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Role of H^+ and Ca^{++} Concentrations on Vorticella stalk Contraction Determination

Amit Kumar Verma[1]* and Ashok Kumar Singh[2]

[1,2] Cell Biol. Res. Lab., P. G. Department of Zoology, Science Faculty, J. P. University, Chapra, Bihar, India.

**Corresponding author: akvjpuz@gmail.com Mob: +91-8804385560*

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Abstract*–Vorticella*** stalks contraction mechanism is based upon the behaviour of novel types of motor proteins involved in the process and thus determined positive sensitive effects when in $[H^+]$ from 14.80×10^{-10} to 7.15×10^{-10} mole whereas $[Ca^{++}]$ from 0.5 to 4.5 mM reflected negative effects on contraction dynamics in terms of birefringence. The protoosmotic applications on the stalk contraction provided information about biochemical nature of motor proteins involved in contraction dynamics of the stalk in terms of frequency and duration profiles in reversible reactions have potential future applications in controlling patterns of structural modifications of motor proteins involved throughout the system.

Key words – H^+ & Ca^{++} concentrations, *Vorticella* stalk, contraction determination, frequencies and durations profiles.

I. INTRODUCTION

Vorticella stalk is a highly contractile device, focussed to determine the nature and patterns of contraction for new information. The helical coiling and rapid rate of contraction provided new ideas for researchers to work in the field of biological dynamics in the light of modern tools and techniques. The spasmins and batonnets are two putative proteins stored inside the stalk on which rate and pattern of stalk contraction depends. The folding dynamics and patterns of organisations of spasmins and batonnets along the length of stalk with different molecular orientations determined helical coiling of the stalk at rapid rate. The stiffening of batonnets brought helical bending patterns during contraction whereas modifications in molecular orientations in spasmins brought rapid rate of sudden contraction [1].

The spring like bending patterns and rubber-like elastic nature were specialised features of the stalk and were based up on the sequencing patterns of amino acid residues found in spasmins and batonnets. The charge neutralisation efficiency and the concentrations of Ca^{++} inside the cytoplasmic matrix of spasmoneme were responsible for their rate of contraction determination. The variation in bond angles' length of spasmins and batonnets of the stalk were the thermodynamic means for stalk contraction in the form of mechanical motion where protoosmotic model generated motive force for stalk contraction by generating positive charges by involving Ca^{++} or H^+ concentration gradients of different strengths around negatively charged protein polymers (spasmins & batonnets) and thus were focused during contraction determination for revealing frequency and duration profiles of contraction at the level of charge neutralisation of associated amino acid residues of spasmins and batonnets at the level of microfilaments (F-actin) based counter flux bioenergetics regulation for the treatment of patho-physiologically determined abnormally performed in other related contractile systems [2], [3].

In the last paragraph of introduction, Section I provides information about the article organization in newly designed format of IJSRBS (isroset) UGC approved journal (64784). Section II contains the related works which are internationally acclaimed and provides new dynamics in the field of contraction dynamics entitled as "Mechanics of Vorticella contraction". The objective designed in this paper is modern and productive. The problem containing biophysical approach with very strong mathematical annotations and thus it has very citation. Section III contains biometric measures of *Vorticella* stalk contraction velocity profiles in frequency and duration 2D format. Section IV contains architecture and essential steps of materials and methods used to obtain results in numerical data. Section V explains the step-wise methodology used in the form of flow chart. Section VI describes results and discussions in a conjectured way to support hypothesis. Section VII contains recommendations of pH and pCa for *Vorticella* stalk performance in antagonistic way. Section VIII concludes research work in the form of applied future perspectives [4], [5].

