Zool. Beitr. N.F. 30: 231 - 247

Proton balance of anaerobic and post-anaerobic metabolism – interrelations with pH regulation in marine invertebrates

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Received November 11, 1986

Summary

Investigations in the marine worm *Sipunculus nudus* have demonstrated a clear quantitative correlation between the net proton balance of anaerobic metabolism and the response of acid-base regulatory mechanisms. Proton equivalent ion movements between muscle tissues, coelomic fluid and ambient sea water reflect a priority assigned to intracellular pH regulation. The coelomic fluid is utilized as a transient sink for protons during long term anaerobiosis, post-anaerobic recovery and muscular activity. Net proton release to the ambient water contributes to acid-base regulation and is mainly observed under aerobic conditions.

Anaerobic metabolic pathways are believed to cause proton loads in animal tissues during muscular activity and during exposure to anoxia. Maintenance of pH, however, may be crucial for the survival of the animal since enzyme function and energy transductions may be negatively affected by either low or high pH values. Some lower marine invertebrates (among molluscs, annelids, sipunculids) inhabiting the intertidal zone are specialized to passively sustain long periods of environmental hypoxia. Several among this group, which are living in the anoxic environment, must also be able to do muscular work without substantial oxygen supply from the surroundings. During recent years investigations of acid-base and metabolic events in the sipunculid worm *Sipunculus nudus* have revealed how anaerobic mechanisms of energy production can support pH regulation and have shown strategies of pH regulation during and after anaerobiosis which may be typical for marine facultative anaerobes.

The Embden Meyerhof pathway in marine anaerobes is modified by reductive condensation of pyruvate with different amino acids, the respective end products being strombine, alanopine, or octopine, depending upon

the amino acid involved (GRIESHABER 1982). This also includes tauropine which has recently been described as a possible anaerobic end product in *Haliotis* (SATO & GÄDE 1986). The opine dehydrogenase reaction mostly relies on pools of the different amino acids which are in dissociation equilibrium with the cell water. Therefore, the net reaction important for the acid-base status is always the formation of pyruvate in anaerobic glycolysis (PÖRTNER 1982, PÖRTNER et al. 1984a).

In the past there have been difficulties in understanding the influence of L-arginine on acid-base balance since in many marine invertebrates L-arginine, phospho-L-arginine and octopine are linked via arginine kinase and octopine dehydrogenase reactions. Fig. 1 shows the pattern of dissociation of these different substances in the range of cell pH, together with the pK values of their functional groups. It is evident that changes in the different pK values of L-arginine in phospho-L-arginine and octopine are too small to cause any significant change in the pattern of dissociation or protonation. This means that if L-arginine is accumulated from phospho-L-

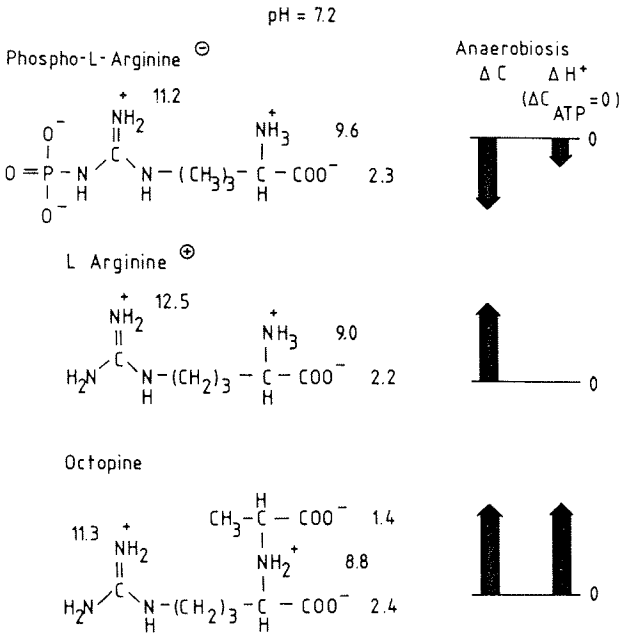

Fig. 1. Pattern of protonation and dissociation of phospho-L-arginine, L-arginine and octopine in the range of cell pH (constants adopted from SOBER 1973). The proton balance (ΔH^+) during anaerobic concentration changes (Δc) results from the fact that dissociation constants of the L-arginine residue remain virtually unchanged in the different binding states. For further explanations see text.

arginine, there is no effect on the acid-base status by L-arginine. If octopine is formed from L-arginine, the same holds true. The only net effect is the proton yield expected from the glycolytic pathway which delivers the pyruvate residue in octopine.

