Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Biotechnology 121 (2006) 442–447
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Biochemical properties of C78SC96S rhFGF-2: A double point-mutated rhFGF-2 increases obviously its activity

Ju Wang [a,*], An Hong [a,**], Jin-song Ren [b], Fen-Yong Sun [a], Ying-jiao Shi [a], Kan Liu [a], Qiu-Ling Xie [a], Yun Dai [a], Zhi-ying Li [a], Yu Chen [c]

[a] *Bio-Engineering Institute of Jinan University, Guangzhou 510632, PR China*
[b] *Changchun Institute of Applied Chemistry, Chinese Academy of Science, Changchun 130022, PR China*
[c] *Biochemistry Department of Chinese University, Hong Kong, SAR, China*

Received 9 March 2005; received in revised form 16 August 2005; accepted 24 August 2005

Abstract

Fibroblast growth factor-2 (FGF-2) is a multifunctional polypeptide that affects many cellular functions and phenomena. The wild-type recombinant human fibroblast growth factor rhFGF-2_W and the mutant C78SC96S rhFGF-2_M were expressed in *Escherichia coli* and their products were purified. The results by the means of fluorescence spectroscopy and CD spectrums, suggested that due to its decreased hydrophobicity rhFGF-2 is not deposited as an inclusion body. The mitogenic activity of the expressed rhFGF-2_M on 3T3 fibroblasts was shown to be 10-fold more than the expressed rhFGF-2_W of which the biological activity was a little less than that of the standard rhbFGF$_W$, indicating that the increased biological activity was due to the change of its secondary structure, dimerization and affinity binding to FGF receptor (FGFR).

Keywords: rhFGF-2; Mutation; Fluorescence spectroscopy; Mitogenic activity

1. Introduction

Human fibroblast growth factor-2 (hFGF-2) belongs to the FGF family synthesized by a variety of different cell types. It is a general regulatory mediator of proliferation, migration and differentiation for endothelial cells and for other cells of mesodermal and neuroectodermal origin (Ornitz and Itoh, 2001; Nugent and Iozzo, 2000; Bikfalvi et al., 1997). The wound-healing activity of hFGF-2 renders it a potential therapeutic agent of industrial importance.

Abbreviations: CD, circular dichroism; FGFR, fibroblast growth factor receptor; rhFGF-2_M, mutant recombinant human fibroblast growth factor; rhFGF-2_W, wild recombinant human fibroblast growth factor

* Corresponding author. Tel.: +86 20 85221983; fax: +86 20 85226616.
** Corresponding author.
E-mail addresses: wangju1688@163.com (J. Wang), gz_hongan@163.com (A. Hong).

0168-1656/$ – see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.jbiotec.2005.08.021

The hFGF-2 is a 18 kDa polypeptide that its overall structure can be described as a trigonal pyramid which is constituted all by β-sheets. Despite the presence of four cysteines, there is no intra-molecular disulphide bond present in native hFGF-2. Cys78 and Cys96 (numbering refers to the 155-amino acid form of hFGF-2) are localized on the surface of hFGF-2, whereas Cys34 is completely buried and Cys101 is partly buried within the folded peptide chain (Eriksson et al., 1993). In light of the key role that hFGF-2 plays in both physiological and pathological conditions, structural–functional relationship studies on this factor are of large interest. Its dimerization and binding to high affinity receptors are considered to be necessary steps to induce FGF receptor (FGFR) phosphorylation and signaling activation. Several reports investigated functionally relevant regions of FGF-2 by analyzing crystallographic structures or by mutagenesis studies (Safran et al., 2000; Herr et al., 1997; Venkataraman et al., 1996). FGF-2 dimer has not been crystallized, and different studies based on molecular simulations indicated different regions possibly lying at the FGF-2 dimer interface.

We report here that the mutant C78SC96S rhFGF-2 expressed in *Escherichia coli* has a high biological activity by 10-fold compared with the wild rhFGF-2 as an initial probe to understand the mechanism of structural–functional relationship between key amino acids (Cys-78 and Cys-96), hydrophobicity, secondary structure and mitogenic activity. This finding of the high activity by double-Cys point-mutation imparts great importance to planning in industrial endeavors.

2. Materials and methods

2.1. Materials

Restriction enzyme *Bam*H I, *Bgl* II, *Nde*/and *Pfu* DNA polymerase were purchased from New England Biolabs. *E. coli* strain BL21 (DE3) plysS, expression vector pET-3c and hFGF-2 clone pUC18-hFGF-2 were all stored in our institute. The standard $rhbFGF_W$ was purchased from National Institute for the Control of Pharmaceutical and Biological Products. Guanidinium chloride and 2-mercaptoethanol (2-ME) were from Sigma. All other reagents were analytical grade. MilliQ water was used throughout.

2.2. Site-directed mutagenesis and protein expression

The complete coding sequence of hFGF-2 was chemically synthesized according to the data from Genbank. For optimizing the expression level according to the paper reported by Song et al. (2002), the nucleotide sequence representing the first 20 amino acids was adjusted as follows: ATG GC(C → T) GC(C → T) GG(G → T) AG(C → T) AT(C → T) AC(C → T) AC(G → T) CTG CC(A → G) GC(C → T) CTG CCG GA(G → A) GA(C → T) GG(C → T) GG(C → T) AG(C → T) GG(C → T) GC(C → T). Emphatically, only the third base of codon had been changed, and these modifications are not alternate the amino acid sequence. For mutation at positions 78 and 96, TGT, the codon for cysteine, was changed into AGC that codes serine; all the other is the same with the wild-type hFGF-2. Both PCR products were cut by *Bgl* II and *Nde* I, and cloned into pET-3C to get the expression vectors $rhFGF\text{-}2_W$ and C78SC96S $rhFGF\text{-}2_M$, that were transformed into host strain BL21 (DE3) plysS, grown in LB medium.

2.3. Purification and bioassay of hFGF-2

Induced with addition of IPTG at mid-log time of cultivation, cells were harvested and extracted. The recombinant proteins were purified from the soluble cell fraction as described previously (Seeger and Rinas, 1996). Mitogenic activity of the expressed $rhFGF\text{-}2_W$, the standard $rhbFGF_W$ and the expressed $rhFGF\text{-}2_M$ on 3T3 fibroblasts was assayed using MTT method. Briefly, Balb/c 3T3 cells were harvested in 0.1% trypsin solution, collected by centrifugation and resuspended with DMEM containing 10% fetal bovine serum, and then seeded in 96-well plates (5.0×10^3 well^{-1}). 12 h later, the medium was removed and replaced by fresh DMEM with 0.4% fetal bovine serum. After 24 h cultivation, rhFGF-2 with different concentrations (100, 25, 6.25, ..., ng/ml, in turn, according to four-fold dilution) was added to cell cultures. The same amount of medium was added in the blank group. Every concentration was repeated three times. The cell number was estimated by MTT assay (570 nm) after 72 h culture. The independent tests were repeated three times.