PROJECT COMPLETION REPORT

Preparation and characterization of peroxo-metal compounds and studies on their biological significance in cellular signalling

DBT Sanction Order No: No.BT/CP/05/NE/TBP/2010
Dated: 25.3.2011

INVESTIGATORS:

Dr. Nashreen S.Islam
Professor, Department of Chemical Sciences,
Tezpur University, Tezpur 784 028

Dr. Gayatri Ramakrishna
Centre for DNA Fingerprinting and Diagnostics (CDFD),Hyderabad
(Present address: Institute of Liver Biliary Science, D1 Vasant Kunj,
Delhi-110070)

FINAL COMPLETION REPORT FOR R&D PROJECT

Section-A : Project Details

A1. Project Title: Preparation and characterization of peroxo-metal compounds and studies on their biological significance in cellular signalling

A 2. DBT Sanction Order No and Date: No.BT/CP/05/NE/TBP/2010
Dated: 25.3.2011

A3. Name of Investigators and Co- PI

Prof. Nashreen S.Islam and Dr. Gayatri Ramakrishna

A4. Institutes: Tezpur University, Assam and Centre for DNA Fingerprinting and Diagnostics (CDFD),Hyderabad

A5. Address with contact Nos. &Email:

Prof. Nashreen Islam, Department of Chemical Sciences, Tezpur University, Assam
Mobile: +919435380222, Email: nsi@tezu.ernet.in

Dr. Gayatri Ramakrishna *, Staff Scientist, Lab of Cancer Biology, Centre for DNA Fingerprinting and Diagnostics (CDFD), Bldg.7, Gruhakalpa 5-4-399/B, Nampally Hyderabad- 500001 Andhra Pradesh,

*Present address: Institute of Liver Biliary Science, D1 Vasant Kunj, Delhi-110070.

A6. Total Cost: Rs.70.03 lakhs

A7. Duration: 2011 to 2014 (extended till March 2015)

A8. Approved Objectives of the project:

Tezpur University Component:

(1). to prepare new macro complexes by attaching peroxo species of vanadium(V), molybdenum(VI) or tungsten(VI) to soluble polymer matrices with appropriate pendant functional groups.

(2) to develop synthetic routes to newer mononuclear and dinuclear peroxo complexes of V(V), Mo(VI) and W(VI) stabilized by appropriate co-ligands of biological relevance.

(3) to characterize the synthesized complexes including the macro complexes on the basis of physico-chemical and spectral studies.

(4) to study the stability of the compounds, decomposition in the solid state as well as in solution.

(5) to examine the effect of catalase on the synthesized compoun

(6). to explore biochemical properties of the polymer-bound as well as free complexes, particularly involving their effect on key enzymes like phosphatases including acid and alkaline phosphatase, PTPase, proteinGl-6-phosphatase and calcineurin.

CDFD component:

Testing of various peroxo-compounds on induction of growth arrest by either programmed cell death or SIPS.

Objective 1: Evaluating the role of Peroxo-compounds in cellular senescence

Objective 2: Role of cytoskeleton reorganization by peroxo-compounds and its implication in growth arrest or cellular senescence :

Objective 3: Peroxovanadate as a tool to study the role of Calcineurin and NFAT2 in mediating senescence

A9. Specific Recommendations made by the Task Force (if any): None

Section-B: Scientific and Technical Progress [For the entire duration of the project]

B.1. Progress made against approved Objectives, Targets & Timelines during the Reporting Period

1. Tezpur University Component:

1.1 Preparation and characterization of new peroxo complexes of d^0 transition metal ions viz., V (V), Nb(V) Mo(VI), W (VI)

We have achieved the synthesis of new peroxo complexes of vanadium (V) (pV) and tungsten (VI) (pW) co-ordinated to water soluble polymers *viz.*, poly(acrylamide) and poly(vinyl sulfonate) by devising a viable synthetic strategy. The macro complexes of the type, $[VO(O_2)_2$-(amide)]–PAm [PAm = poly(acrylamide), $[VO(O_2)_2$-(sulfonate)]–PS [poly(vinyl sulfonate)] and $[WO(O_2)_2$-(sulfonate)]–PS [poly(vinyl sulfonate)] have been generated by reacting V_2O_5 or H_2WO_4 with H_2O_2 and the respective water soluble macromolecular ligand at near neutral pH. The following new macro complex of molybdenum (VI) was synthesized from the reaction of H_2MoO_4 with H_2O_2 and the respective water soluble macromolecular ligand at pH ca.5: **$[MoO(O_2)_2$(sulfonate)]–PS [PS = poly(sodium vinyl sulfonate)] (PSMo).**

Moreover, novel peroxo niobium complexes with nicotinic acid and amino acid co-ligands of the type , $Na_2[Nb(O_2)_3L]$ (L= alanine, valine, arginate or nicotinate) have been synthesized and characterized. We have also achieved the first synthesis of water soluble polymer immobilized $[Nb(O_2)_3$(carboxylate)]-PA [PA = poly(acrylate)] (**PANb**).

1.2 Compound characterization

The compounds were characterized by elemental analysis (CHN and energy dispersive X-ray spectroscopy), spectral studies (UV-Vis, IR, ^{13}C NMR and ^{51}V NMR), thermal (TGA) as well as scanning electron micrographs (SEM) and EDX analysis.

1.3 Stability of the synthesized complexes in solution

Stability of the newly synthesized compounds in solution of varying pH has been explored by chemical analysis as well as spectroscopic methods viz., UV-Vis, ^{51}V NMR, ^{13}C NMR, and ^{95}Mo NMR over a time period of 12 hours. Unlike majority of free monomeric peroxo metal compounds which are usually unstable in solution, the polymer anchored compounds retain their structural integrity in solutions of a wide range of pH values.

