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EFFECT OF DNFB IN COMBINATION WITH DIFFERENT CALCIUM ION CONCENTRATIONS ON VORTICELLA STALK CONTRACTION DYNAMICS

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ABSTRACT
DNFB (2,4-dinitrofluorobenzene) directly or indirectly affected biochemical performances of amino acid residues of the spasmins and batonnets, two different types of proteins found in Vorticella stalk and were responsible for both stalk's contraction and coiling. DNFB exerted chemical stresses on protein conformations and thus produced pronounced effects on stalks' contraction dynamics by means of differential data on frequency and duration of contraction-extension cyclic processes determined significant effects of DNFB concentrations from 1 mM to 5 mM threshold values on live specimens after incubation periods for more than 10 minutes in saline culture solutions and unable to affected rate of protoosmosis along the length of F-actins inside the spasmoneme of the stalk. But, it became able for tension generation on linkage-complexes and thus significantly established new force-pCa relations among protein cable connections above normal threshold values of protic potentials. Hence this study helped in understanding fundamental processes of stalk contraction at molecular level in the light of protoosmosis where energy expense was conserved by the system itself.

INTRODUCTION

The structure of Vorticella consists of a cell body, head or zooid, (30-40 nm in diameter) and a long slender stalk (100 - 200 nm in length and 2 - 3 nm in diameter). Both are connected at specific region called scopula (Amos, W.B., 1972). Nature utilizes a diverse range of designs to generate movements at cellular and molecular levels. Vorticella stalk is extremely rapid, pulling the body towards substratum in less than 10 miliseconds hence considered as fastest biodynamic phenomena (Upadhyay, *et al.*, 2008). The mode, pattern and rate of contraction and coiling of the stalk provide unified features to predict and prepare different research models. Contraction in stalk is very important means of survival of the species which is used to fetch food and bring defensive mechanism against different predators. Stalk is straight in extended state becomes twisted and spring-like helical coil after contraction with six to seven average number of rotations per stalk. Inside the stalk there is crescent shaped helically coiled structure called spasmoneme, containing dispersed and highly contractile putative spasmins and batonnets protein polymers.

Spasmin proteins are negatively charged at both ends. The Ca^{++} dissociation based negative charge-to-charge repulsion makes it extended whereas Ca^{++} association based charge-to-charge neutralization provides biochemical means of contraction (Misra, *et al.*, 2010) with less than 0.5 level of significance. Shrinkage and swelling of the spasmoneme in the presence and absence of Ca^{++} suggests that it has elastic nature similar to polyelectric gel. In the absence of Ca^{++} spasmins filaments thought to be a bundle of negatively charged protein structures, which are roughly parallel becomes weakly cross-linked when Ca^{++} interferes with polymers of spasmin proteins. In the extended state, the tendency of weakly cross-linked polymer networks (Sfi1p and Cdc31p) ready to entropic collapse by Ca^{++} interference guided by electrostatic repulsion forces between the negatively charged filaments (Gogendeau, *et al.*, 2007). The degree of rotation of amino acid residues play important role in protic cable connection which undergoes considerable changes when stalk contraction dynamic processes occur. The works of Allen (1973) and Misra, *et al*. (2010) indicated that there is exchange of energy in contraction-extension cycles of all vorticellids which has their own physiological importance where structural details indicated the link of the spasmoneme and myoneme through the bridges of linkage-complexes in the stalk and thus make possible energy transfer by involving very complex process than the actomyosin system of myonemes.

On one hand DNFB reduces NH^{2+} group of amino acid residues taking part in chemical reactions whereas on the other hand it generates free radicals which forms reactive oxygen species (ROS) in the medium thus exerts poisonous effect on the living system (Raj, *et al.*, 1989). These ROS of the medium produces pronounced effect upon the orientations of cable connection predicted upon protoosmotic model (Amin, 1986) and thus increases expense of energy at the level of ER, dispersed in the free spaces around spasmin proteins. If we correlate the predictions of protoosmotic model

with Vorticella stalk contractility, we get new idea of energy transduction in this complex system where Lohmann reaction does not occur in spasmins' polymers responsible for contraction, but a different mechanism of energy exchange occurs in F-actin along the length of polymers in association with batonnets in the presence of H^+ replaces Ca^{++} sequestrations from ER membrane of linkage-complexes dispersed along the length of stalk upto scopula in which a quite different mechanism is performed than the cross-bridges interactions of actin-myosin of skeletal muscle fibers happens in the presence of ATP while in spasmins' polymers interaction is ATP independent.

Protoosmosis is an electrophysiological phenomenon utilized to generate proton (H^+, an analogue of Ca^{++}) motive force through the process of proton flux mediated negative charge neutralization of sequentially aligned functional groups of known 20 types of amino acid residues of proteins. All the F-actin like proteins of similar/dissimilar family groups as actin, myosin, centrin, calmodulin, caltractin, dynein, kinectin, cytokinin etc work on the same principle for force and power generation to accomplish any related/non-related system associated with contraction-extension cyclic processes of cell movements by involving molecular motors of different kinds. In case of Vorticella stalk contraction-extension cycle, there are repeated sequential events in natural conditions attracting scientists to decipher information in the fields of biological dynamics in relation with future perspective, and thus work was designed to decipher most of the relative information on the basic experimentations, hypothesis and perspective predictions. Thus putative model generated after overall processes provided an excellent approach utilized to understand Vorticella stalk contraction mechanism in a well-experimental design by involving thought based predictions on the basis of frequency and duration profiles by cross scrutiny of available information (Singh & Amin, 1989). The Vorticella stalk involves excitation-contraction coupling reactions of Ca^{++} - Cl^- mediated counter-flux of ionic movements along the length of F-actin in the form of H^+/K^+ coupled reactions and thus generates electro-chemical motive forces along the length of negative charged protein polymers as in myonemes, neurons and algal cells by involving their functional motives and domains of negatively charged amino acid residues for spasmins and batonnets on which the machinery of Vorticella stalk works. This is an excellent model used to know and justify present hypothesis on the basis of biodynamic experimental support for molecular model prediction.

