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Echinococcus granulosus antigen B hydrophobic ligand binding properties

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Abstract

Antigen B (AgB), an immunodominant component of the cestode parasite *Echinococcus granulosus*, presents homology to and shares apparent structural similarities with helix-rich hydrophobic ligand binding proteins (HLBPs) from other cestodes. In order to investigate the fatty acid binding properties of AgB, two of its subunit components (rAgB8/1 and rAgB8/2) were expressed in *Escherichia coli* and purified, and the native antigen was purified from the hydatid cyst fluid by affinity chromatography using a monoclonal antibody raised against rAgB8/1. The interaction of the purified native and recombinant proteins with the fluorescent ligands DAUDA, ANS, DACA and 16-AP was investigated. The palmitic acid derived fluorescent ligand, 16-AP, showed the greatest enhancement in fluorescence when bound to native AgB or to its recombinant subunits, and the dissociation constants for 16-AP binding were determined. Surprisingly, in contrast to HLBPs from other cestodes, interactions with other fatty acids, including palmitic acid, caused an increase in fluorescence instead of competing with 16-AP. Our results suggest that AgB might have evolved different functions in the binding of hydrophobic compounds, dependent on cestode environment.

Keywords: *Echinococcus granulosus*; Antigen B; Hydrophobic ligand binding protein; Fatty acid; 16-(9-Anthroyloxy) palmitate (16-AP)

1. Introduction

Echinococcus granulosus antigen B (AgB) is one of the major antigenic components of the metacestode hydatid fluid (HF) and was first characterized as a lipoprotein of 120–160 kDa [1]. On one-dimensional SDS-PAGE, AgB produces a characteristic ladder-like pattern, consisting of regularly spaced subunits with apparent molecular weights of 8, 16, and 24 kDa, with the relative abundance decreasing asymptotically with the increase of their molecular weight [2]. AgB is an oligomeric protein, composed of at least two different subunits of 8 kDa [3], namely, AgB8/1 [4] and AgB8/2 [5]. A cDNA encoding a third subunit has been recently identified and genomic Southern blot experiments suggest that AgB is encoded by a gene family [6].

AgB is highly immunogenic in human infections, has a high diagnostic value [7], and possibly plays an important role in the biology of the parasite, since it accounts for as much as 10% of the total content of the HF [8]. It has been characterized as a protease inhibitor, with the additional ability to inhibit recruitment of neutrophils [9]. Moreover, it seems to exploit the activation of T helper cells by eliciting a nonprotective Th2 cell response [10]. However, most studies on this antigen have been focused on its relevance in the cystic hydatid disease (CHD) immunodiagnosis and its function remains unclear.

AgB is homologous to proteins belonging to the new group of helix-rich hydrophobic ligand binding proteins

Abbreviations: AgB, antigen B; HLBP, hydrophobic ligand binding protein; HF, hydatid fluid; 16-AP, 16-(9-anthroyloxy) palmitate

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(HLBPs), specific to helminths [11,12]. Putative members of this family were described, among others, in *Moniezia expansa* [13], *Taenia solium* [14], *Taenia crassiceps* [15] and *Hymenolepis diminuta* [11]. Similar to AgB, they are abundant oligomeric proteins composed of small subunits (7–11 kDa). Many of them are highly immunogenic and good candidates for use in immunodiagnosis [12,14,15]. Some were shown to bind fatty acids, with a tryptophan residue involved in the binding [11–13], suggesting that these proteins might be involved in lipid detoxification, transport and metabolism.

In the present work, we investigated the hydrophobic ligand binding properties of the native AgB and of its recombinant subunits rAgB8/1 and rAgB8/2. AgB was affinity purified from HF by immunoaffinity chromatography using an anti-AgB8/1 antibody and the two recombinant subunits expressed in *Escherichia coli.* The native and recombinant forms of AgB were assessed for fatty acid binding by competitive spectrofluorimetry.

