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Zinc Binding to Human Lactogenic Hormones and the Human Prolactin Receptor

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Abstract

Zinc half sites are present in all human lactogenic hormones: human prolactin (hPRL), growth hormone (hGH), placental lactogens (hPL) and the hPRL receptor (hPRLr). The influence of Zn²⁺ as measured by intrinsic fluorescence or FRET in each of these hormones is unique and is affected by the presence of varying stoichiometries of hPRLr. These data show that both Zn²⁺ and hPRLr binding influence hPRL conformers in an interdependent fashion. Although each of these three lactogenic hormones bind hPRLr and induce a biological response that is sensitive to the presence of increasing concentrations of Zn²⁺, each hormone is unique in the mechanistic details of this process.

Keywords

Human; prolactin; growth hormone; placental lactogen; prolactin receptor; zinc; fluorescence; FRET; conformation change

1.0 Introduction

We have previously shown that three structurally similar hormones: human prolactin (hPRL), growth hormone (hGH), and placental lactogen (hPL), undergo a conformation change when binding the extracellular domain of the hPRLreceptor (hPRLr) [1]. Divalent zinc (Zn²⁺) binds a half-site in hPRL, hGH), and hPL [1–3] as well as a second half-site in hPRLr. Depending on the measured end point (cellular binding, site 1 binding, biological activity), μ M concentrations of Zn²⁺ dramatically change hGH binding to the hPRL receptor

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[1, 4, 5] but has little or no effect on hGH binding to the hGH receptor. Zn^{2+} is required for hPL binding to the hPRLr [3]. Finally, the binding of hPRL to hPRLr is modestly diminished by the presence of Zn^{2+} [1].

Zn^{2+} binding will link helices 1 and 4 restricting movements to conformations either more or less favorable for hPRLr binding or biological activity. Binding hPRLr at site 1 of each hormone provides a second half site for Zn^{2+} and may influence Zn^{2+} binding or the stability of the protein complex. In this work we investigate the comparative effects of the presence or absence of Zn^{2+} on hPRL, hGH, hPL, and hPRLr and the interactions of these hormones with hPRLr by intrinsic fluorescence, Förster resonance energy transfer (FRET), and *in vivo* cellular assays in order to describe the effects of Zn^{2+} on hormone structure and function.

2.0 Materials and Methods

2.1 Preparation of Expression Vectors

The pT7-7 phagemid expression vectors for methionyl hPRL, hGH and the extracellular domain of the human hPRL receptor (hPRLr, residues 1–224) were prepared as described [1]. hPL cDNA from the I.M.A.G.E. Consortium (LLNL) cDNA Clones [6] was purchased from Open Biosystems (Huntsville, AL) and cloned into pET-28b(+) (Novagen, EMD Chemicals, Darmstadt, Germany). The vector was cut with Nco I and Hind III, removing the vector's histidine tag and a portion of the poly-cloning site. The hPL cDNA was amplified by PCR using a primer that added a methionyl codon immediately to the N-terminus of the mature protein and a second primer that added a Hind III site 3' to the stop codon of hPL. The PCR product was characterized, double digested with Nco I and Hind III, and ligated into the linearized and modified pET28b(+) plasmid. The hPL expression vector was expanded in XL-10 Gold cells (Stratagene, Cedar Creek, TX) selected by kanamycin resistance, purified, and stored in water at 4°C.

2.2 Mutagenesis

Mutations in the hormones were performed either by the method of Kunkel [7] or by the quick change method (Stratagene)[8]. M158C hPRL, N152C hGH, and N152C hPL by procedures described in [1]. We refer to the N-terminal methionyl residue as residue 0, so the sequence numbering corresponds to that of mature proteins.

2.3 Protein Expression and Purification

Expression of the various proteins was performed in BL20 (DE3) *E. coli* (Novagen). All proteins were expressed as described by Peterson et al. [9] and Voorhees and Brooks [1]. Protein concentrations were determined by the bicinchoninic assay using bovine albumin as a standard [10] or by the calculated molar extinction coefficients ($\epsilon_{280\text{ nm}}$)[11]: hPRL = 21,805 $\text{cm}^{-1}\text{M}^{-1}$ hGH = 17,670 $\text{cm}^{-1}\text{M}^{-1}$ hPL = 17,670 $\text{cm}^{-1}\text{M}^{-1}$ and ex-hPRLr = 66,140 $\text{cm}^{-1}\text{M}^{-1}$.

