Single step recombinant human follicle stimulating hormone purification by peptide affinity chromatography

Juan M. Gurevich Messina¹, Silvana L. Giudicessi¹, Mara C. Martinez-Ceron¹, Nicols Urtasun¹, Guillermina Forno²,
Laura Mauro², Osvaldo Cascone¹, Silvia A. Camperi¹

¹Universidad de Buenos Aires. FFyB. Cát. Biotecnología. CONICET-UBA, Inst. NANOBIOTEC. Junín 956, 1113, Bs As., Argentina
²R&D Zelltek S.A., UNL, FBCB, Ciudad Universitaria, Paraje el Pozo, CC 242, Santa Fe, Argentina

<https://doi.org/10.17952/35EPS.2018.127>

Introduction

Human Follicle Stimulating Hormone (hFSH) is clinically used for ovulation in women and spermatogenesis induction in men, in assisted reproduction technologies (1). As FSH-based biopharmaceuticals are parenterally administered, their purity must be high. Current methods for hFSH purification include several chromatographic steps to reach the required purity. However, these involve a decrease in the hFSH total yield and rises the cost of the process. Affinity chromatography (AC) consists in the specific adsorption of target biomolecules onto ligands immobilized on chromatographic supports. Short peptides have been described as useful ligands for AC because of their low cost, simple chemical synthesis and high stability compared to protein-based ligands (2). The aim of this work was to design an affinity chromatography matrix with an immobilized synthetic peptide for rhFSH purification.

Methods

In a previous work, Sohn *et al.* (3) examined the hFSH receptor interaction with the hormone, testing each amino acid of the exoloop 3 by Ala substitution. Taking into account those works, the mutant with greater affinity: (580)KVPLITVSKAK(590) was selected to design a synthetic ligand for affinity chromatography (AC): Ac-KVPLTVSKAKVAC-NH₂. The peptide was synthesized as amide and was acetylated. The peptide was acetylated to increase its stability against possible attack by proteases present in the crude FSH sample. A Cys was incorporated at the C-termini to facilitate its subsequent immobilization to the chromatographic activated SulfoLink agarose resin. A sample of crude rhFSH was loaded to the peptide affinity column using as adsorption and elution buffers: 20 mM sodium phosphate, 0.5 mM Met, pH 5.6 and 7.2 respectively.

Results and Discussion

The affinity chromatography support with the peptide ligand immobilized was evaluated by loading crude samples composed of host cell proteins from the Chinese hamster ovary (CHO) cell and rhFSH (Fig. 1). A non-reducing SDS-PAGE was done to check rhFSH purification (Fig. 2). The purity obtained after AC purification was 94 % and the yield was 41% (Table 1). The highly glycosylated isoforms, which have the highest *in vivo* potency, were recovered. The identity of the protein band obtained in the SDS-PAGE was checked by peptide mapping analysis using LC-MS/MS. The peptide affinity column was overloaded, and the dynamic capacity obtained was 54.6 mg rhFSH/mL chromatographic resin.

Figure 1: Chromatography of crude samples with host cell proteins and rhFSH using the affinity matrix with the peptide Ac-KVPLTVSKAKVAC-NH₂ immobilized. The mobile phases were: a) adsorption buffer (20 mM sodium phosphate, 0.5 mM Met, pH 5.6); b) elution buffer (20 mM sodium phosphate, 0.5 mM Met, pH 7.2) and c) regeneration buffer (20 mM sodium phosphate, pH 7.2, 2 M NaCl). The arrows indicate the buffer change. The rhFSH eluted when the elution buffer was added.

Table 1: Purification chart of a crude sample of rhFSH with the host cell proteins after chromatography with the affinity matrix with Ac-KVPLTVSKAKVAC-NH₂ immobilized.

Sample	Volume (mL)	Conc. rhFSH (mg/mL)	Total protein (mg/mL)	Purity % (mg/mg)	Fold purification	Yield (%)
Crude sample	17	0.727	1.203	60.4	-	-
Eluate	6	0.845	0.899	94.0	1.56	41

Figure 2: Non-reducing SDS-PAGE of the chromatographic step to check rhFSH purification. Left: Coomassie Blue staining. Right: Silver staining. Lane 1) protein molecular weight marker; Lane 2) standard of pure rhFSH; Lane 3) crude sample of rhFSH; Lane 4) washing fractions; Lane 5) elution fraction; Lane 6) regeneration fraction.

After its purification, rhFSH quality was analyzed. The percentage of oxidized rhFSH was 3.4 % and the percentage of free subunits was 1.2 %, both within the range established by the European Pharmacopeia as also were the sialic acid content and the isoforms profile.

Figure 3: IEF gel for isoforms profile. 1) rhFSH standard; 2) sample of rhFSH purified by AC

Conclusions

A new affinity chromatography matrix with an immobilized peptide was synthesized for rhFSH purification. The method here designed allows obtaining a high quality rhFSH using a low-cost affinity matrix based on a short peptide ligand. The isoforms with high content of sialic acid were recovered by this new chromatographic step. These isoforms have higher plasma half-life and hence higher in-vivo potency. Furthermore, the physicochemical analysis complied with the limits established in European Pharmacopoeia for follitropin containing samples. These results evidence that affinity chromatography with the ligand peptide A c-KVPLTVSKAKV AC-NH₂ is a promising strategy for rhFSH purification from CHO crude extracts. The invention has been presented to the National Institute of Industrial Property, Argentina (Act INPI: No. 2017010281, 2017).

Acknowledgements

S.L.G, M.C.M.C, N.U., O.C. and S.A.C. are researchers of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). This work was partially supported by the Universidad de Buenos Aires (20020170100030B A), the Ministerio de Ciencia, Tecnología e Innovación Productiva de la República Argentina (PICT-2014-1508) and the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (PIP 11220130100119CO).

References

1. Smits J, Wolfenson C, Chappel S, Ruman J. *Reprod. Sci.* 2016; 23: 706-716. DOI: 10.1177/1933719115607992
2. Camperi SA, Martínez-Ceron MC, Giudicessi SL, Marani MM, Albericio F, Cascone O. *En: Methods Mol Biol.* 2014;1129:277-302. DOI: 10.1007/978-1-62703-977-2_22.
3. Sohn J, Ryu K, Sievert G, Jeoung M, Ji I, Ji TH. Follicle-stimulating hormone interacts with exoloop 3 of the receptor. *J Biol Chem.* 2002 Dec 20;277(51):50165-75. DOI 10.1074/jbc.M207646200.