Microbial Cell Factories

Poster Presentation

Open Access

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

Eric Ueda[1], Carlos Soares*[1], Ameae Walker[2] and Paolo Bartolini[1]

Address: [1]Biotechnology Department, IPEN-CNEN, Cidade Universitaria, Sao Paulo, Brazil and [2]Division of Biomedical Sciences, The University of California, Riverside, CA, 92521, USA

* Corresponding author

from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

Microbial Cell Factories 2006, **5**(Suppl 1):P17 doi:10.1186/1475-2859-5-S1-P17

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).

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

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

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).

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

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

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

Conclusion

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

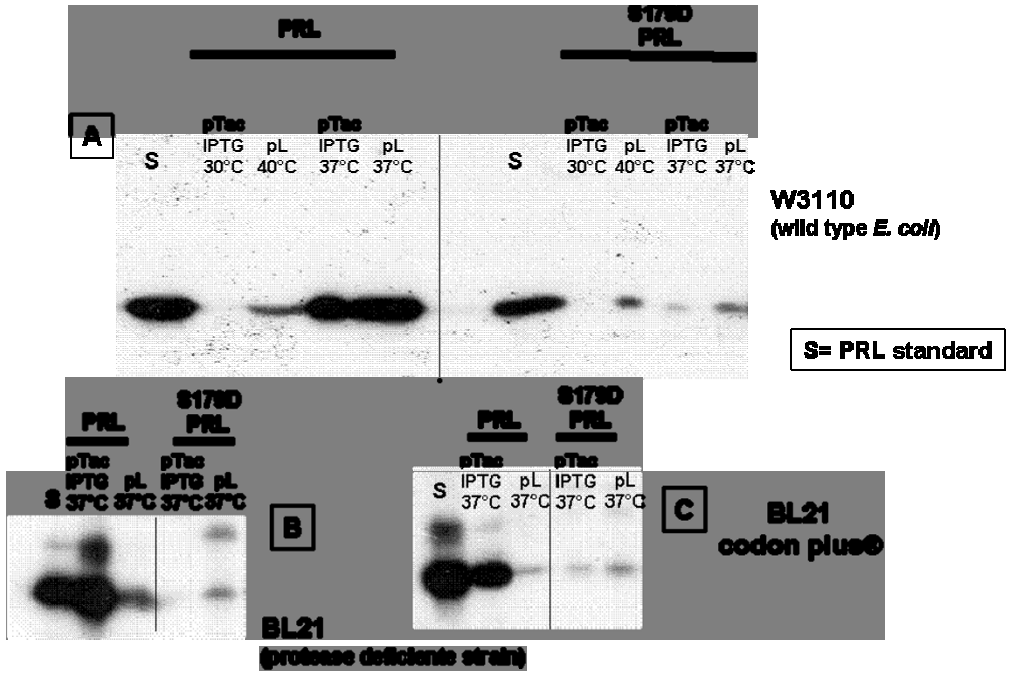

Figure 1
A, B and C, Immunoblots of periplasmic extracts.