2.4 Labeling of hormones with CPM or OG

Hormones (hPRL M158C, hGH N152C, and hPL N152C) containing a free cysteine distal from either the Zn²⁺ or receptor binding sites were labeled with 7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin (CPM, Cat# D-346) or 2'-7'-difluorofluorescein-5-malamide (OG, Cat# O-6034)(Invitrogen, Carlsbad, CA) as previously described [1]. CPM-labeled proteins were used within 24 to 48 hours.

2.5 Measurement of intrinsic fluorescence

One μM concentrations of wild-type hPRL, hGH, hPL, or hPRLr were prepared in 150 mM NaCl, 10 mM Tris, pH 7.4 with varying concentrations (0–40 μM) of ZnSO₄ or Na₂SO₄ and allowed to reach equilibrium by incubating in darkness at room temperature for one hour. Samples were illuminated at 295 nm using a Varian-Cary Eclipse fluorescence spectrophotometer (Varian, Inc., Palo Alto, CA) and emission spectra recorded from 300–450 nm [12].

2.6 Zinc titration of various hormone/hPRLr stoichiometries determined by FRET

Unlabeled wildtype and either CPM- or OG-labeled hormones in 10 mM Tris, pH 7.4, and 150 mM NaCl were mixed to obtain 1 μM concentrations and a fluorescence intensities appropriate for FRET or control studies, respectively. Varying concentrations (0–25 μM) of either ZnSO₄ or Na₂SO₄ were added to the proteins and allowed to bind for 1 hour in the dark at room temperature. Additional sets of CPM-labeled hormones with increasing Zn²⁺ concentrations were combined with various concentrations of hPRLr to obtain 1:0.2, 1:1, and 1:5 ratios. Samples were illuminated at 295 nm using a Varian-Cary Eclipse fluorescence spectrophotometer and emission spectra recorded from 300–550 nm. Finally control experiments binding OG-labeled hormones with or without 15 μM Zn²⁺ were performed to determine the effects on the fluorescence within the vicinity of the extrinsic OG fluochrome independent of FRET coupling.

2.7 Cellular assays for lactogenic activity

FDC-P1 cells stably expressing the human prolactin receptor were provided by Genentech, Inc. (San Francisco, CA) and maintained as previously described [2]. Hormone dose-response studies were performed as previously described [13] using a vital dye method (Alamar Blue, Biomed, Cleveland, OH) [14].

2.8 TPEN Toxicity

FDC-P1 cells were maintained as described and supplemented with no supplement or with 2 μM N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) (Invitrogen)(15). Cells were stained with Trypan Blue at 0, 24, 36, and 48 hours and hemocytometric cell counts recorded total cell count as well living versus dead cells.

Each experiment was performed at least three times. Each experimental replicate provided consistent similar results.

3.0 Results

3.1 Characterization of recombinant proteins

Wild-type and mutant hPRL, hGH, hPL, and wild-type hPRLr were prepared. Each protein was characterized by SDS-containing polyacrylamide gel electrophoresis under reducing conditions and was noted to be >95% homogeneous and run at the appropriate molecular weight (data not shown).

3.2 Effect of Zn²⁺ on the intrinsic fluorescence of hPRL, hGH, hPL, and hPRLr

The addition of Zn²⁺ to hPRL increased the fluorescence intensity at 340 nm (Figure 1). These spectra indicate that the binding of Zn²⁺ to hPRL induces a significant change in the environment of one or both tryptophans and are consistent with changes in hPRL's conformation. Parallel titrations with Na₂SO₄ showed no changes in fluorescence, thus the change of hPRL fluorescence is attributed to the presence of Zn²⁺. In contrast, ZnSO₄ titrations performed with either hGH or hPL showed little or no change in the intrinsic fluorescence when compared to control Na₂SO₄ titrations. These data provide no evidence of a zinc-induced conformation change but do not rule out conformation changes. Finally, although hPRLr contains nine tryptophans, including two tryptophans close to the receptor's Zn²⁺ binding site (D187 and H188), Zn²⁺ titration did not change the intrinsic fluorescence of hPRLr. This observation suggests that Zn²⁺ concentrations up to 40 μM did not influence the conformation of the extracellular domain of the hPRL receptor. The two adjacent residues comprising the receptor's Zn²⁺ half site are unlikely to induce a conformation change because binding does not bring distant residues into proximity inducing significant structural change.

