

The Mouse Salivary Androgen-Binding Protein (ABP) Alpha Subunit Closely Resembles Chain 1 of the Cat Allergen Fel dI

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Androgen-binding protein (ABP) is found in the salivas of a wide variety of rodents and it has been proposed that ABP functions in sex and/or subspecies recognition (Karn and Dlouhy, J. Hered. 82, 453, 1991). This is a report of significant identity between the alpha subunit of mouse salivary ABP and Chain 1 of cat allergen Fel dI (50% identity), as well as with two other proteins that share identity with Chain 1 of Fel dI, rabbit uteroglobin (27% identity with ABP alpha) and human lung Clara 10 (27% identity with ABP alpha). The secondary structure predicted for the mouse ABP alpha subunit is a very good fit with the secondary structure determined by X-ray crystallography for rabbit uteroglobin, a protein that shares with mouse ABP the capability of binding steroid. Fel dI is found in cat saliva, sebaceous glands, and pelt. Its function is not known but it has been proposed to be involved in protecting dry epithelia, a parallel to uteroglobin protecting wet epithelia. Since mice, like cats, lick themselves and each other extensively, coating their pelts with ABP may be part of this or another biological function.

KEY WORDS: mammalian salivary proteins; mouse androgen-binding protein; cat allergen Fel dI; rabbit uteroglobin; human lung Clara 10.

INTRODUCTION

Many unusual proteins have been found in the salivas of mammals. Their functions include digestion, lubrication, maintaining supersaturating concentrations of calcium in saliva, binding dietary toxins, and combating bacterial

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and fungal growth (Karn, 1991). Binding sex steroid hormones is a characteristic unexpected among proteins found in the oral cavity. Mouse salivary androgen-binding protein (ABP) is a family of dimeric proteins produced in the mouse submaxillary gland that constitutes a significant proportion of mouse salivary protein. ABPs are composed of a common, alpha, subunit disulfide-bridged to either a beta or a gamma subunit (Dlouhy *et al.*, 1987). Three separate genes (*Abpa*, *Abpb*, and *Abpg*) have been proposed to account for the subunits (Karn, 1991). No function has been determined for this unusual protein, however, subspecies-specific distribution of genetic variants of the alpha subunit led to the suggestion that it might have a role in sex and/or subspecies recognition (Karn and Dlouhy, 1991).

One means of identifying a protein's function is determination of its amino acid sequence for comparison to those of proteins with known or suspected functions. This is a report of significant structural similarities among mouse ABP, cat allergen Fel dI, rabbit uteroglobin, and human lung Clara 10. It presents the first evolutionary relationship between mouse ABP and other mammalian proteins and should contribute to a better understanding of related biological functions for the group.

MATERIALS AND METHODS

The international protein sequence database (European Molecular Biology Laboratory—Swissprot R26.0, July, 1993; and National Biomedical Research Foundation—Protein Identification Resource, R38.0, September, 1993) was searched for identity to the protein sequence reported for the b variant of the alpha subunit of mouse salivary ABP (Karn and Russell, 1993). Secondary structure predictions were made for ABP using the Hitachi PROSIS program to predict helix and turn regions (Chou and Fasman, 1978; Garnier *et al.*, 1978; Rose, 1978). Hydrophobicity plots were done by the method of Kyte and Doolittle (1982).

RESULTS AND DISCUSSION

The complete putative amino acid sequence of the common (alpha) subunit of ABP has been produced along with a partial sequence for the beta subunit (Karn and Russell, 1993). Partial identity between the N-terminal 34 residues of the beta sequence and the helospectin sequence was reported (Karn and Russell, 1993). No identity with other ABPs was found, which, coupled with the observation that salivary ABP is the only ABP which is an exocrine secretion product, suggested that it has a unique function. A more recent search of an updated version of the database yielded significant identity with Chain 1 of cat allergen Fel dI and two other proteins with

shared identity to Fel dI, rabbit uteroglobin and human lung Clara 10 (Morgenstern *et al.*, 1991). Figure 1 shows that the secreted forms of mouse salivary ABP and cat Fel dI are both 70 amino acid residues in length and are 50% identical. Especially important is the observation that all three Cys residues are identical. Cys is generally considered to be the most conserved amino acid residue among evolutionarily related proteins because it contributes to tertiary/quaternary structure through disulfide bridging and because it may also be involved as cysteine in the functional site of some proteins. In