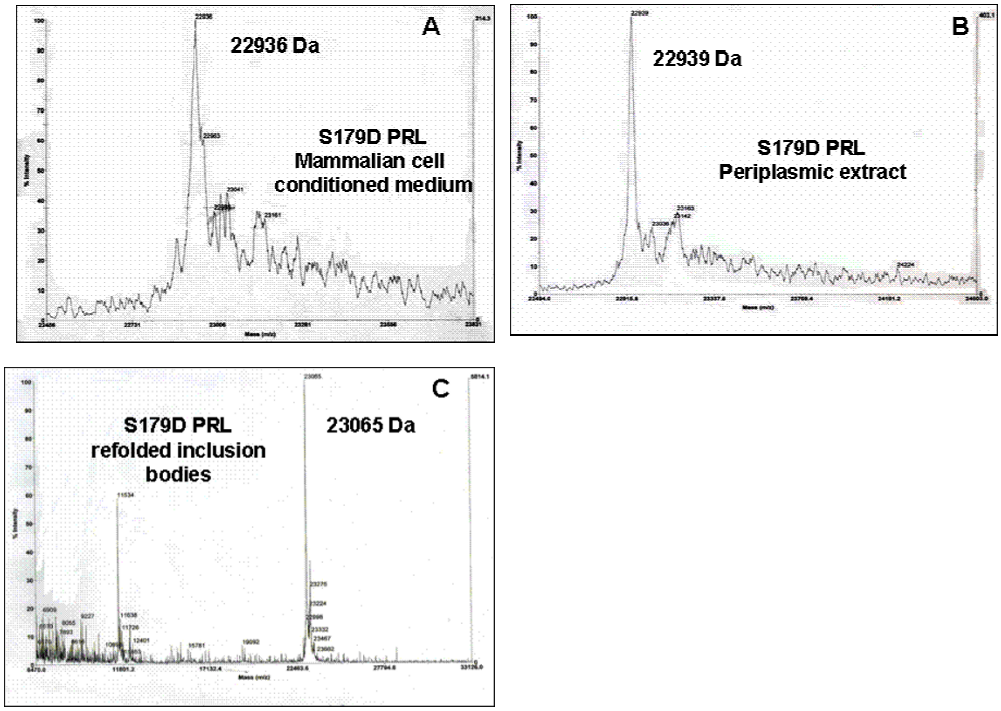

Figure 2
Molecular masses determined by MALDI-TOF-MS.

Table 1: Protein expression yield (μg/mL/OD) and final optical densities (OD_{600}) of different strains with pL promoter.

	*E. coli*strain	Protein yield (μg/mL/OD)	Final OD_{600}
PRL	W3110	1.3 ± 0.2	4.0 ± 0.3
	BL21	1.9 ± 0.4	1.3 ± 0.2
	BL21 codon plus	1.4 ± 0.3	1.0 ± 0.2
S179D PRL	W3110	0.34 ± 0.03	3.8 ± 0.6
	BL21	0.35 ± 0.5	1.3 ± 0.5
	BL21 codon plus	0.40 ± 0.3	1.2 ± 0.1

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].

