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A novel 40 kDa protein from goat mammary secretions: purification, crystallization and preliminary X-ray diffraction studies

P. Kumar, S. Yadav, A. Srinivasan, K. L. Bhatia and T. P. Singh*

Department of Biophysics, All India Institute of Medical Sciences, New Delhi 110 029, India

Correspondence e-mail: tps@aiims.aiims.ac.in

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A novel 40 kDa protein has been purified from dry secretions of the mammary gland of goats. The first 15 N-terminal residues were sequenced and showed a sequence identity of 30% to a novel 39 kDa whey protein from bovine mammary secretions. The protein was crystallized by the microdialysis method. Protein was dissolved to a concentration of 40 mg ml^{-1} in 0.025 *M* Tris–HCl pH 8.0 and equilibrated with the same buffer containing 19%(*v*/*v*) ethanol. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 66.1, b = 107.8, c = 63.2 Å and one molecule per asymmetric unit. Intensity data were collected to 2.9 Å resolution, with a completeness of 95%. Since no similar model is available in the protein structure database, heavy-atom derivatives have been prepared and three-dimensional structure determination using the isomorphous replacement method is in progress.

1. Introduction

The mammary gland is involved in the production of a number of proteins. Its lactational functions occur in cycles, beginning with mammary development and lactogenesis, and concluding with mammary involution. During the period between successive lactations, the mammary glands undergo various functional changes including active involution after cessation of milk removal, followed by re-development and colostrum formation prior to parturition (Smith & Todhunter, 1982).

The concentrations of a number of proteins synthesized by mammary glands in the early part of the lactation period reduce during the later period. In fact, many of them are no longer synthesized. In place of old proteins, synthesis of a number of new proteins takes place. The specific cellular and biochemical events that occur in the mammary glands during the lactation and non-lactation periods have not been fully characterized.

The functions of proteins that are produced during the lactation period include nutrition for neonates and protection against infections. These proteins have been well characterized and most of their functions have been established (Karthikeyan *et al.*, 1999). However, the proteins secreted during the non-lactation period have not been characterized so far and their functions have also not been defined. We have isolated a protein from goat mammary secretions during the non-lactating period. This protein provides a specific marker for mammary function during the non-lactating period. It has been purified and crystallized. It is intended to determine the structure of this protein so that a representative structure of this family of proteins is made available, providing the basis for understanding its functional role. The N-terminal sequence, purification and preliminary X-ray crystallographic studies of this protein are presented here.

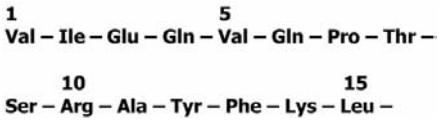
2. Experimental

2.1. Isolation and purification

Mammary secretions were collected from a number of goats after cessation of milk removal. The secretions were diluted with twice their volume of 0.05 *M* Tris–HCl pH 8.0, CM-Sephadex C-50 was added (7 g l^{-1}) and the mixture was stirred slowly with a mechanical stirrer for 1 h. The gel was allowed to settle and milk was decanted. The gel was washed with excess of 0.05 *M* Tris–HCl pH 8.0, packed into a column (25 × 2.5 cm) and washed with the same buffer containing 0.1 *M* NaCl, facilitating removal of impurities. The protein was then eluted with 0.25 *M* NaCl solution in the same buffer. The protein solution was dialyzed against an excess of triple-distilled water and was concentrated using an Amicon ultrafiltration cell. The concentrated protein was passed through a Sephadex G-100 column (100 × 2 cm) using 0.05 *M* Tris–HCl buffer pH 8.0. The purity of the protein was confirmed by SDS–PAGE (Laemmli, 1970) under reducing conditions.

2.2. N-terminal amino-acid sequence

The N-terminal amino-acid sequence of goat 40 kDa protein was determined using a Shimadzu PPSQ-10 system. It gave the following sequence for the first 15 N-terminal amino acids unambiguously.



