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Research paper

De-glycosylation of *Pichia pastoris*-produced *Schistosoma mansoni* cathepsin B eliminates non-specific reactivity with IgG in normal human serum

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Abstract

Production of diagnostic reagents in the yeast *Pichia pastoris* is particularly attractive since this organism is capable of expressing complex eukaryotic proteins in their correctly folded form and is amenable to large-scale fermentation at low cost. The potential of *Schistosoma mansoni* cathepsin B as a diagnostic antigen for human schistosomiasis has been previously established using both native and *E. coli*-derived recombinant proteins. However, when produced in *P. pastoris* we found that recombinant wild-type cathepsin B was preferentially secreted as a heterogeneously glycosylated molecule that migrated at 39 kDa, 41 kDa and a smear of >50 kDa on SDS-PAGE, and was susceptible to treatment with Endo H and PGNase F. The addition of yeast sugars to the cathepsin B caused it to react with IgG in the serum of both normal (non-infected) and schistosome-infected humans in immunoblotting and enzyme linked immunosorbent assays (ELISA). To avoid this non-specific reactivity, a non-glycosylated mutant form of cathepsin B, engineered by disrupting its potential glycosylation site, was produced. The non-glycosylated recombinant cathepsin B migrated as a single band of 39 kDa on SDS-PAGE. Most importantly, the molecule was not reactive with IgG in normal sera and, hence, could be employed in immunoblots or ELISA to specifically detect antibodies in schistosome-infected patients. Addition of oligosaccharides by *P. pastoris* is a potential drawback that needs to be considered before using *P. pastoris*-produced proteins as diagnostic reagents.

Keywords: Schistosomiasis; Cathepsin B; Glycosylation; *Pichia pastoris*

Abbreviations: Endo H, endoglycosidase H; PBS, phosphate-buffered saline; PGNase F, *N*-glycopeptidase F; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

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1. Introduction

Schistosomiasis remains a major public health problem in many tropical and subtropical countries and has significant economic and public health consequences. At least 600 million people are at risk of infection with an estimated 200 million currently infected with schistosomiasis. This places schistosomiasis as the third most important disease within the WHO tropical disease portfolio. Early intervention with chemotherapeutic agents is efficacious for the treatment of schistosomiasis although this relies on the availability of sensitive and specific methods for diagnosis (Doenhoff et al., 1985; Chitsulo et al., 2000).

The detection of parasite eggs in stool and urine samples is the primary method of diagnosis of human schistosomiasis. However, in areas of low transmission, where egg excretion is below 100 eggs/gram of faeces, or where infection has been recent and sampling occurs prior to oviposition, the sensitivity of this test is inadequate (Doenhoff et al., 1985). Immunodiagnostic methods based on the detection of schistosome specific antibodies in sera have proven to be more accurate as indicators of infection (Cesari et al., 1987; El-Sayed et al., 1998). However, many of the current tests employ whole parasite extracts, parasite egg extracts or secreted fractions which have a number of inherent disadvantages, including difficulty in standardisation, high production costs and lack of specificity due to cross-reactivity with antibodies induced by other helminth infections (Cesari et al., 1987; Doenhoff et al., 1985). The use of purified recombinant schistosome antigens would, therefore, avoid these problems.

Cathepsin B (Sm31 antigen), an antigen expressed by maturing and adult schistosome parasites, is highly immunogenic in humans and has been proposed as the most suitable serodiagnostic antigen for detection of infection by *Schistosoma mansoni* (Klinkert et al., 1987), *Schistosoma japonicum* (Ruppel et al., 1987) and *Schistosoma haematobium* (Idris and Ruppel, 1988). The antigen has been produced in *E. coli* but its performance was reduced compared to native enzyme most likely because its expression in this prokaryotic system resulted in the loss of critical antibody-binding epitopes (El-Sayed et al., 1998). Cathepsin B is a secreted protease that is produced as a zymogen (pre-pro-enzyme) and, hence, requires expression in eukaryotic systems for proper processing and folding (Tort et al., 1999).

Production of recombinant proteins in eukaryotic systems such as *Pichia pastoris* may be more appropriate, as this system combines high level of expression with the efficient secretion of correctly folded protein. On the other hand, yeast expression systems are known to introduce post-translational modifications such as glycosylation. Yeast glycans are common in food and nature and have antigenic, immunogenic and immunostimulatory properties in mammals, and serum from normal individuals often contain antibodies which react with these (Podzorski et al., 1990; Letourneur et al., 2001). This could pose a problem for the use of yeast-produced recombinant glycoproteins particularly in terms of their specificity of diagnostic tests.

Cathepsin B possesses a single potential N-linked glycosylation site (Asn^{183}-His-Thr) and in schistosome parasites is glycosylated (Tort et al., 1999). Here we show that the molecule is also glycosylated when expressed in *P. pastoris*, and that the sugar moiety is reactive with IgG in serum of normal non-infected humans. By eliminating the glycosylation site using site-directed mutagenesis we produced a recombinant form of cathepsin B that did not react with sera of normal patients and, hence, can be employed in the development of a future schistosomiasis diagnostic test.

2. Materials and methods

2.1. Preparation of recombinant wild-type and non-glycosylated cathepsin B from P. pastoris

The *S. mansoni* wild-type procathepsin B was amplified by PCR from a *S. mansoni* adult cDNA library (construction described by Smyth et al., 2003) using primers that incorporated a *Sna*BI restriction site at the 5′ end of the gene (5′-GCGGCTACGTACATATTTCAGTTAAGAAC-3′) and an *Avr*II restriction site and His_6-tag sequence at the 3′ end (5′-GCGCCTAGGTTAGTGGTGGTGGTGGTGGTGGGGCCCGTTTATTCGACCGGCTAT-3′). The amplified fragment was inserted in-frame with the yeast alpha-factor at *Sna*BI/*Avr*II site of