II. RELATED WORK

In our previous research work, entitles as "Effect of extracellular pH and Ca^{++} - concentration on the contractility and force generation as verified on *Vorticella* stalk concentration pattern", we have stated concepts for prediction of protoosmotic effects on *Vorticella* as strongly subjected for reverse reaction to explain energy conservation in relation with surrounding administered

culture media on the basis of proton-flux-mediated electroosmotic performances in logarithmic force-pCa curves, threshold value of velocity profiles against pCa pressure gradient for contraction generation and the tension generated on spasmin proteins. The objective of this paper was to explain the frequency profiles of *Vorticella* stalk in the reference with barnacle muscle fibres, kinetics of protein folding dynamics, relation with intact muscle fibres and the elementary release of pCa in relation with the process of protoosmosis [6].

III. METHODOLOGY

Vorticellids are the sessile forms of animalcule, normally attached with the aquatic submerged plants such as *Chara, nitella & Myriophyllum* in fresh water habitat like pools, ponds and ditches. The living *Vorticella* specimens were collected from the shallow water stagnant ponds' places around Chapra (city) in Bihar state of India and were cultured in big potted jars in their natural conditions. The room temperature was suitable for their proper growth. As an alternative experimental medium the specimens were grown in artificial medium. The polished rice with wheat and maize were good medium for specimen survival and thus were used after several successive trials. During experimental trial, it was found that several *Vorticella* individuals grown in the culture medium were settles on the maize kernels and slowly gets fully cover the whole grains with many newly emerged individuals in their cystic forms [7].

The large numbers of specimens were desirable to succeed sub-cultures at the intervals of every two days of intervals and thus we found emerging new trophont individuals, slowly from their cystic form out of the cage. Later on the maize kernels were transferred from old culture medium to fresh culture medium for experimental trials. In another alternate method, purified distil water were purchased from local chemical supplier and then used for APW (artificial pond water) preparation. After the processing of boiling and filtering, distillation water were used to prepare APW by mixing 0.1 mM of NaCl, KCl and $CaCl_2$ in the same concentration. The boiling eggs were used as good nutritive source for emerging individuals in experimental medium. The solidified albumin of hens' egg with a little pinch of ¼ gm was mixed with yolk in grinder bowl had pestle to grind by using adequate amount of distilled water. This paste was mixed with filtered water and stirred for a few minutes for supplementary food utilisation. In this solution of APW with food supplementation was now ready for culture after 2 days of incubation and then tried to grow new batch of *Vorticella* specimens into this growth media. Glassware with high surface volume-area were utilised to grow *Vorticella* individuals as enriched mass-culture. The attachment surfaces of 500 mL of flasks contained 50 mL of media were used for culture the specimen in the laboratory conditions. The entire observations were made under Stereo-microscope (Leitz) with 10 X × 18 X of magnification. Other microscopes used were Magnus MS 24/13 and Olympus ch2ibimf for overall experimental demonstration. The photo-video-graphic camera [Nikon 12.1 150 3200 P/S/A/M (Coolpix)] was used for photography and video graphic recordings. The frequency and duration of stalk contractility of *Vorticella* under known chemical stress conditions in solutions were experimented, recorded and processed at different H^+ and Ca^{++} concentrations. During experimentation, the specimens of *Vorticella* were kept in their standard controlled neutral conditions for 24 hours in APW. Then the cultured *Vorticella* were transferred into sub-culture after every 2 days of intervals. The different Ca^{++} concentrations from 0.5 mM to 5 mM were used for experimental performance. The detergent (Triton-X) treated specimens were prepared under controlled bathing media (pH 7.0) of different Ca^{++} concentrations conditions [8].

