kobe, 650 Japan

Abstract

Glycosylated T₃ was prepared by binding T₃, a thyroid hormone, to glucose. We identified this compound by thin-layer chromatography and high-pressure liquid chromatography. We also demonstrated that glycosylated T₃ could be separated from T₃ by high-pressure liquid chromatography.

A hemoglobin (Hb) component which is formed by a Schiff base linkage of glucose to the N-terminal valine of the β-chain is designated as glycosylated Hb or fast moving Hb (Holmquist et al., 1966; Bookchin et al., 1968). It has been reported that the level of glycosylated Hb is elevated in diabetic patients (Trivelli et al., 1971). Furthermore, it is suggested that the measurement of glycosylated Hb is a good indicator of long-term blood glucose control in diabetic patients (Gabbay et al., 1971). It has been reported that not only glucose but also ascorbic acid and various aldehydes attach to Hb via a Schiff base linkage or Amadori rearrangement (Oimomi et al., 1979; Zaugg et al., 1977). On the other hand, there exists a reaction called a browning phenomenon (Burton et al., 1962), in which sugars are bound to amino acids, in the natural world. Thus, utilizing the reaction in which glucose is linked to the amino group of Hb, we studied whether glucose could attach to the amino group 3′, 5′, 3′ L-triiodothyronine (T₃), a thyroid hormone.

Materials and Methods

One milligram of T₃ was dissolved in 2 ml of absolute ethanol and this solution was divided into halves. To one half of the solution was added glucose in an equimolar with T₃. The mixture was heated in a water bath at 80°C for an hour. After the ethanol had been evaporated, the residue was dissolved in 2 ml of distilled water (Sample 1). The other half of the solution to which glucose was not added was treated in the same manner (Sample 2). Meanwhile, 1 mg of T₃ was preserved in 2 ml of absolute ethanol containing glucose in an equimolar with T₃ (1 mg) at 37°C for one week (Sample 3).

Thin-layer chromatography of these samples was performed using chloroform-tertiaryamyl alcohol-2N-NH₄OH (1:5:6) as a solvent system (Bellabarba et al., 1968).

High-pressure liquid chromatographic separation of Sample 1 and Sample 2 was made on a μ Bondapack C18 column (0.4×30 cm) using 40% acetonitrile: 60% water with paired-ion chromatography B6 as solvent. A flow rate of 0.1 ml per minute was maintained and optical density was read at 254 nm.

Results and Discussion

Thin-layer chromatograms were colored with ninhydrin and periodic acid-benzidine (Knappe et al., 1974) (Fig. 1). Sample 1...
FIG. 1. Separation of triiodothyronine, glycosylated triiodothyronine and thyroxin by thin-layer chromatography. Left: Ninhydrin staining. At each original spot, a red color developed. Right: Periodic acid-benzidine staining. Yellow green spots on a blue ground were produced.

1) Triiodothyronine
2) Triiodothyronine + Glucose (80°C, 1 hour)
3) Triiodothyronine (80°C, 1 hour)
4) Triiodothyronine + Glucose (37°C, 7 days)
5) Thyroxin
6) Glucose

and Sample 3 showed bands with different mobilities and lower RF values than Sample 2. The band colored with periodic acid-benzidine, revealing the presence of glucose, had an RF similar to that colored with ninhydrin, revealing the presence of amino group. This result suggested that this band represented glycosylated T3.

In addition, the high-pressure liquid chromatographic separation of glycosylated T3 showed that its peak was present before that of T3 (Fig. 2). This suggested that T3 and glucose combined to form a substance which was electrically different from T3. The presence of glycosylated T3 is an interesting subject for study because of a possible relationship to the heterogeneity of thyroid hormones in the blood of diabetic patients. The non-enzymatic binding reaction between various amino acids and glucose as observed in the present study seemed to be a common reaction in the living body.

The physiological action of substances produced by such a reaction and their inactivating action on the original substances are worth studying.

References


