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A Study of Salmon (*Oncorhynchus tshawytscha*) Pituitary Gonadotropin Dissociation

B. BRETON

Laboratoire de Physiologie des Poissons, INRA, Campus de Beaulieu, 35042 Rennes, France

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Urea (8 M) treatment of salmon gonadotropin results in the dissociation of the hormone into two subunits. These subunits were separated using DEAE–cellulose chromatography and gel filtration on Ultrogel ACA 54, and studied for their molecular weights, electrophoretic, biological, and immunological properties. Molecular weights of the α and β subunits were, respectively, 12,500 and 17,000 as determined by gel filtration and sodium dodecyl sulfate–gel electrophoresis. In anionic polyacrylamide gel electrophoresis, 7.5%, the α subunit, migrated more rapidly ($R_f = 0.67$) than the native hormone s-GtH ($R_f = 0.38$), and the β subunit did not migrate. The biological activities of the α and β subunits were less than 10% of the GtH. Reassociation allowed a 30% recovery of the initial biological activity. The immunological properties studied on two radioimmunological systems specifically directed against s- and cGtH, show that immunological determinants were connected with the β subunit, which also possesses characteristics of zoological specificity.

That the dissociation of glycoprotein hormone into α and β subunits is a general phenomenon in vertebrates has been clearly demonstrated in mammals with LH and FSH (de la Llosa *et al.*, 1967; Ward *et al.*, 1971, Reichert and Ward, 1974) and in reptiles and amphibians (see review by Licht *et al.*, 1977). There are clear indications that this process also exists in fish, but up to now it has only been demonstrated in two species: a teleostean (carp, Burzawa-Gerard, 1974) and a chondrosteian (sturgeon, Burzawa-Gerard *et al.*, 1975). Some results have suggested hormonal dissociation in salmon (Donaldson *et al.*, 1972; Idler *et al.*, 1975; Salesse *et al.*, 1978), but the subunits have never been separated or their properties studied. This paper presents some results on gonadotropin subunits in salmon (*Oncorhynchus tshawytscha*).

MATERIAL AND METHODS

Gonadotropin purification. Salmon pituitary gonadotropin was purified from pituitaries collected at the Springfield Hatchery in Oregon during the spawning season. The glands were dry-frozen as soon as they were collected.

The method of gonadotropin purification has already

been described (Breton *et al.*, 1978). It derives from that described by Idler *et al.* (1975) and includes buffer extraction, glycoprotein separation using affinity chromatography on a concanavalin A Sepharose (2 × 40-cm column), gel filtration on ACA 54 Ultrogel (5 × 100-cm column), ion-exchange chromatography on DEAE–cellulose (1.6 × 20-cm column), and a second gel filtration on ACA 54 Ultrogel. In the present work, we have added another purification step using affinity chromatography on wheat germ lectin (WGL) 6 MB Sepharose (Pharmacia) (0.9 × 10-cm column). This chromatography, which selectively separates glycoprotein containing N-acetyl glucosamine, was performed in a 0.05 M Tris buffer, pH 7.8, containing 1 mM of CaCl₂, MgCl₂, and MnCl₂.

The proteins retained on the WGL were eluted using the same buffer containing 0.1 M N-acetyl glucosamine (Sigma).

Subunit separation. s-GtH (2.24 mg (0.56 mg/ml)) was dialyzed overnight at 4°C against 0.05 M Tris–HCl buffer, pH 7.8. Solid urea was added to the solution up to 8 M, and the solution was left at room temperature for 24 hr. The resulting mixture was deposited on a DEAE–cellulose Bio-Gel A (0.9 × 10 cm) column equilibrated with the Tris buffer and eluted using an ionic strength gradient (60 ml of 0.05 M Tris–60 ml of 0.6 M NaCl) at a flow rate of 4 ml/hr; 1.5-ml fractions were collected.

The retained and nonretained fractions were pooled and concentrated in an Amicon cell using a UM05 Diaflo membrane, then chromatographed on an ACA 54 Ultrogel column 0.9 × 60 cm. Standardization of the column was obtained by running a mixture of bovine

serum albumin fraction V, ovalbumin, chymotrypsinogen, and cytochrome *c* (Boehringer calibration kit size II).