3.3 Effect of Zn²⁺ on FRET of hPRL, hGH, and hPL

In the absence of Zn²⁺ increased ratios of hPRLr to hormone produced increases in FRET (Figures 2 and Supplemental Figure 1). The pattern of each hormones' hPRLr-mediated increased FRET signal was unique suggesting distinct differences in the receptor binding mechanisms for each hormone.

Zn²⁺ titration in the absence of hPRLr increased hPRL FRET (Figures 2 and 3, Supplemental Table 1); an indication that on average Zn²⁺ binding brought the tryptophans closer to the CPM. Neither hGH nor hPL showed changes in FRET when titrated with Zn²⁺ in the absence of hPRLr (Figure 3 left column, Table 1). In addition titrations with up to Na₂SO₄ did not change the FRET signal for any of the three hormones. These results provide a conclusion similar to that of intrinsic fluorescence measurements; hPRL undergoes a Zn²⁺-induced change in conformation while these methods do not provide evidence of a similar change for either hGH or hPL despite a demonstrated Zn²⁺-dependent change in function.

When hormone/hPRLr complexes were formed with various stoichiometries and titrated with Zn²⁺ the FRET signals for each hormone were unique (Figures 2 and Supplemental Figure 2). These results suggested that binding of both Zn²⁺ and hPRLr influenced each hormone's conformation in a complex fashion and were unique for each hormone. In the

case of hPRL the Zn^{2+} -dependent FRET signal is increased by up to 1:1 hPRLr/hPRL stoichiometries. At a 1:5 stoichiometry the Zn^{2+} -dependent FRET signal is reduced. These changes suggest that the influence of Zn^{2+} on the conformation of hPRL is not only changed by the first hPRLr binding but is further changed (in an opposite direction) by the binding of the second hPRLr.

When hGH bound either equivalent or excess stoichiometries of hPRLr, Zn^{2+} induced an increase in hGH FRET signal up to a 1:1 stoichiometry, but higher stoichiometries produced little further increases in FRET. These results suggest that in the absence of receptor, Zn^{2+} does not change the conformation of hGH, but when site 1 is bound by hPRLr, Zn^{2+} influences hGH's conformation as evidenced by tryptophan approaching the CPM reporter. This change is associated with an increased affinity of hGH for hPRLr at site 1 [1]. Binding hPRLr at site 2 is not associated with further changes in Zn^{2+} -induced hGH FRET.

Finally, Zn^{2+} -dependent changes in the hPL FRET signal were not influenced by the presence of up to equal molar stoichiometries of hPRLr, but when higher hPRLr stoichiometries (1:5, hPL:hPRLr) were present titration of Zn^{2+} produces a modest increase in the FRET signal indicating a Zn^{2+} -dependent change in hPL conformation when bound by hPRLr concentrations (Figures 2 and Supplemental Figure 2) able to saturate both sites 1 and 2.

The lactogens might provide a false-positive FRET signal if Zn^{2+} binding altered the environment surrounding the extrinsic fluoro-chrome and changed the efficiency of the CPM reporter. We covalently linked OG, a non-FRET fluoro-chrome, to the same location as we had previously linked the CPM reporter. When OG replaced CPM the ability of tryptophan to transfer energy to OG was eliminated (data not shown). Thus, OG fluorescence served as a sensitive probe for changes in the extrinsic fluoro-chrome's environment. Fluorescence spectra (Supplemental Figure 3) collected for each lactogen showed a similar pattern where the presence of 15 μM Zn^{2+} produced a modest *decrease* in OG fluorescence, an effect opposite to the Zn^{2+} -induced *increase* in CPM fluorescence observed in our FRET experiments. These results indicate that the small negative changes in the environment of the extrinsic fluoro-chrome could not account for the larger positive changes observed in FRET experiments, but the Zn^{2+} -dependent local quenching will influence FRET making a quantitative analysis ambiguous.