Spasmoneme is extended throughout the length of stalk containing polymeric motor proteins spasmin as the elements of contraction whereas batonnets are responsible for stalk coiling. The well described rubber-like elastic model of contraction over headed by simple damped spring model (Moriyama, *et al*., 1898,1999) provides basic information on contraction-extension dynamic processes in an artificial manner but it is not sufficient to describe the entire molecular mechanisms of stalk contraction, thus protoosmotic model has been selected to resolve some hidden information related with mechanism of structural modifications of spasmin proteins involved in contraction-dynamics processes and to conserve the energy of the system.

MATERIALS AND METHODS

The aim of materials and methods utilised in the experiments to obtain pure results by avoiding aberrations in obtaining data through managing experimental set up at a constant room temperature along with constant and continuous electric supply to get pure results. The tools and techniques used in the experiments were preapproved in the fields of biodynamic experimental data collection and their analyses under differential colloidal non-equilibrium media conditions (Oosawa, 1983). The same methods and procedures were practiced under controlled condition in this case of Vorticella stalk contraction-extension cyclic investigations in the focus of protoosmosis.

This research was designed in the new and nascent format which was step-wise preceded. In which the cultured and sub-cultured specimens (specially mass culture in NPW and APW) were used in isolated form (by zooming camera on individual specimen under microscopes) to view, photography, videography, (by attaching Nikon camera with Magnus & Olympus microscopes one-by-one for clear vision/ images) and data recordings (in the form of videographs) treated with different DNFB ionic concentrations independent as well as in combination with Ca^{++} concentrations from 1 mM to 5 mM for approximate times of repeat on the basis of which settings of the study for further proceedings ($\Delta x/\Delta t$ – distance travelled per unit of time) approached in well determined way. These designs were used in the experimental methods helped to obtain purified data (by involving mean and standard deviation in graphic predictions) for analysis (stock's chart in low, medium and high values software dependent data arrangement and result production), description (literary description of obtained results on the basis of hypothesis determination), discussion (hypothesis prediction on the basis of preplanned ideas and the results obtained) and conclusion (output of the paper). The novelty in this article was designed in the form of hypothetical model/ diagram prediction.

Methods used in the experiments were simple and pre-approved, for that live specimens were collected from seasonal ponds of Chapra city Bihar in India. The collected specimens were cultured and sub-cultured in natural and artificial pond water collected from the same pond and prepared in the laboratory by using 0.1 mM of KCl, NaCl, and NaOH of the same concentrations for that volume-by-weight (v/w) method is applied. For weighing solid chemicals, chemical weighing machine (Wensar weighing limited model number ECB 300) was used.

For the study specimens were kept into watch glass then kept onto the stage of microscopes along with watch glass for the convenience to proceed further processing of biodynamic visualization and recordings. Among the specimens viewed in microscope (Magnus MS 24/13), single specimen was selected for study (Olympus ch2ibimf). For both photography and videography, photo-video-graphic camera [Nikon 12.1 150 3200 P/S/A/M (Coolpix)] was used. After photography and videographic recordings data were transferred to the computer system. By using obtained data manual modification

was performed then computer software was used to prepare graphs on the basis of obtained tables.

Vorticella living specimens were collected from the nearby natural ponds, a very good source of specimen collection for the purpose of study and research. After its collection, the standardized laboratory conditions were used to keep them for years in living natural conditions. To extend the work, the cultured specimens were sub-cultured for 4-5 days of intervals in regular trials and thus we became successful in culturing specimens in both the conditions – NPW & APW. NPW (with normal composition as we know that dry matter – 10^{-3}to10^{-1} %, Organic matter – 10 to 100 mg/kg, Total nitrogen – 1 to 10 mg/kg, Total ammonium nitrate – 0.1 to 10 ppm and Total phosphorus – 0.001 to 1 ppm) (Boyd, 1990) was used after its purification process through boiling, filtration, sterilization, and distillation on one hand whereas APW preparation was used after its preparation in laboratory conditions on another hand. The room temperature (Frederick, 1981) was found suitable for proper growth of the specimen. First of all specimens ware kept and used in NPW then APW laboratory conditions were applied at room temperature. For APW preparation NaCl, KCl and CaCl2 was used in same concentrations (0.1 mM). The boiled eggs were used to provide supplements for the specimen in the form of paste of solidified albumin with a little pinch of yolk 1/4 gm (w/v), then final nutritive supplementary liquid food were prepared for use. Under controlled bathing media, the specimens were immersed for approximately 1 minute in wash solution contained 20 mM KCl, 10 mM EDTA and 10 mM Tris-maleate buffer solution. The live specimens were again washed three times with a wash solution of 50 mM KCl, 2 mM EDTA and 10 mM Tris-maleate buffer for approximately 15 minutes for improved preparation. These steps were followed by reactivation media treatment consisted of 50 mM KCl and 50 mM Tris-maleate in combination with DNFB and pCa. To know the effect of pCa and DNFB, several concentrations of these chemicals from 1 mM to 5 mM were used independently and in combination under preapproved experimental conditions (Kaitoh & Naitoh, 1992).