The washed specimens of *Vorticella* were kept immersed for approximately 1 minute into the solution containing Triton X-100 in combination with: 20 $mmoll^{-1}$KCl, 10 $mmoll^{-1}$ EDTA and 10 $mmoll^{-1}$Tris-maleate buffer at pH 7.0. The treated specimens were then washed three and four times with a washing medium composed of 50 $mmoll^{-1}$KCl, 2 $mmoll^{-1}$ EDTA and 10 $mmoll^{-1}$Tris-maleate buffer solutions at neutral pH. It was kept for 15 to 20 minutes for the extraction of Triton X-100. The washed specimens were kept immersed into the reactivation media of different Ca^{++} concentration from 0.5 mM to 5 mM. The reactivation media were consisted of 50 $mmoll^{-1}$ KCl, 10 $mmoll^{-1}$ Tris-maleate buffer at pH 7.0 had Ca^{++} buffer concentrations of variable strengths. The overall mentioned experiments were performed at temperatures from 20 to 30°C. After the recording of stalk contraction, the contraction-extension cycles of the individual specimens were measured in the form of their stalks' lengths of many *Vorticellids* by measuring linear distances of mechanical motions from the base of the stalks to the scopula (the attachment site of stalk and zooid). The length of stalk after contraction under the influence of different Ca^{++} concentrations represented configurationally changes stalks' patterns by their different unitary lengths, known as fractional stalk length which was expressed in terms of Hill's parameter [9].

The bio-statistical measurements were done in terms of mean and standard deviation, and then obtained data were represented in graphical format of *Stock's chart* (by involving range of data in low, medium and high values arrangement) in pattern of software format (figure - 1) and thus result were obtained in their absolute predicted frequencies and durations which were further used for linguistic discussions for the purpose of abstract and conclusion predictions in their hypothetical determinations.

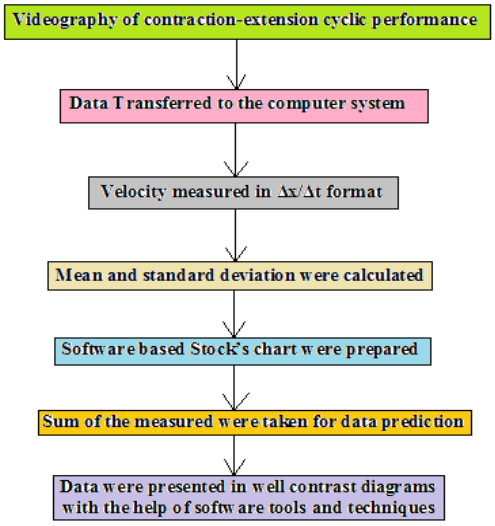

Figure 1: Step-wise technical description used in *Vorticella* stalk contraction-extension cyclic data observation, illustration, analysis and prediction.

IV. RESULTS AND DISCUSSIONS

The neutral pH (7.0 ± 0.5) required for normal rate of protein folding as in myosin, calmodulun, centrin and others. In the same way, when pH of the surrounding medium of spasmins and batonnets changed it affected rate of protein folding dynamics of both the mentioned proteins of the stalk along with myosin of zooid in permeabilised specimen at different experimental conditions reflected different rates of contraction dynamics with variable [H^+] gradients. In overall changed experiments pHs from 5.0 to 9.0, slightly acidic experimental media from 5.0 to 6.8 were most favoured contraction generators if compared with either neutral or alkaline conditions from pHs 7.0 to 9.0. These variable experimental media conditions from 0.5 to 9.0 reflected different rates of contraction velocity - frequency and duration profiles in terms of equation of motion ($\Delta x/\Delta t$) in both the conditions as in the presence as well as in the absence of EDTA at defined concentrations of experimental media compositions in association with triton-x 100 detergent (figures – 2 & 3) [10].

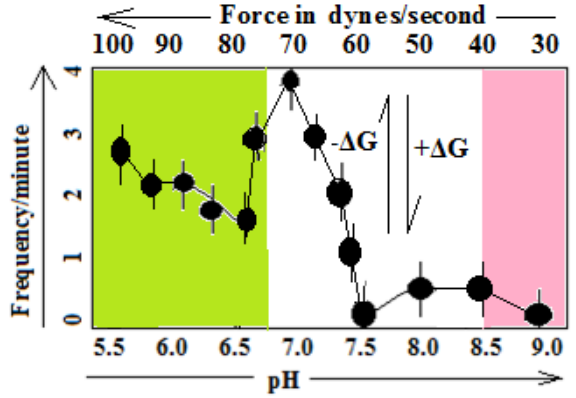

Figure 2: Frequencies of H^+ concentration dependent *Vorticella* stalk contraction in terms of thermodynamics where N = 8 and S. D. = ± 0.02. Here, green colour is high sensitive zone; pink colour is low sensitive zone while white colour is thermodynamically protected zone for contraction.