1.4 Effect of catalase on the peroxotungsten compounds

In line with the primary objective of the present work it was considered important to examine the fate of the compounds in presence of catalase vis-a-vis H_2O_2, its natural substrate. There is a search for peroxide derivatives easily formed and stable to degradation, yet efficient in their action, that can substitute for H_2O_2 at far lower doses in order to investigate how the small concentrations of H_2O_2 generated in cells will function in presence of abundant catalase.

On incubation with catalase, each of the water soluble polymeric compounds which were otherwise ascertained to be stable in solution of a wide range of pH values, were found to be degraded slowly with the loss of peroxide. In contrast, addition of catalase to a phosphate buffered solution of H_2O_2 released a half-equivalent (molecular basis) of oxygen, as expected from disproportionation reaction, which will be completed within 2 min. The rate of degradation of hydrogen peroxide under the effect of catalase was reported to be 430 μM/min from a solution of H_2O_2 of 0.1 mM concentration (Table 1). Thus action of catalase on the newly synthesized compounds was found to be a slow process compared to H_2O_2. From the Table 1 it is evident that the macromolecular complexes are at least 20-30 times weaker as substrate to catalase compared to H_2O_2

Total peroxide loss from each of the compound solution having equivalent concentration of co-ordinated peroxide (0.4 mM) was recorded to be *ca.* 0.4 mM, indicating a ratio of 1:2 for peroxide : peroxotungstate which are in excellent agreement

Table 1 Catalase dependent oxygen release from pW compounds

Sl. No.	Compound	Concentration (mg/mL)	Peroxide Content (mM)	Loss of peroxide (μM/min)
1	PAmW (3.4)	0.156	0.4	14.41
2	PSW (3.5)	0.085	0.4	15.11
3	H_2O_2	----	0.1	430.00

1.5 Inhibition of acid and alkaline phosphatases by the peroxotungsten compounds

Using the established enzyme assay system and p-NPP as substrate, the effect of the polymer anchored compounds upon the activity of the membrane associated proteins, rabbit intestine ALP as well as wheat thylakoid membrane ACP was examined vis-a-vis the effect induced separately by the previously reported free mononuclear or dinuclear pW compounds and tungstate anion.

The following compounds were investigated : **[$WO(O_2)_2$(carboxylate)]–PA (PA = poly (sodium acrylate) (PAW), [$WO(O_2)_2$(carboxylate)]–PMA [PMA = poly (sodium methacrylate)] (PMAW), [$WO(O_2)_2$(amide)]–PAm [PAm = poly (acrylamide)] (PAmW), and [$WO(O_2)_2$(sulfonate)]–PS [PS = poly (sodium vinyl sulfonate)] (PSW).**

Phosphatase activity was assayed spectrophotometrically by using p-NPP as a substrate. The continuous production of p-nitrophenol (p-NP) was determined at 30 °C by measuring absorbance at 405 nm in a reaction mixture containing the enzyme in incubation buffer. The inhibitory potential of the inhibitor species were quantified by determining the half-maximal inhibitory concentration (IC_{50}) for each inhibitor, (Table 1). It is notable that the ACP activity was efficiently inhibited by the pW compounds at doses lower than 1 μM.

The mode of inhibitory action of the complexes on the activity of the model enzymes used were evaluated by determining the kinetic parameters, K_m and V_{max} were determined by using Lineweaver-Burk double reciprocal plots. As demonstrated by the L.B. plots. An increase in concentration of each of the polymeric complexes led to a

substantial decrease in V_{max} although K_m remained unaffected suggesting a non-competitive type of inhibition by these complexes. The free monomeric complex **MWG** as well as dinuclear complex **DWG** exerted mixed inhibitory effects combining competitive and non-competitive modes. Tungstate also exhibited non-competitive type inhibition on ACP activity under these conditions.

All compounds exhibited affinity for the ACP as well as ALP in a close order of magnitude as observed from the K_i and K_{ii} values (Table 4). The inhibitors could thus be arranged in the following order of potency: **DWC > MWG > PSW > PMAW > PAmW > PAW > Na_2WO_4**. Polymer bound and neat complexes act via distinct mechanisms. Polymer bound and neat complexes act via distinct mechanisms. Each of the macromolecular compounds is a classical non-competitive inhibitor of ALP. In contrast, the action of neat pW compounds on the enzyme function is consistent with mixed type of inhibition. The pW complexes, irrespective of being supported or free, displayed more than 50 fold greater affinity as inhibitor for ACP than ALP.

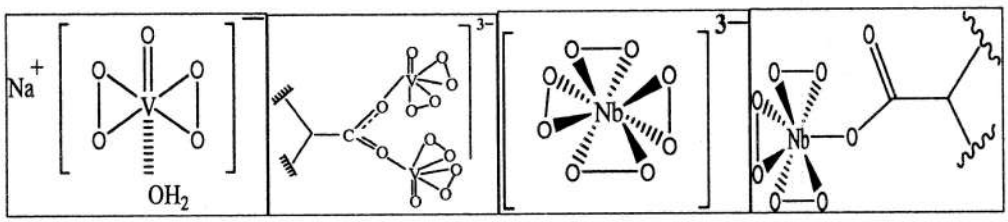
Table 2 Half-Maximal Inhibitory Concentration (IC_{50}) and Inhibitor Constants (K_i and K_{ii}) Values for pW Compounds and Other Inhibitors against ACP and ALP

