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Protein acyltransferase function of purified calreticulin. Part 1: characterization of propionylation of protein utilizing propoxycoumarin as the propionyl group donor

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We have earlier reported that an endoplasmic reticulum luminal protein calreticulin (CR) mediated the acetylation of certain receptor proteins such as glutathione S-transferase (GST) by polyphenolic acetates, leading to irreversible inhibition. This function of calreticulin was termed calreticulin transacetylase. In this communication, we have demonstrated for the first time the ability of the purified recombinant calreticulin of a parasitic nematode *Haemonchus contortus* to transfer propionyl group from 7,8-Dipropoxy-4-methylcoumarin (DPMC) to recombinant *Schistosoma japonicum* glutathione S-transferase (rGST). Calreticulin transacetylase exhibited hyperbolic kinetics and yielded K_m (140 μM) and V_{max} (105 units) when the concentration of DPMC was varied keeping the concentration of rGST constant. rGST thus propionylated was found to positively interact with anti-acetyl lysine antibody. Also, the nanoscale LC-MS/MS analysis identified the propionylation sites on three lysine residues: Lys-11, -180 and -181 of rGST. These results highlight the transacylase function of calreticulin (CRTAase).

Keywords: anti-acetyl lysine antibody/calreticulin/*Haemonchus contortus*/propoxycoumarins/protein acylation.

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; CR, calreticulin; CRTAase, calreticulin transacylase; DAB, diaminobenzidene; DAMC, 7,8-diacetoxy-4-methylcoumarin; DHMC, 7,8-dihydroxy-4-methylcoumarin; DPMC, 7,8-dipropoxy-4-methylcoumarin; GSH, reduced glutathione; GST, glutathione S-transferase; HAT, histone acetyltransferase; NOS, nitric oxide synthase; PA, polyphenolic acetates; 7-PMC, 7-propoxy-4-methylcoumarin; PVDF, polyvinylidene difluoride; rGST, recombinant glutathione *S*-transferase; rhCRTAase, recombinant *Haemonchus contortus* calreticulin transacylase; SDS–PAGE, sodium dodecylsulphate–polyacrylamide gel electrophoresis.

Post-translational modifications plays pivotal role in regulation of cell processes. One of the major post translational modifications is lysine modification by way of methylation, phosphorylation and acetylation. Recently, propionylation and butyrylation of lysine is reported in histones mediated by histone acetyltransferase (HAT) such as p300/CBP (*1*).

Our laboratory has concentrated on the novel role of calreticulin (CR) in mediating the acetylation of certain functional proteins such as cytochrome P-450, cytochrome P-450 reductase, nitric oxide synthase (NOS) and glutathione S-transferase (GST) utilizing polyphenolic acetates (PA) as the acetyl group donors (*2–6*). We delineated certain biological effects such as the anti-mutagenic action (*7*), enhancement of intracellular levels of nitric oxide (NO), inhibition of ADP-induced platelet aggregation and inhibition of protein kinase C in the asthmatic patients arising out of the transacetylase action of CR on PA (*8*, *9*).

In the present communication, we have attempted to study acyltransferase function of CR utilizing propoxycoumarin as a propionyl group donor. Recently, we have reported the placental CR mediated acetylation of neuronal NOS by DAMC and the concomitant autoacetylation of CR by using mass spectrometry (*5*, *10*). The analysis of post-translational modifications of protein by mass spectrometry has gained popularity because of its sensitivity and rapid characterization (*11*, *12*). We have here observed CR mediated propionylation of the receptor protein, a recombinant GST of *Schistosoma japonicum* (rGST) by 7,8-Dipropoxy-4-methylcoumarin (DPMC) using nanoscale-tandem mass spectrometry (MS/MS). Hence, calreticulin protein acetyltransferase is now assigned the generalized term calreticulin transacylase (CRTAase).

Materials and Methods

Cloning, expression and purification of recombinant calreticulin transacylase (rhCRTAase) from H. contortus

rhCRTAase was cloned in pPRO EX TM expression system. The clone was bulk cultured and the bacterial cell lysate were prepared. rhCRTAase was purified to homogeneity as described earlier in detail (*13*).

Assay of rhCRTAase

rhCRTAase was assayed (*14*) using DAMC/DPMC and rGST as the substrates as per the details given in our earlier report (*4*). The assay mixture consisted of 0.25 M potassium phosphate buffer (pH 6.5), rhCRTAase (5 µg protein), DAMC/DPMC (100 µM) added in 50 µl DMSO, purified rGST (5 µg protein) and water to make up 0.8 ml. The contents of the tube, scaled up as per requirement were preincubated at 37°C for various periods. The aliquots were removed periodically into a spectrophotometer cuvette containing CDNB and GSH to make up their concentration (1 mM) in a total volume of 1 ml and the progress of the rGST activity was followed at 340 nm using a Cary spectrophotometer (Cary Bio100). Reactions wherein substrate *i.e.* DAMC/DPMC or rhCRTAase were omitted, respectively, were used as controls. The unit of TAase was expressed in terms of percentage inhibition of rGST under the conditions of the assay and ensured that the reaction was linear with respect to enzyme concentration and incubation time.