3.4 Biological Assays

FDC-P1 cells transfected with the human prolactin receptor were assayed without ($\approx 1 \mu M$ endogenous Zn^{2+}) [26, 27] or with the addition of 15 μM $ZnSO_4$ (Figure 3, Table 1). The addition of 15 μM Zn^{2+} did not influence the ED_{50} of the hPRL bioassay. In contrast, the addition of Zn^{2+} to the hGH bioassay increased the biological activity of hGH by reducing the ED_{50} by 5-fold. The ED_{50} for hGH is several-fold less than that of hPRL. Finally, the supplementation of the endogenous Zn^{2+} with 15 μM Zn^{2+} reduced the hPL ED_{50} 2-fold. The ED_{50} s of hPL was between 16- and 33-fold greater than that of hPRL.

We attempted to reduce free Zn^{2+} concentrations by addition of TPEN, a high affinity chelator of Zn^{2+} [15]. Addition of 2 μM TPEN effectively eliminated endogenous Zn^{2+} .

Exposure to this concentration of TPEN for up to 48 hours did not affect cell viability as determined by trypan blue dye exclusion or by the cells ability to replicate (data not shown). But when hPRL-stimulated cellular proliferation was measured by the Alamar Blue vital dye method the basal rate of resazurin reduction [14] was reduced and hPRL failed to effect cellular proliferation despite our knowledge that Zn^{2+} is not required for hPRL binding to the hPRLr (Figure 3). Zn^{2+} is critical to numerous cellular processes and it appears that treatment of cells with TPEN removes the intracellular pool of Zn^{2+} leaving the cells unable to respond to lactogenic hormones. Based on these results we could not determine the effects of Zn^{2+} reduction on *in vivo* lactogen action.

4.0 Discussion

hPRL receptor binding kinetics for each of the three human lactogenic hormones previously was shown to be influenced by Zn^{2+} [1] and suggests that Zn^{2+} binding may induce changes in the conformation of each lactogen. Data presented in our current work confirms the hypothesis that Zn^{2+} induces conformational changes in each of these hormones and that these changes are unique for each lactogenic hormone and influenced by hPRLr binding. A linked relationship of Zn^{2+} and hPRLr binding to each of the three lactogenic hormones is not surprising when one considers that the Zn^{2+} binding site is shared between the hormone and hPRL receptor.

In contrast to hPRL, neither intrinsic fluorescence nor FRET *in the absence of hPRLr* revealed a Zn^{2+} -dependent conformation change in either hGH or hPL. These observations do not rule out Zn^{2+} -induced conformation changes. FRET studies of hGH or hPL in the presence of hPRLr indicated that Zn^{2+} was associated with conformation changes. Functional studies show that Zn^{2+} changes both receptor binding [3–5] and biological activities [16] of hGH and hPL, this may indicate that the effect of Zn^{2+} is only apparent when both the hormone and receptor half sites are present or that the geometry of CPM and the single tryptophan in hGH and hPL does not allow observation of a Zn^{2+} -induced conformation change. We investigated this idea by hormone-based FRET assays where Zn^{2+} titrations were performed with various stoichiometries of hPRLr. We observed that each of the three lactogenic hormones showed a unique Zn^{2+} -induced change in their FRET signal with sub-saturating or saturating stoichiometries of hPRLr.

When one considers the role that Zn^{2+} may play in the mechanism of lactogen binding and activation of the hPRL receptor a complex set of reactions need to be considered. *In vivo* Zn^{2+} may bind each of the lactogens, the hPRL receptor, and each lactogen/receptor complexes. Although the lactogens' half site binds Zn^{2+} with micromolar affinities [2, 17], the metal affinities for either the hPRLr half site or lactogen/receptor complexes have not been determined. Thus, additional affinities will need to be measured to understand Zn^{2+} 's role. In target tissues the concentration of extracellular Zn^{2+} is largely determined by the serum concentration. The total serum Zn^{2+} concentration is believed to be approximately 15 μ M [18], but the free zinc concentration is believed to be in the sub-nanomole range [19]. Our Zn^{2+} titrations for both hormones and for hormone/hPRLr complexes were performed in the μ M range and showed a graded response in this concentration range. This suggests that lactogenic hormones in blood and extracellular fluid may have few molecules bound by