The sequence was analyzed by NCBI BLAST (Altschul *et al.*, 1997) using entries of over 687 743 known sequences in the protein database. It showed 30% sequence identity to a 39 kDa whey protein from bovine mammary secretions collected during the non-lactating period (Rejman & Hurley, 1988). This novel protein provides a specific marker for mammary function during the non-lactating period. The structures of proteins from mammary secretions and related families have so far not been determined. This protein was found to be immunologically distinct from other milk-associated proteins or serum proteins and also from cytoskeletal proteins. Its low sequence identity with sequences of known proteins suggests that it may have a novel role in the mammary gland. Even though the precise role of this protein has not yet been established, its presence in mammary secretions during involution and the non-lactating period makes it an important marker for mammary function. The structural details of this protein are expected to provide further insight into the function of the mammary gland as it undergoes involution.

2.3. Crystallization

The purification protocol developed for the 40 kDa protein is very similar to that used for the purification of other milk proteins such as lactoferrins and lactoperoxidases from the colostrum (Raman *et al.*, 1993; Kumar *et al.*, 1995). The crystallization conditions include an organic solvent as the precipitating agent. Crystals suitable for X-ray diffraction were obtained by the microdialysis method. Protein solution at a concentration of 40 mg ml^{-1} in 0.025 *M* Tris–HCl was equilibrated against the same buffer containing 19%(*v*/*v*) ethanol at pH 8.0. Crystallization experiments were carried out at 277 K. Colourless irregular-shaped crystals grew to dimensions of 0.6 × 0.4 × 0.3 mm in about three weeks (Fig. 1).

3. X-ray analysis

3.1. Data collection

The X-ray intensities were measured at 288 K using a MAR Research imaging-plate scanner with a diameter of 300 mm. The crystal-to-detector distance was kept at 250 mm. Monochromatic Cu $K\alpha$ radiation was produced with a graphite monochromator mounted on a Rigaku RU-200 rotating-anode X-ray generator operating at 40 kV and 100 mA with a focal point of 0.3 × 0.3 mm. 110 images, each of 1° rotation, were collected. A complete data set was collected using only one crystal. The crystals diffracted well to 2.9 Å resolution. The *HKL* package (Otwinowski, 1993; Minor, 1993), *MARXDS* and *MARSCALE* (Kabsch, 1988) were used for the determination of unit-cell parameters, data processing and scaling of the data. The crystals belong to the orthorhombic system, space group $P2_12_12_1$, with one molecule in the asymmetric unit. The unit-cell parameters were found to be a = 66.1, b = 107.8, c = 63.2 Å, with one molecule per asymmetric unit; this corresponds to a crystal volume per unit molecular weight (V_M) of 2.89 Å^3 Da^{-1}, given the molecular weight of 40 kDa for the protein. This is within the range of the values expected for most protein crystals (Matthews, 1968) and corresponds to a solvent content of 57%. R_{sym} for the 9757 unique reflections to 2.9 Å resolution was 6.8% (12% for the highest resolution shell 3.4–2.9 Å). The multiplicity for all the data was 3.6. $I/\sigma(I)$ in the last resolution shell was 10.1. The overall completeness of the data is 95% (92% in the highest resolution shell 3.4–2.9 Å).

4. Discussion

While the complete sequence determination of the protein from the cDNA from mammary gland of goat is in progress, we have attempted to obtain the structure by molecular-replacement method on a trial basis using models of proteins with similar molecular weight whose structures are available in the PDB. Simultaneously, we have prepared several heavy-atom derivatives in order to determine the structure by multiple isomorphous replacement methods.

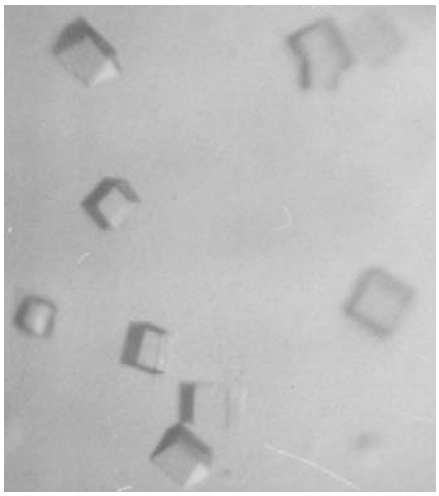


Figure 1
Single crystals of the 40 kDa protein from goat mammary secretion obtained by the microdialysis method.

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