Enzyme	Compound	IC_{50}(μM)	K_i(μM)	K_{ii}(μM)	K_{ii}/K_i	Types of inhibition
ACP	PAW	0.81	0.83	0.82	0.98	Non-competitive
	PMAW	0.67	0.72	0.70	0.97	Non-competitive
	PAmW	0.71	0.78	0.77	0.99	Non-competitive
	PSW	0.64	0.67	0.61	0.92	Non-competitive
	MWG	0.36	0.20	0.56	2.80	Mixed inhibition
	DWC	0.17	0.10	0.34	3.40	Mixed inhibition
	Na_2WO_4	15.23	10.45	10.10	0.96	Non-competitive
	Free polymer	---	---	---	---	---
ALP	PAW	19.33	17.54	17.13	0.97	Non-competitive
	PMAW	16.12	15.55	15.08	0.96	Non-competitive
	PAmW	17.97	16.23	16.01	0.98	Non-competitive
	PSW	15.95	14.95	14.43	0.96	Non-competitive
	MWG[9a]	14.20	14.70	48.20	3.20	Mixed inhibition
	DWC[9c]	8.20	6.46	19.10	2.95	Mixed inhibition
	Na_2WO_4[9c]	31.68	17.15	---	---	Competitive
	Free polymer	---	---	---	---	---

1.6 Effect of pV (peroxo-vanadium) and pNb (peroxo-niobium) compounds on the activity of calcineurin

Calcinurin is Ca^{2+} /calmodulin dependent protein phosphatase that belongs to the family of protein phosphatase 2B and is widely distributed in many mammalian organs. This enzyme plays important roles in the central nervous system and immune system and

in the cell death mechanism. It has been demonstrated that H_2O_2 induces dose dependent loss of calcinurin activity which has been related to the H_2O_2 induced oxidative stress. We have studied the *in vitro* effect of different concentrations of $[V_2O_2(O_2)_4(carboxylate)]$-PA [PA = poly(acrylate)] **(PAV)** and $[Nb(O_2)_3(carboxylate)]$-PA [PA = poly(acrylate)] **(PANb)** as well as $Na[VO(O_2)_2].H_2O$ **(DPV)** and $Na_3[Nb(O_2)_4]\cdot 13H_2O$ **(NaNb)** compounds on the activity of calcineurin vis-à-vis H2O2, the natural inhibitor of the enzyme. The compounds under investigation are-



(a) DPV (b) PAV (c) NaNb (d) PANb

Calcineurin activity was assayed spectrophotometrically by measuring the hydrolysis of p-NPP or R II peptide as a substrate at 30° C. The R-II peptide is the natural substrate of the enzyme. The activity was measured by following malachite green assay. The phosphate release from the substrate, forming malachite green phosphomolybdate complex with malachite green reagent (4.2% ammonium molybdate in 4N HCl + malachite green + tween-20) was measured spectrophotometrically at 620-650 nm. The effect of pV and pNb compounds were assessed by adding different concentration of the compounds on the assay. IC_{50} values were determined graphically as the half maximal inhibitory concencentration of the compounds.

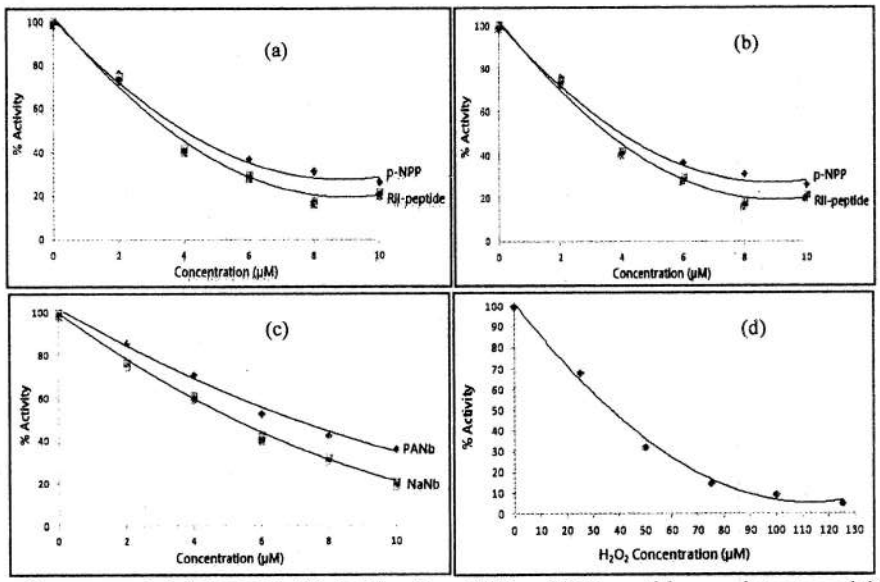


Fig 1 Effect of compounds (a) NaDPV, (b) PAV with p-NPP and RII-peptide as substrate and (c) NaNb and PANb with RII-peptide as substrate and (d) H_2O_2 with p-NPP as substrate on the activity of calcineurin.

It was reported that H_2O_2 inhibits calcineurin dependent signaling, with a 50% inhibitory concentration (IC_{50}) of 30-40 μM.[1] We recorded the IC_{50} value for H_2O_2

inhibition as 37.06 μM, whereas for the pNb and pV compounds the values were within the range of 3.7 -6.8 μM. The inhibitors are arranged in increasing order of their efficacy- **H_2O_2 < PANb < NaNb < DPV < PAV**. The results indicate that the pV and pNb compounds are nearly 10-fold more potent inhibitors of the enzyme, compared to H_2O_2.

The mode of inhibition of the compounds was evaluated by determining the kinetic parameters K_m and V_{max} by using Lineweaver-Burk double reciprocal plots. An increase in concentration of each of the compounds led to decrease in V_{max} as well as k_m value. As both $1/K_m$ and $1/V_{max}$ changes to the same extent, so the plot will consist of parallel lines, hallmark of uncompetitive inhibition. The affinity of the enzyme for the inhibitor can be measured by inhibitor constant. Kiu is the inhibitory constant for uncompetitive inhibition. It was calculated by plotting the intercepts obtained from Lineweaver plots against inhibitor concentration. The plots of 1/Km and 1/Vmax against [I] are found linear. Accordingly, both pV and pNb compounds have been established as uncompetitive inhibitors of calcineurin with Kiu values within the range of 1.1 to 2.8 μM.

