

## Point mutation of serine 179 in the human Prolactin (PRL) affects recombinant protein expression, folding and secretion, abolishes PRL nickel (II)-binding and increases heparin binding capacities

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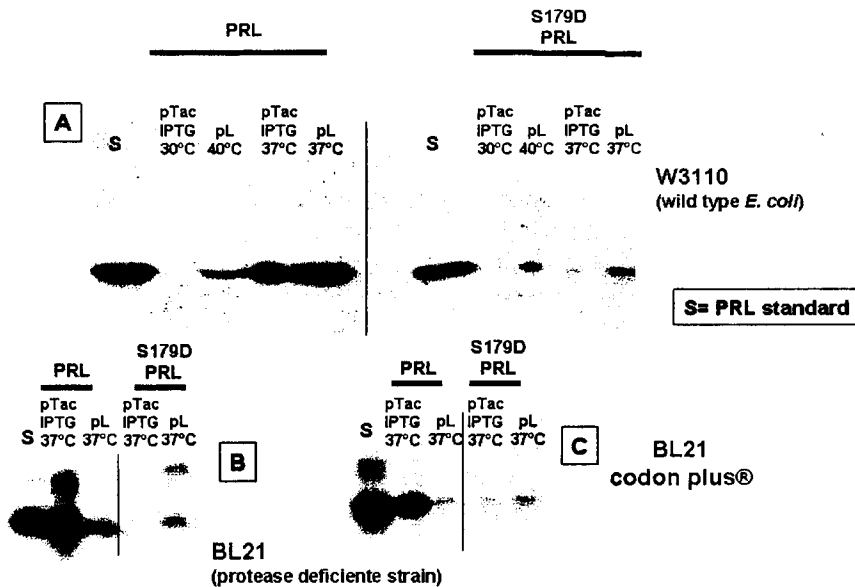
### Background

S179D prolactin (S179D PRL) is a pseudophosphorylated form of human prolactin (PRL) which has inhibitory effects on tumor growth [1] and angiogenesis [2]. The S179D PRL preparations used for these experiments consisted of properly refolded inclusion bodies (IB) from *Escherichia coli* [3]. Trying to attain a better folded mutant, we used secretion expression based systems. However, single point mutations can affect protein periplasmic expression [4], and secretion from mammalian cells [5]. We observed that upon a mutation of Serine 179 to an Aspartate, expression was nearly abolished when compared with PRL in *E. coli* periplasm, while the cytoplasmic product was more prone to proteolysis. Using eukaryotic cells we were able to produce preparations comparable to IBs in terms of bioactivity. We also demonstrated that this mutant had a higher affinity for heparin and lower binding capacity towards divalent metals (M(II)).

### Results

S179D PRL periplasmic expression was very low when compared to PRL. Use of different promoters, different signal peptides or different activation temperatures had no effect (figure 1).

