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Research Article

EXPERIMENTS ON *VORTICELLA* STALK CONTRACTION DYNAMICS

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ABSTRACT

Vorticella stalk is a highly contractile device exclusively used to know the behavior of motor proteins (spasmins & batonnets) for new information in the light of thermodynamics. For the same purpose preapproved materials and methods were used to reflect enzyme – kinetics by work, power and force measurement through applying equation of motion, mean and standard deviation, where in case of *Vorticella* stalk contraction, V_{max} became constant at initial stages of mechanical motion but presented upper-limit burst prediction in the presence of DNFB (2, 4 – dinitro – flouro – benzene) from 1 mM to 5 mM. Birefringence variation was 5 to 8 % for pCa. Binding affinity variables were 1 to 2% for batonnets in comparison to spasmins, whereas it was 5 to 6% for myosin in the presence of DNFB. Denominators and numerators were well designed coordinates for geometric explanation with equation of motion. K_{cat} was prevented by reversible reactions at pHs 5 to 6.8.

Keywords: *Vorticella* stalk, Mechanical motion, Birefringence, K_{cat}.

INTRODUCTION

Vorticella stalk is a power-actuator mechano-chemical artificial-machine. It is auto-catalysed by Ca^{++} - addition and removal across the ER of *linkage-complexes* (Allen, 1973; Katoh & Naitoh, 1992; Ryu *et al*., 2012). The Ca^{++} - addition with spasmins and batonnets generates enormous power for sudden contraction of *Vorticella* stalk through which signal for contraction traverse at the rate of thousands time of the stalk length per second at more rapid rate than their relative groups of stalked ciliates. The high-speed-video-graphic demonstration confirmed the isometric contraction to the stalk where Ca^{++} - addition generated power for subsequent contraction of stalk in their fractional stalk signal propagation and get extended after Ca^{++} removal across ER membrane by involving ATP – hydrolysis for the active transport of pCa through active feed-back effect of pCa regurgitation into the luminal compartments of ER based linkage – complexes. This mechano-chemical biological performance generated multiple of models in the light of computer based mathematical simulations (France *et al*., 2017; Misra *et al*., 2010; Upadhyaya *et al*., 2008) for applied implications in the fields of biological studies and research.

The fractional stalk length predictions in the light of equations of motion were well-expressed in the reference with Stoke's formula for damped-harmonic-oscillations. In these physiological performances by involving Hookean force and Reynold's number, volumetric variations represented geometric constrains along the axis of x, y and z for rubber-like elastic repeated contractions (Moriyama *et al*.,1998; Moriyama *et al*., 1999). In this mechano-chemical signal-transduction, contraction preceded along the length of stalk from zooid to base involving membrane depolarization (Shiono & Naitoh, 1997). Thus the new dynamics for enzyme – kinetics were approached by involving DNFB and [Ca^{++}] inhibitory effects independently and conjecture.

MATERIALSAND METHODS

The specimens collected for laboratory culture and experiments were *Chara, Nitella* and *Myriophyllum* (aquatic sub-merged plants of local ponds and pools) of fresh-water habitat had several vorticellids, found attached with substratum in microscope based laboratory search for their laboratory culture. The laboratory culture was performed in NPW (natural pond water) of same pond (Page, 1981), followed by APW (artificial pond water) of

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0.1 mM of NaCl, KCl & NaOH of same concentrations (Singh & Amin, 1991). The pastes of solidified albumin and egg-yolk in the concentration of ¼ (v/w) were the food materials of vorticellids for successive culture in laboratory conditions at 30ºC and were isolated after enriched culture by using microscopes of variable magnifications. For controlled conditions HCl and KOH were used in the presence of pCa & [DNFB] independently and in combinations (1 mM to 5 mM) at 25 to 37ºC by using sterilized glassware and equipments. The photo-graphic and video-graphic demonstrations were performed by using Magnus MS 24/13 Olympus India Pvt. Limited New Delhi (magnification – 10x × 5x & 10x × 10x), Olympus chi2ibimf India, Pvt. Ltd. (magnification – 10x × 15x & 10x × 18x) along with Nikon camera (12.1 150 3200 P/S/A/M - Coolpix).The data were analysed biostatistically by using equation of motion, then extrapolated to describe enzyme – kinetics in the reference of spasmins and batonnets in the light of modern investigations (Li *et al*., 2006).

RESULTS AND DISCUSSION

In case of *Vorticella* stalk contraction, V_{max} becomes constant at initial stages of mechanical motion whereas at later stages $1/V_{max}$ exclusively altered after 10 minutes of DNFB introduction into the experimental medium. If V_{max} was compared with K_m, adverse expression was pronounced and was strong supportive of V_{max} expression. DNFB expressions were not affected the reversibility of the reactions at pHs 5.5 to 5.6 which remained constant for spasmins and batonnets in controlled conditions [Figure 1, equation - (1), Table 1].

