Malaria parasites tolerate a broad range of ionic environments and do not require host cation remodeling

Supplemental Information

Ajay D. Pillai, Rachel Addo, Paresh Sharma, Wang Nguitragool, Prakash Srinivasan, and Sanjay A. Desai*

From the Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD 20852

* Email: sdesai@niaid.nih.gov

Tel.: (301) 435-7552; Fax: (301) 402-2201

Table S1. Basal medium: constituents present in all media and their concentrations (mM) prior to addition of serum.

Ca(NO ₃) ₂ • 4H ₂ O	0.42
MgSO ₄	0.41
L-Arginine	1.15
L-Asparagine	0.378
L-Aspartic acid	0.150
L-Cysteine	0.416
L-Glutamic acid	0.136
L-Glutamine	2.05
Glycine	0.133
L-Histidine	0.097
Hydroxy-L-proline	0.153
L-Isoleucine	0.381

L-Leucine	0.381		
L-Lysine • HCl	0.219		
L-Methionine	0.101		
L-Phenylalanine	0.091		
L-Proline	0.174		
L-Serine	0.285		
L-Threonine	0.168		
L-Tryptophan	0.024		
L-Tyrosine • 2Na • 2H ₂ O	0.111		
L-Valine	0.171		
D-Biotin	0.00082		
Choline Chloride	0.0215		
Folic Acid	0.00227		
•	•		

myo-Inositol	0.194		
Niacinamide	0.0082		
p-Amino Benzoic Acid	0.0073		
D-Pantothenic Acid, Ca salt	0.000524		
Pyridoxine• HCl	0.0049		
Riboflavin	0.00053		
Thiamine • HCl	0.0030		
Vitamin B-12	0.0000037		
D-Glucose	11.1		
Glutathione, reduced	0.00326		
HEPES, free acid	24.9		
Hypoxanthine	0.03		

Table S2. Constituents that differ between media and their concentrations (mM) prior to addition of serum.

	RPMI 1640	full-K⁺	4suc:6KCl	zeroK	full-K + 50 sucrose	NaCl:KGluc	7suc:3KCl	4suc:3KCl:3KGluc	Sucrose medium + 5.4 mM K
NaCl	102.7	0	0	108	0	64.8	0	0	0
KCI	5.4	108	64.8	0	108	5.4	32.4	32.4	5.4
NaHCO ₃	28.6	0	0	28.6	0	28.6	0	0	28.6
KHCO ₃	0	28.6	28.6	0	28.6	0	28.6	28.6	0
Na ₂ HPO ₄	5.64	0	0	5.64	0	5.64	0	0	5.64
K ₂ HPO ₄	0	5.64	5.64	0	5.64	0	5.64	5.64	0
Sucrose	0	0	84.3	0	50	0	147.5	84.3	216
K gluconate	0	0	0	0	0	64.8	0	32.4	0

Table S3. Nominal concentrations of key ions, calculated osmolarities, and ionic strength of each medium. Values include contributions from basal medium, but not those resulting from addition of serum.

	RPMI 1640	full-K⁺	4suc:6KCI	zeroK	full-K + 50 sucrose	NaCI:KGluc	7suc:3KCI	4suc:3KCI:3KGluc	Sucrose medium + 5.4 mM K
[Na ⁺], mM	142.8	0.2	0.2	148.1	0.2	104.9	0.2	0.2	40.1
[K ⁺], mM	5.4	147.9	104.7	0	147.9	70.2	72.3	104.7	5.4
[Cl ⁻], mM	108.3	108.2	65.0	108.2	108.2	70.4	32.6	32.6	5.6
osmolarity*, mosm	335.9	335.7	333.6	335.7	385.7	389.7	332.0	333.6	346.5
ionic strength**, mol/L	163.8	163.7	120.5	163.7	163.7	190.7	88.1	120.5	61.1

^{*} medium osmolarity was calculated assuming activity coefficients of 1 for all solutes and 100% dissociation of salts.

Video S1. Normal merozoite egress and successful invasions of two erythrocytes in RPMI 1640 medium. Note the marked deformation of the erythrocytes upon completed merozoite invasion.

Video S2. Defective egress and reduced invasiveness of free merozoites in 7suc:3KCl medium. Although interactions of freed merozoites and two erythrocytes are apparent, these did not lead to successful invasion, as indicated by absence of merozoite internalization or erythrocyte deformation.

^{**} ionic strength, a weighted measure of the total concentration of ions in solution, is calculated according to $I = \frac{1}{2} \sum_{i=1}^{n} b_i z_i^2$ where b and z are the concentration and valence of each cation or anion in the medium.