Figure 1

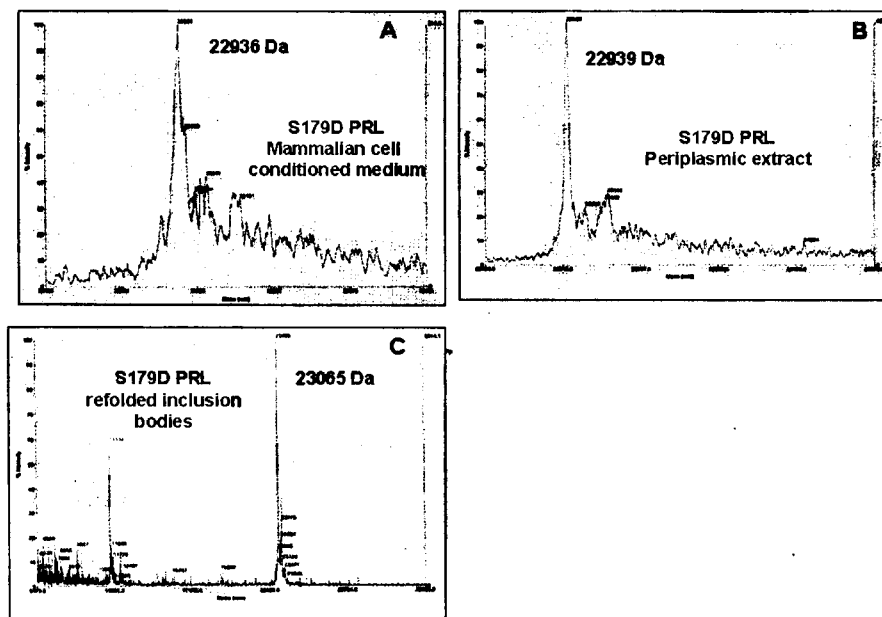


A, B and C, Immunoblots of periplasmic extracts.

MALDI-TOF spectrometry was carried out for identity of S179D PRL in the extracts (figure 2).

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Figure 2



Molecular masses determined by MALDI-TOF-MS.

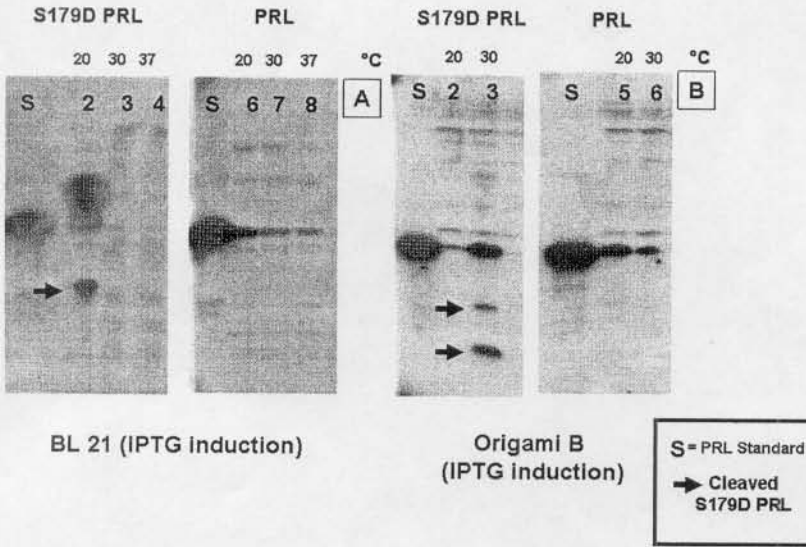
BL21 strain was used (figure 1B) without improvements for S179D PRL expression (table 2).

**Table 1.** Protein expression yield ( $\mu\text{g}/\text{mL}/\text{OD}$ ) and final optical densities ( $\text{OD}_{600}$ ) of different strains with pL promoter.

	<i>E. coli</i> strain	Protein yield ( $\mu\text{g}/\text{mL}/\text{OD}$ )	Final $\text{OD}_{600}$
PRL	W3110	$1.3 \pm 0.2$	$4.0 \pm 0.3$
	BL21	$1.9 \pm 0.4$	$1.3 \pm 0.2$
	BL21 codon plus	$1.4 \pm 0.3$	$1.0 \pm 0.2$
S179D PRL	W3110	$0.34 \pm 0.03$	$3.8 \pm 0.6$
	BL21	$0.35 \pm 0.5$	$1.3 \pm 0.5$
	BL21 codon plus	$0.40 \pm 0.3$	$1.2 \pm 0.1$

We used BL 21 codon plus® in order to investigate the GC-, AT-rich sequence of the PRLs influence on expression. This strain did not rescue expression of S179D PRL or PRL (figure 1C). pTac induction at lower temperatures should encourage protein solubility and folding in the cytoplasm [6]. We carried out cytoplasmic expression with an Origami B strain, in which cytoplasm folding is facilitated [7]. Surprisingly, when S179D PRL was produced in soluble form, unlike PRL, low molecular forms were observed (figure 3A and 3B), and also in BL21, cleaved forms and soluble high molecular aggregates were present (figure 3A). pL constructs had very low yields for both PRLs (figure 3).