zinc. The affinities of the lactogen/receptor complexes for Zn^{2+} may be much stronger than the μM affinity observed for the lactogens' half site and may represent the species where biologically significant Zn^{2+} binding occurs. Alternatively, in the absence of sufficient Zn^{2+} concentrations to allow hPRL receptor binding, hGH may be restricted to actions through the hGH receptor. Micromolar Zn^{2+} concentrations promote association with hormones in secretory granules [20, 21]. Perhaps the primary role of Zn^{2+} is to package and store these hormones in secretory granules [22].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations

hPRL	Human prolactin
hGH	human growth hormone
hPL	human placental lactogen
hPRLr	extracellular domain of the human prolactin receptor
Zn^{2+}	divalent zinc
FRET	Förster Resonance Energy Transfer
CPM	7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin
OG	Oregon Green; 2',7'-difluorofluorescein
TPEN	N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine
DTT	dithiothreitol

References

- Voorhees JL, Brooks CL. Obligate ordered binding of human lactogenic cytokines. *J. Biol. Chem.* 2010; 285:20022–20030. [PubMed: 20427283]
- Sun Z, Li PS, Dannies PS, Lee JC. Properties of human prolactin (PRL) and H27A-PRL, a mutant that does not bind zinc. *Mol. Endocrinol.* 1996; 10:265–271. [PubMed: 8833655]
- Walsh STR, Kossiakoff AA. Crystal structure and site 1 binding energetics of human placental lactogen. *J. Mol. Biol.* 2006; 358:773–784. [PubMed: 16546209]
- Somers W, Ultsch M, De Vos AM, Kossiakoff AA. The X-ray structure of a growth hormone-prolactin receptor complex. *Nature.* 1994; 372:478–481. [PubMed: 7984244]
- Cunningham BC, Bass S, Fuh G, Wells JA. Zinc mediation of the binding of human growth hormone to the human prolactin receptor. *Science.* 1990; 250:1709–1712. [PubMed: 2270485]
- Lennon GG, Auffray C, Polymeropoulos M, Soares MB. The I.M.A.G.E. consortium: an integrated molecular analysis of genomes and their expression. *Genomics.* 1996; 33:151–152. [PubMed: 8617505]
- Kunkel TA, Bebenek K, McClary J. Efficient site-directed mutagenesis using uracil-containing DNA. *Meth. Enzymol.* 1991; 204:125–139. [PubMed: 1943776]

