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A glycoprotein from mammary gland secreted during involution promotes apoptosis: Structural and biological studies

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ABSTRACT

Secretory signalling glycoprotein (SPX-40) from mammary gland is highly expressed during involution. This protein is involved in a programmed cell death during tissue remodelling which occurs at the end of lactation. SPX-40 was isolated and purified from buffalo (SPB-40) from the samples obtained during involution. One solution of SPB-40 was made by dissolving it in buffer containing 25 mM Tris-HCl and 50 mM NaCl at pH 8.0. Another solution was made by adding 25% ethanol to the above solution. The biological effects of SPB-40 dissolved in above two solutions were evaluated on MCF-7 breast cancer cell lines. Free SPB-40 indicated significant pro-apoptotic effects while ethanol exposed SPB-40 showed considerably reduced effects on the apoptosis. SPB-40 was crystallized in the native state. The crystals of SPB-40 were soaked in four separate solutions containing 25% acetone, 25% ethanol, 25% butanol and 25% MPD. Four separate data sets were collected and their structures were determined at high resolutions. In all the four structures, the molecules of acetone, ethanol, butanol and MPD respectively were observed in the hydrophobic binding pocket of SPB-40. As a result of which, the conformation of Trp78 was altered thus blocking the binding site in SPB-40 leading to the loss of activity.

1. Introduction

Mammary gland works as a dynamic bioreactor in which a number of proteins are synthesized. The production of proteins differs significantly during different functional stages of mammary gland beginning with mammary gland development, lactogenesis and mammary gland involution [1]. At the end of lactation, extensive changes occur in the mammary tissues leading to the process of mammary involution [2]. It results in a rapid loss of tissue due to programmed cell death [3,4]. During the process of involution, a novel 40 kDa signalling protein called, SPX-40 is over expressed [4–7]. The preliminary studies have indicated a pro apoptotic role of SPX-40 [8]. The crystal structures of SPX-40 in the native unbound state from a number of species are known [5–7]. The structures of a few of its complexes are also known [9–11]. These studies have helped in defining the binding site in the form of sub sites from −5 to +1 [9,10]. The side chain of Trp78 in the native state occupies a position that separates the sub site −1 from sub site +1 and is assumed to occupy the 0 position [6,7]. However, it undergoes conformational changes to occupy the sub site, −1 [11] as well as sub site, + 1 [9,10]. This kind of flexibility allows Trp78 to play an important role in the functioning of this protein [9–11]. In spite of several studies, the exact biological function of this protein is not yet fully understood.

It is well known that the apoptotic stimulation occurs through a distinct signalling cascade [12,13]. The intrinsic or mitochondrial pathway integrates signals generated by a variety of stressor. SPB-40 is expected to induce apoptosis by intrinsic pathway. The members of Bcl-2 family which include both pro-apoptotic Bax [14], Bak [15], Bid [16] and Bim [17] and anti apoptotic BCL-2, BCL-xL and MCL-1 [18] proteins have played a dominant role in the mitochondrial pathway of apoptotic signalling. These proteins translocate to the mitochondria, or change their conformations and interaction partners on the mitochondria in response to various death stimuli. This leads to permeabilization of mitochondrial outer membrane which is considered a milestone in induction of apoptosis by intrinsic pathway [19,20]. The death is considered to be committed at this point of permeabilization of mitochondrial outer membrane. The mitochondrial outer membrane permeabilization (MOMP) causes the diffusion of numerous proteins to the cytosol that normally reside in the space between the outer (OMM) and inner (IMM) mitochondrial membranes. Cytochrome *c* is one such protein which subsequently activates the executioner caspase, caspase-3 [21,22]. The caspases (cysteine-dependent aspartate-directed

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Table 1
Data collection and refinement statistics for four complexes of signalling protein from buffalo (SPB-40).

