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Screening for soluble expression constructs using cell-free protein synthesis

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Abstract

The SH2 domain of STAT6 was chosen to test the in vitro protein synthesis as a screening tool. Goal of the screening was to obtain constructs which produce soluble protein in *E. coli*. The expression of 70 different constructs using an *E. coli* based cell-free system revealed two constructs, which give partly soluble protein. The introduction of two mutations, which had been suggested by a structural based alignment of 20 different SH2 domains lead to increased solubility. The expression of both constructs in *E. coli* followed by an affinity and size exclusion chromatography resulted in milligram quantities of highly purified protein.
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Keywords: Structural based approach; Multiple constructs; Cell-free translation; Screening for soluble expression

1. Introduction

For structural studies, milligram quantities of highly purified proteins are required. It has been established by pilot studies that soluble protein expression is one of the bottlenecks in structural biology [1]. It is still not possible to predict the structure of a soluble expression construct and it is absolutely impossible to predict the structure of a construct, which provides good diffracting protein crystals.

Due to fast growth, easy handling, and low cost, *E. coli* is the principle expression system of choice [2,3]. However, recombinant proteins produced in *E. coli* often accumulate as insoluble aggregates [4,5]. In some cases changing parameters such as temperature, additives, induction conditions, or the addition of affinity tags may alter the behavior of the recombinant proteins and therefore help to overcome this problem [6]. In a lot of other cases it is helpful and time saving to start with a large number of different expression constructs in order to end up with at least one construct which provides soluble protein for further experiments. Therefore, a fast and effective screening method for soluble expression is necessary. A faster and more convenient approach to screen a large number of expression constructs as well as expression conditions of recombinant proteins compared to classical in vivo systems is the cell-free protein expression [7,8]. A main advantage of this method is the fact that linear templates generated by PCR can directly be used for in vitro expression of the encoded protein and thereby prevent time-consuming cloning steps [9–11]. One open question is still the correlation of the in vitro expression based on an *E. coli* lysate and the expression in *E. coli*. To answer this question we have carried out a systematic screening of 70 different SH2 domain constructs for solubility using an *E. coli* based cell-free system followed by transfer of the obtained results to *E. coli*.

Since the published domain borders often do not represent ideal expression constructs in terms of solubility we have chosen a structural based approach to define proper borders of the SH2 domain. Therefore 20 structures of different SH2 domains had been aligned and this three-dimensional alignment was used to correct a sequence alignment produced with ClustalX. Afterwards the structural as well as the sequence alignment were employed to define constructs of the STAT6 SH2 domain.

Abbreviations: *E. coli*, *Escherichia coli*; SH2, src homology domain 2; STAT, signal transducer and activator of transcription; IL, interleukin; PDB, Brookhaven Protein Data Bank; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis; AP, alkaline phosphatase; LB, luria broth; BCCP, biotin carboxylase carrier protein; MALDI-MS, matrix-assisted laser desorption/ionization-mass spectrometry; NMR, nuclear magnetic resonance; SPR, surface plasmon resonance

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STAT6 is a transcription factor that is activated by IL-4 and 13 and mediates most of the IL-4/IL-13 induced gene expression [12,13]. Both cytokines play a crucial role in the pathophysiology of asthma and allergic diseases [14,15]. Inhibition of STAT6 should reduce the common IL-4/IL-13 associated features of asthma. Therefore STAT6 is an interesting target for the pharmaceutical industry. We are focussing on the SH2 domain of STAT6 because all compounds found during the primary and secondary high-throughput screens are active in a SH2 domain binding assay.

2. Methods

2.1. Three-dimensional alignment of SH2 domains

The PDB (status 18th of March 2003) was searched for sh2 or "src homology 2 domain". From the 121 structures found, a subset with less than 90% sequence identity was chosen (36 structures). This subset was inspected for "false-positives" and the remaining 20 structures (Table 1) were aligned with the program LSQMAN and the superimposed structures were visualized with O (Fig. 1).