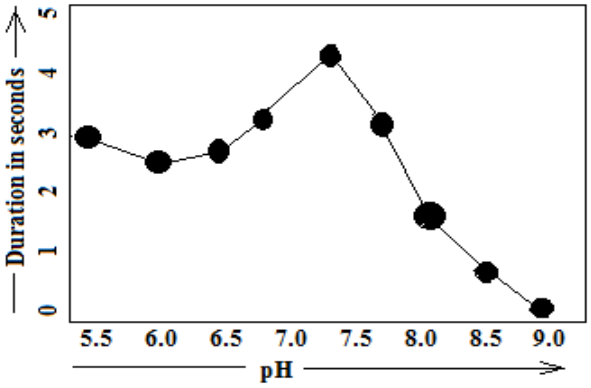

Figure 3: H^+ concentrations dependent duration of *Vorticella* stalk contraction.

In overall experiments, more significant changes were predicted in their velocity based frequency and duration profiles of contraction. When both the data were compared, then the data of duration were more tilted than the frequency profiles during early stages of mechanical motion than the later stages of curve tilt. It indicated that at pHs 5.5 to 6.5, there were most significant variation in threshold value confirmed very strong motive force generation by acidic media, thus protoosmosis played significant role in their frequency and duration graphical shift in the form of present data with differential rate modifications when rate of contraction velocity travelled from left to right in reversible conditions at the fixed pHs. Thus electroosmotic potential was functional to generate differential power potential for contraction by supporting charge neutralisation of negatively charged R-group of amino acid residues of spasmins and batonnets in the same way as occurs in actin, actinin, myosin, calmodulin, centrin, dynine, Sfi1p, Cdc31, Bcl2, T40750, KIAA0542 and other related proteins but the mode and pattern of frequency and duration among these proteins were quite different and distinct. Thus these all proteins have different rate of force and power generation in respect of [H^+] gradients in all related or non-related, same or different family members proteins responsible for different types of cell motilities [11], [12], [13].

At H^+ 3.16×10^{-9} to 12.10×10^{-10}, Ca^{++} threshold value was lower than 9.1×10^{-8} $moll^{-1}$ and was well perfect for stalk contraction. At this H^+ concentration, Ca^{++} established significant force-pCa relation and thus proteins were significantly promoted for structural modifications in spasmins and batonnets of the stalk inside the spasmoneme in terms of second law of thermodynamics along the axis of x, y and z for their sequence based molecular orientation in their 3D predictions in terms of bioinformatics predictions. Here in this case 50% structural modification was obtained at Ca^{++} concentrations from 1.9×10^{-6} mol to 1.0×10^{-7} mol in the frequency and duration profiles which reflected referential modifications in patterns of stalks' orientation with decreasing lengths of the stalk and increasing number of

coils along with the alignment of amino acids residues of spasmins and batonnets in their composite manner along the length of proteins polymers with variation in their radius of gyration in relation with their potential thermodynamic variations in their potential into kinetic forms of radiation profiles in the terms of bonds formation and their dissolution throughout their active states.

At H^+ fixed concentration 6.14×10^{-9} mol in association with variable Ca^{++} concentrations from 0.5 mM to 4.5 mM, velocity profiles reflected insignificant changes in their frequency data prediction if compared with pCa concentration at 5.0 mM. Thus Ca^{++} reflected slight change in frequency profile rather than duration (figures – 4 & 5). Even then EDTA were incorporated in triton-x 100 permeabilised solutions of Ca^{++} concentrations from 4 mM to 10 mM at controlled condition the velocity of stalk contraction reflected sudden but little change in frequency profile by bringing variation in tension generated at the level of functional motives and domains of spasmins and batonnets. At H^+ concentration 12.10×10^{-10} mol in combination with Ca^{++} concentration from 0.5 mM to 4 mM, permeability of ER membrane modified at certain limits thus the rate of stalk contraction were promoted same as in skeletal muscles' contraction if it was compared with barnacle muscle fibres contraction dynamics [14].