Measurement of kinetic constants for rhCRTAase utilizing acyloxycoumarins

The enzyme was assayed as per the procedure described above. The concentrations of DAMC and DPMC were varied from 10 to 200 µM keeping the concentration of rGST constant during preincubation with rhCRTAase for 10 min followed by the addition of GSH and CDNB for GST assay. The reciprocal of the initial rate of CRTAase expressed in terms of % inhibition of GST under condition of assay was plotted (Lineweaver–Burk) against the reciprocal of the concentration of DPMC/DAMC to deduce the K_m and V_{max} values.

Demonstration of rhCRTAase catalyzed acylation of rGST by DPMC/DAMC (western blot)

Polyclonal anti-acetyl lysine antibody was used for the recognition of propionylated lysine residues of receptor protein. Purified rhCRTAase (10 µg) was incubated with rGST (30 µg), DPMC/DAMC (100 µM) in 10 mM potassium phosphate buffer (pH 7.2) incubated for 30 min at 37°C in a shaking water bath. After the completion of reaction, sample buffer (loading dye) was added in the reaction mixture to stop the reaction. This reaction mixture was used to detect the propionylation of rGST by western blot technique. The reaction mixture devoid of rhCRTAase was chosen as an appropriate control to elucidate the role of CRTAase in catalyzing the enzymatic propionylation of the receptor protein. After electrophoresis, the separated protein on the gel was transblotted to a polyvinylidene difluoride membrane (PVDF) at 300 mA for 3.5 h at 4°C. PVDF membranes were kept in blocking reagent (5% blotto) to block the non-specific sites. The primary antibody was diluted (1:1,000) in *tris*-buffer saline with 0.05% Tween-20 detergent (TBST) containing 1% BSA and was incubated with the blot for 1 h at 4°C with moderate agitation. The PVDF membranes were extensively washed with TBST, followed by incubation with secondary antibody (goat anti-rabbit HRP conjugate) for same time duration at room temperature. The membrane was washed extensively with TBST/TBS and the transblotted acylated protein band was visualized by treating with diaminobenzidene (DAB) and hydrogen peroxide.

Demonstration of propionylation of rGST by nanoscale LC-MS/MS

In test sample purified rhCRTAase (10 µg) was incubated with rGST (30 µg), DPMC (100 µM) in 10 mM potassium phosphate buffer (pH 7.2) incubated for 30 min at 37°C in a shaking water bath. The sample devoid of purified rhCRTAase served as control. These mixtures were then boiled in denaturing buffer prior to SDS–PAGE. After electrophoretic separation, the gel was stained with coomassie brilliant blue dye. Gel bands corresponding to the expected rGST molecular weight (26,000 M_r) were excised and digested according to Schevchenko *et al.* (*15*). Briefly, sliced gel pieces were washed with 100 mM NH_4HCO_3 and HPLC-grade acetonitrile (Sigma-Aldrich) (1 : 1, v/v) (buffer A). Then, the protein was in-gel reduced, and subsequently alkylated with iodoacetamide (Sigma). After a washing step with buffer A, the gel pieces were dried in a vacuum centrifuge, and then rehydrated at 4°C in digestion buffer (50 mM NH_4HCO_3, 5 mM $CaCl_2$) containing 25 ng/µl trypsin. After overnight incubation, the peptides were extracted from the gel using three separate washings with a mixture of acetonitrile/water/formic acid 70/25/5 (v/v/v). The extracts were combined and dried down in a vacuum centrifuge.

Chromatography was performed on an Ultimate nano LC system from Dionex (Sunnyvale, CA), using a valveless setup (*16*, *17*). The peptide extracts were redissolved in 40 µl of loading pump solvent (see below) and 10 µl were loaded onto an in-house packed 100 µm i.d. Integra Frit^{TM} (New Objective, Cambridge, MA) trapping column (packing bed length 1.5 cm) at 10 µl/min flow of loading pump solvent, consisting of H_2O/acetonitrile/trifluoroacetic acid (TFA) 97.95:2:0.05 (v/v/v). After 4 min of column washing, the trapping column was switched on-line to the analytical column: an in-house packed 50 µm i.d. Pico Frit^{TM} column (New Objective), filled with the same stationary phase used for the trapping column packing: 3 µm C_{18} silica particles (Dr Maisch, Entringen, Germany). Peptide separation started at 100 nl/min using a binary gradient. Mobile phase A was H_2O/acetonitrile/formic acid/TFA 97.9:2:0.09:0.01 (v/v/v/v); mobile phase B was H_2O/acetonitrile/formic acid/TFA 29.9:70:0.09:0.01 (v/v/v/v). Gradient was from 5 to 45% B in 40 min. After 10 min at 95% B, the column was re-equilibrated at 5% B for 20 min before the following injection.

