



The reactive centre loop of corticosteroid-binding globulin (CBG) is a protease target for cortisol release



John G. Lewis*, Peter A. Elder

Steroid & Immunobiochemistry Laboratory, Canterbury Health Laboratories, Christchurch, New Zealand

ARTICLE INFO

Article history:

Received 13 June 2013

Received in revised form 25 November 2013

Accepted 6 January 2014

Available online 11 January 2014

Keywords:

Corticosteroid-binding globulin

CBG

Serpins

Chymotrypsin

Elastase

Cortisol

ABSTRACT

Corticosteroid-binding globulin (CBG) binds more than 90% of circulating cortisol and is a non-inhibitory member of the family of serine protease inhibitors (SERPINS) with an exposed elastase sensitive reactive centre loop (RCL). At sites of inflammation neutrophil activation can release elastase which may cleave the RCL and result in cortisol release from CBG. The RCL sequence also has two theoretical chymotrypsin cleavage sites and we used a monoclonal antibody with specificity for the RCL to investigate chymotrypsin cleavage of CBG. Here we show, for the first time, rapid chymotrypsin cleavage of the RCL of CBG, resulting in undetectable levels of intact CBG, whereas total CBG levels were unchanged. Coincident with both chymotrypsin and elastase cleavage there was an increase in the free cortisol fraction of serum to levels similar to when CBG had been inactivated by heat indicating total cortisol release from CBG. These findings demonstrate a new mechanism for cortisol release from its binding globulin.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Corticosteroid-binding globulin (CBG) is a monomeric circulating glycoprotein comprising 383 amino acids with 6 consensus glycosylation sites (Hammond et al., 1987) of which 5 may be occupied and variably glycosylated (Sumer-Bayraktar et al., 2011) thus accounting for its observed heterogeneity. It normally binds up to 90% of circulating cortisol with high affinity and, in concert with albumin, helps to regulate the distribution of free cortisol to tissues (Lewis et al., 2005). Another alternative delivery mechanism is based on the observation that CBG may interact directly with cell membrane receptors (Strel'chyonok and Avvakumov, 1991). Furthermore CBG is a non-inhibitory member of the family of serine protease inhibitors (SERPINS) and possesses an exposed reactive centre loop (RCL) which can be cleaved by elastase released from activated neutrophils. Following cleavage, CBG undergoes a transition from the stressed high affinity cortisol binding state, to the relaxed conformational, low cortisol binding affinity, state thereby releasing cortisol at sites of inflammation (Pemberton et al., 1988). The elastase cleavage site is between Val344 and Thr345 within the exposed RCL which itself comprises 22 amino acids between residues 333–354 (Lin et al., 2009). Following

elastase cleavage the 4-kDa C-terminal fragment is normally retained within the protein but can be released from CBG following denaturing electrophoresis (Pemberton et al., 1988). Recently we raised a monoclonal antibody directed at the RCL of human CBG using a synthetic peptide-conjugate and the epitope consensus sequence was STGVTLNL corresponding to amino acids 341–348. The antibody recognizes this intact sequence but recognition is lost following elastase cleavage of either this peptide or CBG (Lewis and Elder, 2011). We have used this antibody, in concert with other antibodies, to provide a measure of the intact RCL, designated “intact CBG”, and total CBG and showed that both cleaved and uncleaved CBG may coexist in circulation (Lewis and Elder, 2013). Of interest is that the epitope consensus sequence (STGVTLNL) contains 2 leucine residues which are the only potential chymotrypsin cleavage sites within the RCL. This raised the possibility that the RCL of CBG could be sensitive to chymotrypsin cleavage. We sought to investigate this using these antibodies and show that a synthetic peptide, comprising the consensus sequence, with a proline leash, as well as CBG in serum can be rapidly cleaved by chymotrypsin without affecting total CBG levels. Furthermore, following elastase and chymotrypsin cleavage of CBG, a molecular weight loss of 4-kDa is evident on electrophoresis. There was also an increase in the percent free cortisol fraction to levels similar to when CBG has been inactivated by heat. This indicates RCL cleavage and total cortisol release from CBG. These findings show, for the first time, another mechanism for the release of cortisol from its binding globulin.

* Corresponding author. Address: Steroid & Immunobiochemistry Laboratory, Canterbury Health Laboratories, P.O. Box 151, Christchurch, New Zealand. Tel.: +64 3 3640 877; fax: +64 3 3640 889.

E-mail address: john.lewis@cdhb.health.nz (J.G. Lewis).

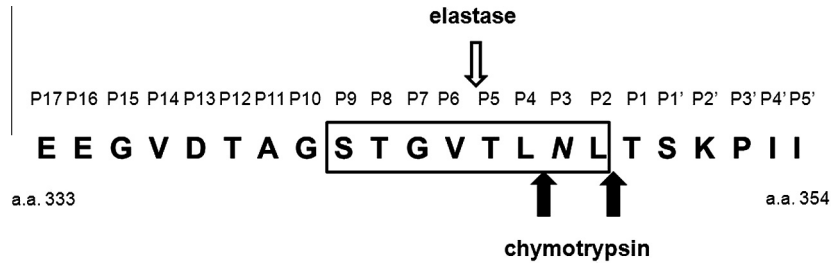


Fig. 1. Amino acid sequence of the RCL of human CBG from amino acids 333–354, corresponding to P17 and P5' respectively. The elastase cleavage site and the 2 chymotrypsin cleavage sites are arrowed and the epitope sequence for the RCL specific antibody, 9G12, is boxed. The carbohydrate attachment site at Asn 347 (P3) is shown in italics.