In clinical conditions, DNFB extrapolated reaction-kinetics in the presence of Ca^{++} - concentrations (1mM to 5mM) and thus generated poisonous effects on the system 3.5 to 6 folds by producing DNP in case of myosin but not in case of spasmins and batonnets, thus unable to produce dinitrophenyl (DNP) in *Vorticella* stalk. Hence in case of *Vorticella* stalk, Lohmann-reaction was not pronounced.

Thus, DNFB binds with spasmins and batonnets other than active sites and thus altered 3D orientations of spasmins and batonnets by affecting enzyme-kinetics at ryanodine-receptors. Here, inside the spasmoneme, DNFB influenced the binding affinities of functional motifs and domains with their isotypes (Sfi1p and Cdc31p) (Gogendeau et al., 2007), and thus K_m and V_{max} subsequently altered. Here molecular diagnosis reflected isomeric inter-conversion (cis – trans isomerization) for changed motifs and domains at defined concentrations of HCl, KOH, $CaCl_2$ and DNFB. In this reaction kinetics, the addition and removal of pCa and $[H^+]$ coordinates orientations along the axis of x, y and z referred geometric orientation of the stalk in respect of functional motives and domains of spasmins and batonnets which was well explained in the form of birefringence modifications (5 to 8%) (Weis-Fogh & Amos, 1972) [equation – (ii) & (iii)].

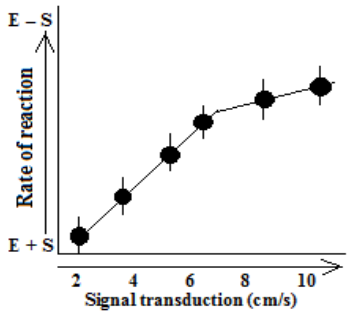

Figure 1. DNFB dependent signal transduction during *Vorticella* stalks contraction.

Equations at a glance:

$$\left.\begin{aligned} ax + by = c \\ dx + ey = f \end{aligned}\right\} \text{ — (i)} \qquad x = \frac{\begin{vmatrix} c & b \\ f & e \end{vmatrix}}{\begin{vmatrix} a & b \\ d & e \end{vmatrix}} \qquad y = \frac{\begin{vmatrix} a & c \\ d & f \end{vmatrix}}{\begin{vmatrix} a & b \\ d & e \end{vmatrix}} \text{ — (ii)}$$

$$\left.\begin{aligned} x = \frac{ce - bf}{ae - bd} \\ y = \frac{cd - af}{bd - ae} \end{aligned}\right\} \text{ — (iii)}$$

$$V = \frac{k_{cat}/(1 + [I]/K_I) \times [E]_t\,[S]}{K_M + [S]} = \frac{K_{cat}^{app}\,[E]_t\,[S]}{K_M + [E]} \text{ — (iv)}$$

$$V \longrightarrow V_{max}^{app} = k_{cat}^{app}\,[E]_t = \frac{k_{cat}\,[E]_t}{1 + [I]/K_I} \text{ — (v)}$$

Table 1. Force - generated in nano-Newton where N = 5, S. D. = ± 0.02.

pH	DNFB 1 mM	DNFB 2 mM	DNFB 3 mM	DNFB 4 mM	DNFB 5 mM
5.5	304	253	212	131	110
6.0	293	212	191	120	89
6.5	282	171	160	109	78
7.0	221	150	139	97	67
7.6	180	129	118	86	56
8.0	159	108	97	75	55
8.5	138	97	75	44	38
9.0	116	86	54	43	33

They involved hydrogen bonding and van der Waal forces of attraction, and thus involved ϕ and ψ limitations for amino acid sequences in terms of Ramachandran plots and the Lavinthal's paradox where thermodynamic harmony were expressed in terms of hydrophobic and hydrophilic patches (Lijnzaad *et al.*, 1996) or metal shadow casting (Bras, 2002). In these reaction – kinetics, functional groups of amino acid residues under gone formative displacements in the form of two pendulant on a common support as P + S (at K_m : 10^{-8} mol l^{-1}) and P – S (at K_m : 10^{-5} mol l^{-1}) [equation – (iii)]. On the basis of this analysis we can say that the presence of DNFB modified k_{cat}. It distorted the catalytic efficiency upto 10% for protein folding and thus there was birefringence variations were observed in optical expressions where E was equal to ES with the same value of K_1 (5 to 8) in new format [figure – 2, table – 2, equation – (iv)]. In this equation (iv), $k_{cat} = K_2$ and the result was exactly that of expectation. Thus it was clear that, the K_M was relatively influenced by DNFB concentrations but k_{cat} remained constant with increasing [DNFB]. There was V_{max} modified at slightly acidic at pH 5.5 to 6.5 [equation – (v)].