MS detection was performed on a QSTAR XL hybrid LC-MS/MS from Applied Biosystems (Foster City, CA) operating in positive ion mode, with nESI potential at 1,300 V, curtain gas at 15 units, CAD gas at 3 units. Information-dependent acquisition (IDA) was performed by selecting the two most abundant peaks for MS/MS analysis after a full TOF-MS scan from 400 to 1,600 m/z lasting 2 s. Both MS/MS analyses were performed in enhanced mode (2 s/scan). Threshold value for peak selection for MS/MS was 30 counts.

Data analysis

MS/MS data were converted to Mascot Generic Format (mgf) by the Analyst software 1.1 (Applied Biosystems). Data were searched on the Mascot search engine (www.matrixscience.com), version 1.9 (*18*), against the MSDB protein database (accessed on October 2007) using the following parameters: MS tolerance 20 ppm; MS/MS tolerance 0.3 Da; fixed modifications carbamidomethyl cysteine; variable modifications methionine oxidized, propionyl (K); enzyme trypsin; max. missed cleavages 2 (*19*).

Autoacylated rhCRTAase as a stable intermediate

For the preparation of autopropionylated/autoacetylated rhCRTAase, the protein (100 µg) was incubated separately with DPMC/DAMC (100 µM) for 30 min and run on Native-PAGE under non-denaturing condition at 4°C. Negative staining was done to locate the bands of autopropionylated/autoacetylated rhCRTAase, the bands were cut and protein was eluted by crushing the gel in PBS. For the proper elution of protein, the crushed gel pieces were kept in PBS for overnight at 4°C. The autopropionylated/autoacetylated rhCRTAase so obtained was incubated with purified rGST separately for 30 min at 37°C. Further, western blotting was performed as described above to identify acylated rGST.

Results

Immunoblot identification of rhCR and rGST

The rhCR and rGST purified (Figs 1A and 2A) to homogeneity were identified as evident from immunoreactivity of the former with anti-calreticulin antibody (Fig. 1B) (1:1,000, v/v; Stressgen) and the latter with anti-GST peroxidase antibody (Fig. 2B) (1:2,500, v/v; Sigma).

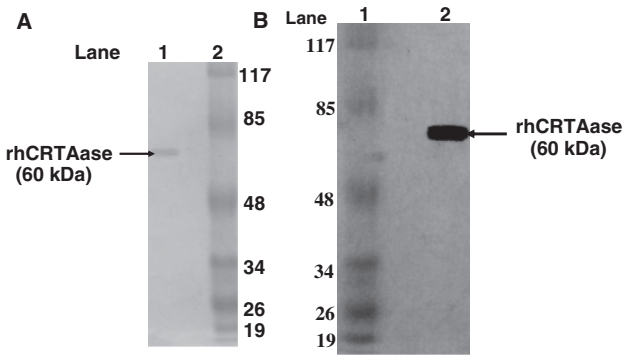


Fig. 1 Purity of rhCR and its immunological identification using anti-calreticulin antibody. (**A**) SDS–PAGE analysis of purified rhCR. Lanes: 1, Purified rhCR (60,000 M_r); lane 2, M_r standards (β-galactosidase 117,000 M_r, bovine serum albumin 85,000 M_r, ovalbumin 48,000 M_r, carbonic anhydrase 34,000 M_r, β-lactoglobulin 26,000 M_r, lysozyme 19,000 M_r). (**B**) Western blot was carried out using anti-calreticulin antibody (1: 1,000, v/v; Stressgen). Lane 1, M_r standards (β-galactosidase 117,000 M_r, bovine serum albumin 85,000 M_r, ovalbumin 48,000 M_r, carbonic anhydrate 34,000 M_r, β-lactoglobulin 26,000 M_r, lysozyme 19,000 M_r); lane 2, rhCR (10 μg).

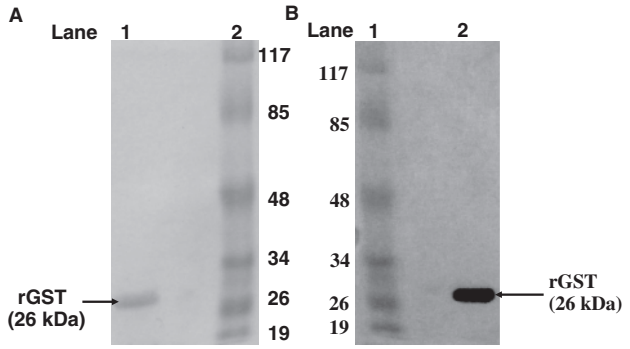


Fig. 2 Purity of rGST and its immunological identification using anti-GST peroxidase antibody. (**A**) SDS–PAGE analysis of purified rGST. Lanes: 1, Purified rGST (26,000 M_r); lane 2, M_r standards (β-galactosidase 117,000 M_r, bovine serum albumin 85,000 M_r, ovalbumin 48,000 M_r, carbonic anhydrase 34,000 M_r, β-lactoglobulin 26,000 M_r, lysozyme 19,000 M_r). (**B**) Western blot was carried out using anti-GST peroxidase antibody (1: 2,500, v/v; Sigma). Lane 1, M_r standards (β-galactosidase 117,000 M_r, bovine serum albumin 85,000 M_r, ovalbumin 48,000 M_r, carbonic anhydrate 34,000 M_r, β-lactoglobulin 26,000 M_r, lysozyme 19,000 M_r); lane 2, rGST (15 μg).