2. Materials and methods

2.1. Synthetic peptide and chymotrypsin sensitivity

A synthetic peptide within the RCL region corresponding to P9–P1 (amino acid residues 341–349) of human CBG was synthesized with 4 additional proline residues and a cysteine residue at the C-terminus. This sequence, STGVTLNLT-PPPP-C, was synthesized and purified by Mimotopes Pty Ltd., Vic, Australia and spanned the epitope recognition sequence of antibody 9G12 (STGVTLNLT) and included the C-terminal threonine prior to the proline residue leash. The peptide was dissolved in phosphate buffered saline (1 mg/mL) containing 0.01% NaN₃. Peptide sensitivity to chymotrypsin was assessed by coating 96 well microtitre plates overnight with peptide solution in 6 M aqueous guanidine hydrochloride (0.25 µg/well). The following day the plate was washed and chymotrypsin sensitivity assessed by incubation of the adsorbed peptide with varying dilutions of chymotrypsin for varying times at 37 °C. Peptide digestion was terminated by washing the plate, and peptide cleavage assessed using the CBG intact RCL specific antibody 9G12 with conditions similar to those described in Section 2.5.

2.2. Serum

Serum for the enzyme digestion experiments and percent free cortisol determinations was obtained from a single consenting local individual at 1000 h and stored in 0.5 mL portions at –20 °C.

The plasma cortisol was 261 nmol/L. For each experiment an aliquot was thawed and used only once. Some aliquots were heated at 60 °C for 60 min to inactivate CBG. These were stored similarly. Serum was diluted in 0.1 M Tris–HCl, pH 7.5 (1 in 50) immediately prior to use.

2.3. Enzymes

Human sputum elastase was purchased from Elastin Products Co. Inc., MI, USA and reconstituted in 0.05 M acetate buffer, pH 5.0 containing 0.1 M NaCl and 50% glycerol and stored as a stock solution at –20 °C (875 units/mL) and used at a dilution of 1 in 50 in 0.1 M Tris–HCl buffer pH 7.5 containing 0.5 M NaCl and 0.01% NaN₃. Chymotrypsin (>40 units/mg, type II from bovine pancreas) was obtained from Sigma–Aldrich, NSW, Australia. It was freshly prepared as a stock solution, 1 mg/mL, and routinely used at a dilution of 1 in 50 in 0.1 M Tris–HCl buffer pH 7.5 containing 0.5 M NaCl and 0.01% NaN₃. For timed digestions of human serum, by elastase or chymotrypsin, activity was terminated by the addition of 10 mM PMSF in isopropanol (Trainor, 1987) and promptly diluted in the ELISA assay buffer. Enzyme treatments were at 37 °C unless otherwise stated.

2.4. Percentage serum free cortisol

The percentage serum free cortisol was measured by an established ultrafiltration/ligand binding method (Lewis et al., 2005). Diluted serum (0.5 mL of a 1 in 50 dilution) was equilibrated with

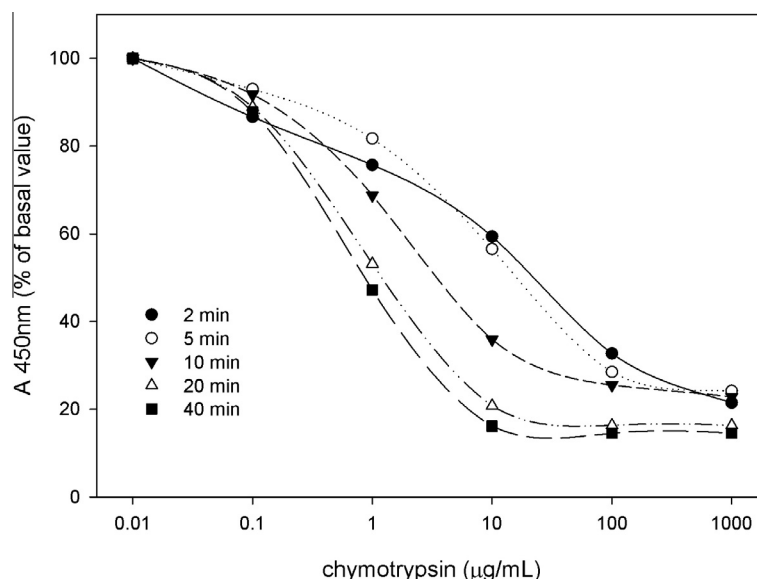


Fig. 2. The effect of immobilized peptide STGVTLNLT-PPPPC, spanning the epitope sequence of 9G12, treated with varying doses of chymotrypsin at varying times at 37 °C. Following treatment the plate was washed and probed with RCL specific antibody 9G12 and the absorbance at 450 nm expressed as percent of the basal value.

Download English Version:

<https://daneshyari.com/en/article/2196051>

Download Persian Version:

<https://daneshyari.com/article/2196051>

[Daneshyari.com](https://daneshyari.com)