Proton consumption, however, can be observed during phospho-L-arginine hydrolysis. This is generally observed during cleavage of any phosphagen since proton consumption is caused by the pK change of the phosphate group in these substances between bound and free inorganic phosphate (MEYERHOF & LOHMANN 1928, PÖRTNER et al. 1984 a).

In addition to proton consumption during phosphagen hydrolysis, inorganic phosphate accumulation also occurs. During anaerobiosis cell pH decreases from a value above 7 towards the pK of inorganic phosphate (a value of 6.81). Therefore, a drastic increase in the non-bicarbonate buffer value of the tissue can be expected, depending upon the amount of phosphagen cleaved. In *Sipunculus nudus* a 30 % increase in the total buffer value could theoretically result in a 25 % reduction of the intracellular acidosis during anaerobiosis (fig. 2).

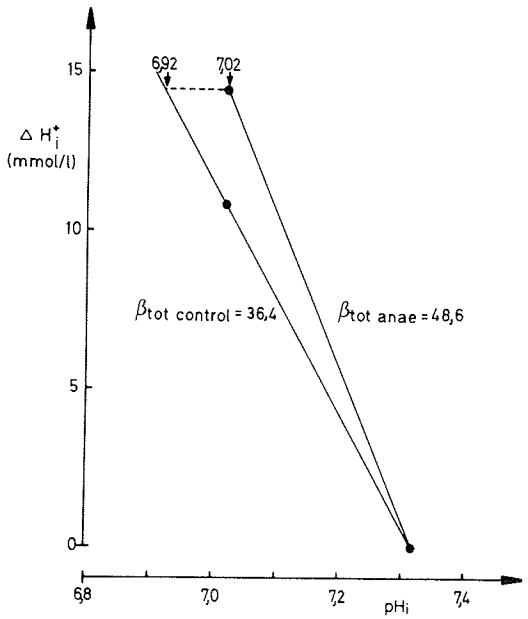

Fig. 2. Changes in the intracellular total buffer value β_{tot} (non-bicarbonate + bicarbonate buffer values) of the body wall musculature expected from the accumulation of inorganic phosphate during anaerobic phospho-L-arginine hydrolysis. The decrease of pH_i during 24 h of anaerobiosis was theoretically reduced by 0.1 pH units (based on PÖRTNER et al. 1984 a).

During intensive muscular activity the phosphagen pool is the first to be utilized and anaerobic glycolysis starts to accumulate protons and the end product. Accordingly, an alkalosis was observed during the first contractions of an isolated molluscan muscle following by an acidosis during long term activity (CHIH & ELLINGTON 1985). Muscular activity in *Sipunculus nudus* in vivo leads to the formation of two end products of anaerobic glycolysis, strombine and octopine (PÖRTNER et al. 1984b). The presented data (table 1) originate from experiments where pressure changes in the coelomic cavity were measured during digging. The pressure times time integral is taken as a measure of the work done by the animals and the mean pressure represents a measure of the power output (PÖRTNER 1982). Increasing muscular activity leads to an increase in glycolytic end product, esp. octopine formation as well as a more pronounced utilization of the phosphagen. Concomitantly, muscular activity causes an extracellular acidosis, which is also more pronounced at higher work loads. The non-respiratory part of the acidosis results mainly from strombine formation but is increased by an enhanced octopine formation, when the animals become more and more active. The buffering effect of phospho-L-arginine, however, also depends on the work load of the animals and counteracts the proton accumulation by glycolysis.

It is surprising that the pH_i is regulated at a constant level after it has decreased somewhat during muscular activity (table 1 and 2). More than 90 % of the metabolic protons appear in the coelomic fluid after intensive digging and thereby the metabolic proton load of the tissue is reduced even when compared to lower work loads (table 2). An increased formation of CO_2, however, is responsible for keeping pH_i constantly low. The reduction of the metabolic tissue proton load, therefore, could be the regulatory answer to the pronounced respiratory acidosis in intensively working animals. In terms of pH regulation it is also interesting to note that the period of digging was obviously long enough in these animals to allow for efficient proton transfer from the tissues to the coelomic plasma despite a continuous H^+ production in the musculature (based on results obtained by PÖRTNER, GRIESHABER & HEISLER unpubl.). The pH_i values in table 1 and 2 are calculated values, however, based on the assumption that a quantitative interrelationship exists between acid-base and metabolic events (see below, table 4).