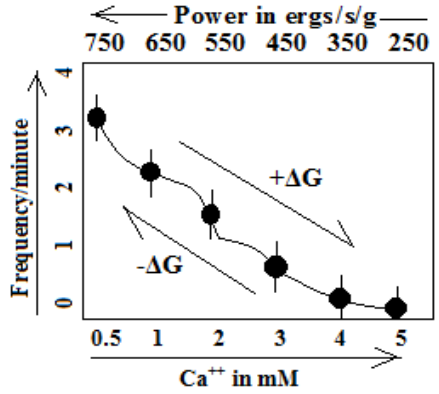

Figure 4: Frequencies of Ca^{++} concentrations dependent *Vorticella* stalk contraction in terms of thermodynamics where N = 8 and S. D. = ± 0.02.

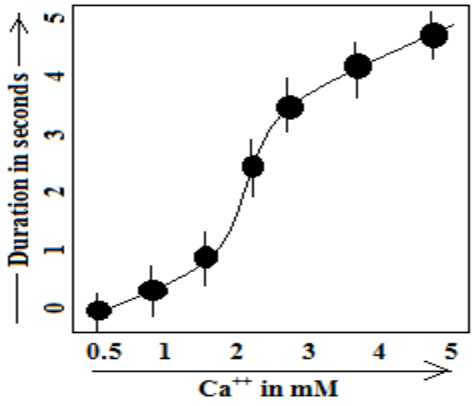

Figure 5: Ca^{++} concentrations dependent duration of *Vorticella* stalk contraction.

The decreased H^+ concentrations reduced the probability of Ca^{++} movements across the ER membrane receptor channels (ryanodine or ryr) in barnacle muscle fibres but in other cases consistencies of probability for Ca^{++} transport into the cytoplasmic matrix of spasmoneme around spasmins and batonnets inside the stalk was maintained by negative feedback effect without ATP hydrolysis but the case was different in other protein related contraction mechanism which involves different methods of molecular mechanics such as in sliding – filament molecular performance of acto-myosin, dynine related cilia, flagella and the neuronal transport systems, etc where ATP hydrolysis is required. Thus the spasmins and batonnets like novel proteins were taken in concern with their molecular nature for contraction dynamics, hence, the Protein folding kinetics of spasmins and batonnets were greatly concerned with their bioenergetics in relation with protoosmotic power potentials generation, and thus the *Vorticella* stalks contraction dynamics were affected by H^+ concentration gradients by regulating Ca^{++} discharge from ER intra-tubular lumens of the stalk. Thus it is clear from frequency profiles that sift in force-pCa relations were governed by $[H^+]$ same as in skeletal muscles fibres through the mechanism of mechano-chemical regulations by controlling motive force generation along the length of actin-myosin contractile systems [15].

V. CONCLUSION

Thus it is concluded that hydrogen ion concentration gradients generated motive force for contraction dynamic force and power generation by replacing Ca^{++} sequestration from intra luminal at their ER storage sites and thus protoosmosis is controlling protein folding dynamics inside the *Vorticella* stalks in relation with their frequency and duration profiles.

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AUTHORS' CONTRIBUTION

The complete research work was conducted in Cell Biology Research Laboratory, P. G. Department of Zoology, Jay Prakash University, Chapra, Bihar, India, by Amit Kumar Verma under the guidance and technical assistance of Ashok Kumar Singh.