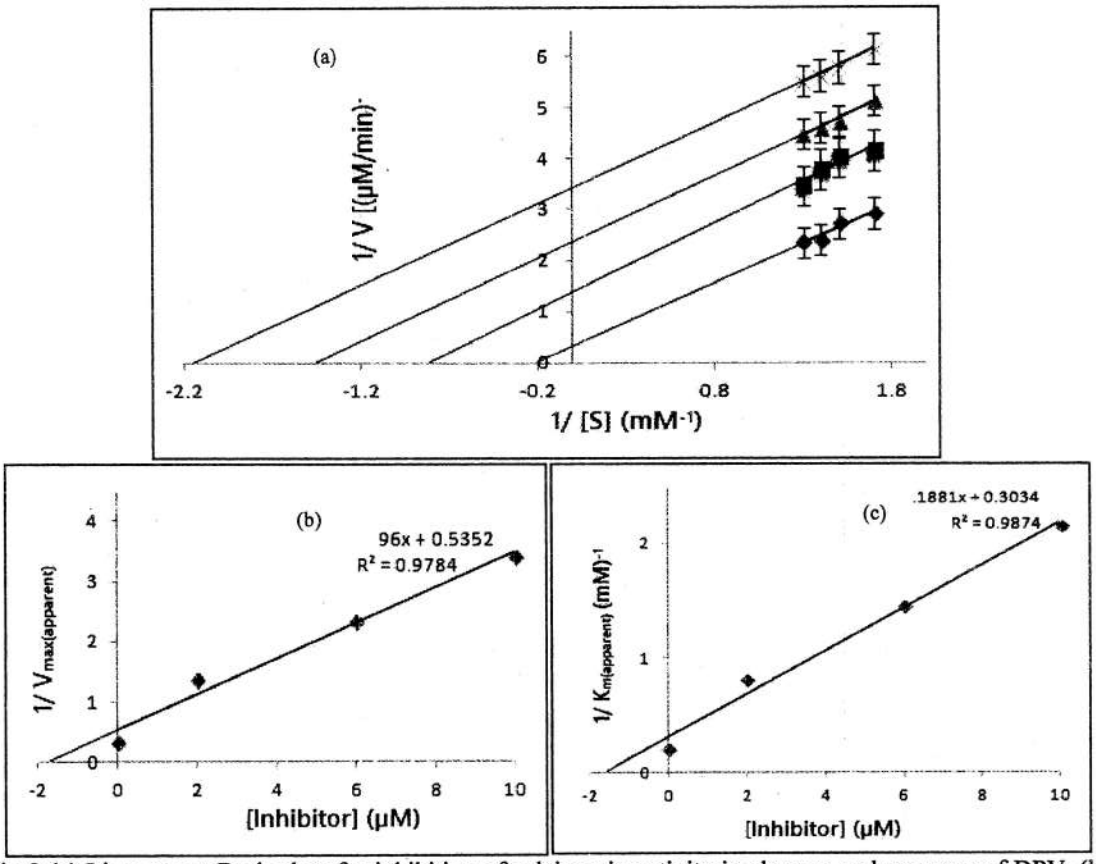


Fig 2 (a) Lineweaver-Burk plots for inhibition of calcineurin activity in absence and presence of DPV, (b) and (c) represent secondary plot of initial kinetic data of Lineweaver plot. (b) $1/ V_{max}$ were plotted against inhibitor concentrations and K_{iu} values were obtained from x-intercepts of these plots, (c) $1/ K_m$ values were plotted against inhibitor concentrations.

B1. CDFD:

Background : Treatment of cells with a variety of cytotoxic agents usually at sub-lethal doses can induce premature senescence. These include oxidizing agents, ionizing radiation, DNA-damaging agents, microtubule-damaging agents, retinoids, mitogens, cytostatic agents. H_2O_2 is the most preferred oxidant for studying SIPS and is also considered a unifying ageing mediator. However, studies on the cellular effects of H_2O_2 are constrained by the need for high concentrations and long duration of treatment because cells are abundantly equipped with catalase and glutathione peroxidase that rapidly deplete intracellular H_2O_2. Earlier it has been reported that single pulse of sub-lethal dose (150–250 mM) led to cell cycle arrest/apoptosis/senescence in human cells. Prolonged exposure to low levels of H_2O_2 also led to SIPS-like state. Availability of sufficient peroxide in intracellular milieu appears to be a prerequisite for cellular ageing. H_2O_2 forms a stable peroxo- complex with orthovanadate, and its peroxo groups are relatively slowly degraded by catalase. If DPV can act as a good oxidant because of an active and stable peroxo-group , we hypothesized that peroxovanadate can substitute for H_2O_2 mediated SIPS at much lower concentrations. Earlier we reported that Diperoxovnadate can induce features similar to senescence in the mouse cells (Chatterjee et al, 2011, Mechanism of Ageing and Development). We have now extended this work to human fibroblasts and reported in the last year progress report that that peroxovanadate, DPV, is more efficient than H_2O_2 in inducing growth arrest and cytomorphological changes in both mouse (NIH3T3) and human fibroblasts (WI38).

In the current year we did a comparative evaluation on effects of DPV and another peroxo compound viz., polyacrylic acid sodium salt peroxovanadate (PAAV) in inducing senescence in lung cancerous epithelial cell viz., A549.