For the experimental work, first of all Chara, Nitella and Myryophyllum like aquatic submerged plants were collected from nearby existing seasonal ponds of Chapra-city in Bihar-state of county-India. These aquatic submerged plants are good source of Vorticella confinement along the length of plants' body as branches and twigs in clear shallow water where specimens are found in fixed conditions. For the culture of living specimens as found Vorticellids under microscopic observations of Magnus MS 24/13 (monocular) and Olympus ch2ibimf (binocular) along with attached Nikon photo-video-graphic camera 12.1 150 3200 used in both normal and zoomed view for mass and single specimen observations on the basis of need to measure contraction-extension cyclic processes of specimens' stalks under predetermined chemical stress conditions of DNFB in independent and Ca^{++} combined conditions.

For experimental solution preparations, simple technique was used on the basis of density gradients and molecular weights of planed and available chemicals in v/v (DNFB, NaOH, HCl & KOH) and v/w (NaCl, KCl & CaCl2) ratio conditions. Each time during experiment for chemical pick-up new pipette was used for different specimens for several times of experimental applications in a fix and predetermined chemical stress solutions. The standardized method used for APW (artificial pond water) preparation was to mix KCl, NaCl and NaOH in 0.1 mM same concentrations.

For controlled experiments, pCa and DNFB solutions were prepared from 1 mM to 5 mM in different glass flasks which were marked for their identification. The pCa solutions were prepared in the ratio of volume/weight whereas DNFB, HCl and KOH solutions were prepared in the ratio of volume/volume in the light of mole concept and volumetric determinations of theoretical and practical concepts of chemistry textbooks (physical and organic both).

For APW preparations, NaOH, KCl & $CaCl_2$ were mixed in a ratio of 0.1 mM in their liquid solution forms. The cultured specimen in NPW washed by APW for experimental culture trial, and then transferred into APW experimental culture medium, in which animal based supplementary nutrients a mixture of albumin and yolk with water in the form of solutions were used in the ¼ ratio. After 24 hours of culture conditions in APW had food supplements, we had enriched vorticellids in culture medium for use which were achieved to precede the experimental trial through photographic and videographic technical implementations.

To continue experiments after 24 hours of cultured experiments in APW, specimens were transferred on to the surface of watch glass along with twigs of Chara, Nitella or Myriophyllum branches (Verma, 2020) of 1 Cm in length under thorough microscopic observations, and then DNFB of different chemical strengths were supplied to observe contraction-extension cycles under Magnus MS microscope. The specimens were then transferred to Olympus for zoomed view, photography and video recording with attached Nikon photo-video-graphic camera. Photographs and video-graphs were taken. Photo-graphs were developed whereas video-graphs were transferred to computer system for quantitative recording. The quantitative recordings of velocity of stalk contraction were measured in the form of $\Delta x/\Delta t$. Then biodynamic measurements were followed by biometric methods utilizations. The data obtained were plotted in the form of frequency and duration parameters. These data were compared and matched with the findings of Mahadevan and Matsudaira (2000) then we have well-excuted tables (1 & 2) in their relative forms.

The 1 mM to 5 mM chemical strength solutions were designed to know the effect of each chemical's strength on the velocity and acceleration profiles of Vorticella stalk contraction dynamics. These experiments were confirmed spasmins' and batonnets' based resistance capabilities of the system for DNFB concentrations by controlling pCa regurgitation from natural ER stalks' tubular house present around spasmins and batonnets inside the spasmoneme of the stalk. The reversibility were maintained by acidification from pHs 6.8 to 5.5 and thus it became artificial regulatory method to control the DNFB toxicity in both pCa independent and in association medium via different trials. Thus it reflected very strong justification for pCa independent along with associative combinations in the

form of replacements or occurrence of pCa by [H^+]/proton via flux regulation operating system.

For culture preparation, Borosilicate glass-wares were used in high-volume-surface (HVS) ratio of 50 mL, 100 mL and 500 mL for the purpose of solution preparation of different chemical strength concentrations which was completely desired and needful for handling and utilization of used glassware during research work.

Before experimentation, specimens were kept in standardized controlled culture media then transferred into sub-cultures after every 2 days of intervals. Then solutions of different Ca^{++} concentrations and DNFB concentrations were used for the experiments and the experiments were performed at 25 to 30ºC to observe contraction-extension cyclic processes of the stalk. The lengths of stalks of many Vorticellids were measured by linear distances from the base of the stalk by using simple equation of motion ($\Delta X/\Delta T$ where ΔX indicates distance travelled in ΔT time intervals), where it was assumed that n moles of pCa and DNFB bonded with the contractile filaments of spasmins and batonnets in the contracted state of spasmoneme and the stalk where the binding brought contraction in the stalk at differential rates of fractional stalks at different time intervals (Ochiai, *et al.*, 1979) in ms (miliseconds) which can be better expressed in the form of following equation:

$$K = K_m n = \frac{[s]\left[pCa/DNFB/pCa^{+}DNFB\right]^n}{\left[S.pCa_n/DNFBn/pCa_n{}^{+}DNFB_n\right]}$$

Where, K is the rate of reaction, $K_m{}^n$ is the equilibrium constant, [S] is the contracting element in the extended state, pCa is the factor bringing contraction during extended state whereas DNFB is the factor bringing reduction in the rate of contraction in its time dependent specifications.

