STUDIES ON PROTEIN ACETYLTRANSFERASE
FUNCTION OF CALRETICULIN

ABSTRACT

SUBMITTED TO THE UNIVERSITY OF DELHI FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY

SUBMITTED BY

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NOVEMBER, 2012
ABSTRACT

Acetylation is the most common post-translational modification of a protein. Biological protein acetylation can be enzymatic and non-enzymatic. The enzymatic protein acetylation independent of acetyl CoA was unknown till our group discovered an enzyme termed acetoxy drug: protein transacetylase (TAase), catalyzing the transfer of acetyl group from polyphenolic acetates (PA) to receptor proteins (RP), resulting in the modulation of the biological functions of RP. TAase was identified as calreticulin (CR), an ER luminal protein in the eukaryotic tissues. Hence, CR was appropriately termed Calreticulin Transacetylase (CRTAase). Proteins such as glutathione S-transferase (GST), cytochrome c-reductase and nitric oxide synthase (NOS) were found to function as RP for the action of CRTAase. We have also provided tacit proof furnishing the results of mass spectrometry for the CRTAase catalyzed acetylation of neuronal NOS (nNOS) by DAMC. Similar activation of NOS and other receptor proteins in rat tracheal smooth muscle cells (TSMC), human peripheral blood mononuclear cells (PBMC) etc has also been well documented.

In the present work we have carried out ex vivo studies on transacetylase function of calreticulin, providing results in support of ex vivo acetylation by DAMC of GST and calreticulin in rat TSMCs. Also the effect of various PA on NOS activity has been studied ex vivo. CRTAase activity was found to increase with increasing concentration of DAMC and also found to be linear with the concentration of DAMC. Various PA were found effective in the following order as substrate to the TSMC CRTAase in catalyzing the inhibition of GST: DAMC > 7-AMTC > 7-AMC = 7-AAMC > 6-AMC > 5-AMC. Similar concentration dependent increase in NADPH cytochrome c reductase activity, NOS activity and cGMP levels were observed with increasing concentration of DAMC. These results are also found to be in conformity with the specificity of CRTAase to the various PAs. These results provide evidence in support of the ex vivo acetylation of receptor proteins i.e., GST, NADPH cytochrome c reductase and NOS by DAMC mediated by CRTAase. The findings of these studies have relevance from the point of view of enhancement of NO in airway cells by DAMC. Since NO is known to relieve the exacerbation of airways in diseases such as...
asthma and chronic obstructive pulmonary diseases (COPD), DAMC may find therapeutic applications.

Investigations on increased calreticulin expression during hypoxia and its impact on acetylation and activity of various receptor proteins like GST, NOS were studied. TSMCs isolated from rats exposed to hypobaric hypoxia were found to show high CRTAase activity in comparison with the cells isolated from rats in normoxic conditions. The incubation of DAMC with TSMC CRTAase from rats exposed to hypoxia resulted in the modulation of GST, NADPH cytochrome c reductase and NOS to a much greater extant as compared to that from rats exposed to normoxia. Here again a concentration dependent change with increasing concentration of DAMC was observed. The results were also in tune with the specificity of CRTAase to various PAs. The effect of DAMC on expression profile of NOS isoforms was analysed by quantitative real-time PCR. Approximately two fold increase was observed in the expression of endothelial NOS (eNOS) and inducible NOS (Inos) of normoxic cells treated with DAMC. This expression was found to be further enhanced in case of TSMC isolated from hypoxia-exposed rats. However, there was no significant change in the expression of nNOS of DAMC treated TSMC isolated from both normoxic and hypoxia-exposed rats. These findings may be useful in the management of hypoxia and resulting pulmonary hypertension and other respiratory ailments.

We also examined the impact of DAMC on the Vascular endothelial growth factor (VEGF) isoforms 121, 165, 189 in human PBMC. The increased NO production in human PBMC treated with DAMC and other PA resulted in enhanced thioredoxin activity of these cells. DHMC, the dihydroxy derivative of DAMC failed to bring about the change thus supporting acetylation of thioredoxin (TRX). All these results were also found to be in tune with the specificity of CRTAase to various PA and increased in a concentration dependent manner. As a result of the increased production of NO, an increase in the expression of TRX, thioredoxin reductase (TRXR) and decrease in the expression of thioredoxin interacting protein (TRXIP) was observed. This further led to the increased expression of the various VEGF
isoforms. The cells incubated with DAMC or other PA counteracted the production of reactive oxygen species (ROS) induced by D.D.T. This was due to the upregulation of the various genes of the TRX family which are involved in maintaining the redox state of the cell. The increase in the expression of TRX and VEGF isoforms may find many applications such as in treatment of tissue ischemia, preventing lung injury and treating mucosal damage caused by ROS.

Further efforts were made to elucidate the active site residue of CRTAase. It is well documented now that P-domain of CRTAase is responsible for the transacetylase activity and lys-206, 207, 209, 238 of P-domain of CR are getting autoacetylated during this reaction. Hence, we introduced the mutation at lysine residues of P-domain of CR, where lysine was replaced with alanine (K→A). For this, site-directed mutagenesis using the megaprimer method was followed. Recombinant mutants were cloned using specific primers and protein was purified. Immunoblotting with antiacetyl lysine revealed Lys-207 as the active site while the other two mutants wherein lys-207 was replaced with alanine, failed to show the transacetylase activity. rP-domain TAase demonstrated hyperbolic kinetics. \( K_m \) and \( V_{max} \) for rP-domain TAase at varying DAMC concentrations (concentration of rGST was kept constant) as derived from the double reciprocal plot were found to be 52\( \mu \)M, 134units and 59\( \mu \)M, 129 units for the wild-type P-domain (\( P_{WT} \)) and mutant-1 (\( P_{MT-1} \)-wherein the Lys-207 was intact ) recombinant protein respectively. The substrate specificity of \( P_{WT}/P_{MT-1} \) to various PA was in the following order: DAMC > 7-AMTC > 7-AAMC = 7-AMC > 6-AMC > 5-AMC.

These results provide the tacit proof for Lys-207 to be the active site residue of CRTAase.