1. Summary of the work done in current reporting year:

Our results indicated that diperoxovanadate (DPV) can induce features of senescence viz. flattened morphology, up-regulation of p21, PAI-1 and HMGA2 in mouse fibroblasts (NIH3T3) at much less dose (25μM) compared to H_2O_2 (150μM). In addition, we report altered localization of cyclin-D1 to cytoplasm in the senescent cells. However, our attempts to induce senescence in lung carcinoma cell line A549 using the similar doses of DPV were not successful. Hence, another peroxovanadium compound polyacrylic acid sodium salt peroxovanadate (PAAV), which is more catalase resistant and a stronger oxidant than DPV was tried. We report here that peroxovanadate when anchored to polyacrylic acid (PAPV) becomes a highly potent inhibitor of growth of lung carcinoma cells (A549). The early events associated with PAPV treatment included cytoskeletal modifications, accumulation of the reactive oxygen species, increase in GTPase activity of Rac, but not other small GTPases viz., Ras and cdcd25, and also increase in phosphorylation of H2AX (γH2AX), a marker of DNA damage. These effects persisted even 24 hours after removal of the compound and culminated in increased levels of p53 and p21 and growth arrest. PAPV-mediated growth arrest was could be pretreated

and p21 and growth arrest. PAPV-mediated growth arrest was could be pretreated effectively by pretreatment with the ROS scavenger, N-acetyl-L-cysteine, or with Rac1 knocked down by siRNA, or with NADPH oxidase activity inhibited by DPI. In conclusion our results show : (a) PAPV specifically activates the axis of rac1-NADPH oxidase leading to growth arrest and (b) PAPV is an efficient tool in the peroxide-modulated redox regulation of rac1-small GTPases. The work has been now communicated and under review.

2. Details of the Progress made against the Approved Objectives, Targets & Timelines during the reporting Period

(a) PAPV inhibited growth by activating the axis of rac1-NADPH in A549 cells

(b) The small GTPase Rac-1 is effectively modulated by PAPV

3. Results:

3 Results:

PAPV increases the activity of small GTPase Rac1

Small GTPases of the rho family are known to influence the cytoskeleton structure, and of these rac1 and cdc42, are implicated in formation of lammelipodia and filopodia like projections, respectively, Formation of such actin protrusions in PAPV-treated cells prompted a check for the activation of the three small GTPases viz. ras, rac1 and cdc42. The pull down assay method is based on the principle that active GTPases in GTP bound form will interact with their specific downstream effectors: Rhotekin (for rhoA), p21-activated kinase (for rac1 and cdc42) and raf binding domain (for ras) which in turn can be identified by immunobloting with specific antibodies to ras, rac1 and cdc42. A significant increase in rac-1 GTPase activity was noted in the cells one hr after PAPV treatment, but not ras and cdc42 GTPase activities (Figure 1A and 1B). With increasing dose of PAPV (5 μM to 15 μM (supplementary figure 1A) an incremental increase in rac 1 GTP activity was also noted. Intriguingly, the increase in rac1 activity by PAPV was an early event seen within 1 hr of PAPV treatment and then it was sustained for 24 hr even after removing PAPV from the medium. Further, rac1-GTP activity was significantly higher in PAPV-treated A549 cells compared to cells treated with 150 μM dose of H_2O_2.

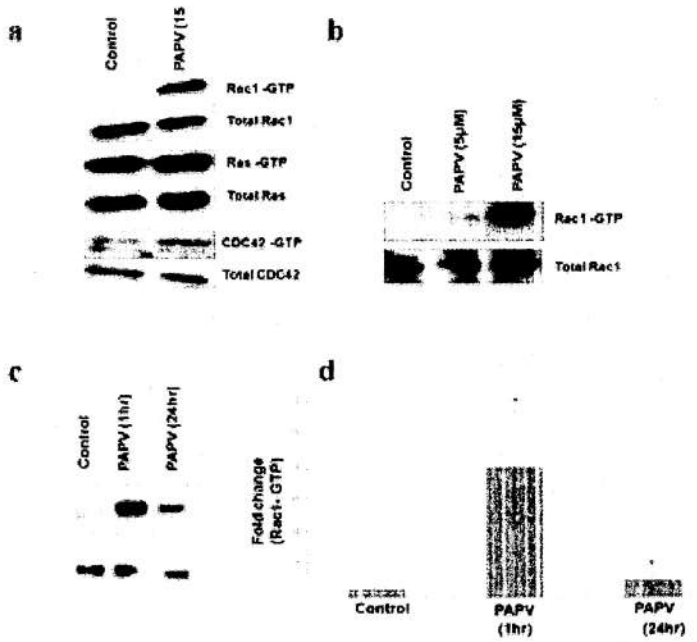


Fig. 1 (A,B) PAPV specifically activated small GTPAse Rac-1 in A549 cells. (C,D). The Rac-1 activity was maximum following 1hrs of PAPV treatemnet and remains higher than non-treated cells upto 24hrs following the removal of PAPV.

Growth arrest caused by PAPV treatment is due to elevated ROS and DNA damage.