The length of stalk after contraction under the influence of different pCa and DNFB concentrations represented changes in stalk configuration at known intervals of fractional stalk lengths and thus the fractional stalk length expressed differential molecular orientations of spasmins and batonnets proteins polymers under the influence of protoosmosis under different acid-base balanced conditions used to conserve the energy of the system. On the basis of energy transduction processes protoosmotic flux reflected unified molecular hypothetic desired prediction for the description of entire processes involved in Vorticella stalk contraction processes.

The parameters used in these experiments provided new information in the light of energy compensation along the length of the stalk as differential rates after predetermined chemical strengths' treatments. Throughout the experiments as described in this paper is based upon the use of live specimens. The tick marks in figures and graphs were not applied to hide turn-over assessments and thus the spaces were left blank for quantitative justification in the way of practical approach to reflect the technical expression for experimental clarification in a regular way and thus it reflects the smooth pictures of frequency and duration profiles which may be frequently utilized to know concept of protein folding on the basis of numbers unitary area of proteins confinements in the spasmoneme of the stalk.

The negatively controlled experiments on frequency and duration profiles of DNFB independent were used to reveal present data in absolute format of the velocity and accelerations of stalks' contraction and were controlled by HCl and KOH in terms of repetitive reversible reactions of Vorticella stalks' contraction dynamics at the range of pHs 6.5 to 5.5 by utilizing single electrode based pH meters of Systronic company and the functional affirmatives of the instrument were cross-checked before its utilizations by applying different buffer tablets of Merck company and thus the results we have reflected positive approach for hypothesis predictions on live specimens.

The biometric calculations used in these experiments were mean and standard deviation followed by data arrangement in the format of stock's chart preparation for their sophisticated predictions in the form of figures after table arrangements reflected purity in that we have presented in nascent format.

RESULTS AND DISCUSSION

Effect of DNFB treatment

The concentrations of DNFB (1 to 5 mM) exerted very mild effect on living specimen. Initially it exhibited spontaneous contractions at very low frequency which was negligible and different from the frequency in standard normal saline solution of controlled experiments. Determining the rate of contractility after each successive 1 minute of time-intervals, frequency decreased slightly if compared with alternate repetitive contractions. The conditions remained similar for 1 mM to 5 mM of DNFB concentrations. The apparent decrease in frequency was observed gradually after 10 minutes of time-intervals in controlled saline solutions incubation (Figure - 1). In contrast the duration of contraction was halted upto 70 to 80% with maximum degree of stalk coiling. Its rate along with increased dimension of stalk decreased immediately after application of DNFB and this remained unchanged at defined concentrations (Figure - 2). This decreased duration was mostly

Table 1: DNFB concentration based biodynamic phenomena in paradigm shift of Vorticella stalk contractility where S.D. = ±0.02 and N = 4.

[DNFB] in mM	Frequency /minute	Contractility in μm/s	Force in dynes/s	Power in ergs/s/g
1	0.51	2.28	7.68	53.7
2	1.83	4.42	16.31	117.8
3	2.52	11.42	38.9	131.7
4	3.54	15.44	51.92	375.4
5	0	0	0	0

Table 2: DNFB and pCa combination based biodynamic paradigm shift of Vorticella stalk contractility where S.D. = ±0.02 and N = 4.

[DNFB] + pCa in mM	Frequency /minute	Contractility in μm/s	Force in dynes/s	Power in ergs/s/g
1	0.92	4.71	13.44	95.21
2	1.98	8.28	27.84	203.95
3	2.54	11.42	37.47	267.12
4	3.63	15.85	52.81	384.23
5	3.81	16.56	55.68	405.91

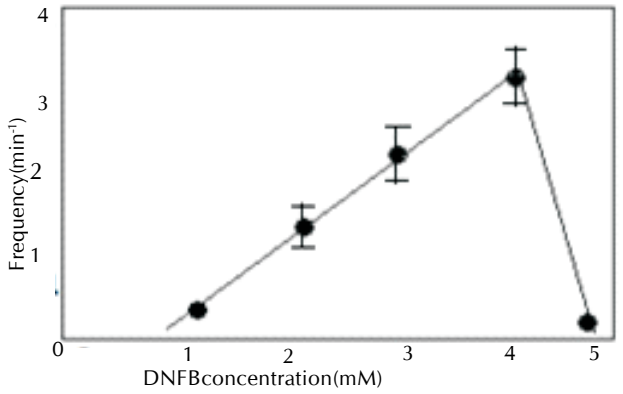

Figure 1: DNFB concentration based frequency profile on Vorticella stalk contractility.