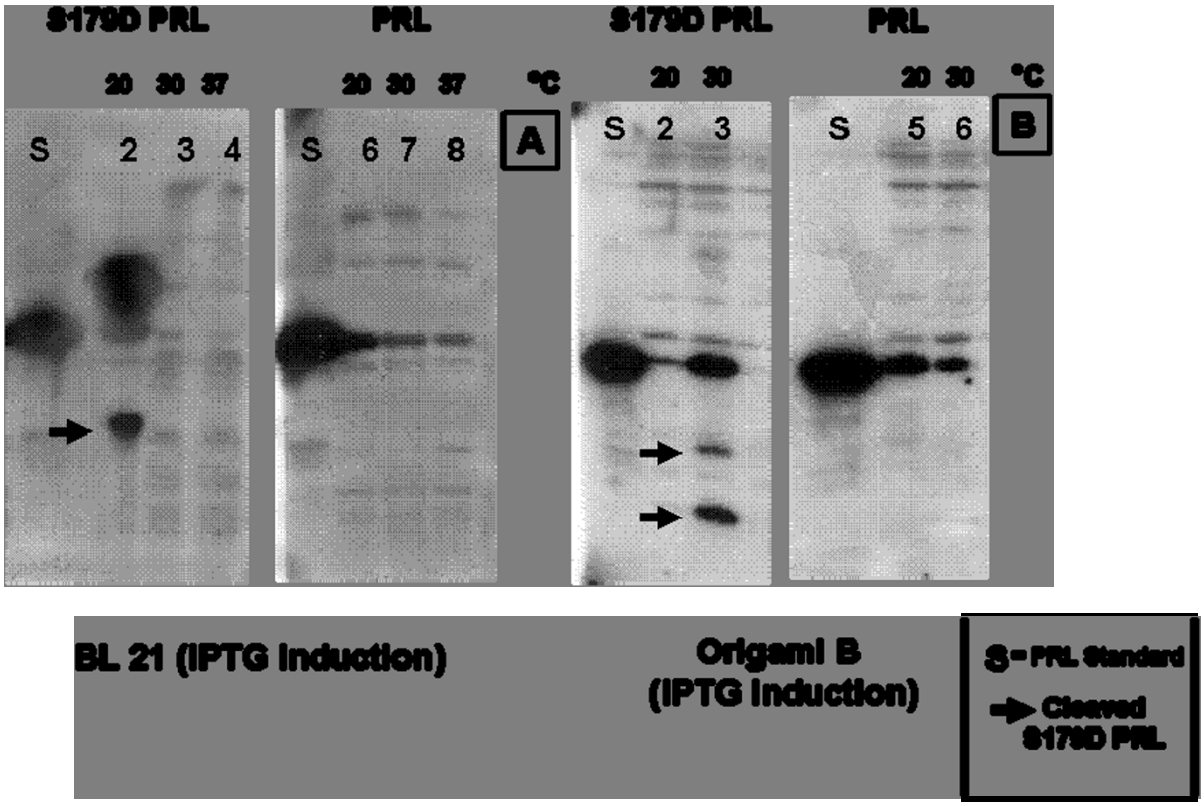

Figure 3
Immunoblots of soluble fractions of *E. coli* lysates.

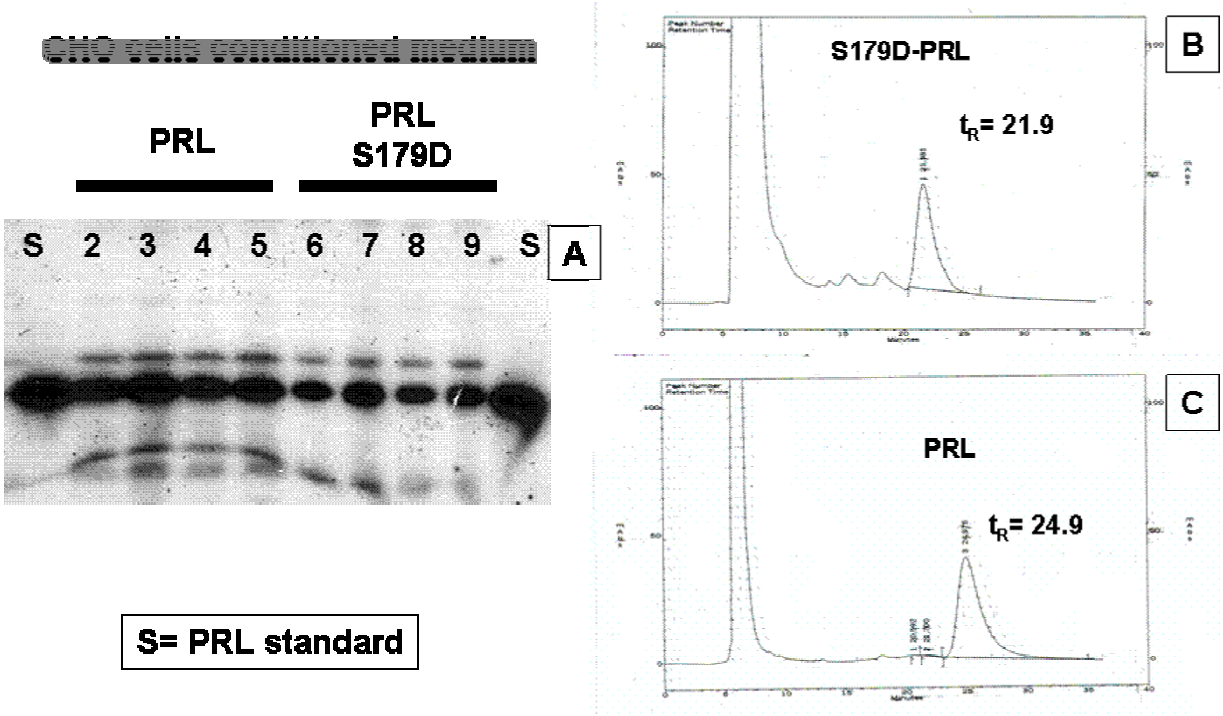

Figure 4
Immunoblot of conditioned medium. **B, C,** RP-HPLC analysis.