Increase in ROS in the A549 cells on adding PAPV was monitored by increase in fluorescence of DCF oxidation product in the cells preloaded with DCFH-DA, both by flow cytometry and fluorescence microscopy. A surge in ROS occurred as early as 5 min after addition of PAPV and continued to remain high for 1 hr (Figure 2A and 2B). At this time the medium was replaced and ROS level was again monitored for 24 hr. The ROS levels remained high even at 24 hr following the treatment. The cellular imaging showed an increase in the number of fluorescent- positive cells, supporting the flow cytometry data. These results clearly indicated that PAPV, with four peroxo groups (supplementary is acting as a strong oxidant. The surge in ROS inturn lead to DNA damage (Fig.3A) and treatment with N-actylcysteine led to attenuation of PAPV-mediated growth arrest and by preventing the DNA damage (Figure 3);

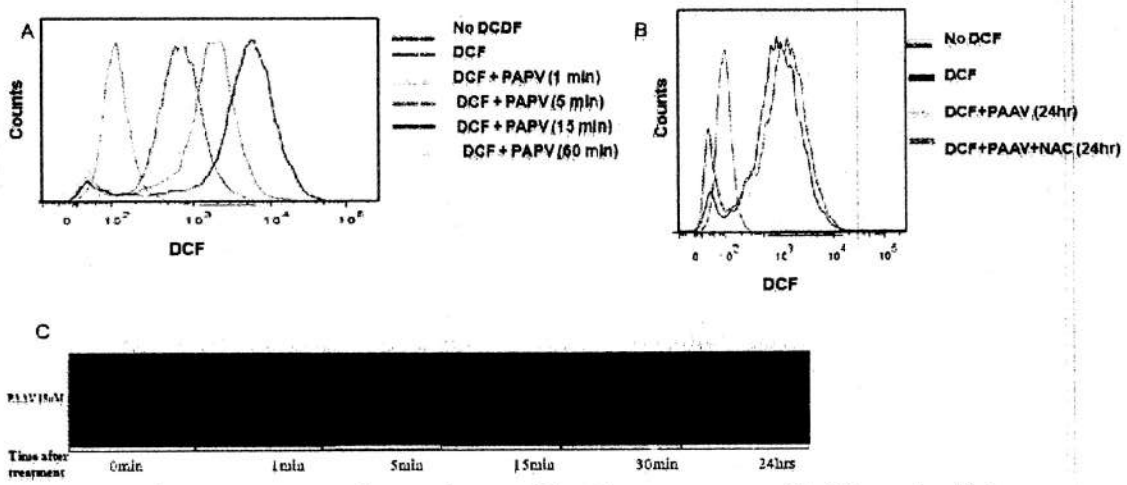


Fig. 2 Reactive oxygen species as detected by flow cytometry (A,B) and cellular imaging (C) following treatment with PAPV

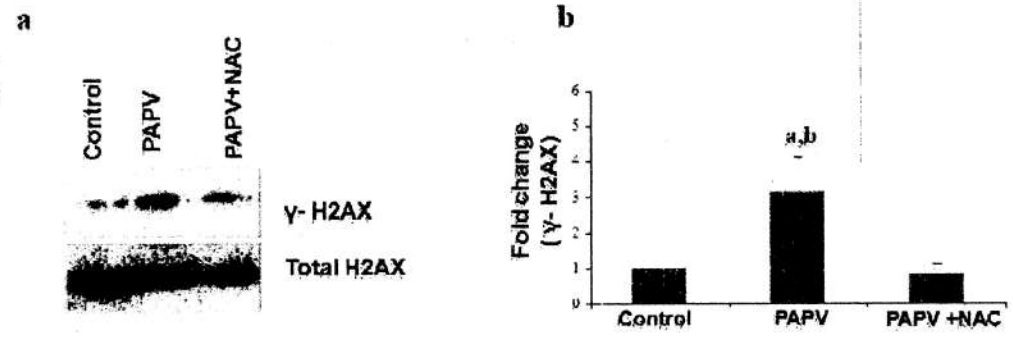


Fig. 3 (A) DNA damage, as monitored by yH2AX, a DNA damage marker and (B) tretamenet with N-acetylcysteine (NAC) prevented the DNA damage

Role of Rac1-NADPH oxidase pathway in PAPV mediated growth arrest

To evaluate the mechanism by which PAPV resulted in increase in ROS level in cells, we evaluated the role of Rac1- NADPH oxidase system. The NADPH oxidase activity was evaluated by a colorimetric method based on the reduction of ferrocytochrome to ferricytochrome. A549 cells treated with PAAV showed three fold higher NADPH oxidase activity compared to the untreated cells and the activity remained high even after removal of PAPV (Figure 4A). To investigate the role of NADPH oxidase in ROS production leading to PAPV mediated growth arrest, cells were treated with its inhibitor viz., diphenylene iodonium (DPI). Cells were treated with DPI (100nm) for 30 minutes, followed by PAPV treatemnet for 1hour. DPI treatment partially reversed the PAAV mediated growth arrest. DPI alone had no effect on growth of A549 cells. (figure 4B).

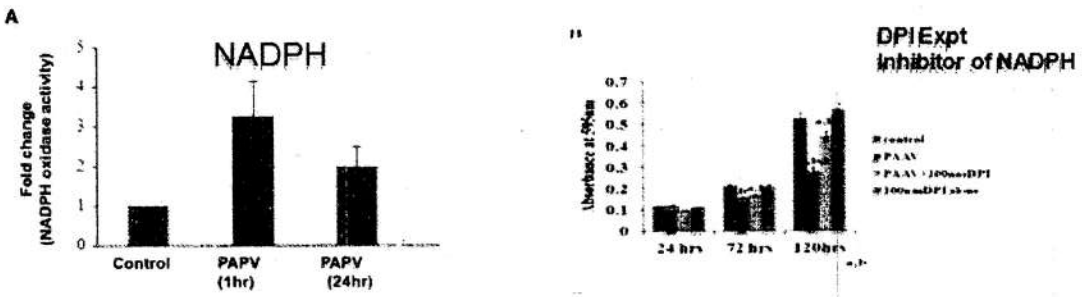


Fig. 4 (A) NADP activity in cells treated with PAPV and (B) DPI an inhibitor of NADPH oxidase could rescue growth in PAPV treated cells.