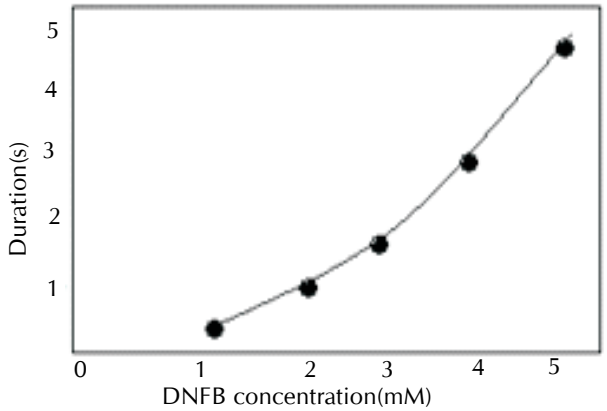

Figure 2: DNFB concentration based duration profile on Vorticella stalk contractility

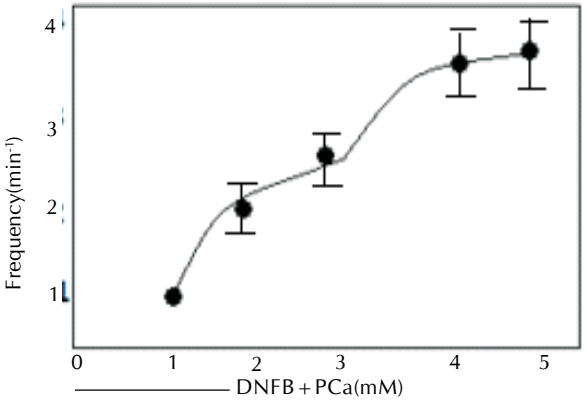

Figure 3: Effect of DNFB concentration in combination with Ca^{++} concentration representing frequency profile of Vorticella stalk contractility.

due to the prolonged phase of patterns modification in terms of DNP productions.

To test the effect of DNFB in more precise and standard manner, both frequency and duration of contraction were determined at the values of 3 minutes of time intervals where S.D. was ± 0.02 and N was 4 after introduced DNFB at different concentrations ranging from 0.5 mM to 5.0 mM. In that situation, both frequency and duration reduced slightly with increasing concentration gradients above respective assumptions where experimental threshold value was 1.0 mM for the prompted frequency and 2.5 mM for the duration variation (Table - 1).

In our earlier experiments (Verma and Singh, 2017a&b) we have found that the effects of Ca^{++} ion concentrations in the external medium for living specimens did not reflect significant effect on frequency and duration profiles of stalk contraction. The DNFB on the other hand reflected mild effect on the frequency and duration profiles of contraction variations in these present observations. The condition was found to be similar from 1 mM to 5 mM, but apparent decrease was found only when the specimen was incubated for 10 minutes or more. This examination confirmed the positive sensitive effects of both Ca^{++} and DNFB concentrations in independent conditions as well as together with in association and thus reflected modifications in spasmin proteins folding dynamics which was compensated by time dependent bioenergetics phenomena in terms of second law of thermodynamics.

Combined effect of DNFB and Ca^{++} concentrations

The application of DNFB & Ca^{++} in combined state reflects significant effect on longer stay in saline solutions for both frequency and duration which is referred in both contraction and duration data format which was affected with increasing concentrations gradients of DNFB applications, but when the Ca^{++} concentrations were used in combinations, performance altered at 5 mM concentration in maximum thermodynamic variable sense in the applied saline solutions, and thus the suppression was halted at projected frequencies and durations as resumed for contraction. Thus it is clear that the inhibitory effect of DNFB was ameliorated biodynamic phenomena of stalk contraction along the length of the stalk in light of protoosmotic molecular dynamics in terms of provided data (Table – 2, Figures – 3 & 4) which can be further interpreted in recently approached model (figure - 5) in conjectured way at advanced implications.

The apparent decrease in frequency was observed in logarithmic terms in respect of time of 10 minutes at 1 to 4 mM DNFB concentrations which was same for the linear graph at 5 mM of concentration. If DNFB of same concentrations were applied with same value of concentration gradients of pCa from 1 mM to 5 mM, there was sudden fall in acceleration potentials which could be prevented by pCa inactivation thus the reactions in terms of enzyme kinetics were easily regulated through HCl and KOH involvement at pHs 5.5 to 6.8 and not for upper range of pHs 7.8 to 9.0 to where protoosmotic regulatory mechanism was insignificant (figure 1 & 2). During these reaction kinetics, the process of contraction-extension cycles were halted upto 70 to 80% in terms of speed of contraction and coiling of the stalk at different rates, paths and patterns from DNFB independent concentrations and in combinations with pCa concentrations gradients.

When DNFB was independently applied in experimental culture conditions, it reflected positive significant effect on contraction dynamics where HCl and KOH was used as

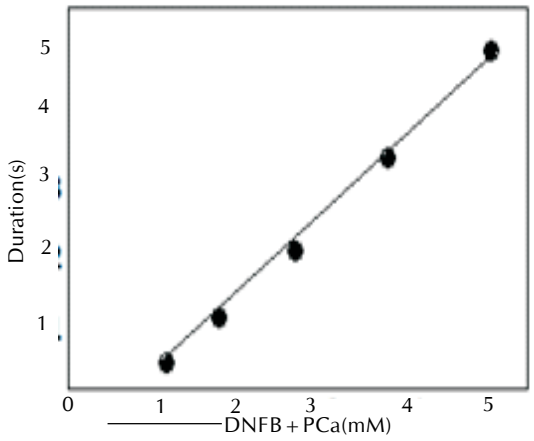

Figure 4: Effect of DNFB concentration in combination with Ca^{++} concentrations representing duration profile on Vorticella stalk contractility.

negative feedback control system in case of DNFB concentration gradients in combined conditions of pCa concentration gradients in same concentrations as 1 mM, 2 mM, 3 mM, 4 mM and 5 mM combinations. In these cases, DNFB frequency profiles indicated positive sensitive effects, when HCl and KOH were used as positive and negative reversible reactions index controller, whereas DNFB in combination with Ca^{++} concentration as DNFB regulatory system and comparative indices for physiological performance of Vorticella stalk worked in association enzyme-kinetics indices. In these applications of DNFB, binding affinities of ryanodine receptors and folding dynamics of spasmins and batonnets were affected at different rates as in the form of frequency and duration experimental data (figures – 1 to 4, tables – 1 and 2). Ca^{++} along with DNFB in same concentration from 1 mM to 5 mM reflected differential pictures of frequencies and durations, thus referred a divergent path for protein based enzyme-kinetics functional performances.

