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Partial deglycosylation of α subunit modifies sturgeon gonadotropin function✪

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Chemical deglycosylation (dg) of sturgeon *Acipenser gueldenstaedti* Br. (αGTH) resulted in the loss of 83% of its initial carbohydrate content. It altered also recombinant dgαGTH + βGTH dimer molecule, reducing its immunoreactivity by 30%, and fully blocking the hormonal function. CD spectroscopy showed that deglycosylation led to changes in the secondary structure of dgαGTH and in the α-β recombinant. The sugar moiety of sturgeon αGTH is suggested to play an important role in maintaining the biological function of the hormone dimer molecule.

Sturgeon gonadotropic hormone (GTH) is a typical representative of the glycoprotein hormone family. The heterodimer molecule of sturgeon GTH (26 kDa) is formed from complementary noncovalently bonded α and β subunits [1]. The content of asparagine-linked carbohydrate in sturgeon GTH is about 30%, of which about two-thirds is localized on αGTH, and one-third on βGTH [2]. Carbohydrate analysis has shown that, like in mammalian gonadotropins [3], the sugar moiety of sturgeon GTH contains mannose, galactose, fucose, aminosugars, and sialic acids [2].

Studies of mammalian GTH showed that the sugar fraction is important both as a structural and functional component of the hormone, affecting its conformation and biological function [3], as well as its half-life in the bloodstream [3, 4]. However, little is know about the role of sugar components of the

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Abbreviations: GTH, gonadotropic hormone (gonadotropin); αGTH and βGTH, native subunits of GTH; dg, deglycosylated, deglycosylation; dgαGTH, deglycosylated αGTH; CD, circular dichroism.

complementary subunits in fish GTH, particularly of αGTH, which contains the bulk of carbohydrates of the hormone.

The aim of the present work was to use selective chemical deglycosylation to determine the role of the sugar component associated with the α-subunit on the biological function of the dimer molecule of sturgeon GTH.

MATERIALS AND METHODS

Hormone. Highly purified preparation of sturgeon GTH was isolated from acetone-dried pituitary glands of the fish using gel-filtration and ion-exchange chromatography on DEAE-cellulose columns, according to the procedure described earlier [5, 6].

Subunits. Individual α and β subunits of GTH were isolated by ion-exchange chromatography on SE-Sephadex C25 in 0.025 M acetate buffer at pH 4.9, after GTH dissociation in 8 M urea according to Hennen *et al.* [7].

Reassociation of subunits was carried out in saline (12 h) at room temperature at a total protein concentration of about 0.2%, using counterpart subunits at an equimolar ratio.

Immunotests. The double immunodiffusion test in 1% agar gel (Bacto-agar Difco, U.S.A.) in veronal buffer (ionic strength 0.05, pH 8.6) was performed by Ouchterlony's method [8] using polyclonal monovalent antisera, raised in rabbits, against highly purified sturgeon GTH or αGTH.

Bio-tests. The frog *Rana esculenta* L. oocyte *in vitro* maturation test [9] was used to evaluate the gonadotropic activity of the purified hormone and subunit recombinants. The gonadotropic activity was expressed as the minimal dose of the hormone preparation that produced 100% oocyte maturation.

Chemical deglycosylation. Selective deglycosylation (dg) of sturgeon αGTH was carried out under an atmosphere of nitrogen for 5 h at 0°C as described by Kalyan & Bahl [10] using trifluoromethane sulfonic acid (99% purity, Aldrich, U.S.A.) and anhydrous anisol as a scavenger. By-product contamination was eliminated by dialysis (Visking tubing 8/32, Serva, Germany) and gel-filtration.

The residual sugars in the dgαGTH were detected by the classical colorimetric methods described for the detection of amino-sugars [11] and neutral sugars [12].