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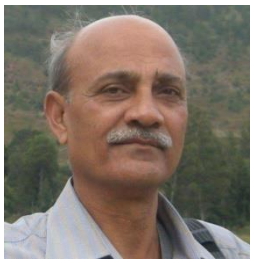
AUTHORS' PROFILE

Amit Kumar Verma: He has completed his B.Sc. in Zoology honours from Rajendra College, Chapra, Bihar; B. Ed. with pedagogy in biology from Gorakh Singh College, Maharajganj, Siwan, Bihar; M. Sc. in Zoology with Fish & Fisheries specialization from Rajendra College, Chapra, Bihar; SRF in ICMR research project at S.S. Hospital & Research Centre at Patna, Bihar; Ph. D. in Zoology from P. G. Department of Zoology, J. P. University, Chapra, Bihar. He is an active researcher and tutor, worked as a part-time/full-time involvements in different educational institutions as Mahendra Misra Central School, Jalalpur, Saran, Bihar; Don Bosco High School, Baishakhi, Siwan, Bihar; Bhagwat Vidya Peeth, Chapra, Bihar; Vigyanand Kendriya Vidyalay, Chanaur, Siwan, Bihar; Don Bosco Paramedicals, Siwan Bihar; Rajendra College, Chapra, Bihar; Bharti Institute of Paramedicals and Research, Chapra, Bihar; Turning Point Medical Institute, Pachim Vihar, New Delhi; S. S. Hospital & Research Centre, Patna, Bihar; etc. He has actively participated in six seminars and conferences out of which, one was in MP (ERW, Kajuraho), four was in Bihar (NLS, Jalalpur, Chapra, Saran; P.G. Dept. of Zool. JPU, Chapra, Saran; BIT, Patna; TMB, Bhahalpur, Bihar) and one was in Delhi (NIPGR), and received certificates from there, except TMB, Bhagalpur. He has published his three research papers on *Vorticella* stalk contraction dynamics. Out of these three papers, two publications are in association with his guide Ashok Kumar Singh whereas last publication is individually authored. For his last publication, he received Franklin membership from the chief author Dr. Wael, A. in LJRS: Natural and Formal, U. K. He is waiting for publication of currently communicated conference paper entitled as "H^+ - integrated *Vorticella* stalk contraction performance" in 7^{th} annual national conference organized by ESW Khajuraho, Madhya Pradesh, India. Other three research papers are under review, communicated to Indian Journal of Experimental Biology (NISCAIR), Indian Journal of Biochemistry and Biophysics (NISCAIR) and The Journal of Basic and Applied Zoology (Springer Open).



Ashok Kumar Singh: He has completed his B.Sc. (Zoology, Honours) in 1973 & M. Sc (in Zoology) in 1976 from Magadh University, Bogh Gaya, Bihar. He was awarded with Ph. D. Degree in Zoology subject (1988) from School of Life Sciences, JNU, New Delhi. He has 13 publications and under his guidance, 7 students obtained their Ph. D. Degree. His popular articles were published on AIDS (two publications) and MCQ in Zoology (two publications). He is the author of three books entitled on Actins-myosin system, Histology and Vertebrate Systems. He attained three international seminars in Austria. He is the expert of Zoology, Cell biology & Biophysics. He has delivered more than 30 lectured on different topics in biology subjects in other universities. He held following positions during his work periods as H.O.D. of Zoology, Chair person in Board Studies, Dean of Science Faculty, Member of Academic & Executive Councils, Member of Professional & Academic Bodies, Academic Councillor, Dean of Student's welfare, and Convener of B. Ed. Syllabus Formulation Committee. He has worked as lecturer, reader and professor of Zoology in L. N. College, Bodh Gaya, (BRA University), Bihar; Amity University (Department of Biotechnology for Biophysics), NOIDA; Jagdam & Rajendra College (U.G. & P.G. Zoology Departments) and the University P. G. Departments in J. P. University, Chapra, Bihar. He has hosted two seminars and participated in more than fifty seminars organized in different universities in India. Now he is a retired professor of Zoology from J. P. U. Chapra, Bihar, currently leading his life in New Delhi, India.