In this work, key parameters used to know the effects of DNFB effects independently and in combinations with pCa on all respective biodynamic processes associated with Vorticella stalk contraction dynamics, especially on frequency dependent protein folds, DNFB and pCa binding kinetics and the path and patterns of protoosmosis along the length of F-actins dependent spasmins and batonnets molecular polymers isotypes as Sfi1p and Cdc31p in a linear format. The ideas of the work plan and discussions of the results came from the previous works as throughout in references. The force and power utilized in the tables 1 & 2 are the relative values in respect of velocity, frequencies, durations, and force – power relative profiles. The frequency based findings of the tables have same values of force and power, and were adopted from the work of Mahadevan and Matsudaira (2000). Thus on the basis of tables presented we can correlate our findings with others in a respective way. These data can further be extended upto the levels of bioenergetics and thus we have thermodynamic approach for protein folding regulation responsible for Vorticella stalk contraction extension cycles and thus the relative values of protein based machinery in biomechanical ways, and thus the complete biodynamics associated with Vorticella stalk contraction dynamics can be better understood in a comparative way of other related systems on which overall biodynamic cell physiological operations depend.

The logistic approach for the discussions of the work tells us molecular physiology of the Vorticella stalk contraction dynamics in a complex way to reveal its multidimensional approach towards the works and workers contributions in the same relative fields that the students and the scientists can further extend their future plan in another relative or non-relative ways, hence a comprehensive approach of discussion has adopted to deal all possible mechanisms of molecular physiology of Vorticella stalk in a new versatile dimension. During this logistic approach of discussions, all publications mentioned in references played equal and independent significant approach for the discussions extended to predict the hypothesis in a nascent justified linguistic description. Among all these approaches, protoosmotic approach for energy conservation presented thermodynamic view to the process in respect of DNFB concentrations, thus, the discussions contain multitudes of approach rather than a single straight way prediction.

Frequency decreases at 5 mM in figure 1 due to over expression of DNFB contamination due to increased concentrations of fluoride in the medium and thus, the DNP (dinitrophenyl) production at the level of amino acid sequencing of proteins (spasmins and batonnets) affected the molecular orientation feasibility of different types of associated amino acid residues in terms of bond angles which became distorted up to limited range as a result there is sudden fall in frequency profile of DNFB mediated contraction dynamics. The contraction speed of Vorticella stalk was very slow due to very fast binding affinities of DNFB molecules if it was independent in comparison to the pCa association, while DNFB and pCa free media in natural condition without involving any chemical in culture media, reflected the rate of stalk contraction at very fast rate which could be predicted only by using high speed camera involvement (Moriyama, *et al*., 1998).

The duration of contraction specifically revealed the average values of time intervals during Vorticella stalk contraction cyclic processes. These durations projected in diagrams, figures and tables reflecting tentative approach towards the capacity of tolerance for DNFB toxicity by the proteins involved in the process, and thus on the basis of positive efficiency of work capabilities of the system, durations were found and predicted. It further explains the binding affinities of receptor mediated enzyme-kinetics involved in the process on the basis of concentration gradients of DNFB independently and in conjecture with pCa concentrations. Thus we have resistant power of spasmins and batonnets for ROS generated in the system, fluoride based toxicities and DNP based protein folding dynamics which can be sequentially predicted in the light of Lavinthal paradox (Martinez, 2014) in terms of Ramachandran plots by determining phi (Õ), psi (ψ theta θ omega ω and tau (∂) bond angles in terms of radius of gyration along with involving coarse grained model prediction (Korkut and Hendrickson, 2013; Jayaram, *et al*., 2013; Bansal, 2001; Bhattacharjee and Bansal, 2005; Vijayan, 2016).

In all these observations, maximum velocity (Vmax) was better

understood in modified version of Briggs-Haldane equation. Here initial velocity (V_0) for both pCa dependent and independent conditions in the presence of DNFB were same but Vmax/2 indicated upper turnover number for most of the reactions at pH 5.5 to 6.5 where energy compensation (ΔG^0) at the level of P-S (proton-spasmin) complex is equal to 0.5 in terms of Nernst equation potential where P (protons) approaches 20 to 70% that of Km at steady-state for few minutes (about 5 minutes) (Table 1& 2). Here $V_0 = K_2$ and Vmax = K_2E_0 and thus K2 becomes Vmax/E_0. It indicated strong turnover number of protons in the transient states (P.S) (Figures - 1 & 3) which was later on converted into the product (P-S) at the rates of per unit time. In this case, most of the reactions were accomplished at the concentration of 8.9×10^{-8} to 1.9×10^{-5}mol/l/s and thus only few protons represented positive sensitive effect on stalk contraction in slight variation in terms of frequency and duration at the concentration 1.9×10^{-6} mol/l/s (Figure - 1; Table - 1).