These results indicate that the utilization of anaerobic metabolism during digging in the anoxic substratum may lead to only minor tissue acid-base disturbances. The coelomic fluid, which represents approximately 50 % of the animal (PÖRTNER 1982), can be utilized efficiently as a sink for metabolic protons. The coelomic cells, which exhibit a low metabolic rate especially during hypoxia or anoxia (PÖRTNER 1982), very likely contribute to proton

Table 1. Comparison of metabolic and acid-base changes during digging activity depending on the amount of "work" and "power" output ($\bar{x} \pm SD$, n = 3 or 5, respectively). Underlined pH_i values have been calculated (see Table 2 and text; PÖRTNER, GRIESHABER & HEISLER, unpubl.).

"work" (cm H_2O × min)	"power" (cm H_2O)	Time (min)	PLA	Octopine	Strombine	Succinate	ATP	pH_e	$[HCO_3^-]_e$ (mmol l^{-1})	P_{CO_2} (Torr)	pH_i
			(μmol g^{-1} fresh weight)								
0 (3)	0	0	46.64 ±0.64	0.10 ±0.05	1.67 ±1.18	0.05 ±0.01	3.27 0.09	7.98 ±0.05	7.36 ±0.58	1.62 ±0.28	7.34
482 (5) ±124	10.6 ±1.9	45.6 ±9.1	43.38 ±1.65	0.59 ±0.29	7.22 ±3.58	0.13 ±0.07	3.26 ±0.32	7.76 ±0.06	5.53 ±1.57	2.19 ±0.44	<u>7.29</u>
1158 (3) ±119	17.3 ±1.2	67.3 ±9.9	34.75 ±5.23	2.45 ±0.61	9.37 ±3.42	0.17 ±0.06	3.01 ±0.45	7.54 ±0.14	4.70 ±1.93	3.14 ±0.21	<u>7.29</u>

Table 2. Comparison of the amounts of non-respiratory protons in the extracellular space (ΔH^+_e non-resp.) and the extent of proton production by metabolism (ΔH^+_e met. max., calculated for the extracellular space) during digging activity. The metabolic (non-respiratory) proton load of the tissues (ΔH^+_i non-resp.) was estimated from the surplus protons in metabolism. pH_i values after activity were calculated from non-respiratory and respiratory proton accumulation (based on data from PÖRTNER, GRIESHABER & HEISLER, unpubl.).

"work" (cm H_2O × min)	"power" (cm H_2O)	ΔH^+_e non-resp. (mmol l^{-1})	ΔH^+_e met. max. (mmol l^{-1})	ΔH^+_i non-resp. (mmol l^{-1})	ΔH^+_i resp. (mmol l^{-1})	pH_i
0 (3)	0	–	–	–	–	7.34
482 (5)	10.6	+2.87 (81%)	+3.55	+1.78	+0.46	7.29
1158 (3)	17.3	+4.70 (94%)	+4.99	+0.75	+1.16	7.29

buffering, but can be excepted to only negligibly increase the extracellular proton load by their own anaerobic metabolism. It is tempting to assume that the regulation of intracellular pH is consistent with the mode of life of *Sipunculus nudus*. The animal regularly starts voluntary excursions into the substratum for feeding purposes. pH regulation may help to delay fatigue and, thereby, ensures that it is always able to return to the surface of the substratum in order to obtain oxygenated water.

During environmental hypoxia the glycolytic pathway and phosphagen hydrolysis are typical of the first hours of adaptation to anaerobiosis (KREUTZER et al. 1985). Aspartate is involved as a second substrate for succinate and propionate formation. During this time, however, metabolism shifts to the utilization of the succinate-propionate pathway involving a carboxylation of phospoenolpyruvate or pyruvate in glycolysis (see PÖRTNER et al. 1984b for a metabolic scheme).

The proton balance of these pathways cannot simply be explained by proton release via ATP hydrolysis since proton relevant reactions, which do not directly include ATP synthesis, are involved. Also, some reactions deliver energy for ATP synthesis but do not affect the acid-base status. For each metabolite the proton yield can be determined, however, depending upon the turnover of ATP, CO_2 and bicarbonate (PÖRTNER 1982, 1986a, PÖRTNER et al. 1984a). The amount of protons generated per ATP formed is reduced during succinate, propionate and acetate formation due to the involvement of oxidative decarboxylation reactions. Reoxidation of accumulated NADH is required, however, for redox balance, which succinate formation from fumarate may not be solely responsible for (PÖRTNER 1982). This question of redox balance has not yet been solved but the other mechanisms of NADH reoxidation are not expected to include the generation of further metabolic protons i.e. acidic end products (PÖRTNER 1986a). In general, this analysis demonstrates that the proton balance of a pathway is equivalent to the amount of formed or consumed carboxyl groups (PÖRTNER 1982, PÖRTNER et al. 1984a).