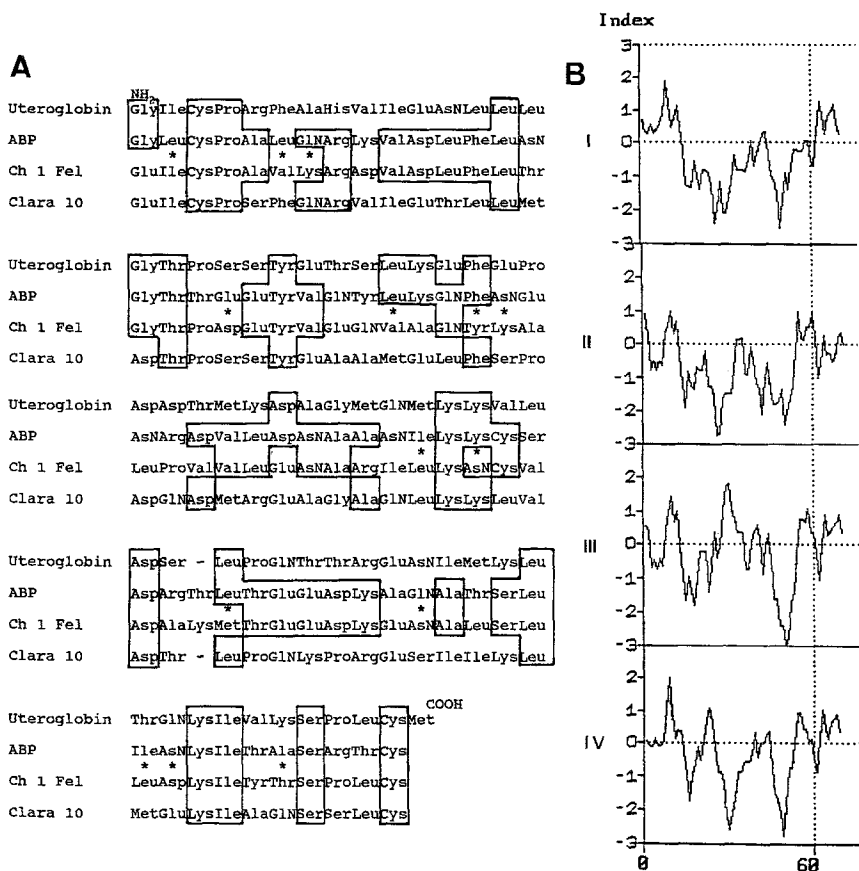


Fig. 1. Primary and secondary structural similarities among four proteins. (A) Alignment of mouse salivary androgen-binding protein (ABP) with cat Fel dI, rabbit uteroglobin, and human lung Clara 10. Identities between the ABP sequence and those of the others are boxed. Asterisks indicate conservative substitutions between mouse ABP and cat Fel dI. (B) Hydrophobicity indices for the four sequences: I, rabbit uteroglobin; II, mouse salivary ABP; III, cat Fel dI; and IV, human lung Clara 10. Positive peaks indicate hydrophobic regions; negative peaks indicate hydrophilic regions.

this case, both proteins are heterodimers with disulfide-bridged subunits, requiring that at least one Cys be involved in dimer formation. There is less identity between the alpha subunit of mouse ABP and rabbit uteroglobin (27%) and human lung Clara 10 (27%) but two of the three Cys residues in ABP are shared with these proteins.

Figure 1 also shows hydrophobicity plots derived from the amino acid sequences of the four proteins. These reinforce the impression of significant identity, particularly between mouse ABP and cat Fel dI.

The secondary structure predicted for mouse ABP is a good fit with the secondary structure determined by X-ray crystallography for rabbit uteroglobin (Mornon *et al.*, 1980). Figure 2 shows that four alpha helices are predicted for both proteins and that they align relatively well between the two sequences. The most interesting observation, however, is that 17/19 identical amino acid residues flank the helices, most falling into shared regions of beta turns. That suggests that both the turns and the helices are conserved between the two proteins. A similar secondary structure analysis for Chain 1 of Fel dI gave four helices with locations roughly similar to those shown for uteroglobin and ABP (not shown). Again, the identities flanking and within the beta turns were more striking: Leu¹⁴, Gly¹⁶, Thr¹⁷, and Pro¹⁸ for Turn 1; Leu²⁵/Val²⁵ (conservative substitution) and Phe²⁸/Tyr²⁸ (conservative substitution) for Turn 2; Lys⁴² and Asp⁴⁶ for Turn 3; Lys⁴³, Ile⁴⁴, and Ser⁶⁷ for Turn 4; and Ser⁶⁷ and Cys⁷⁰ for Turn 5.