For the planning of new constructs of the STAT6 SH2 domain only those structures with an isolated SH2 domain were chosen (Table 2). The three-dimensional alignment (Fig. 1) was used to correct a sequence alignment produced with ClustalX (Fig. 2). The numbering is the same as in STAT3 (PDB entry code 1BG1).

Table 2
List of the 14 structures which were used for the sequence alignment

PDB ID	Experimental technique	Source	Chain length
1AYA	X-ray diffraction	Mus musculus	101
1BLJ	NMR, 20 structures	Mus musculus	114
1CSY	NMR, 20 structures	Homo sapiens	112
1D4T	X-ray diffraction	Homo sapiens	104
1FU5	NMR, minimized average structure	Rattus norvegicus	111
1JU5	NMR	Homo sapiens	109
1JWO	X-ray diffraction	Homo sapiens	97
1JYR	X-ray diffraction	Homo sapiens	96
1LKK	X-ray diffraction	Homo sapiens	105
1LUI	NMR, 20 structures	Mus musculus	110
1MIL	X-ray diffraction	Homo sapiens	104
1QAD	X-ray diffraction	Bos taurus	111
1SHA	X-ray diffraction	Rous sarcoma virus	104
2PLD	NMR	Bos taurus	105

For the planning of new constructs of the STAT6 SH2 domain only those structures with an isolated SH2 domain were chosen for the sequence alignment shown in Fig. 2.

2.2. Generation of linear expression templates by PCR

The generation of PCR products for in vitro transcription/translation reactions is based on a two-step PCR procedure and was performed with the EasyXpress linear template kit according to the manufacturer's protocol (Qiagen, Hilden, Ger-

Table 1
List of 20 structures containing SH2 domains

PDB ID	Descriptor	No. of amino acids used for the alignment	Rmsd (Å)
1A81	SYK kinase, T-cell surface glycoprotein CD3 epsilon chain	46	1.730
1AYA	Tyrosine phosphatase SYP (N-terminal SH2 domain) (PTP1D, SHPTP2) (E.C.3.1.3.48) complexed with the peptide PDGFR-1009	46	1.787
1BF5	STAT-1/DNA complex	57	1.445
1BLJ	P55 BLK protein tyrosine kinase	46	1.841
1CSY	SYK protein tyrosine kinase, ACETYL-THR-PTR-GLU-THR-LEU-NH2	41	1.709
1D4T	Protein (T-cell signal transduction molecule sap)	46	1.757
1FU5	Phosphatidylinositol 3-kinase regulatory alpha subunit, doubly phosphorylated middle T antigen	31	2.103
1JU5	n/a	44	1.686
1JWO	CSK homologous kinase	44	1.622
1JYR	Growth factor receptor-bound protein 2, peptide: PSPYVNVQN	42	1.932
1K9A	n/a	39	1.448
1LKK	Human P56 tyrosine kinase, phosphotyrosyl peptide AC-PTYR-GLU-GLU-ILE	48	1.720
1LUI	n/a	41	1.837
1MIL	SHC adaptor protein	38	1.498
1QAD	PI3-kinase P85 alpha subunit	42	1.947
1QCF	Haematopoetic cell kinase (HCK)	46	1.702
1SHA	V-SRC tyrosine kinase transforming protein (phosphotyrosine recognition domain SH2) (E.C.2.7.1.112) complex with phosphopeptide A (TYR-VAL-PRO-MET-LEU, phosphorylated TYR)	48	1.734
2ABL	ABL tyrosine kinase	37	1.807
2CBL	Proto-oncogene CBL, ZAP-70	38	1.701
2PLD	Phospholipase C-gamma-1 (E.C.3.1.4.11) (C-terminal SH2 domain comprising residues 663–759) complexed with a phosphopeptide from the platelet-derived growth factor	42	2.017

The PDB was searched for "sh2" and this subset of structures with less than 90% sequence identity was chosen for an alignment with the program LSQMAN.

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