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Crystal Structure of Human Prostate-Specific Antigen in a Sandwich Antibody Complex

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Human prostate-specific antigen (PSA or human kallikrein-related peptidase 3) present in small quantities in the sera of healthy men becomes elevated in prostate cancer (PCa) and other prostate disorders. The ability to identify the free PSA fraction associated with PCa could increase the reliability of the PSA diagnostic test. Here we present the crystal structure of human PSA from seminal fluid in a sandwich complex with two monoclonal antibodies (mAbs). MAb 5D5A5 captures total PSA with exceptionally high affinity, and mAb 5D3D11 selectively discriminates between free PSA subforms that are more abundant in sera from patients with PCa. Although the antigen is not of seric origin, several insights into cancer diagnosis can be discerned from this complex. MAb 5D3D11 recognizes a PSA conformation different from that previously reported. Interacting with the kallikrein loop, the PSA *N*-linked glycan attached to asparagine 61 is an uncommonly complex sialated triantennary chain. *O*-linked glycosylation is observed at threonine 125. The description of how PSA subforms in prostatic fluid can be discriminated using pairs of antibodies is a first step in the design of new strategies that are capable of real discrimination among PSA subforms, which will lead to the formulation of more reliable diagnostic tests. In a companion article [Muller, B. H., Savatier, A., L'Hostis, G., Costa, N., Bossus, M., Michel, S., et al. (2011). *In vitro* affinity maturation of an anti-PSA antibody for prostate cancer diagnostic assay. *J. Mol. Biol.*], we describe engineering efforts to improve the affinity of mAb 5D3D11, a first step towards such goal.

Introduction

Prostate-specific antigen [PSA or human kallikrein-related peptidase (KLK) 3], in the crystal structure described here, is a 237-residue glycoprotein of the kallikrein serine protease family[1] obtained from purified pooled multidonor human seminal fluid. Secreted by the prostate epithelium and activated by KLK2, its physiological role is the liquefaction of the coagulum consisting of semenogelin I, semenogelin

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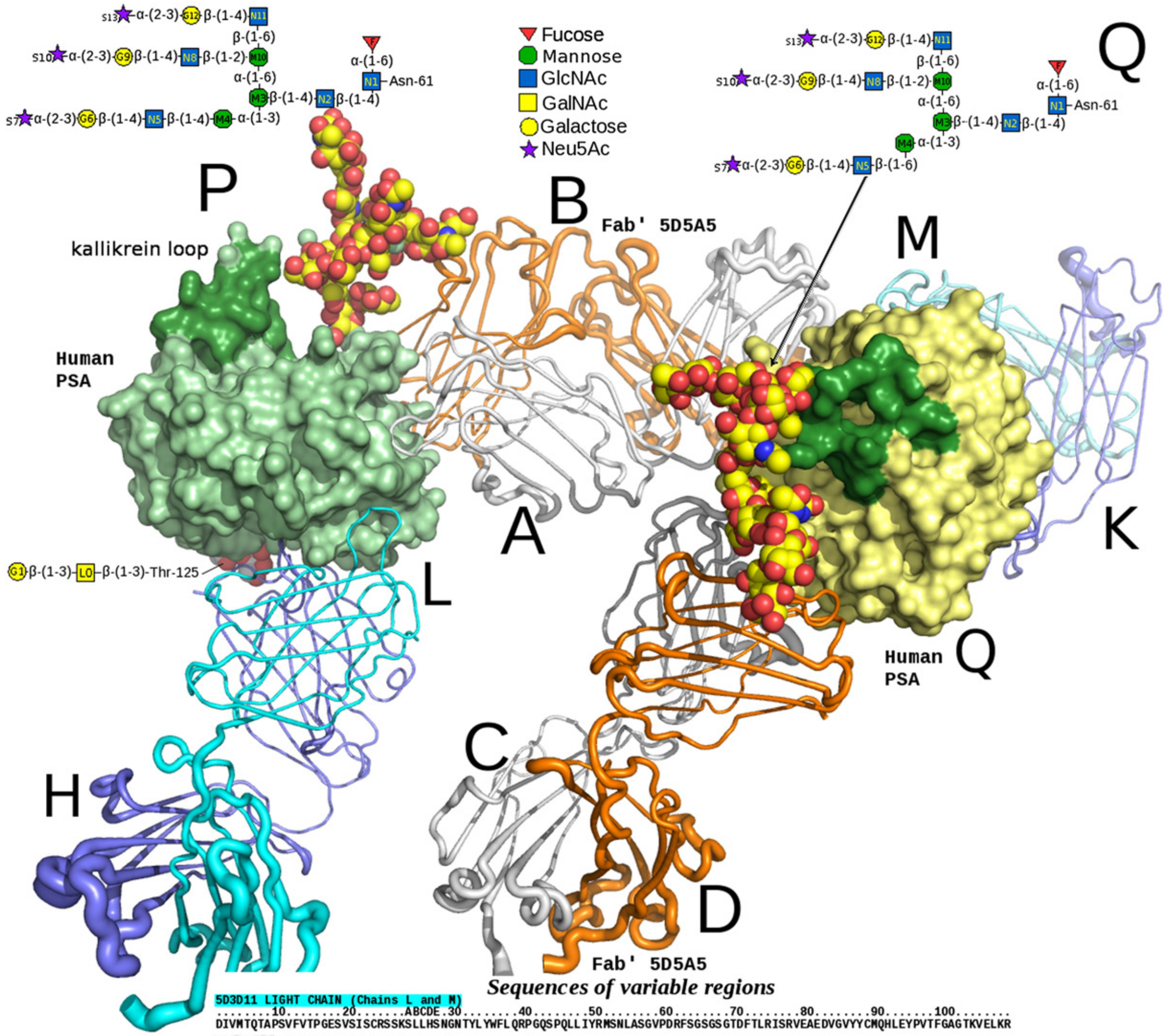
Abbreviations used: PSA, prostate-specific antigen; PCa, prostate cancer; mAb, monoclonal antibody; KLK, human kallikrein-related peptidase; Neu5Ac, *N*-acetylneuraminic acid; GlcNAc, *N*-acetyl glucosamine; Fuc, fucose; CDR, complementarity-determining region; PDB, Protein Data Bank; V_L, light-chain variable; V_H, heavy-chain variable; PEG, polyethylene glycol; ESRF, European Synchrotron Radiation Facility.