Specificities of transacylase to propoxy derivative of coumarins

The transacylase activity of rhCR (recombinant *H. contortus* calreticulin) was assayed by the method of Raj *et al.* (*4*) similar to the assay of transacetylase. The relative specificities of propoxy and acetoxy derivative of 4-methylcoumarins were studied to compare the ability of acyloxy homologues to act as donor for CRTAase mediated protein acylation. For this purpose, DAMC and DPMC were preincubated with CRTAase and rGST followed by assay of GST as described earlier (*4*) in order to measure the CRTAase activity. The time dependent preincubation of reaction mixture showed a linear enhancement in CRTAase activity. To further understand the effect

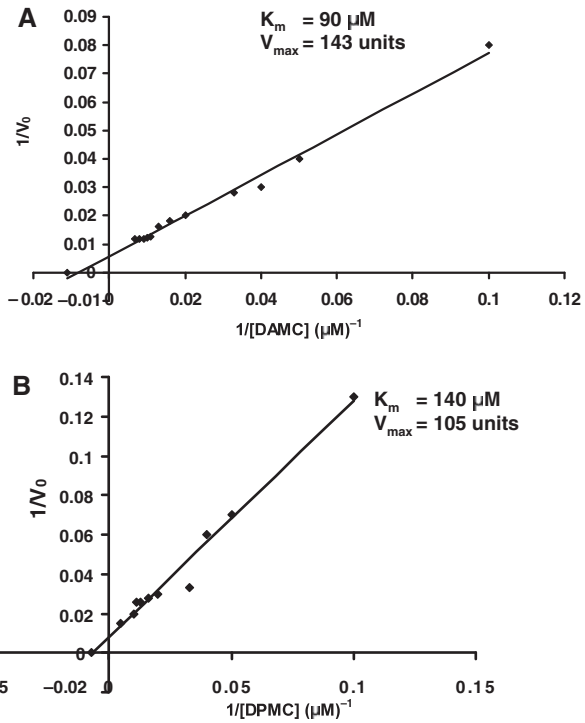


Fig. 3 CRTAase assay. Substrates DAMC and DPMC were separately preincubated (37°C, 10 min) with rhCRTAase and rGST in potassium phosphate buffer (pH 6.5) followed by the addition of GSH and CDNB. The absorbance was measured at 340 nm. Initial reaction velocities of CRTAase were determined at varying substrate concentrations (10–200 μM). (**A**) Lineweaver–Burk plot of initial velocities in the presence of varying DAMC concentrations. (**B**) Lineweaver–Burk plot of initial velocities in the presence of varying DPMC concentrations.

of acetoxy and propoxycoumarins in CRTAase activity, we investigated the kinetic parameters of the rhCRTAase in more detail. Initial reaction velocities were determined at varying substrate concentrations (10–200 μM), and Lineweaver–Burk (double-reciprocal) plots were produced and the K_m and V_{max} values were subsequently determined for each substrate. The K_m and V_{max} obtained for DAMC were 90 μM and 143 units, respectively (Fig. 3A) while K_m and V_{max} values for DPMC were 140 M and 105 units, respectively (Fig. 3B).

Immunoblot identification of CRTAase catalyzed acylation of rGST by DAMC/ DPMC

Receptor protein like rGST was used as the substrate for CRTAase catalyzed acylation (acetylation and propionylation) by DAMC and DPMC. The rGST was separately preincubated with the substrates and rhCRTAase followed by western blot using anti-acetyl lysine antibody. Figure 4 (lanes 1 and 6) clearly shows acylated rGST bands as well as the autoacylation of CRTAase when either of the acyl group donors (DAMC and DPMC) were used as substrates.

Autoacylated CRTAase as the stable intermediate in the protein acyltransferase reaction

The autoacylated CRTAase prepared by incubation with DAMC and DPMC was isolated on Native Gel. The isolated autoacylated CRTAase (Fig. 5, lanes 1

and 5) was found capable of acylating rGST independent of acyl group donors DAMC/DPMC as demonstrated by the immunoreactivity of the acylated rGST with anti-acetyl lysine antibody (Fig. 5, lanes 2 and 4). Further, by immunoreactivity with anti-acetyl lysine antibody it is evident that the autoacylated rhCRTAase upon interaction with rGST resulted in the transfer of acyl groups as revealed by faint band intensity of the autoacylated rhCRTAase during western blot (Fig. 5, lanes 2 and 4).