CD spectra. The spectra were registered on a Jasco J-710 spectropolarimeter over the wavelength range from 180 to 260 nm at 22–25°C, in phosphate buffer, pH 7.0. The spectopolarimeter was calibrated by the standards of epiandosterone and 10-camphorsulphonic acid. For recording of spectra, 0.1–0.01 cm quartz cells of Helma were used. The concentration of proteins in the solution was 10^{-3}–10^{-4} M. CD data are given in units of molar ellipticity per amino-acid residue of an average relative molecular mass 108. The content of various secondary structures in the preparations was calculated with use of VARSELEC [13, 14]. The underlying method uses matrix techniques and CD spectra are expressed as vectors containing digitized data. Singular value decomposition is used to create orthogonal CD basis vectors from the CD spectra of proteins with known secondary structure. In our case CD spectra of 22 proteins were used for calculation of the secondary structure elements.

RESULTS AND DISCUSSION

Deglycosylation removed 83% of the sugar fraction of αGTH, including all neutral sugars and sialic acids, and half of aminosugars. However, in the double immunodiffusion tests with anti αGTH antiserum, dgαGTH showed a rather high immunoreactvity (Table 1), and revealed antigenic identity with intact αGTH. This indicates that partial deglycosylation did not alter the covalent structure of the subunit or its antigenic determinants, while it might have an indirect effect on its conformation (see below). The comparative immunotests of the recombinant

dgαGTH + βGTH, containing αGTH with a reduced sugar moiety associated with native βGTH, showed that the immunoreactivity of the recombinant was decreased by 30% in comparison with that of the native GTH, while the typical hormonal activity was completely lost (Table 1).

Table 1. Biological activity of the native and modified sturgeon GTH preparations

Preparation	Immuno-reactivity (%)	Hormonal activity* (%)
GTH	100	100
αGTH+βGTH	100	75
dgαGTH+βGTH	70	0
αGTH	100	0
dgαGTH	80	0

*Each figure represents the mean value of the gonadotropic activity of the preparations at different doses (0.1–0.5 μg/ml) tested in three replicates.

CD spectra shown in Figs. 1 and 2 indicate quite clearly that partial removal of the sugar component of the α-subunit influenced not only its own conformation, but also produced a change in the spatial structure of the whole dgαGTH + βGTH dimer molecule.

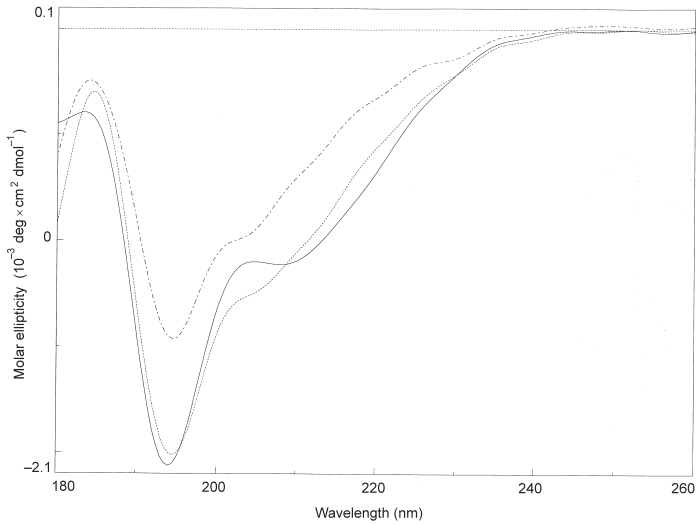

Figure 1. CD spectra of the sturgeon GTH (—) and recombinants αGTH + βGTH (. . .) and dgαGTH + βGTH (- - -) in phosphate buffer, pH. 7.0, 180–260 nm.

Variances of molar ellipticity: for dgαGTH + βGTH ± 5%, for the other four compounds ± 1–3%.