II, and fibronectin, components of the semen that form the sperm-entrapping gel, leading to the liberation of spermatozoa.[2] PSA is a chymotrypsin-like serine protease with a typical His-Asp-Ser triad and a catalytic domain similar to those of other kallikrein-related peptidases. Outside its physiological role, PSA (alone or in conjunction with other kallikrein-related peptidases) may participate in the processes of neoplastic growth and metastasis.[3,4] While PSA isolated from seminal plasma is composed mainly of the catalytically active free form, in serum, the majority is complexed with endogenous inhibitors, predominantly α1-anti-chymotrypsin and α2-macroglobulin.[5] Sera of patients with prostate cancer (PCa) have been reported to contain both complexed PSA and free PSA, which includes mature inactive and active forms of free PSA. The first crystal structure of PSA was obtained in complex with an anti-total-PSA monoclonal antibody (mAb), 8G8F5, which is able to enhance the



Sequences of variable regions

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5D3D11 LIGHT CHAIN (Chains L and M)
........10........20.......ABCDE.30........40........50........60........70........80........90.......100........
DIVMTQTAPSVFVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLRISRVEAEDVGVYYCMQHLEYPVTFGAGTKVELKR
5D5A5 LIGHT CHAIN (Chains A and C)
........10........20.......ABCD .30........40........50........60........70........80........90.......100........
DIVLTQSPPSLAVSLGQRATISCRASESIDL YGFTFMHWYQQKPGQPPKILIYRASNLESGIPARFSGSGSRTDFTLTINPVEADDVATYYCQQTHEDPYTFGGGTKLEIKR

5D3D11 HEAVY CHAIN (Chains H and K)
........10........20........30........40........50..A......60........70........80..ABC......90.......100........110
QVQLQQSGPELVKPGASVKISCKVSGYAISSSWMNWVKQRPGQGLEWIGRIYPGDGDTKYNGKFKDKATLTVDKSSSTAYMQLSSLTSVDSAVYFCARDGYRYYFDYWGQGTALT
5D5A5 HEAVY CHAIN (Chains B and D)
........10........20........30........40........50..A......60........70........80..ABC......90........101.......110
QVQLQQSGAELAKPGASVKMSCKTSGYSFSSYWMHWVKQRPGQGLEWIGYINPSTGYTENNQKFKDKVTLTADKSSNTAYMQLNSLTSEDSAVYYCARSGRLY-FDVWGAGTTVT
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Fig. 1. Overall structure for the sandwich complex and sequences of the V_L and V_H domains of 5D3D11 and 5D5A5. The asymmetric unit in the crystal consists of two sandwich complexes and a total of 10 polypeptide chains. The two PSA molecules P and Q are shown in surface representation in green and yellow, respectively, with the kallikrein loops shown in dark green. Fab′ 5D3D11 light chains (L/M) are shown in cyan, and heavy chains (H/K) are shown in blue. Fab′ 5D5A5 light chains (A/C) and heavy chains (B/D) are shown in white and orange, respectively. The sugars are represented in space-filling spheres, and the sugar composition is shown diagrammatically. The V_H and V_L sequences of the two mAbs are shown aligned with each other.