The metabolism of amino groups may also influence the proton yield of anaerobic metabolism. Transamination reactions are not linked to net proton release or consumption (PÖRTNER et al. 1984a). If reductive amination or ammonia accumulation occurs, as has been assumed for facultatively anaerobic invertebrates (DE ZWAAN & VAN MARREWIJK 1973, ZURBURG & DE ZWAAN 1981, PÖRTNER et al. 1986a), it has to be taken into account that NH_4^+ formation is almost always linked to proton consumption (PÖRTNER 1986a, only the release of ammonium by the glutaminase reaction is neutral for the acid-base status).

The question arises whether pH changes observed during environmental hypoxia are consistent with these considerations. A rigorous quantitative

analysis in living animals is difficult to perform since it requires measurement of all experimental parameters in the same animals, considering all body compartments and acid-base regulation.

Table 3 shows the concentration changes of different metabolites in the body wall musculature of *Sipunculus nudus* during 24 h of anaerobiosis as well as the extent of concomitant proton production or consumption. For quantitative analysis metabolite concentrations in the plasma and changes in the intra- and extracellular acid-base status were also considered (PÖRTNER 1986b). In the whole animal non-respiratory changes in the acid-base status amount to 1.4 mmol $\cdot$ l^{-1} body water after 24 h of anaerobiosis (table 4), whereas 1.8 mmol $\cdot$ l^{-1} body water are expected from metabolism. Twenty-two % of the protons produced in the animals, therefore, cannot be explained by the changes in the acid-base status (PÖRTNER 1986b). In separate experiments, however, it was demonstrated that the animals release metabolic protons to the ambient water. The amount during 24 h of

Table 3

Proton balance of anaerobic metabolism as evaluated from concentration changes of metabolites in the body wall musculature (concentrations in µmol $\cdot$ g^{-1} fresh weight, $\bar{x} \pm SE$, for further explanations text).

	aerobic ($n = 10$)	anaerobic (24 h) ($n = 10$)	Δc	ΔH^+
octopine	0.07 ± 0.01	1.17 ± 0.15	+ 1.10	+ 1.10
strombine	1.05 ± 0.24	5.18 ± 0.34	+ 4.13	+ 4.13
alanopine	0.10 ± 0.02	0.09 ± 0.01	–	–
pyruvate	< 0.02	< 0.02	–	–
glutamate	1.04 ± 0.03	1.05 ± 0.10	–	–
glutamine	0.36 ± 0.08	0.25 ± 0.05	− 0.11	− 0.22
α-ketoglutarate	< 0.02	< 0.02	–	–
aspartate	1.04 ± 0.06	0.52 ± 0.03	− 0.52	− 1.04
alanine	1.40 ± 0.15	3.03 ± 0.25	+ 1.63	+ 1.63
malate	0.04 ± 0.01	0.13 ± 0.01	+ 0.09	+ 0.18
succinate	0.06 ± 0.01	0.90 ± 0.09	+ 0.84	+ 1.68
propionate	0.10 ± 0.02	0.47 ± 0.03	+ 0.37	+ 0.37
acetate	0.30 ± 0.02	0.27 ± 0.02	–	–
NH_3	2.63 ± 0.27	3.91 ± 0.20	+ 1.28	− 1.28
$-NH_2$ (reductive amination)			+ 0.89	− 0.89
phospho-L-arginine	44.8 ± 0.6	31.3 ± 1.7	− 13.5	− 3.95
L-arginine	10.0 ± 0.6	22.2 ± 1.7	+ 12.2	–
ATP	3.36 ± 0.16	3.21 ± 0.14	–	–
ADP	0.44 ± 0.02	0.47 ± 0.02	–	–
				+ 1.71

Table 4.

Comparison of proton generation by metabolism and non-respiratory (non-resp.) changes in the acid-base status after 24 h of anaerobiosis. The surplus protons expected from metabolism very likely have been released to the ambient water by ionic exchange mechanisms ($\Delta H^+_{e \rightarrow w}$; PÖRTNER, 1986b).

	ΔH^+ non-resp. 0-24 h of anaerobiosis (mmol l^{-1} body water)
metabolism:	+1.79
acid-base status:	+1.39
discrepancy:	+0.40 (22%)
$\Delta H^+_{e \rightarrow w}$:	0.3-0.5

anaerobiosis seemed quite independent of the anaerobic metabolic rate and equalled 0.3 to 0.5 mmol · l^{-1} body water (PÖRTNER & HEISLER unpubl.). This covers the observed discrepancy quite well. Finally, this analysis demonstrates that the extent of anaerobic changes in the acid-base status clearly depends on the net amount of protons generated by metabolism (PÖRTNER 1986b). It may be even more important that during anaerobiosis the amount of acidic equivalents formed is described by the known end products.