Can Rac-1 GTPase specifically influence the the process of PAPV mediated growth arrest? To answer this Rac1 was depleted in the A549 cells by transfecting cells with specific siRNA. An almost 50% decrease in Rac1 level was achieved when cells were treated 100nm Rac1 siRNA cocktail (Figure 5A). Cells treated with non target siRNA (NT) served as control. Rac1 depletion alone had no significant change in the growth of A549 cells as monitored by crystal violet method. A549 cells transfected with either non-target or with Rac-1 siRNA in general showed decline in growth when treated with PAAV in comparison to untreated cells . However, it has to be noted that the growth rate was more in PAPV treated Rac1 depleted cells compared to the non target siRNA transfected cells. Further this growth difference was statsically significant (Figure 5B). Additionally depleting Rac1 also reduced the ROS generation in the PAAV treated cells especially at 24 hours post treatment (Fig. 5C,D). These results clearly indicated the role of Rac1-NADPH oxidase-ROS axis in growth inhibition of PAPV treated A549 cells.

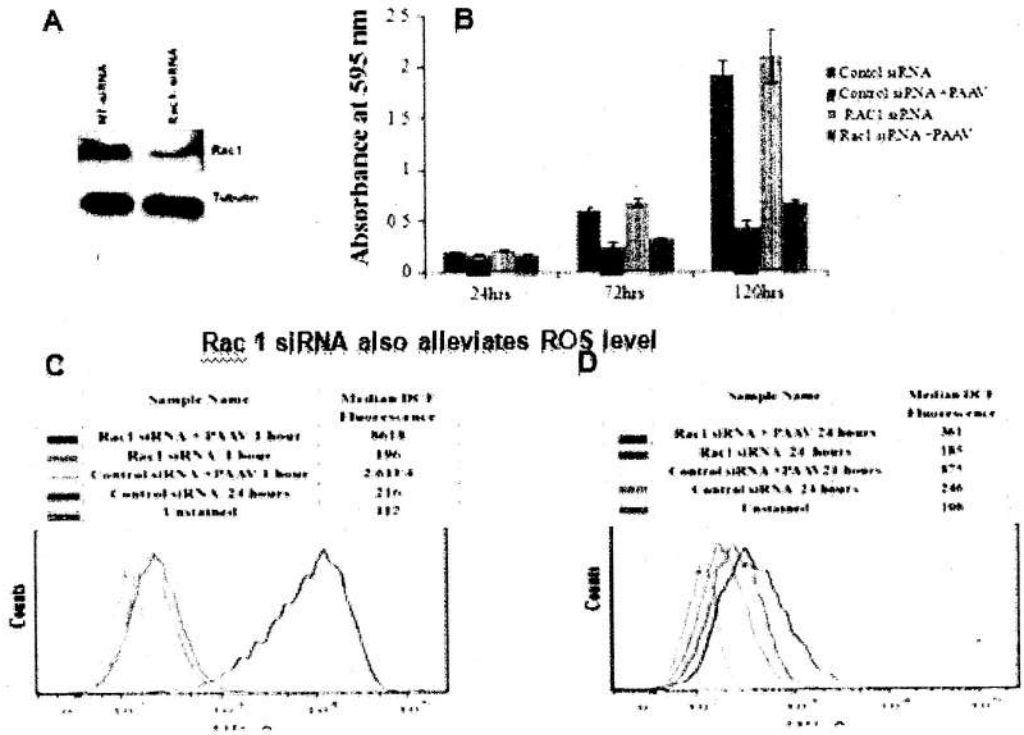


Fig.5 Effect of Rac1 knockdown (A) on growth of cells (B) and its effect on the ROS levels (C,D).

B2. Summary and Conclusions of the Progress made so far (Tezpur and CDFD)

Tezpur University

1. The polymeric compounds, as well as a pair of previously reported monomeric and dinuclear pW complexes are effective inhibitors of membrane bound phosphatases viz., wheat thylakoid membrane acid phosphatase (ACP) and rabbit intestine alkaline phosphatase (ALP).
2. The kinetic data enabled us to group the complexes into two classes on the basis of their mechanistic preferences as inhibitors. The polymeric pW complexes behave as classical non-competitive inhibitors for ACP as well as ALP while the free heteroligand pW compounds show mixed-type of inhibition combining competitive and non-competitive pathways.
3. A new peroxomolybdenum(pMo) polymeric compound has been synthesized and characterized.
4. The pMo compound are potent inhibitor of PTPase 1B.
5. Peroxovanadium as well as peroxoniobium complexes inhibit the activity of calcinurin, the serine/threonine protein phosphatse with a nearly 10 fold greater

potency then H2O2, the natural inhibitor of the enzyme. The mode of inhibition was found to be uncompetitive.

CDFD: The tow lead finding of the work are:

1. **PAPV effects growth of cells by activating the Rac-NADPoxidase leading to DNA damage and growth inhibition**
2. **Our work has shown that PAPV can be used as a tool as effective tool to activate specifically the small GTPAse Rac-1**

B3. Connectivity of the parenting institutes (Institute wise achievements to be given separately for each objective)

Tezpur University

Objective 1 & 2: We have prepared some new macro complexes by attaching peroxo species of vanadium(V), molybdenum(VI) or tungsten(VI) to soluble polymer matrices.

Objective 3: The synthesized compounds have been characterized by physic-chemical methods.

Object 4: The hydrolytic stability of the compounds of the compounds have been ascertained..

Objective 5: The polymer immobilized compounds were found to be relatively resistant to catalase action.

Objective 6: Effect of the of the polymer-bound as well as free complexes on key enzymes such as catalase, ACP, ALP, PTPase and calcinurin, the Ca2+/calmodulin dependent protein phosphatase have been investigated.

The project has been completed and work has been communicated.

