

AN FT-IR APPROACH TO HUMAN GRANULOSA CELLS

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1. Introduction

Endometriosis is a common, benign, chronic, gynaecological disease that affects approximately 10% of women of reproductive age, with up to 50% of those being infertile. The association of endometriosis with infertility is unequivocal; however, the mechanisms involved remain unclear. Some studies evidenced a clear involvement of the granulosa cells, somatic cells surrounding the oocyte, which undergo relevant molecular and metabolic changes. All these changes seem to worsen oocyte quality affording to sterility. In fact, the relationship between the oocyte and its granulosa cells is complex and represents a determining factor of later developmental competence. The aim of this study is to evaluate how endometriosis affects macromolecular composition and metabolism of these cells.

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2. Results and Discussion

A significant number of granulosa cells, achieved from idiopathic (n. 5 samples) and endometriotic (n. 7 samples) patients, were deposited on silicon supports for IR transmission analysis. For all the samples, the microphotographs were first obtained, on which the chemical maps were acquired. All the spectral data were submitted to multivariate analysis, affording for each cluster a representative spectrum. The vibrational analysis evidenced different spectral profiles for the two experimental groups, above all in the proteic pattern, in carbohydrate content and in nucleic acids composition (Figure 1).

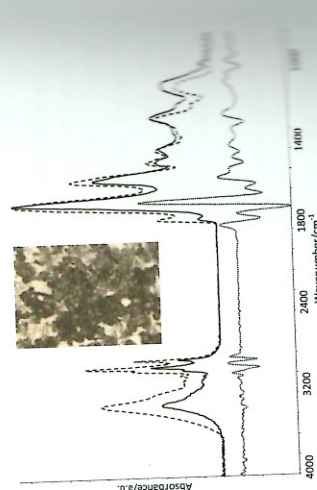


Figure 1. Representative spectra of granulosa cells from idiopathic and endometriotic patients and related difference spectrum.

Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajeir N, Agarwal A, Fert Ster 2008; 90: 247-257
McKenzie LJ, Pangas SA, Carson SA, Kovanci E, et al. Hum Reprod, 2004; 19: 2869-74.
Carnevali O, Conti C, Ferraris P, Garavaglia MG, Giocchini G, Giorgini E, Rubini C, Sabbatini S, Tosi G, J Endocrinol, 2009; 938: 207-213.

STRUCTURE AND DYNAMICS OF SS-BOND STABILIZED MINIPROTEINS

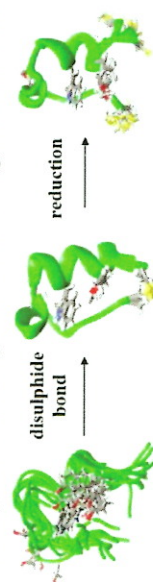
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Exendin-4 (Ex-4) is a short (39 aa.) but folded polypeptide used recently to treat Type 2 diabetes^[1]. The C-terminus of Ex-4 (20-39) is called as the "Trp-cage" foldamer supposed to stabilize the central α -helix (10-28) of the macromolecule, which secondary structural element plays a key role in binding to GLP1 receptor at a nanomolar affinity. We have recently designed and synthesized new disulphide-bond stabilized Trp-cage analogues to reduce their internal mobility and to promote their crystallization. Homo- and heteronuclear NMR spectroscopic methods were used to decipher structural and dynamics changes of disulphide bond containing variants as function of their chemical composition and state. Both the oxidized and the reduced forms of H2 and H5 were characterized and compared to the parent H2 and H5 miniproteins.

As long as H2 is largely unfolded, presenting high internal dynamics on the ns-ps time scale of motion, H5 has a nice fold easy to characterize with a single 3D structure in a time average manner. Thus, the introduction of a smartly placed disulphide bridge could induce larger structural changes and stabilization for the less folded H2 then for the more folded H5 nanosystem. Following the resonance assignment and NOE based

characterization of both the reduced and oxidized forms of H2_{SS} and H5_{SS} we have found cysteine residues of both H2_{SS} and H5_{SS} stabilize the



molecules even in their reduced forms. Thus, the interaction of Cys results in an increased structural stability even via their SH groups. These findings are also supported by MD simulations. Local structure stabilizing effects such as H-bond between the SH and the C-terminal carboxyl group could play a role. These H-bonds with additional structure stabilizing effects could have a role in modulating backbone dynamics of H5_{SS}(red) so that specific slow dynamics appear. Fast (ns-ps) and slow (ms) backbone motions detected by R_1 , R_2 , hetNOE and CPMG measurements of the present nano-system will be presented.

[1] Neidigh et al., *Biochemistry*, 40, 13188-13200, 2001.

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