Subunit studies. The biological activity of the products was measured using the *in vitro* trout oocyte maturation assay (Jalabert *et al.*, 1974).

All the fractions were studied for their electrophoretic properties in 7.5% polyacrylamide gel electrophoresis at pH 9.1 according to Ornstein (1964) and in sodium dodecyl sulfate–gel electrophoresis standardized with the same protein mixture as the ACA 54 Ultrogel column. All the fractions were deposited in the same quantities on the gels (75 μ g).

The subunits were reassociated by mixing equal quantities (0.5 mg) of the two DEAE–cellulose chromatographic fractions in 0.05 M phosphate buffer, pH 7.6, for 1 hr at a final concentration of 1 mg/ml.

We also studied the immunological properties of s-GtH and its subunits by competition in two specific radioimmunological assays developed for carp (Breton *et al.*, 1971) and trout (Breton *et al.*, 1975) GtH. Using covariance analysis (Snedecor and Cochran, 1957), we compared the slope of the displacement curves for each compound to those obtained with the hormone reference, and we also determined the dose which induced 50% of binding inhibition.

RESULTS

Biochemical properties. Affinity chromatography on the WGL of the s-GtH preparation showed a 2.25-fold increase in biological activity. Before this chromatography, polyacrylamide gel electrophoresis revealed one major ($R_f = 0.38$) and one minor ($R_f = 0.44$) strip. This strip which represented about 5% of the total protein deposited disappeared after WGL chromatography. After 8 M urea treatment, the major strip remained thin, and a more electropositive component ($R_f = 0.67$) appeared.

DEAE–cellulose ion-exchange chromatography of the 8 M urea-treated s-GtH gave two peaks: a first nonadsorbed peak (1) and a second peak (2) eluted at 0.13 M ionic strength. With the same chromatographic conditions, the native s-GtH was eluted at about 0.1 M.

The two DEAE–cellulose chromatography components run separately on the same ACA 54 column (0.9 $\times$ 60 cm) gave only one peak whose K_d corresponded to an ap-

parently equivalent molecular weight of between 16, and 17,000. Peak 1 of these components revealed only one strip with polyacrylamide gel electrophoresis ($R_f = 0.67$); peak 2 did not migrate under our electrophoretic conditions. When compared with the results obtained on carp GtH (Burzawa-Gerard, 1974), the first peak would correspond to the α -sGtH subunit and the second to the β subunit.

Sodium dodecyl sulfate electrophoresis showed the molecular weight of the α and the β subunits to be 12,500 and 17,000, respectively.

Polyacrylamide gel electrophoresis of the α - and β -subunit mixture presented two strips of equal intensity, corresponding to those of the native hormone and the α subunit. This result indicates incomplete recombination. The percentage of recombination was not determined.

Biological and immunological properties. Table 1 shows the biological activity of the native hormone and its dissociation and recombination products. The results are expressed as the dose needed to induce 50% of maturation in the *in vitro* trout oocyte maturation assay.

The α and β subunits did not present a significant biological activity (5.5 and 8.4%, respectively, of the native sGtH). After recombination, only 30% of the biological activity was regenerated.

The competition curves of sGtH and [125 I]cGtH binding to their specific antibodies against the native hormone and its subunits are shown on Figs. 1 and 2. In the salmonid model, the slopes of these curves are not significantly different for native sGtH, β subunit, and the mixture of α and β subunits (Table 2). On the contrary, the α -subunit curve was not parallel as compared to that of the native hormone. The immunological activity expressed by the dose inducing a 50% inhibition was the same for sGtH, β -sGtH, and $\alpha + \beta$ -sGtH.

In the carp RIA, no curves were parallel to those obtained with the standard cGtH,

TABLE 1
EFFICIENT DOSE (D_{50}) WHICH INDUCED 50% OF
TROUT OOCYTE MATURATION *IN VITRO* MEANS AND
95% CONFIDENCE LIMITS IN ng/ml

sGtH	α -sGtH	β -sGtH	$\alpha + \beta$ -sGtH
36	661	433	125
40	727	476	132
45	798	524	147

although β -sGtH gave a value of 0.812, similar to the reference slope which was 1.115 (Table 2). The immunological activities of the salmon hormone preparations were also very low (Table 2).