8. Wang W, Malcolm BA. Two-stage PCR protocol allowing introduction of multiple mutations, deletions and insertions using QuikChange™ site-directed mutagenesis. *BioTechniques*. 1999; 26:680–682. [PubMed: 10343905]
9. Peterson FC, Anderson PJ, Berliner LJ, Brooks CL. Expression, folding, and characterization of small proteins with increasing disulfide complexity by a pT7-7-derived phagemid. *Protein Expression Purif*. 1999; 15:16–23.
10. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NK, Olson BJ, Klenk DC. Measurement of protein using bicinchoninic acid. *Anal Biochem*. 1985; 150:76–85. [PubMed: 3843705]
11. Pace CN, Vajdos F, Fee L, Grimsley G, Gray T. How to measure and predict the molar absorption coefficient of a protein. *Protein Sci*. 1995; 4:2111–2423.
12. Lakowicz, JR. Principles of fluorescence spectroscopy. 3rd. Springer; 2006.
13. Duda KM, Brooks CL. Human growth hormone site 2 lactogenic activity requires a distant tyrosine 164. *FEBS Lett*. 1999; 449:120–124. [PubMed: 10338116]
14. O'Brien J, Wilson I, Pognan F, Orton T. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Europ J Biochem*. 2000; 267:5421–5426. [PubMed: 10951200]
15. Arslan P, Di Virigilio F, Beltrame M, Tsien RY, Pozzan T. Cytosolic Ca²⁺ homeostasis in Ehrlich and Yoshida carcinomas. A new, membrane-permeant chelator of heavy metals reveals that these ascites tumor cell lines have normal cytosolic free Ca²⁺. *J. Biol. Chem*. 1985; 260:2719–2727. [PubMed: 3919006]
16. Dattani MT, Hindmarsh PC, Brooks CGD, Robinson ICAF, Kopchick JJ, Marshall NJ. G120R, a human growth hormone antagonist, shows zinc-dependent agonist and antagonist activity on Nb2 cells. *J. Biol. Chem*. 1995; 270:9222–9226. [PubMed: 7721840]
17. Permyakov EA, Veprintsiv DB, Deikus GY, Permyakov SE, Kalinichenko LP, Grishchinko VM, Brooks CL. pH-induced transition and Zn²⁺-binding properties of bovine prolactin. *FEBS Lett*. 1997; 405:273–276. [PubMed: 9108303]
18. Cousins, RJ. Present knowledge in nutrition. Bowmen, BA.; Russell, RM., editors. Washington, DC: International Life Sciences Inst; 2006. p. 445-457.
19. Magneson GR, Puvathingal JM, Ray WJ. The concentrations of free Mg²⁺ and free Zn²⁺ in equine plasma blood. *J. Biol. Chem*. 1987; 262:11140–11145. [PubMed: 2956262]
20. Greenan J, Lorenson MY, Conconi MV, Walker AM. Alterations in *in situ* prolactin secretory granule morphology and immunoreactivity by thiols and divalent cations. *Endocrinol*. 1990; 126:512–518.
21. Lorenson MY, Patel T, Liu J-W, Walker AM. Prolactin (PRL) is a zinc-binding protein. Zinc interactions with monomeric PRL and divalent cation protection of intragranular PRL cysteine thiols. *Endocrinol*. 1996; 137:809–816.
22. Keeler C, Hodsdon ME, Dannies PS. Is there structural specificity in the reversible protein aggregates that are stored in secretory granules? *J. Mol. Neurosci*. 2004; 22:43–49. [PubMed: 14742909]