Nanoscale LC-MS/MS

In order to confirm covalent propionylation of the target protein and to pinpoint as many sites of modification as possible, the incubation mixtures were separated by SDS–PAGE. The protein bands corresponding to rGST protein in the control sample and in the putatively propionylated sample were subjected to in-gel digestion by trypsin and nanoscale LC-MS/MS analysis as described in the MATERIALS AND METHODS section. The protein was identified with a Mascot score of over 300 in all samples, and an overall sequence coverage of 28%. Interestingly, two peptides bearing in total three covalent propionylations on three different lysines were identified by MS/MS database search. The overall list of identified peptides, with special emphasis on modified ones, is reported in Table I.

Propionylated peptides were only detectable in test sample and not in the control sample. This could be appreciated by drawing selected ion chromatograms (SICs) for each of the two propionylated peptides detected. Figure 6 shows SICs for one of the two peptides, as an example. It can be appreciated that the intense peak identified by data-dependent MS/MS as being, propionylated IKGLVQPTR (retention time 30 min, m/z 534.3) could not be found back in the SICs referring to the control sample. This indicated that the specific modifications detected by MS/MS were only present in the incubation mixture containing the propionyl donor and the corresponding enzyme.

Figure 7A and B reports the MS/MS spectra of the two precursor ions which allowed for the identification of three propionylation sites. Propionylation is not expected to produce any intense neutral loss fragment, similarly to what recently reported for lysine acetylation. In fact, peptide sequences and the covalent

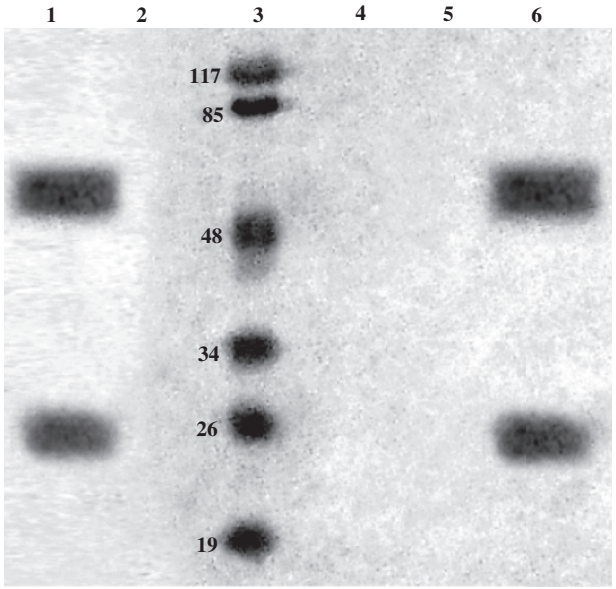


Fig. 4 rhCRTAase catalyzed acetylation and propionylation of rGST by DAMC/DPMC: western blot analysis using anti-acetyl lysine antibody. Lane 1, rhCRTAase + DPMC (100 μM) + rGST; lane 2, DPMC (100 μM) + rGST (control); lane 3, M_r standards (β-galactosidase 117,000 M_r, bovine serum albumin 85,000 M_r, ovalbumin 48,000 M_r, carbonic anhydrase 34,000 M_r, β-lactoglobulin 26,000 M_r, lysozyme 19,000 M_r); lane 4, rhCRTAase + DMSO + GST (control); lane 5, DAMC (100 μM) + GST; lane 6, rhCRTAase + DAMC (100 μM) + GST. Details are given under the MATERIALS AND METHODS section.

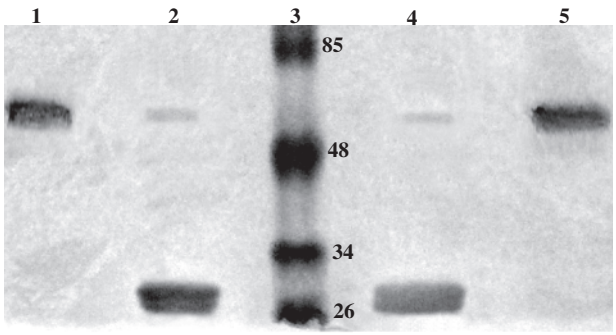


Fig. 5 Autoacylated rhCRTAase catalyzed acylation of rGST. Autoacylated rhCRTAse isolated from Native PAGE (as described under the MATERIALS AND METHODS section) was incubated with rGST and western blot was performed using anti-acetyl lysine antibody. Lane 1, isolated autoacetylated rhCRTAase; lane 2, autoacetylated rhCRTAase + rGST; lane 3, M_r standards (bovine serum albumin 85,000 M_r, ovalbumin 48,000 M_r, carbonic anhydrase 34,000 M_r, β-lactoglobulin 26,000 M_r); lane 4, autopropionylated rhCRTAase + rGST; 5, isolated autopropionylated rhCRTAase. Details are given under the MATERIALS AND METHODS section.

Table I. Peptides from rGST of *Schistosoma japonicum* identified by nanoscale LC-MS/MS and Mascot database search.