Other evidence demonstrating that there is a clear correlation between the anaerobic metabolic rate and the extent of acidosis provoked during anaerobiosis is shown on table 5. A comparison of metabolic and acid-base events in animals collected during different times of the year demonstrates that the extent of extracellular acidosis during anaerobiosis is correlated with the amount of protons produced in metabolism (PÖRTNER et al. 1986a, b). An increase in metabolic rate or proton production, depending upon the season, is mainly reflected by changes in the rate of strombine accumulation, but also involves higher amounts of octopine and volatile fatty acids (esp. acetate) being produced. Since acid-base changes follow the extent of metabolic proton production, the intracellular proton load for October animals can be calculated. As expected, the anaerobic change of intracellular pH is also more pronounced in October as compared to the March animals. The change in intracellular pH resulting from these considerations is consistent with earlier observations (PÖRTNER et al. 1984c).

Furthermore, table 5 shows that with increasing metabolic rate the extracellular space contributes increasingly to the storage of non-respiratory (= metabolic) protons during anaerobiosis. During digging activity of the animals almost all metabolic protons are found to be released from the

Table 5. Comparison of acid base and metabolic changes during 24 h of anaerobiosis in animals collected during March and October. In March, 35% of the non-respiratory (n.r.) protons were found in the extracellular compartment, and in October, 63%. Underlined values are calculated values assuming a quantitative correlation between anaerobic changes in metabolism and in the acid-base status (based on data from PÖRTNER et al. 1986a, b and PÖRTNER, 1986b; M: Musculature, CP: Coelomic Plasma).

		pH_e	$[HCO_3^-]_e$ (mmol l^{-1})	P_{CO_2} (Torr)	$\Delta H^+_{e\,nr}$ (mmol l^{-1})	$\Delta H^+_{i\,nr}$ (mmol l^{-1})	pH_i	Strombine M (μmol g^{-1} fresh weight)	Octopine M (μmol g^{-1} fresh weight)	Acetate M (μmol g^{-1} fresh weight)	Acetate CP (mmol l^{-1})
March	Control	8.09	5.50	0.88			7.27	1.05	0.07	0.30	0.07
	Anaer.	8.20	4.86	0.50	+0.65 (35%)	3.22	7.19	5.18	1.17	0.27	0.36
October	Control	7.97	4.42	1.02			7.27	4.5	1.0	0.36	0.27
	Anaer.	7.42	0.71	0.73	+6.24 (63%)	9.61	7.01	12.8	6.7	0.81	1.25

musculature to the coelomic fluid (table 2). This could be due to intensive perfusion or "rinsing" of the tissues, caused by the movements of the body wall. As will be dicussed below, the efficiency of pH regulation also seems to depend upon the availability of oxygen, which during digging activity in these animals is facilitated by taking a hemerythrin bound oxygen store with them in the coelomic cavity when they enter the anoxic substratum.

It becomes increasingly clear from these results that there is an exchange of protons between intra- and extracellular compartments which could be due to an exchange of organic acids. The only acids which are found to accumulate in the plasma, are succinate, propionate, and acetate. The opines, quite opposite to the behaviour of lactate, do not leave the tissues at all. Fig. 3 a shows that during long term anaerobiosis the organic acid anions present in the coelomic plasma do not cover the amount of non-respiratory protons. During the period of extracellular alkalosis, which is caused by proton consumption during phospho-L-arginine and aspartate degradation being in excess over proton production by glycolysis, there is already an accumulation of organic acid anions in the plasma. Evidently, protons and organic acid anions are distributed between intra- and extracellular compartments according to different equilibria and kinetics (PÖRTNER et al. 1984 c). Acid-base and metabolite concentration changes measured during recovery confirm this conclusion (fig. 3 b, PÖRTNER et al. 1986 a, b). There is even an increased extracellular accumulation of non-respiratory protons during the initial recovery period, while at the same time the concentration of organic acid anions is already reduced.

Phosphagen repletion, which rapidly occurs during initial recovery, could be demonstrated to cause the extracellular acidosis. The protons consumed during anaerobiosis have to be released during resynthesis of the phosphagen. This occurs during initial recovery when oxygen is available to the tissue and is obviously linked to an efficient regulation of intracellular pH (PÖRTNER et al. 1986 a, b). The latter observation supports the conclusion that the presence of oxygen supports pH_i regulation since the rate of proton production by metabolism is higher during initial recovery than during anaerobiosis. The release of these protons from the tissues is required because the equilibrium of arginine kinase is pH sensitive and a high pH is necessary for complete repletion of the phosphagen. pH_i regulation obviously determines the extent of proton exchange between intra- and extracellular compartments independent of the movements of metabolites.