Connectivity with CDFD: The compounds, DPV and peroxovanadate incorporated poly(acrylate) (PAAV) are being tested for their efficacy in growth arrest, calcinurin-NFAT activation etc as mentioned in the report of Dr.Gayatri.

CDFD

1. We have tested two compounds sent by Dr. Nashreen on senescence induced changes in two different cell lines

2. Two research students from Tezpur visited CDFD and have learnt standardization on calcineurin assay which has now been successfully being done in Tezpur University.

3. A part of this work has been jointly presented in Keystone Sympoisum on Ageing which was recently held in Japan.

4. **The project has been completed and work has been communicated.**

B4. New Leads Obtained, if any:

1. The polymeric metal compounds synthesized by us are the first known examples of non-competitive inhibitors of phosphatases. The compounds may serve as excellent selective probes of the non-competitive site of the model systems investigated. (Tezpur)

2. PAAV can induce growth arrest in cancerous cell like A549 (CDFD).

3. Peroxo vanadium and peroxo niobium compounds have been established as uncompetitive inhibitors of calcineurin which are at least ten times stronger inhibitors compared to H2O2, the natural inhibitor of calcinurin.

B5. Details of publications & Patents, if any: *Synthesis and characterization of peroxotungsten(VI) complexes bound to water soluble macromolecules and their interaction with acid and alkaline phosphatases*
S.P.Das, S. R. Ankireddy, Jeena Jyoti Boruah and Nashreen S. Islam
RSC.Advances, 2 (2012) 7248-7261

Nirupama Chatterjee[1], Nashreen Islam[2], T. Ramasarma[3] and Gayatri Ramakrishna[1] **Peroxovanadium compounds as an alternate strategy to hydrogen peroxide in promoting stress induced premature senescence . (Paper presented in The Keystone Symposium on Ageing and Diseases of Ageing held in Japan, 2012** October 22–27, 2012)

The paper has been now communicated and under review

Manuscript under preparation

1. *Peroxovanadium(V) and peroxoniobium(V) complexes as highly potent inhibitors of Ca^{2+}/ calmodulin dependent calcinurine activity*

2. *Kinetics of inhibition of acid phosphatase by peroxoniobium(V) compounds. Interaction of peroxoniobates with catalase*

B6. The training undertaken by the NER PI and the recruited manpower at the Collaborating Institutions (Details of personnel trained, duration of training undertaken)

i) Mr. A. Seshadri Reddy, JRF working under this project was sent to the Laboratory of Cancer Biology, CDFD, Hyderabad, to receive training under Dr. Gayatri Ramakrishnan, the collaborating PI, for two weeks, in December, 2011. He received training to work with various enzyme assays relevant to the present work.

ii) The PI (NER) visited Department of Biochemistry, IISC,Bangalore to hold discussion with Prof.T.Ramasarma, advisor to the present project, on project related work during December, 26-31, 2012. She also delivered a talk on biorelevant activity of polymeric peroxometallates in the Department of Biochemistry, IISC, during this visist.

B7. The Details of visits of the collaborating institutes PI and personnels to NER

The two participating institution are actively collaborating. The CDFD counterpart did not visit NER, but we have been actively interacting via telephonic calls and email. CDFD hosted the visit of two students from Nashreen's group for learning the calcineurin assay in the laboratory of Gayatri Ramakrishna at CDFD. The students visited the group for 10 days and learnt the standardization of the protocol.

Additionally both the groups jointly presented a part of the work at the Key stone symposium (abstract attached---Annexure 1)

Section-C: Details of Grant Utilisation
(Tezpur University)
(UC and other documents have already been submitted to DBT)

C1. Equipment Acquired or placed Order with actual cost:

Equipment	Cost(Rs.)
Deep freezer	17,000
N-gas cylinder with regulator and moisture trap	31,050
Analytical Balance	1,44,599
UV-Visible spectrophotometer	10,12,669 (revised)
pH meter	2,77,862
Absorbance Reader	2,61,750
Total	17,44,930

C 2. Manpower Staffing Details (In the financial year wise manner)

Manpower Staffing Details (In the financial year wise manner)

NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR (1/4/2011 to 31/3/2012)	TOTAL SALARY PAID DURING PROJECT PERIOD
Mr.Ben Raj	**JRF**	**12/7/11**	**31/8/11**	**12,000/-**	**19,200**	
Mr.A.Seshadri Reddy	**JRF**	**24.10.12**	**16/9/13**	**12,000/-**	**63,639/-**	
					FINANCIAL YEAR (1/4/2012 to 31/3/2013)	
Mr.A.Seshadri Reddy					1,44,000/-	
					1/4/2013 to 31/3/2014	
1.Mr.A.Seshadri Reddy 2. Ms. Gangutri Saikia	**JRF** **JRF**	 **1.10.13**	**16.9.13** [illegible]	**12,000/-** **12,000/-**	**1,38,000/-**	**3,64,839/-**
					1/4/2014 to 30/9/2014	
Ms. Gangutri Saikia	**JRF**	**-do-**	**1.10.2014**		**72,000/-**	**4,36,839/-**

C3. Details of Recurring Expenditure (in Rs.) (*Final consolidated statement of expenditure is enclosed)*

Item	Grants received from DBT during the period	Unspent balance carried forward from previous year [A] Rs.	Expenditure incurred during the entire period Rs.	Balance (A –B) Rs.
Human resource	4,83,000	NA	4,36,839	46,161
Consumable	9,93,739	NA	9,47,819	45,920
Travel	89,000	NA	1,30,086	-41, 086
Contingency	75,000	NA	77,439	-2439
Overheads	1,92,331	NA	1,92,312	19
Total	18,33,070	NA	17,84,495	48,575

C4. Financial Requirements for the Next Year (3rd year) with Justification: Nil

(Project has been completed).