Observed	M_w	M_w	Delta	Missed	Mascot	Peptide sequence
513.79	1025.58	1025.58	0.00	0	59	IEAIPQIDK
516.79	1031.57	1031.58	−0.01	0	55	LTQSMAIIR
531.81	1061.6	1061.61	0.00	2	47	LVCF**KK**R +2 Propionyl
591.85	1181.68	1181.68	0.00	1	49	RIEAIPQIDK
657.85	1313.69	1313.69	0.00	1	67	IAYSKDFETLK
534.33	1066.64	1066,65	−0.01	1	40	I**K**GLVQPTR + Propionyl

Peptide fragments with high Mascot scores are provided here (adequate explanations are given in the RESULTS section). Modified lysines (propionylated) are underlined and in bold.

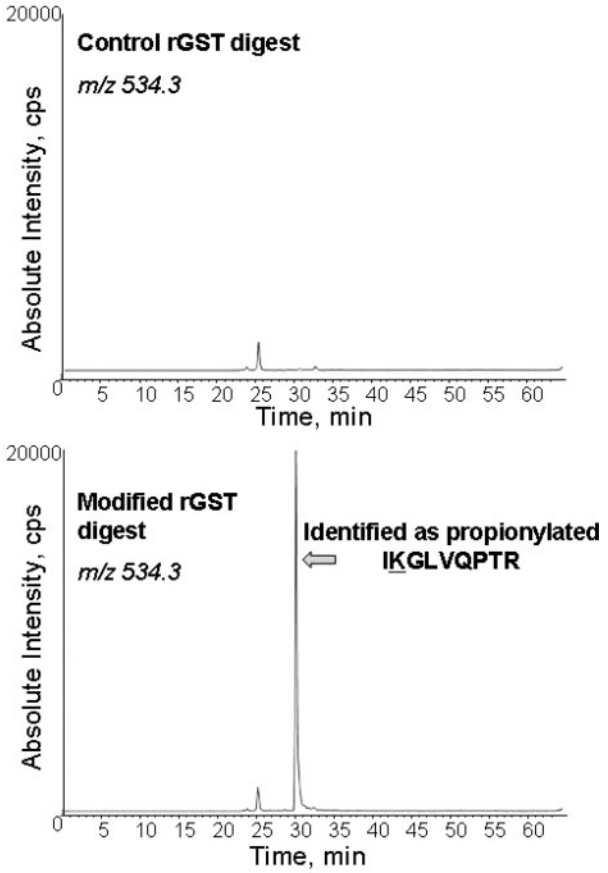


Fig. 6 Selected ion chromatograms for *m/z* 534.3. Chromatographic peak identified in test sample, as propionylated IKGLVQPTR (bottom panel) is absent in the control sample (top panel).

modification sites were assigned based on strong ion scores obtained by intact b and y ions observable in the spectra. High Mascot scores for the MS/MS spectra strongly support the covalent propionylation of amino acid sequence. These data fully supports the propionylation of three lysine residues of rGST, Lys-11, -180 and -181.

Discussion

During the course of our persistent investigations on the biochemical action of substituted 4-methylcoumarins, the role of liver microsomes in mediating the acetylation of some functional proteins by acetoxycoumarins such as DAMC, resulting in the modification of their activities was postulated. The existence of a transacetylase (TAase) capable of transferring the acetyl group from DAMC to a receptor protein such as GST culminating in the inhibition of glutathione conjugation activity was envisaged (*4*, *20*–*22*). The irreversible inhibition of cytosolic GST caused by incubation of DAMC with rat liver microsomes served as a convenient procedure for the assay of TAase (*4*). TAase was purified to homogeneity utilizing this assay procedure and was identified as an endoplasmic reticulum luminal protein calreticulin (CR). The protein acetyltransferase activity of CR was termed CRTAase (*21*). CRTAase catalyzed reaction was found to be bimolecular in nature and the formation of 7,8-dihydroxy-4-methyl coumarin (DHMC), a deacetylated product of the CRTAase catalyzed reaction was identified by us (*4*, *22*). Our earlier studies established that CR isolated from rat liver and human placenta catalyzed acetylation of receptor protein GST 3-3 (*21*, *23*) by DAMC as observed by interaction of acetylated GST 3-3 with anti-acetyl lysine antibody. Further, we have also demonstrated CRTAase catalyzed acetylation of GST and NOS by DAMC using mass spectrometry. Since we have observed that GST 3-3 and GST from *S. japonicum* share >60% amino acid sequence similarity, we are now routinely using recombinant GST from *S. japonicum* (rGST) as the receptor protein. In this communication, we have extended the investigations to explore the ability to transfer propionyl group to the receptor protein rGST by rhCRTAase utilizing propoxycoumarin as the propionyl group donor. By the application of nanoscale LC-MS/MS, the localization of N-ε-propionylated lysine residues in rGST could be examined. Accordingly, the modified lysine residues were surface exposed and distributed randomly over the sequence of rGST of *S. japonicum*. The propionylated rGST could be identified using polyclonal anti-acetyl lysine antibody as acetylated and propionylated lysines share structural similarity. Accordingly, Zhao *et al*. demonstrated the HAT catalyzed propionylated histones utilizing propionyl CoA using anti-acetyl lysine antibody (*1*). It is worth mentioning that this observation pertains to the *in vivo* acylation of protein wherein the receptor protein (Histone) underwent acetylation as well as propionylation by the endogenous acetyl group and propionyl group donors' namely acetyl-CoA and propionyl-CoA, respectively. In the present work, we have utilized propoxycoumarin as the exclusive propionyl group donor and the product of CR-catalyzed propionylation of rGST was shown to interact with anti-acetyl lysine antibody for the first time. In addition, the product of propionylation mainly the propionylated rGST was also identified by mass spectrometry. It is pertinent to state that HAT such as HAT1, CBP and p300 were also found to accommodate higher acyl group donors such as propionyl CoA and butryl CoA without steric hindrance to mediate the transfer of acyl group to histones (*1*). Although the acyloxycoumarin was found to be effectively acted upon by transacylase, the thioacetates such as acetyl CoA and propionyl CoA, the biological acyl group donors, were found to be the poor substrates (unpublished data). This observation is reminiscent of the enzymatic acetylation of arylamine utilizing *p*-nitrophenylacetate as the substrate for the arylamine N-acetyltransferase which exhibited a pretty high V_{max} (140-fold higher) as compared to acetyl CoA when used as the acetyl group donor (*24*). The LC-MS/MS results confirmed the identity of propionylated rGST from *S. japonicum*. The spectra obtained when analyzed by proteome search engine MASCOT (*18*), indicated three distinct peptide with significant score as propionylated on lysine residues (Table I). Three lysine residues Lys-11, -180 and -181, were found to be propionylated