The ionic regulation of intracellular pH could cause part of the observed increase in metabolic rate during recovery (PÖRTNER et al. 1986 b). On the other hand the decrease in metabolic rate during anaerobiosis, which in *Sipunculus nudus* occurs by a factor of about 20 as compared to the ATP turnover under normoxia (PÖRTNER 1982), could imply a regulatory reduc-

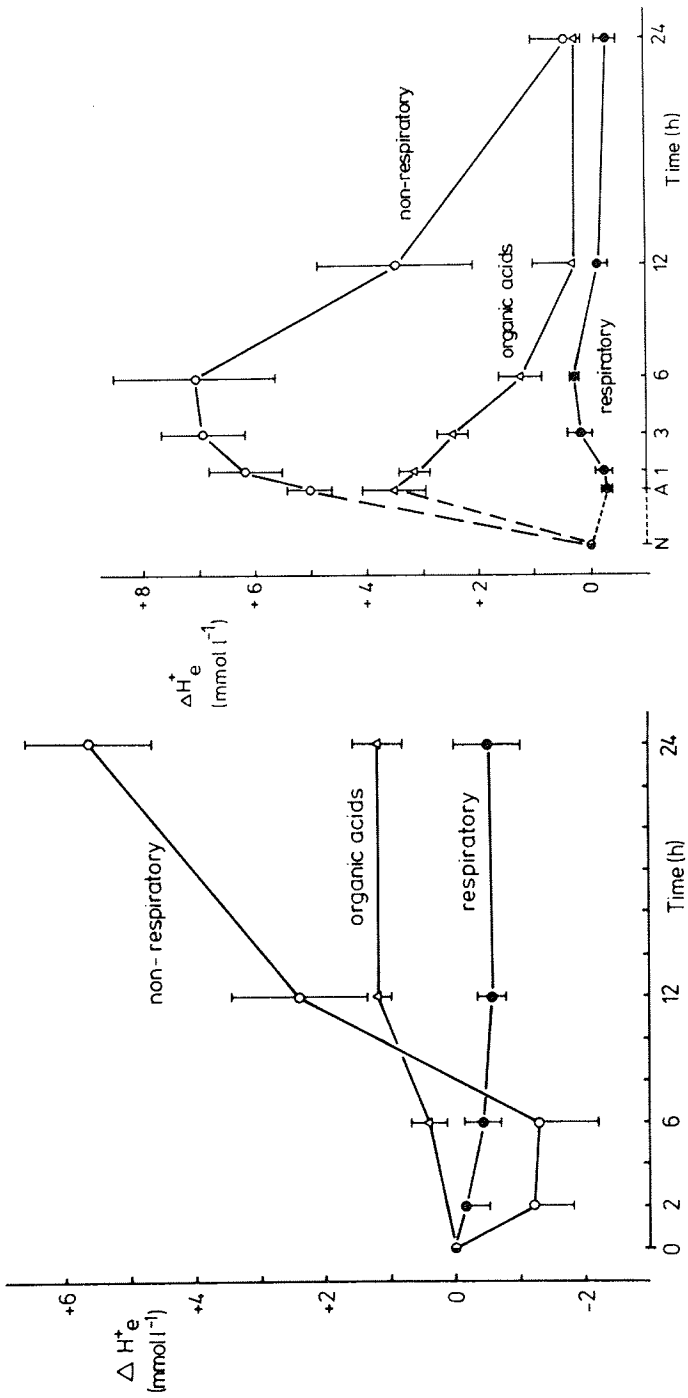

Fig. 3. Respiratory and non-respiratory changes in the extracellular acid-base status compared with the amount of protons expected from organic acids present in the plasma (succinic, propionic, and acetic acid) (a) during 24 h of anaerobiosis, (b) during postanaerobic recovery (N: Normoxia, A: Anaerobiosis). Proton movements between intra- and extracellular compartments depend upon the regulation of pH_i and not upon the movements of metabolites (PÖRTNER et al. 1984c; PÖRTNER et al. 1986b).

tion in the ionic exchange between tissues and coelomic fluid. This would explain the insufficient regulation of pH_i under these conditions. Part of the lower degree of anaerobic acid-base regulation could also be due to a decreasing muscular activity of the animals which leads to an insufficient perfusion of the tissues, thereby reducing the capability for pH_i regulation. Perfusion or "rinsing" of the tissues is obviously sufficient in ventilating animals recovering from anaerobiosis.