DISCUSSION

Unlike Idler (Idler and Ng, 1979), we found that salmon GtH can be retained on an immobilized lectin, such as wheat germ lectin. This resulted in the increase, by more than twofold, of the biological activity.

Our results concerning urea salmon GtH dissociation confirm those obtained on carp (Burzawa-Gerard, 1974) and sturgeon

(Burzawa-Gerard *et al.*, 1975) GtH. Dissociation of sGtH can be obtained by 8 M urea treatment. Physicochemical and biological properties of the products obtained after dissociation indicated that they correspond to α and β sGtH subunits similar to those described in two other species of fishes (Burzawa-Gerard *et al.*, 1975). The estimated molecular weight of α -sGtH was about 12,500 and that of β -sGtH between 16, and 17,000. As in cGtH (Jolles *et al.*, 1977), the molecular weight of the β subunit was higher than that of the α subunit. In the same species, Donaldson *et al.* (1972) reported a molecular weight of 12,500 for sGtH after an acidic treatment which induced dissociation. Our findings are close to these values but gel filtration did not allow detection of any difference between the molecular weights of the α and β components. However, a more sensitive technique, such as SDS-gel electrophoresis, could.

In the present study, the electrophoretic properties were also similar to those of

FIG. 1. Displacement curves obtained on trout RIA system. Samples were assayed in triplicate.

FIG. 2. Displacement curves obtained on carp RIA system.

cGtH where only the α subunits migrate in the anion system; α and β aci-GtH subunits, however, would behave very much alike in such a system (Burzawa-Gerard *et al.*, 1975, 1976). As is the case for gonadotropins in mammals (Papkoff, 1972), amphibians, reptiles (Licht *et al.*, 1977), and fish, such as carp and sturgeon (Burzawa-

Gerard *et al.*, 1975), the biological activity of the s-GtH subunits was very low, while that of the β -sGtH subunit was slightly higher than that of the α -sGtH. After incubation, we recovered only 30% of the initial biological activity; this value was lower than that obtained in carp and aci-GtH. In fact, we cannot relate this recovery to the

TABLE 2
SLOPES OF INHIBITION CURVES ELICITED BY sGtH AND ITS SUBUNITS IN TWO RIA SYSTEMS

Trout RIA system			Carp RIA system	
Product	Slope of the displacement curves	D ₅₀ ^a (ng)	Slope of the displacement curves	D ₅₀ (ng)
sGtH	-0.896	0.32	-0.409**	<5
α -sGtH	-0.447**	<5	0**	<5
β -sGtH	-0.768 N.S. ^b	0.40	-0.812**	<5
$\alpha + \beta$ -sGtH	-0.829 N.S.	0.42		
cGtH	-0.309**	<5	-1.115	0.38

^a D₅₀, dose which inhibited 50% of binding of the labeled hormone to its specific antibody.

^b N.S., not significant.

***P* < 0.005.

reassociation rate, which was not determined. Instead, we can assume that it was incomplete since electrophoresis of this recombination showed two strips corresponding to native and α -sGtH. In cGtH, the reassociation constant is about 160-fold more rapid than that of mammalian LH (Marchelidon *et al.*, 1978, 1974), whereas in sGtH, it is about 5- to 10-fold faster than in oLH (Salesse *et al.*, 1978). Thus, sGtH seems to be more stable than cGtH which dissociated spontaneously (Burzawa-Gerard, 1973). The biological activity of sGtH was retained for 6 months in a solution (Donaldson *et al.*, 1978). It is possible that this stability is related to slower dissociation-association kinetics than in carp. The 1-hr incubation time for sGtH subunit reassociation would not be sufficient to allow total recombination. In addition, we mixed the same quantity of the two subunits (whose molecular weights were different), inducing an excess of one of them, and we did not allow complete recombination.

Immunological results on the carp RIA system confirm our earlier findings concerning the immunological specificity of fish gonadotropins (Breton *et al.*, 1973). This specificity is related to the β subunit as in mammals (Kerdelhue *et al.*, 1972) and in carp (Burzawa-Gerard and Kerdelhue, 1978). The cross-reaction between the β -sGtH and antibody directed against cGtH seems to indicate that when the GtH's from different species have some common antigenic determinant, they are probably associated with the β -subunit. The same is true for carp and β -pLH (Dufour *et al.*, 1979). The α subunit would be more closely related to the zoological specificity.

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