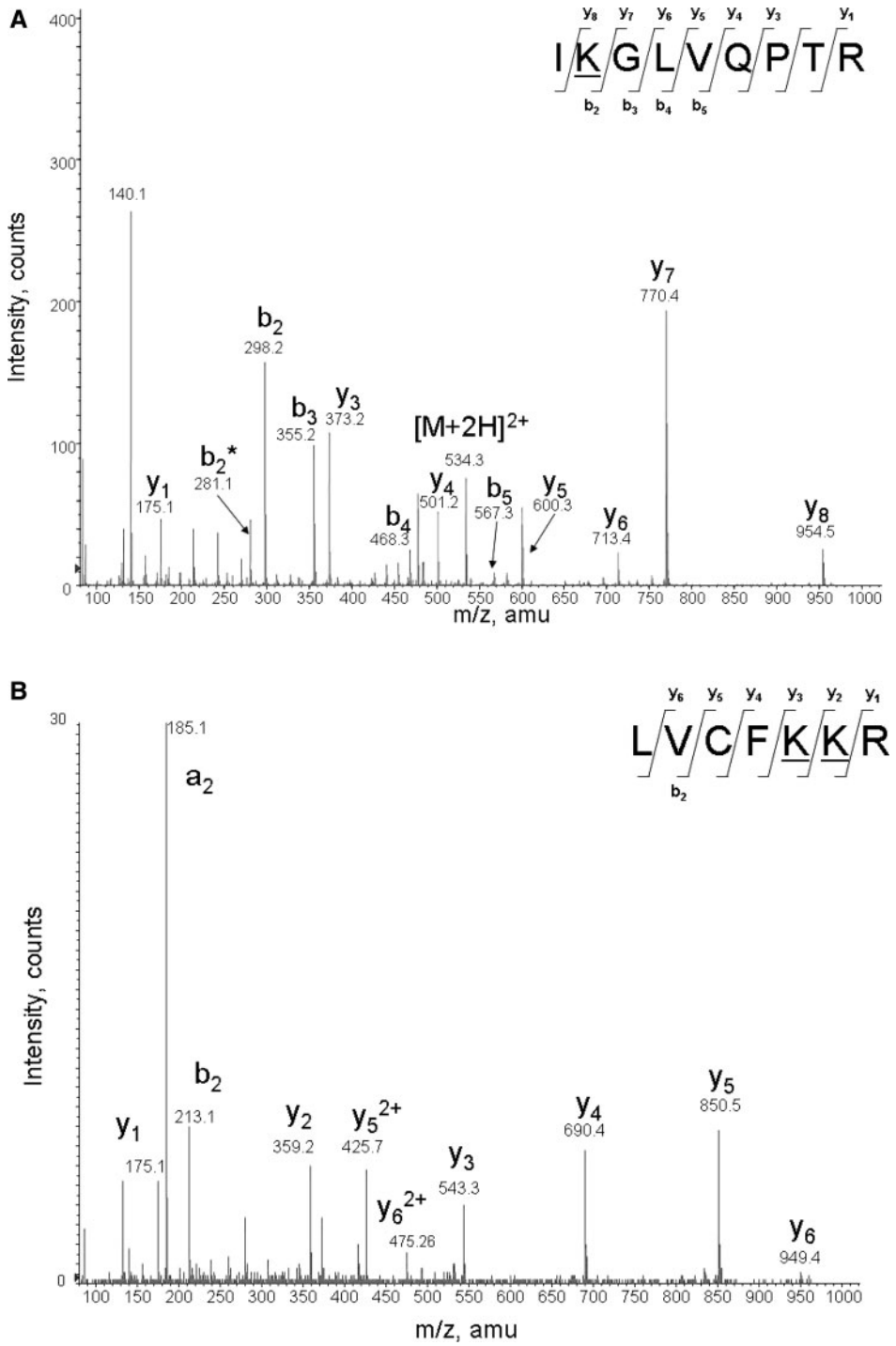
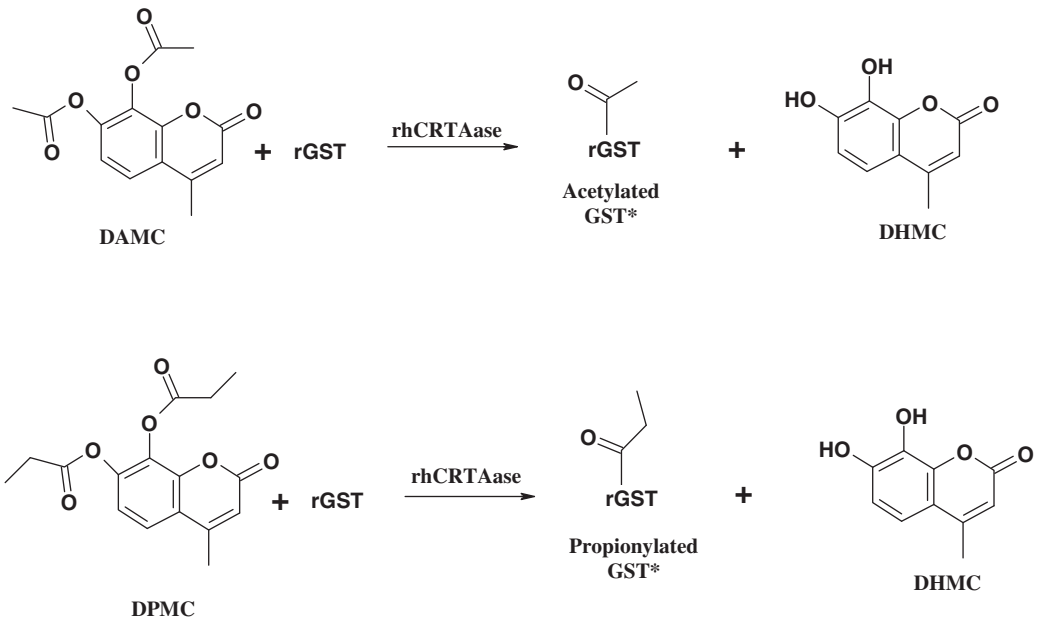