2. Materials and methods

2.1. Protein purification

Native AgB was purified by immunoaffinity chromatography from an HF parasite enriched fraction. HF of fertile cysts were collected from cattle infected viscera. HF was then concentrated and a parasite enriched fraction was prepared according to the protocol of Oriol et al. [1]. A specific monoclonal antibody raised against the recombinant AgB8/1 subunit [7] was coupled to a cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the manufacturer. HF enriched fraction was then passed through the column and bound AgB eluted with 100 mM tris-glycine pH 3.5. The eluate was dialyzed against double-distilled water and concentrated. The presence of the subunits AgB8/1 and AgB8/2 in the eluate (purified native AgB) was confirmed by immunoblot using specific antibodies (data not shown). Recombinant proteins, rAgB8/1 and rAgB8/2, were expressed in *E. coli* as a fusion with GST. The GST moiety was removed by thrombin treatment during the purification process and the recombinant proteins purified as previously described [7]. At this stage, proteins were over 95% pure. Purified proteins were delipidated using Lipidex LH-20 (Sigma) at 37 °C as previously described [16].

2.2. Spectrofluorimetry

Fluorescent measurements were made at 20 °C in a Shimadzu RF-530/PC spectrofluorimeter using 2-ml samples in a quartz cuvette as previously described [11,12]. The fluorescent probes 16-(9-anthroyloxy) palmitate (16-AP) (Molecular Probes), 11-[(5-dimethylaminonaphthalene-1-sulfonyl) amino] undecanoic acid (DAUDA), 8-anilino-1-naphthalene sulfonic acid (ANS) and dansyl-DL-alfa-aminocaprylic acid (DACA) were used to examine the AgB hydrophobic binding site.

The fluorescent ligands were stored as stock solutions (10 mM) in ethanol in the dark at −20 °C and freshly diluted to 0.1 mM with 50 mM potassium phosphate buffer (pH 7.4) immediately prior to use. Fluorescent titrations were carried out as previously described [11,12]. The fluorescent ligands were added in small aliquots (2–5 μl) to 2 ml of protein in 50 mM potassium phosphate buffer (pH 7.4). Fluorescent measurements (excitation, $E_{x\ max}$ 360 nm, emission, $E_{m\ max}$ 450 nm) were corrected for dilution and solvent effects and for the fluorescent contribution of the unbound ligand. 16-AP binding sites were characterized as previously described [17] and data subjected to Scatchard analysis [18] for determining the estimates of the apparent dissociation constants (K_D) and the number of binding sites (n) per monomer. In the calculations, the molecular mass of native AgB was taken to be that of the monomer (8 kDa). Bovine serum albumin (BSA) (Sigma) was always used as a positive control.

The binding of non-fluorescent ligands to native AgB and recombinant proteins was evaluated by measuring the displacement of bound 16-AP.

3. Results

3.1. Protein purification and delipidation

Native AgB was purified by immunoaffinity chromatography from a HF parasite enriched fraction using a monoclonal antibody raised against the recombinant subunit AgB8/1 (Fig. 1). SDS-PAGE of the purified native AgB showed, in addition to the 8, 16 and 24 kDa components, bands corresponding to larger components, increasing in size by about 8 kDa and forming the ladder-like pattern previously described [2]. The presence of both subunits,

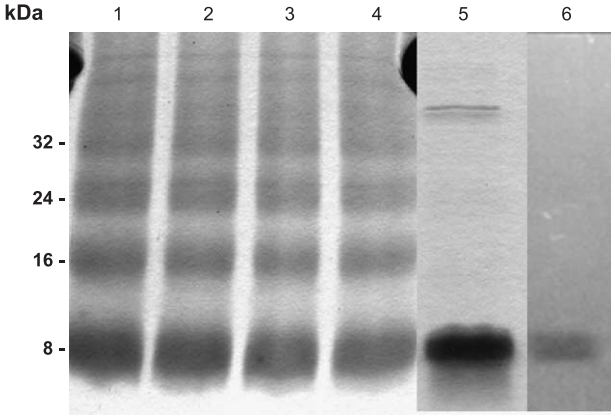

Fig. 1. Coomassie blue-stained SDS-PAGE 15% showing the purified proteins. Native AgB (1) and AgB delipidation on ice (2), at room temperature (3) and at 37 °C (4), rAgB8/1 (5) and rAgB8/2 (6). Molecular weight markers are shown on the left.