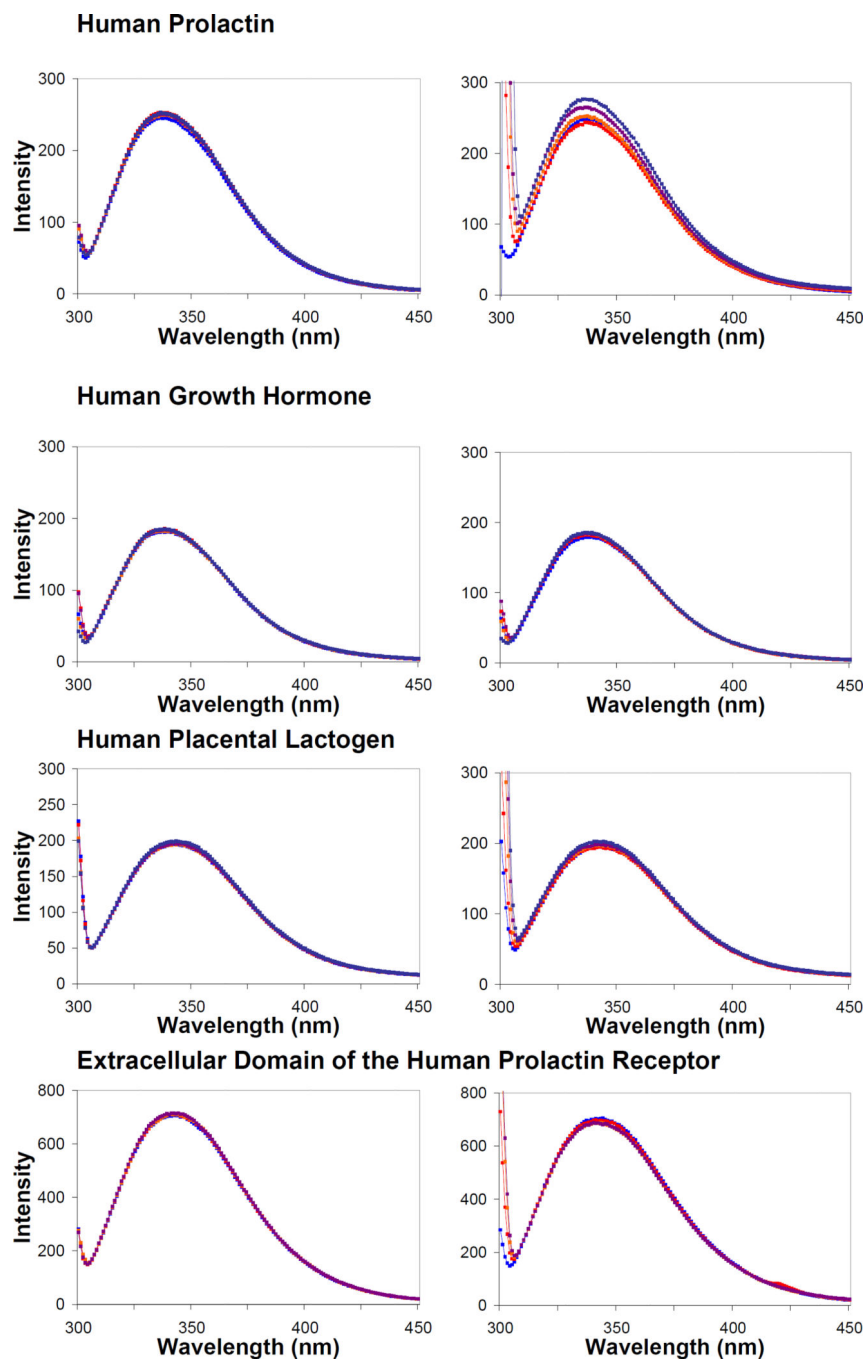


Figure 1. Zn^{2+} dependence of intrinsic fluorescence of lactogenic hormones and the extracellular domain of the hPRL receptor. Wild-type hPRL (row A), hGH (row B), hPL (row C), or hPRLr (row D). One μM proteins in 150 mM NaCl, 10 mM Tris, pH 7.4 and varying concentrations (0–40 μM) of Na_2SO_4 (left column) or ZnSO_4 (right column) were excited at 295 nm and emissions recorded between 300–450 nm. One of three independent experiments.