Fig. 7 MS/MS spectra of propionylated peptides. Assignment of the site of propionylation was confirmed by corresponding b and y ions. (**A**) b_2 and y_8 ions confirms propionylation of Lys-11. (**B**) y_3 and y_2 ions confirms propionylation of Lys-180 and -181, respectively.

(Chart 1). The fragment ions corresponding to b and y ions of the peptides as shown in Fig. 7A and B clearly indicates propionylated lysine residues.

CRTAase-catalyzed protein acetylation by DAMC was found to be accompanied by autoacetylation of CRTAase (*10*, *23*). Likewise, the autopropionylation of CRTAase was also encountered (Fig. 4, lane 1). The autoacylated CRTAase was found to transfer acyl groups to the receptor protein rGST (Fig. 5). We have earlier observed an increased net negative charge in autoacetylated CR (*10*) in comparison with native CR. The acetylation of lysine ε-NH_3^+ groups in α-amylase was reported to offer stability by increase in net negative charge leading to decrease in rate of unfolding by anionic surfactant and also diminished protein aggregation (*25*). Accordingly, the autoacylated CR can be expected to be a stable intermediate in the protein acyltransferase function of CR as reported here (Fig. 5). Several acetyltransferases such as HATs and transcription factors (TFIIB) are known



*** Represents lysine N-epsilon acylation (Details given in the text)**

Scheme 1 Protein transacylase function of calreticulin.

1 mspilgywki **k**glvqptrll leyleekyee hlyerdegdk wrnkkfelgl efpnlpyyid

61 gdvkltqsma iiryiadkhn mlggcpkera eismlegavl dirygvsria yskdfetlkv

121 dflsklpeml kmfedrlchk tylngdhvth pdfmlydald vvlymdpmcl dafpklvcf **k**

181 **k**rieaipqid kylksskyia wplqgwqatf gggdhppk

Chart 1 Sequence of rGST of *Schistosoma japonicum* (swissprot accession no. Q540A3). Propionylated lysines are highlighted in bold.

to undergo autoacetylation utilizing acetyl CoA as the acetyl group donor (*26–29*). The relative stability of autoacylated CR and its effectiveness as a stable intermediate to mediate the protein acylation as described here may amount to provide a tangible evidence for the proposed mechanism of action of aforedescribed protein acyltransferases. The result of our investigation has offered tacit proof for the protein acyltransferase function of CR (Scheme 1).

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Conflict of interest

None declared.

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