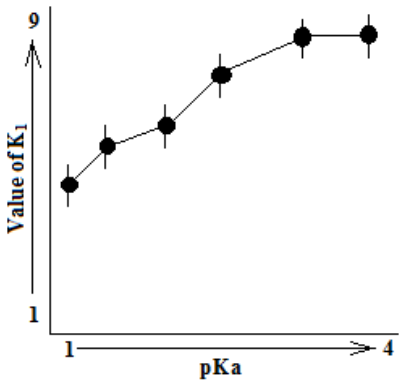

Figure 2. pKa value in relation with Ca^{++}- stress conditions.

Table 2. Power – generated in nano-Watt where N = 5, S. D. = ± 0.02.

pH	pCa 1 mM	pCa 2 mM	pCa 3 mM	pCa 4 mM	pCa 5 mM
5.5	0.95	0.75	0.60	0.55	0.18
6.0	0.76	0.68	0.58	0.50	0.15
6.6	0.64	0.65	0.49	0.35	0.14
7.0	0.58	0.46	0.46	0.34	0.13
7.5	0.49	0.38	0.39	0.24	0.12
8.0	0.38	0.26	0.36	0.22	0.10
8.5	0.26	0.21	0.27	0.21	0.08
9.0	0.25	0.18	0.24	0.20	0.06

It includes well expressed enzyme – kinetics in terms of Lineweaver – Burk plot. In this plot, where the frequencies of ryanodine receptor binding sites of spasmins and batonnets for pCa in non-mutated conditions was very large that of DNFB binding sites, but still, DNFB represented positive sensitivity for spasmins and batonnets, hence for K_M as denominator and numerator, the velocity decreases 3 to 6 folds in the presence of DNFB where V_{max} was equal to [S] at initial stages of mechanical motion where [S] = K_M and $v = V_{max}/2$ and under specific conditions $k_2 >> k_3$. Thus $K_M = k_2/k_1$ and the dissociation constant for ES were the value of K_M represented binding affinities of terminal domains and motifs along the axis of x, y and z for equation of motion. Here the value of k_{cat} was much higher for spasmins than the batonnets and myosin (1 to 2 folds that for batonnets whereas 3 to 6 folds that of myosin) (Hadad *et al*., 1999). In these reactions kinetics, DNFB affected binding affinities of amino acid residues of proteins at lower range in comparison with pCa affinity at controlled conditions (Figure 3 & 4, Table 3).

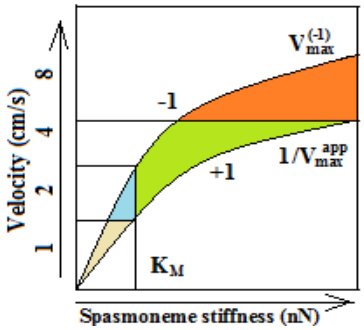

Figure 3. Velocity profile in reference with spasmoneme stiffness.

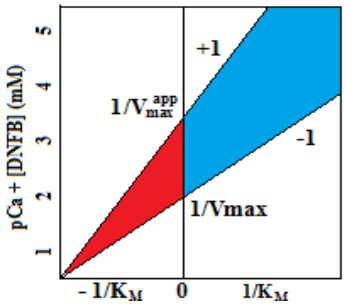

Figure 4. Lineweaver – Burk plote in relation with pCa and DNFB concentration gradients.

Table 3. Work done in pico-joule where N = 4, S. D. = ± 0.02.

pH	pCa + DNFB 1 mM	pCa + DNFB 2 mM	pCa + DNFB 3 mM	pCa + DNFB 4 mM	pCa + DNFB 5 mM
5.5	1.98	1.50	1.02	0.75	0.49
6.0	1.87	1.30	0.95	0.70	0.48
6.5	1.75	1.00	0.85	0.63	0.46
7.0	1.61	0.95	0.83	0.58	0.44
7.5	0.95	0.85	0.80	0.53	0.42
8.0	0.85	0.76	0.75	0.48	0.39
8.5	0.70	0.60	0.65	0.38	0.27
9.0	0.60	0.55	0.50	0.35	0.25

CONCLUSION

This work helped to understand the biochemical nature of spasmins & batonnets in comparison with myosin at the level of patterns of protein folding in respect of force, power and energy utilisation.

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