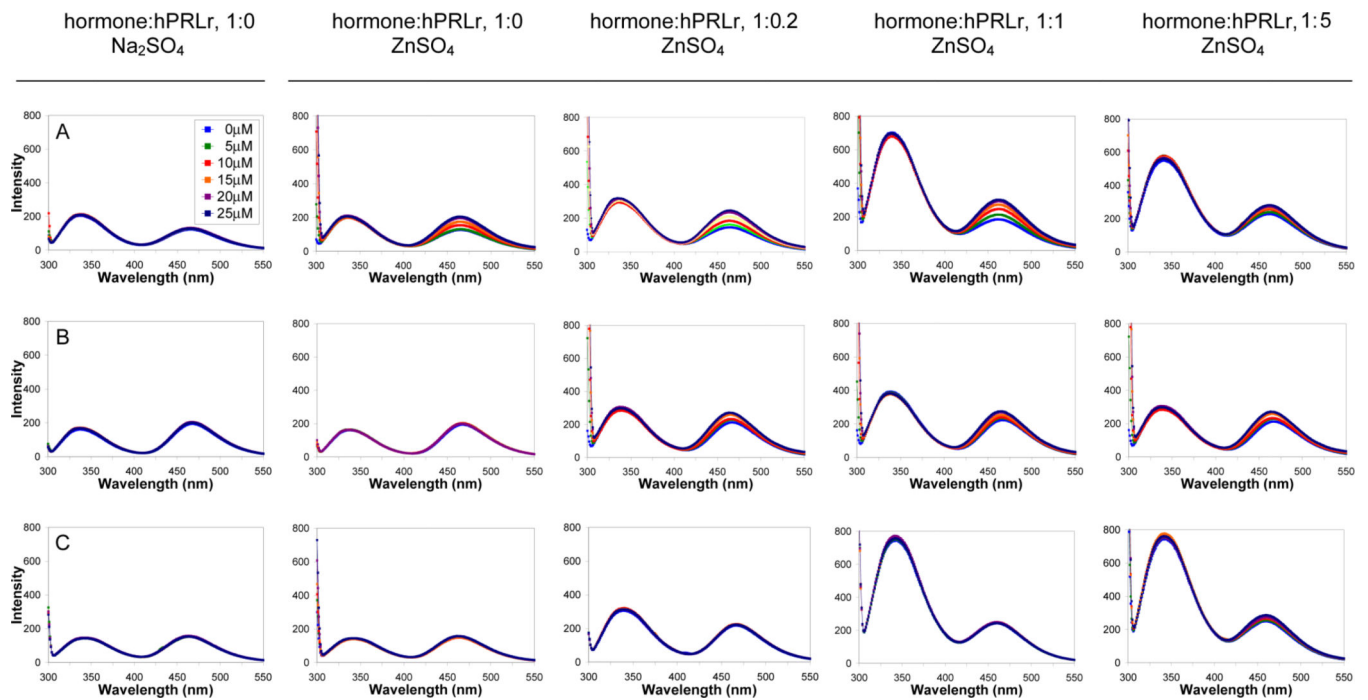


Figure 2.

Zn^{2+} -dependence of FRET spectra from lactogenic hormones in the absence or presence of hPRLr. hPRL (row A), hGH (row B), and hPL (row C) solutions were prepared in 150 mM NaCl, 10 mM Tris, pH 7.4 with varying concentrations of Na_2SO_4 (left column) or $ZnSO_4$ (0 to 25 μ M, right columns) and increasing stoichiometries of hPRLr (1:0, 1:0.2, 1:1, and 1:5), excitation 295 nm and emission 300–550 nm. One of three independent experiments.

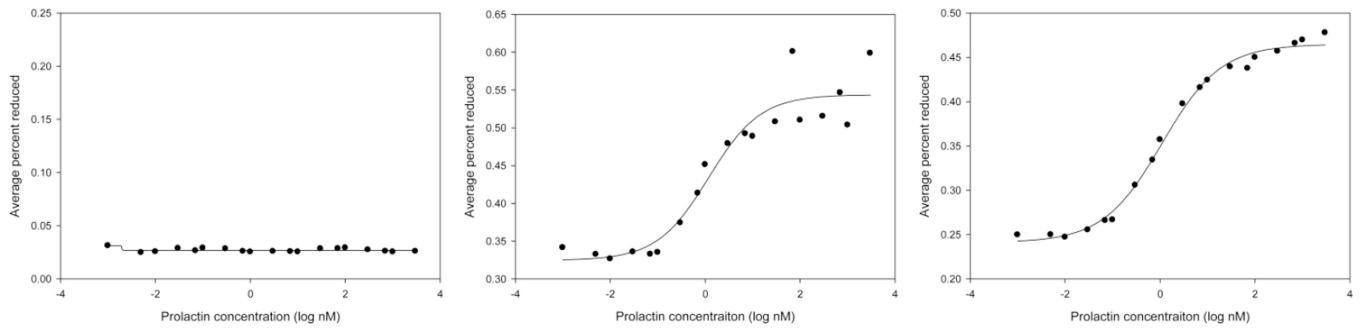


Figure 3.

Effect of increased Zn^{2+} concentrations on the biological activity of human prolactin. Biological assays assessing the cytokine-induced response were performed in the presence of increasing concentrations of hPRL in media supplemented with 2 μM TPEN (left), no supplementation (center), or 15 μM $ZnSO_4$ supplementation (right). One of two independent experiments. Significant changes in ED_{50} s are achieved with a 2 to 3 fold change in value.

Table 1ED₅₀ for lactogen-stimulation of growth with varying concentrations of Zn²⁺

	<u>Zn²⁺ Concentrations</u>			<u>Zn²⁺-dependent increase in ED₅₀</u> (1 μM to 16 μM)
	<u>0 μM</u>	<u>1 μM</u>	<u>16 μM</u>	
hPRL	N/A *	0.85 nM	0.81 nM	0.95
hGH	N/A *	0.33 nM	0.06 nM	5.50
hPL	N/A *	26.68 nM	10.10 nM	2.64

* not applicable, no cell growth observed without Zn²⁺

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