Rabbit uteroglobin is a homodimer with subunits disulfide-bridged as antiparallel chains (Mornon *et al.*, 1980) and is capable of binding steroid (Beato and Baier, 1975). Crystallography shows it to be a globular protein with a hydrophobic pocket where progesterone is thought to bind (Mornon *et al.*, 1980). Comparisons at the tertiary structure level will require X-ray crystallographic analysis of ABP, however, the shared steroid-binding capability and similarities in secondary structure suggest that its tertiary structure is probably not significantly different from that of uteroglobin. Thirteen of fifteen residues shown to line the hydrophobic pocket of uteroglobin are either identical or conserved substitutions in mouse ABP. Given the apparently conserved locations of beta turns in Chain 1 of Fel dI, it may also have a tertiary structure similar to uteroglobin.

Although all four proteins compared here are dimers composed of disulfide-bridged subunits, the second subunit varies widely among them. Rabbit uteroglobin and human Clara 10 are homodimers (Mornon *et al.*, 1980; Singh *et al.*, 1988), while cat Fel dI (Morgenstern *et al.*, 1991) and mouse ABP (Karn and Russell, 1993; Karn, 1991) are heterodimers. The N-terminal sequence of the ABP beta chain [30/ca. 70 residues (Karn and Russell, 1993)] and its amino acid composition [Ala rich: 11% (Dlouhy *et al.*, 1986)] suggest that it is very different from Chain 2 of Fel dI. In this context



Fig. 2. Proposed secondary structure similarities for mouse salivary ABP and rabbit uteroglobin (Mornon *et al.*, 1980). Primary structure identities are boxed. Large open boxes indicate helical regions; black bars indicate turns.

it is interesting to reflect on the identity between the ABP beta subunit and helospectin, a member of the glucagon superfamily (Karn and Russell, 1993).

The lack of identity of Chain 2 of Fel dI with either the alpha or the beta subunit of ABP is all the more striking given the 50% identity between Chain 1 of Fel dI and the ABP alpha subunit, the shared salivary gland origins of the two proteins, and the observation that both proteins have a disulfide-

bridged heterodimeric structure. Distinct genes have been characterized for Chains 1 and 2 of Fel dI (Griffith *et al.*, 1992) and three separate genes have been proposed for the alpha, beta, and gamma subunits of ABP (Dlouhy *et al.*, 1987). There are two isoforms of Chain 2 of Fel dI: Ch2L, composed of 92 amino acids and preferentially expressed in the salivary glands of the cat; and Ch2S, composed of 90 amino acids and preferentially expressed in the skin. Griffith *et al.* (1992) have proposed that Ch2L is encoded by an exon not contained within their genomic clone.

Bond *et al.* (1993) described the disulfide-bridged, antiparallel quaternary structure of Fel dI and posited at least two disulfide bridges, one at each end of the dimer. They suggested that a third bridge might exist between the center Cys residues but could not be as sure of the data for that one. It is particularly interesting in this context that rabbit uteroglobin and human lung Clara 10 share the two Cys residues found at positions 3 and 70 in mouse ABP and cat Fel dI but not the middle Cys residue. Might that indicate that the disulfide bridges at each end of the dimeric structure are similar in all four dimers?

Fel dI is found in cat saliva, sebaceous glands, and pelt (Morgenstern *et al.*, 1991). Currently it has no known function but has been proposed to be involved in protecting dry epithelia, a parallel to uteroglobin protecting wet epithelia (Morgenstern *et al.*, 1991). Mouse salivary ABP has been proposed to play a role in sex and/or subspecies recognition by binding androgens, perhaps in the urines of territorial markings, and then interacting with an oral/olfactory tissue such as the vomeronasal gland (Karn and Dlouhy, 1991; Karn and Russell, 1993). Since mice, like cats, lick themselves and each other extensively, coating their pelts with ABP may be part of this or another biological function. In any event, it seems likely that mouse ABP may have allergenic properties analogous those of cat Fel dI. This may have significance for the immunology of allergic responses in humans.

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