Parameters	Complex with acetone	Complex with ethanol	Complex with butanol	Complex with MPD
Resolution range (Å)	56.32–1.49 (1.53–1.49)	56.50–1.79 (1.84–1.79)	56.37–1.65 (1.69–1.65)	55.9–1.65 (1.68–1.65)
Wavelength (Å)	0.97	0.97	0.97	0.97
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)	a = 60.46, b = 66.52, c = 105.85	a = 61.07, b = 66.74, c = 106.07	a = 61.14, b = 66.55, c = 106.01	a = 60.87, b = 66.42, c = 105.69
Number of molecules in the asymmetric unit	1	1	1	1
V_m (Å^3/Da)	2.6	2.7	2.7	2.6
Solvent Content (%)	53	54	54	53
Total number of reflections	274,890	162,553	271,366	243,248
Number of unique reflections	65,450	38,703	50,253	52,135
Overall completeness (%)	99.0 (99.0)	99.9 (90.4)	99.9 (99.0)	99.9 (99.7)
R_{sym}	0.031 (0.286)	0.062 (0.415)	0.052 (0.309)	0.035 (0.465)
R_{cryst}	0.147	0.150	0.165	0.143
R_{free} (3% data) (%)	0.176	0.186	0.205	0.195
Number of protein atoms	2884	2874	2878	2903
R. m. s. deviations				
Bond length (Å^2)	0.03	0.02	0.02	0.01
Bond angles (°)	2.9	2.3	2.3	1.5
Dihedral angles (°)	22.0	23.1	24.0	16.6
Mean B factor (Å^2)				
Wilson B-factor	20.4	22.5	22.9	18.9
Main chain atoms	25.2	24.4	25.7	20.4
Side chains and water molecules	30.5	34.0	34.4	30.7
Acetone, ethanol butanol and MPD	33.5	39.0	45.0	34.8
Overall	27.8	30.7	29.4	26.5
Ramachandran plot statistics				
Residues in the fully allowed regions (%)	97.0	96.1	96.4	96.6
Residues in the additionally allowed regions (%)	3.0	3.6	3.3	3.1
Residues in the generously allowed regions (%)	0.0	0.3	0.3	0.3
PDB ID:	**5Z05**	**5Z4W**	**5Z3S**	**5ZV4**

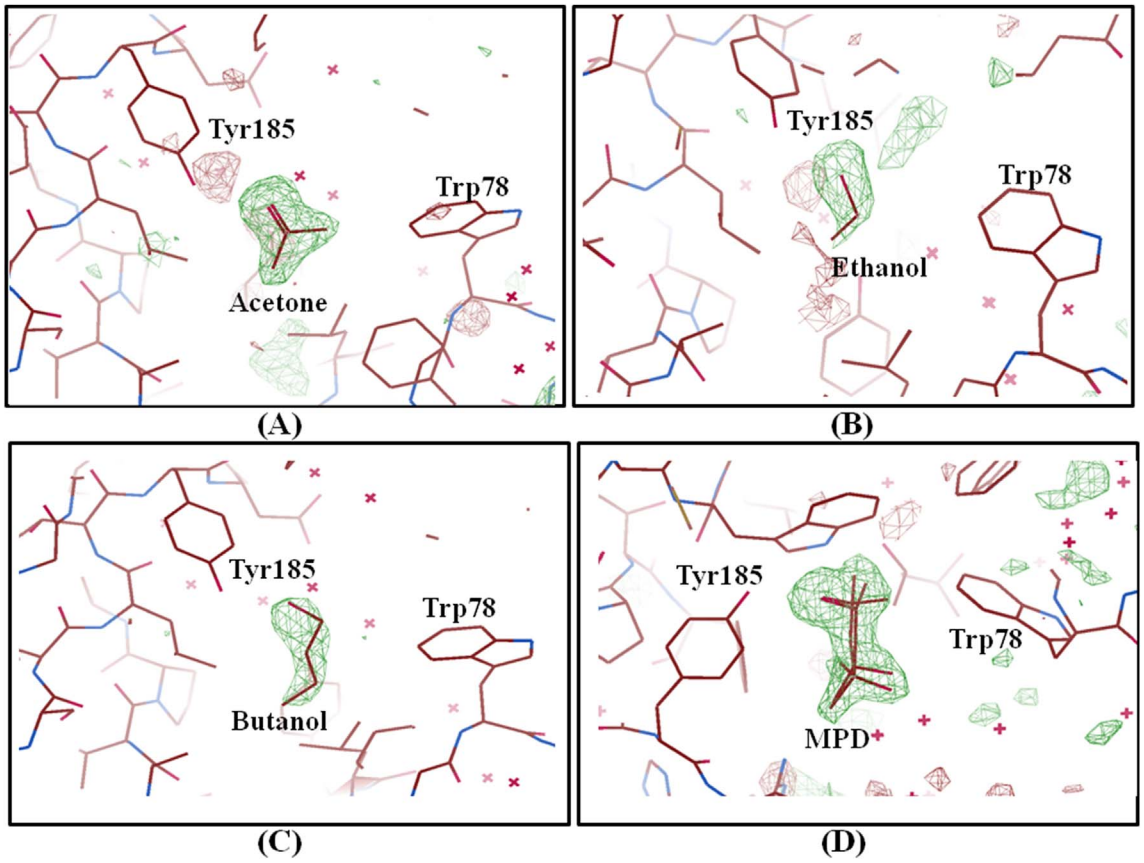

Fig. 1. The omit (Fo - Fc) electron density maps at 3.0σ for **(A)** acetone, **(B)** ethanol, **(C)** butanol and **(D)** MPD. The maps were drawn using COOT [28].