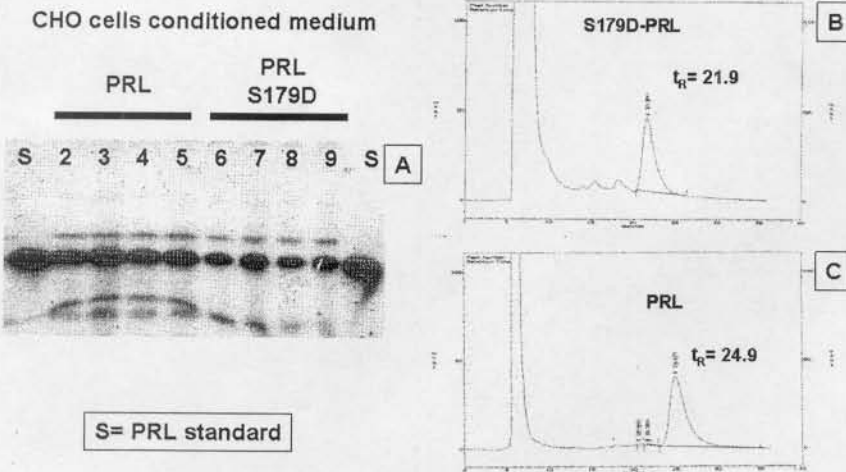
Figure 3



Immunoblots of soluble fractions of *E. coli* lysates.

An eukaryotic expression system was chosen to successfully produce soluble, monomeric, recombinant S179D PRL.

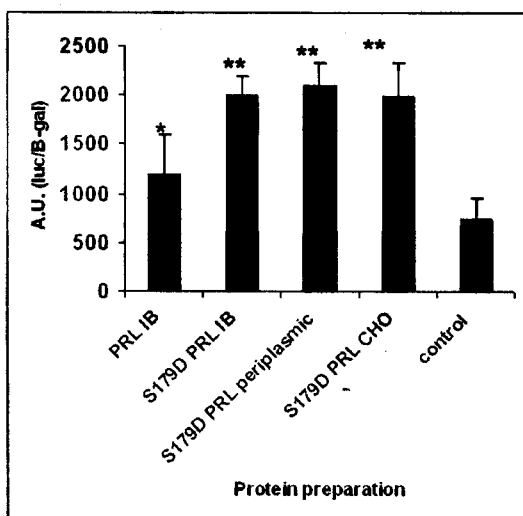
Figure 4



Immunoblot of conditioned medium. B,C, RP-HPLC analysis.

B-casein bioassays were carried out to check S179D PRL folding. (figure 5).

Figure 5



β-casein bioassay. \*  $p < 0.05$  versus control; \*\*  $p < 0.01$  versus control. AU, arbitrary unit.

Moreover S179D PRL had a decreased affinity towards Ni (II) and Zn (II). On the other hand it had an increased affinity towards heparin.

### Conclusions

We tried to produce a correctly folded form of S179D PRL, already obtained as refolded IBs [3]. Unexpectedly, this point mutation of PRL impaired protein expression, and was not related to the strain, protease degradation of our protein, or preferential codon usage (figure 1). To avoid proteolysis and misfolding we used lower temperatures during protein production [8], but it failed to produce S179D PRL. Low levels of S179D PRL were only detected by immunoblots (figure 1) and by immunoassay (table 1). Expression of soluble S179D PRL in the cytoplasm of *E. coli* was not efficient either, as denoted by soluble aggregates and cleaved S179D PRLs. Eukaryotic expression systems have a better folding machinery, being difficult-to-fold proteins more easily expressed [9]. Thus, we successfully produced S179D PRL at RP-HPLC detectable levels (figure 4). MALDI-TOF analysis showed that all samples had the expected molecular weight (figure 2). RP-HPLC demonstrated that S179D PRL had a different folding than PRL. The bioactivity assay showed that all preparations of S179D PRL were correctly folded. S179D PRL also showed physical-chemical differences, having a lower M (II)-affinity and a higher heparin-affinity. This confirms reports of PRL mutants with low Zn (II) affinity that are poorly secreted [4] and also could account for its anti-angiogenic effect [2,10].

### References

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