Above this concentration, protons reflected negative role on stalk contraction. Typically, Km values of P (protons) were in the range of 1 mM to 4mM where P was a small fraction of Km. In this case, rate of reaction was not expected as in 1st order reaction but it was in second order reaction. The rate of reactions became zero at higher pHs above 8.0 at the for the DNFB concentration 1.9×10^{-8} mol/l/s. It indicates that there was great variation in the protoosmolarities due to the variation in enzyme-kinetics (Figures - 1 & 3) thus ameliorated the following processes:

Ca^{++},-flux at the level of ER,

Binding, kinetics of excitation-contraction coupling through ryanodine receptors (Ryr) at their functional domain,

The, spasmin proteins binding kinetics and folding kinetics inside the spasmoneme of the stalk,

Generation, and effects of protonation-deprotonation potentials on stalk dynamics

Membrane, potentials,

Modification, in Hookean force and the Raynolds' number,

Bioenergetics ,of motive-force production *etc*.

Myonemes as well as spasmonemes of the head and stalk are two contractile and force generating micromachines (Figure - 5). Their activity was inhibited when different concentrations of DNFB were introduced in experimental culture media. Ryanodine-receptor sites in both head and stalk of SR and ER of linkage complexes represented molecular mechanisms of pCa dependent biodynamic performances at different rates. The affected mechanism brought variations in ryr binding kinetics in respect of associated molecules of DNFB. The contractility of head and Vorticella stalk is halted by DNFB upto limited range in experimental medium perhaps from 30 to 50% of contraction process (Figures - 1 & 2) *i.e* equal to 3.6 to 6 folds that of load free shortening of actomyosin contractile machines. pCa and DNFB worked independently in the contractility process of actomyosin, spasmins and batonnets protein polymers of myonemes and spasmonemes (Figures - 1, 3 & 5) of both stalks and the zooid of Vorticella stalks and the vertebrates skeletal muscles systems of biomechanics. There was slight variation observed in this contractile biodynamic process in different pCa and DNFB time-dependent concentration processes upto the range of experimental data which affected the rate of contractility upto their measurable range (Table - 1 & 2) for spasmins, batonnets and actomyosin in differential cases on the basis of their density gradients in different living systems. The data obtained shows that pCa variation reduces its S.D. This established purity in observations with increasing pCa thus brought variations in their threshold value, shift in force-pCa curve and the tension generated by chemical stresses representing their positive sensitive effect on contraction dynamic process of Vorticella stalk. This was perfect and absolute for DNFB treatment when experimented on spasmins, battonets and actomyosin systems of contractile systems, but does not ameliorated Lohmann reactions as the case in spasmin proteins' binding phenomena if it was compared with muscle's physiology. In case of spasmin proteins' Ca^{++} sequestration rate at the level of SR and ER linkage complexes, thus affected Lohmann reaction process which could not be predicted. Thus we can say that H^+- flux mediated protoosmotic potentials directly interferes with Lohmann reactions and thus utilized to conserve the energy compensation system which was ATP independent. The same rates and results were found in time-dependent DNFB ameliorated rates of contraction dynamics in spasmins, batonnets and actomyosin dependent contraction dynamics where in case of Vorticella stalk and zooid, H^+-flux along the length of the stalk is greatly concerned with the linkage complexes of ER and SR membranes throughout the biodiversity. Along the length of spasmin proteins, Sfi1p and Cdc31p binding kinetics represented differential folding states in relation with associated molecules in case of Vorticella stalks' biodynamic phenomena. When the stalk contracts the folding and binding rates were determined by concentration dependent time-interval data which were obtained and measurement for stalk contraction rate data analysis by biometric methods in terms of frequencies and durations profile where protein structural conformations changes in terms of stereochemistry to accomplish the work of contraction dynamics in the light of protoosmosis (Figure - 5). Such biodynamic phenomenon occurs along the length of SR and ER membrane of the system and works on the basis of protoosmosis (Hadad, *et al*., 1999).

The molecular bending of Sfi1p and Cdc31p along the length of the stalk is accomplished by pCa incorporation which was little bit affected by DNFB incorporation. This phenomenon was completely independent of ATP hydrolysis at the level of ER around the spasmoneme of the stalk. This phenomenon used to accomplish the bridge of *â*-pleated sheet by Ca^{++} incorporation in the form of cable chain connection and thus the protoosmotic flux conductivity along the length of the stalk facilitated force and power generation in terms of proper conduction of H^+ and K^+ counter-flux mechanism, and thus generated power and force along the length of F-actin of actomyosins, spasmins and batonnets (Table - 1; Figures - 1 & 5). Here, DNFB is known to modify the ryr binding kinetics in sub-milimolar concentrations at the ER membranes of stalks and zooid and thus developed pronounced higher affinities by blocking pCa intra-tubular release (Table - 2; Figure - 3) by external pCa incorporation of the experimental media and thus DNFB inhibited crystalline ATP incorporation into the