During recovery from anaerobiosis in the natural environments the animals have access to oxygenated sea water which by means of ionic exchange is utilized as a sink for metabolic protons (fig. 4a,b; PÖRTNER & HEISLER, unpubl.). Ion exchange with the ambient water, however, already responds to the changes in the acid-base status during anaerobiosis as evaluated from the changing rates of both ammonia and bicarbonate accumulation. The reduction in ammonia production during anaerobiosis very likely represents the cessation of aerobic protein catabolism and the switch to glycogen as a substrate. After anaerobiosis the control rate of ammonia release or rate of aerobic protein metabolism is achieved again. The net proton equivalent ion transfer rates resulting from these changes was only slightly negative during the control period. During anaerobiosis, proton or bicarbonate exchange reflects the extracellular pH changes, a base release during initial extracellular alkalosis turning into a net proton release during the later period of progressive metabolic acidosis.

During recovery the net release of protons is accelerated. This is obviously linked to the recovery induced acidosis (cf. PÖRTNER et al. 1986b). During long term recovery a proton gap results from these changes, since the cumulative proton transfer does not return to the normoxic control rate. This gap amounts to about 4 $mmol \cdot kg^{-1}$ body weight and can be quantitatively explained by the accumulation of end products during anaerobiosis which is not reversed during recovery. Mainly strombine and acetate are found to be slowly metabolized during recovery (PÖRTNER et al. 1986a). Restoration of the aerobic acid-base status is possible, however, due to the release of the respective protons to the ambient water (PÖRTNER & HEISLER, unpubl.).

Considering the rates of proton transfer to the ambient water, it is evident that this rate is stimulated at the onset of recovery (fig. 4b). Since pH_i is kept constant during that period (PÖRTNER et al. 1986b), the proton or bicarbonate transfer to the water could depend mostly on the extracellular acid-base status. pH_i is still below aerobic values, however, and could contribute or alternatively even cause the observed proton equivalent ion movements. The rate change could not only be due to the extent of intra- and/or extracellular acidosis but also to the availability of oxgen, which enables an increase in metabolic rate. The P_{O_2} change stimulus must be very efficient,