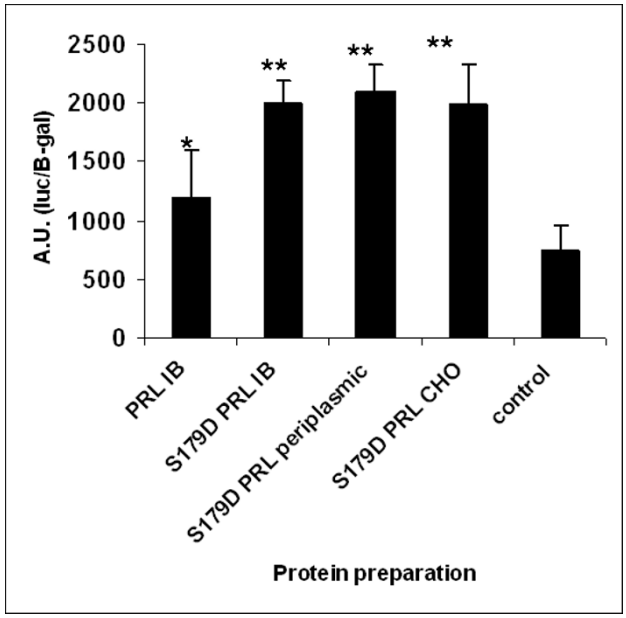

Figure 5
β-casein bioassay. * $p < 0.05$ versus control; ** $p < 0.01$ versus control. AU, arbitrary unit.

References

1. Xu X, Wu W, Williams V, Khong A, Chen YH, Deng C, Walker AM: **Opposite effects of unmodified prolactin and a molecular mimic of phosphorylated prolactin on morphology and the expression of prostate specific genes in the normal rat prostate.** *Prostate* 2003, **54:**25-33.
2. Ueda E, Ozerdem U, Chen YH, Yao M, Huang KT, Sun H, Martins-Green M, Bartolini P, Walker AM: **A molecular mimic demonstrates that phosphorylated human prolactin is a potent anti-angiogenic hormone.** *Endocr Relat Cancer* 2006, **13:**95-111.
3. Chen TJ, Kuo CB, Tsai KF, Liu JW, Chen DY, Walker AM: **Development of recombinant human prolactin receptor antagonists by molecular mimicry of the phosphorylated hormone.** *Endocrinology* 1998, **139:**609-16.
4. Duenas M, Ayala M, Vazquez J, Ohlin M, Soderlind E, Borrebaeck CA, Gavilondo JV: **A point mutation in a murine immunoglobulin V-region strongly influences the antibody yield in *Escherichia coli* .** *Gene* 1995, **158:**61-6.
5. Sun Z, Lee MS, Rhee HK, Arrandale JM, Dannies PS: **Inefficient secretion of human H27A-prolactin, a mutant that does not bind Zn2+.** *Mol Endocrinol* 1997, **11:**1544-51.
6. Reyes LF, Sommer CA, Beltramini LM, Henrique-Silva F: **Expression, purification, and structural analysis of (HIS)UBE2G2 (human ubiquitin-conjugating enzyme).** *Protein Expr Purif* 2006, **45:**324-8.
7. Bessette PH, Aslund F, Beckwith J, Georgiou G: **Efficient folding of proteins with multiple disulfide bonds in the *Escherichia coli* cytoplasm.** *Proc Natl Acad Sci USA* 1999, **96:**13703-8.
8. Makrides SC: **Strategies for achieving high-level expression of genes in *Escherichia coli* .** *Microbiol Rev* 1996, **60:**512-38.
9. Ellgaard L, Helenius A: **Quality control in the endoplasmic reticulum.** *Nat Rev Mol Cell Biol* 2003, **4:**181-91.
10. Ricard-Blum S, Feraud O, Lortat-Jacob H, Rencurosi A, Fukai N, Dkhissi F, Vittet D, Imberty A, Olsen BR, van der Rest M: **Characterization of endostatin binding to heparin and heparan sulfate by surface plasmon resonance and molecular modeling: role of divalent cations.** *J Biol Chem* 2004, **279:**2927-2936.