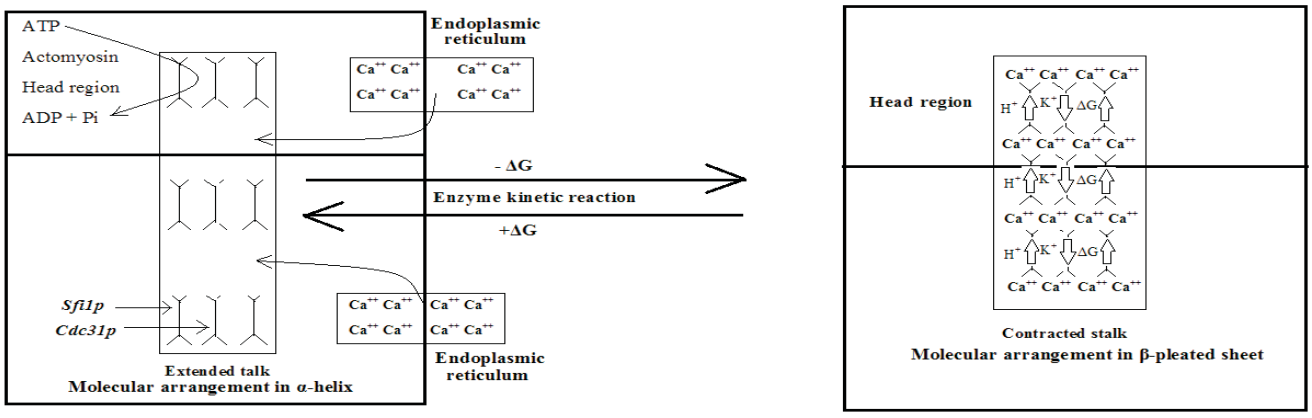

Figure 5: Proposed model of Vorticella stalk to represent contractility in light of protoosmotic model.

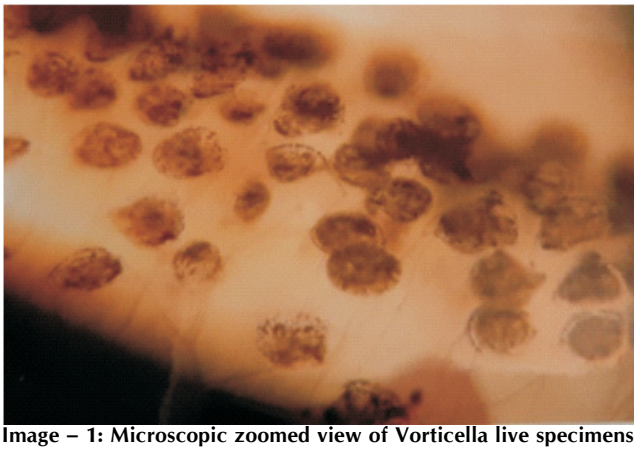

Image – 1: Microscopic zoomed view of Vorticella live specimens under laboratory culture conditions.

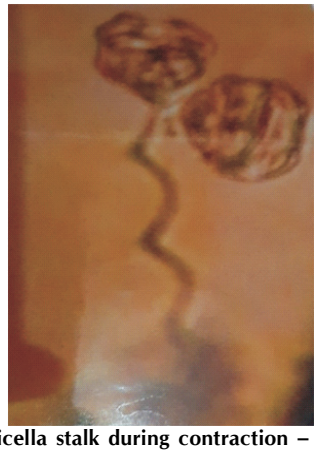

Image – 2: Vorticella stalk during contraction – extension cyclic processes representing rotational dynamics in clockwise helix formation with old and newly formed both the types of zooid bells.

musclular and Vorticella stalk and zooid systems, when the hypothesis was applied in above threshold value of concentrations. But in case of Vorticella stalk, the contractility played the role of Ca^{++} sequestration at the level of SR and ER membranes but does not involve in the process of contraction. This description has been supported by the given model for stalk contraction (Figure - 5) in light of protoosmosis. Thus, the energy harvesting process utilized the protons gradients to generate force and power in terms of ionic conduction along the length of F-actin protein polymers.

In this paper, we have fixed results to discuss elaborative explanations on the basis information cited in the form of figures and tables. Thus we have used new, nascent and elaborative ways of explanations rather than straight and forward approach, hence, the straightforward discussions has molded into multidimensional networking format as in signal transduction, used by most of the recent scientist to expose the complex processes in a conjectured way, thus, the model given in the forms of figures, tables and hypothetical diagrams are integrally very important in communicative and correlative way for model and hypothesis prediction.

In this correlative way, we are able to reflect molecular physiology of Vorticella stalk contraction dynamics in the light of protoosmosis based protein folding dynamics of spasmins, batonnets and actomyosin systems of contraction in relation with Sfi1p & Cdc31p. Thus we have an extraordinary modern hypothesis related with Vorticella stalk contraction dynamics in terms of ideas of fractional stalk lengths of energy transmission on the basis of DNFB and pCa implications in an independent and in conjectured way. which determined differential physiological properties of Vorticella stalk contraction dynamics in terms of patho-physiological regulations in terms of negative regulation of pCa sequestration at the level of ER luminal compartments (figure – 5, table – 1 & 2) on the basis of ryr binding-kinetics, DNP production and negative feedback regulation of actin-myosin, Sfi1p-Cdc31p and Spasmin-spasmin interactions as in centrin, calmodulin, dynin, kinectin, cytokinin and much more than this.

On the basis of protoosmotic model, diagrams, figures and tables given in the paper, we can understand power of DNFB resistance in the presence and absence of pCa differential concentrations for actomyosins, spasmins and batonnets in terms of stalk contraction dynamics. Thus, the presence and absence of pCa concentrations in different concentrations regulated intra-tubular pCa sequestration and regurgitation at the level of luminal compartments of ER inside the spasmoneme of the stalk as in myoneme, spasmoneme and other related systems. Thus, it is clear that the process of Lohmann reactions and protoosmotic processes are independent and work at two different placed which cannot be conjecture but can be discuss independently at two different

places as occurs at the level of ER luminal compartments, external media solutions, ryr binding affinities and protein folding predictions.

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