In the CD spectra of the first group of GTH preparations (Fig. 1) a broad negative band around 195 nm and a shoulder at 210 nm are well expressed for native GTH and recombinant αGTH+βGTH. In the spectrum of recombinant dgαGTH+βGTH, the 195 nm band is weaker and the 210 nm shoulder less pronounced. The negative Cotton effect around 195 nm is close to the well known negative band of the random coil at 198 nm; while the shoulder at about 215 nm could arise from the contribution of both the β-pleated sheet structure, characterized by a negative Cotton effect at 218 nm, and the α-helix with a negative CD band at 222 nm [15–17]. The results of the calculations of the content of various secondary structures in the preparations, shown in Table 2, allowed us to conclude that the spatial structures of the native GTH and the native recombinant αGTH+βGTH were quite similar, while those of the native GTH and the modified recombinant dgαGTH+βGTH containing deglycosylated αGTH differed appreciably: in the modified recombinant there was no α-helix or parallel β-strand, only 10% of the β-turn, 38% of the random coil and 44% of the anti-parallel β-strand. The secondary structures of dgαGTH and αGTH also were slightly different (cf. Fig. 2 and Table 2) CD spectra of Fig. 2 reveal a large negative band at 198 nm typical for random coil structures and a weak shoulder at about 215–220 nm reflecting a possible contribution of the β-strand as mentioned above. The calculated content of random coil was higher for the deglycosylated α-subunit (40%).

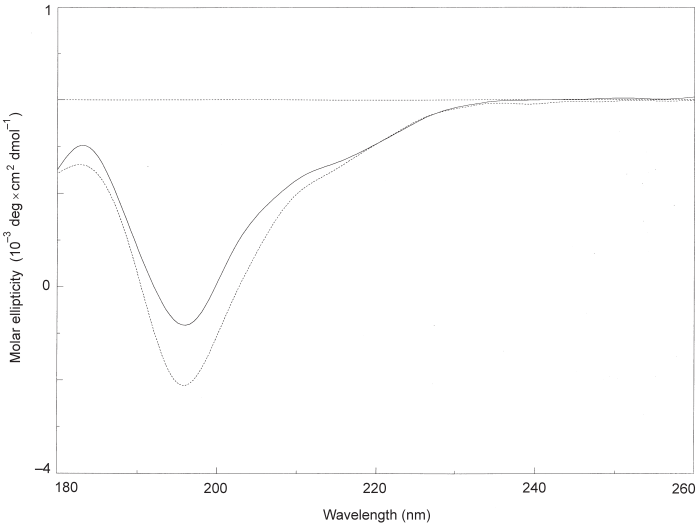

Figure 2. CD spectra of the sturgeon dgαGTH (—) and αGTH (. . .) in phosphate buffer, pH 7.0, 180–260 nm.

Variances of molar ellipticity as in Fig. 1.

Our results indicate that partial selective reduction of the carbohydrate moiety of the sturgeon GTH α-subunit produces a negative effect on both immunoreactivity and specific gonadotropic function of the recombinant heterodimeric molecule dgαGTH+βGTH. The immunoreactivity of the modified recombinant was lowered in comparison with that of the native GTH, while its hormonal function was completely lost (Table 1). As shown by analysis of CD spectra, the partial deglycosylation of the αGTH caused specific conformational changes not only in the modified α-subunit but also in the whole dimer recombinant molecule of the hormone (Fig. 1). Consequently, it may be concluded that these changes in the molecular conformation lead to a decrease in the steric complementarity between the hormone effective groups and membrane receptors, preventing the hormonal signal transduction into the hormone-sensitive test-oocytes.

Table 2. Types of secondary structure of native and modified sturgeon gonadotropic hormone (GTH) preparations

	Structure* (%)				
Preparation	α	β_a	β_p	β_t	R
αGTH	3	38	3	19	36
dgαGTH	3	36	3	17	40
GTH	4	41	2	17	35
αGTH+βGTH	2	43	2	17	34
dgαGTH+βGTH	0	44	0	10	38

*α, α-helix; β_a and β_p, antiparallel and parallel β-strand; β_t, β-turn; R, random coil.

The study showed that the sugar moiety localized on the α-subunit of sturgeon GTH is not an inert component of the hormone molecule but plays an important role stabilizing both its structure and function. The integrity of the sugar moiety is a prerequisite for the fish sturgeon GTH molecular conformation that is required for eliciting of the normal cellular response to the hormone.

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