NOTE

Glycosylation of 3, 5, 3'-L-Triiodothyronine and its Biological Activity

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Abstract

The formation of glycosylated Hb by the nonenzymatic reaction between Hb and glucose suggests that glycosylation is a general reaction in the living body. We demonstrated by means of TLC and HPLC that T₃ was glycosylated when reacted with glucose in absolute ethanol for a week. We demonstrated in a metamorphic study in tadpoles that the biological activity of thus glycosylated T₃ was less than about 1/10 of that of T₃.

These findings suggest that glycosylation of substances with amino group other than serum proteins including Hb may occur and that glycosylated substances may differ in biological activities from the original substances.

Materials and Methods

Glycosylated hemoglobin formed by glycosylation between glucose and hemoglobin (Hb), which increases in patients with diabetes mellitus, has attracted attention as an indicator of diabetic control (Gabbay et al., 1977). Since glycosylation is a nonenzymatic reaction, it may occur with other various proteins and peptides. It has recently been demonstrated that serum albumin (Dolhofer and Wieland, 1980), lens crystallins (Stevens et al., 1978) and red cell membrane protein (Miller et al., 1980) are glycosylated. In the present study, we investigated glycosylation of hormones using 3, 5, 3'-L-triiodothyronine (T₃). We prepared glycosylated T₃ by chemical reactions. Then, we purified glycosylated T₃ from the chemical reactants and studied its biological activity.
logical activities of T₃ and glycosylated T₃ were assayed, lyophilized T₃ and glycosylated T₃ were submitted to HPLC.

Biological activities of T₃ and glycosylated T₃ were assayed by taking advantage of metamorphosis of tadpoles (Rana nigromaculata) obtained by artificial fertilization. Tadpoles at developmental stages 31-33 according to Gosner's stage table of anuran development (Gosner, 1960), were placed in 200 ml of T₃ or glycosylated T₃ solution at concentrations of 3.1×10⁻⁴ to 3.1×10⁻⁷ mM.

After 24 hours, the animals were transferred into a cistern holding approximately 1 liter of water without T₃ or glycosylated T₃. Water was changed every day. Observation was made on the length of tail fin and the course of metamorphosis.

Results

Samples 1, 2 and 3 as well as T₃, thyroxin (T₄) and glucose as controls were submitted to TLC. Duplicate chromatograms were prepared, one being developed with ninhydrin and the other with periodic-acid benzidine (Knappe, 1974). The chromatograms of samples 1 and 3 showed a band for T₃ and a band with lower Rf than T₃ when colored with ninhydrin. Also with periodic-acid benzidine, a band with lower Rf than T₃ appeared. (Fig. 1)

The presence of glycosylated T₃ in the reaction mixture was identified by HPLC. T₃ and glycosylated T₃ were separated from samples 1 and 3 by HPLC on a column (Fig. 2). The T₃ and glycosylated T₃ fractions were lyophilized. These fractions were dissolved in a small quantity of absolute alcohol, and then diluted with distilled water. HPLC was performed to determine the concentrations of T₃ and glycosylated T₃. (Fig. 3).

Isolated T₃ and isolated glycosylated T₃ showed single peaks. It was thus confirmed that the two isolated substances were puri-
fied T₃ and glycosylated T₃, respectively. From the graph in Fig. 3 at left, a calibration curve for T₃ was constructed, and the concentrations of isolated T₃ and glycosylated T₃ were estimated.

The biological activity of the thyroid hormone was assayed using tadpoles. Observation was made on the metamorphosis and length of tail fin every day.

Figures 4 and 5 show the changes of tadpoles at 3 days after 24-hour exposure to T₃ or glycosylated T₃. At the concentration of \(3.1 \times 10^{-6}\) mM, the biological activity of glycosylated T₃ was about 1/10 and 1/13 as potent as that of T₃ in terms of the developmental stage and length of tail fin, respectively.

T₃ and glycosylated T₃ had no biological effect as a thyroid hormone at concentrations of less than \(3.1 \times 10^{-8}\) mM and less than \(3.1 \times 10^{-7}\) mM, respectively.

Discussion

HbA1, a glycosylation of Hb, which increases in diabetic patients, has been thought to be produced at \(\beta\)-chain N-terminal valine (Holmquist and Schroeder, 1966; Bookchin and Gallop, 1968; Bunn et al., 1975). Furthermore, it has been demonstrated that glycosylation of Hb occurs at the \(\alpha\)-chain N-terminus as well as the \(\varepsilon\)-amino group of a lysine residue of both \(\alpha\) and \(\beta\)-chains of Hb.
Schiff-base linkage and Amadori rearrangement which are required for glycosylation can occur. Glycosylation is, as described above, a nonenzymatic reaction and a widely-seen reaction in the natural field as a browning phenomenon (Burton et al., 1962). Meanwhile, recent reports have described the occurrence of glycosylation of various proteins in the living body. In order to demonstrate our assumption that some hormones also are glycosylated, we selected T₃, an active thyroid hormone, from among thyroid hormones with relatively long half-lives. Since the binding of T₃ to glucose is considered to be due to the browning phenomenon, T₃ was allowed to react with glucose not only at 37°C, i.e. under physiological conditions but at 80°C so as to accelerate the reaction. The presence of an amino acid was shown by the ninhydrin reaction, and that of glucose by the periodic-acid benzidine reaction.

The reaction product showed spots corresponding to T₃ and glucose and another spot. The latter spot was colored with both ninhydrin and periodic-acid benzidine, suggestive of the formation of glycosylated T₃. The change in solubility of T₃ suggests that T₃ is glycosylated when allowed to react with glucose on heating.

T₃, T₄ and glycosylated T₃ were analyzed by HPLC. T₃ and glycosylated T₃ were separated by HPLC on a high precision column, and were submitted to bioassay. For the comparison, activity was expressed as the ratio of glycosylated T₃ to T₃.

The biological activities of T₃ and glycosylated T₃ were assayed in terms of metamorphosis of tadpoles, mainly shortening of tail fin and change of legs. Tadpoles at stages 31-33 at which hind limbs began to come out were used for the study. Glycosylated T₃ showed only less than 1/10 of the activity of T₃. Decreased oxygen dissociation of Hb due to glycosylation has been reported (Bunn et al., 1979; Shapiro et al., 1980).
considered that the browning phenomenon decreases the activity of a substance or changes its nature. In other words, glycosylation decreases or changes the function of the substance. It follows that glycosylation takes part in inactivation of not only T₃ but also various hormones.

The results of the present study suggested that glycosylation might be involved in inactivation of hormones i.e. a decrease in biological activity of hormones. Taking into account the fact that glycosylation depends on glucose levels, it appears that special attention should be paid to heterogeneity of hormones in diabetic patients.

References