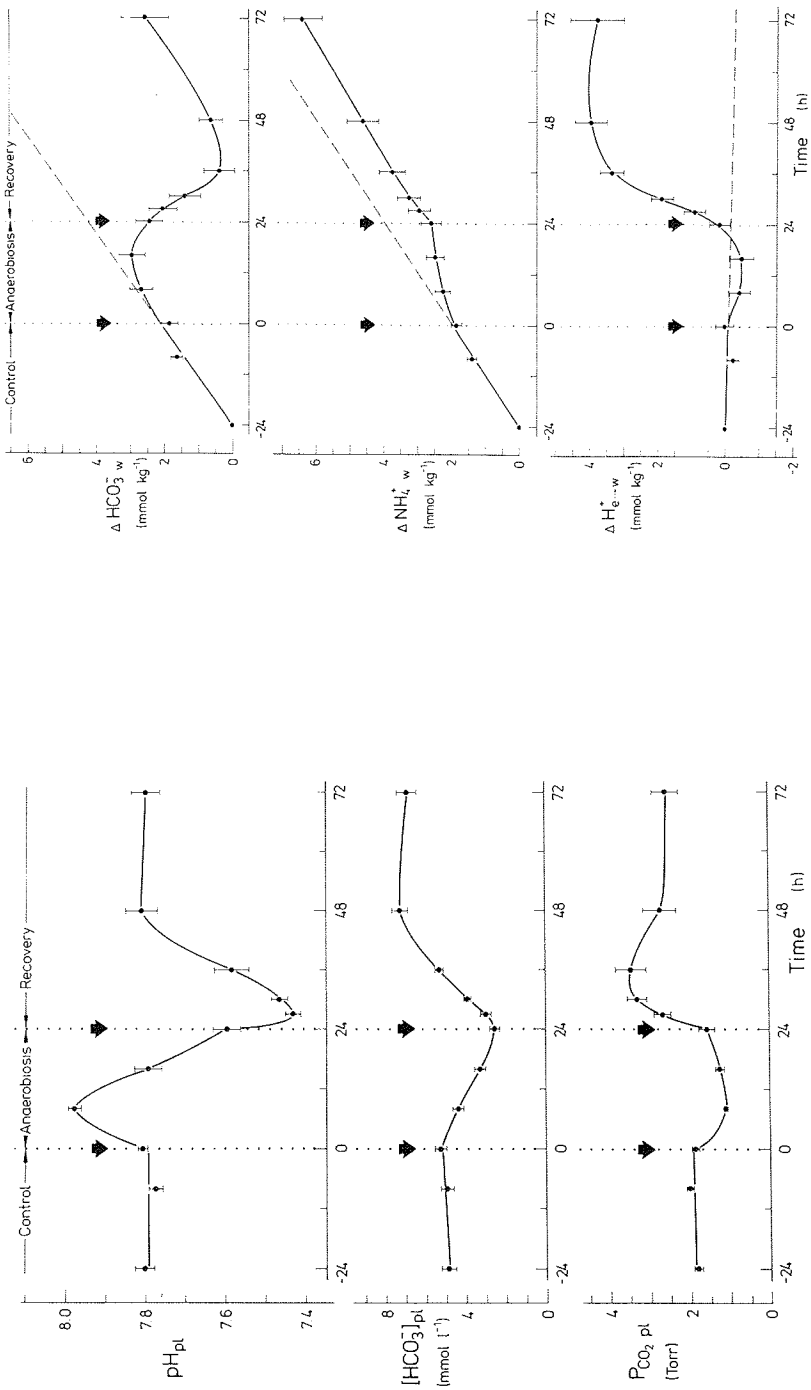

Fig. 4. Changes in the extracellular (pl. = plasma) acid-base status (a) and concomitant proton equivalent ion transfer ($\Delta H_{e\rightarrow w}^+$) between animals and ambient water, calculated from the rates of water ammonia (ΔNH_{4w}^+) and bicarbonate (ΔHCO_{3w}^-) concentration changes (b), under control conditions during 24 h of anaerobiosis and during subsequent recovery (PÖRTNER & HEISLER unpublished results).

since the resulting rate of net proton transfer is higher than under hypercapnia when there is a more pronounced fall in extracellular pH (PÖRTNER & HEISLER unpubl.).

In conclusion, changes in the acid-base status of *Sipunculus nudus* during anaerobiosis follow the net proton yield of metabolism (besides following P_{CO_2} variations) and can thereby be quantitatively explained. Ion transfer between intra- and extracellular compartments and ambient water responds to the respective metabolic (and/or respiratory) proton loads. During long term anaerobiosis the efficiency of ionic pH regulation is reduced. Under these conditions pH regulation primarily means a minimization of proton accumulation by metabolism. This implies proton consumption during phosphagen depletion and accumulation of inorganic phosphate as an additional buffer substance. The stoichiometric amount of protons generated per mole of ATP turned over is reduced from unity to a lower value. In addition, some end products like acetate or propionate may leave the animal in protonated form, thereby reducing the metabolic proton load. An important mechanism assuring long term survival is the reduction of the anaerobic metabolic rate. Ionic regulation of pH during anaerobiosis occurs primarily as an exchange between intra- and extracellular compartments but only slightly reduces the proton load of the total organism. During functional and environmental hypoxia the extracellular compartment is utilized as a sink for metabolic protons. The ambient water only becomes important during recovery, an observation which is at least valid for environmental hypoxia.

Finally, efficient pH regulation in *Sipunculus nudus* during metabolic acidosis requires oxygen to be present. Oxygen enables the regulation of intracellular pH and secondarily causes high rates of ionic exchange with the ambient water. Perfusion or "rinsing" of the tissues (an expression, which is more adequate for the situation in *Sipunculus*) can be assumed to help in facilitating the release of protons from the tissues to the plasma. A low metabolic rate during long term anaerobiosis and a high metabolic rate during recovery could reflect regulatory changes in the rate of ionic exchange and thereby influence the efficiency of pH regulation.

Zusammenfassung

Untersuchungen am Spritzwurm *Sipunculus nudus* haben gezeigt, daß eine Netto-Protonenbildung im anaeroben Stoffwechsel in quantitativer Beziehung zu den Änderungen von Parametern des Säure-Basen Haushaltes steht. Die Nettobewegungen von Protonenäquivalenten zwischen Muskelgewebe, Coelomflüssigkeit und umgebenden Seewasser lassen auf eine vorrangige Regulation des intrazellulären pH schließen. Während extremer Muskelaktivität, langfristiger biotopbedingter Anaerobiose und anschließender Erholung werden saure Äquivalente vorübergehend aus dem Gewebe

in die Coelomflüssigkeit abgegeben. Die Nettoabgabe von Protonen an das Seewasser, die vorwiegend unter aeroben Bedingungen zu beobachten ist, trägt zur Regulierung des Säure-Base Status bei.

Acknowledgements

The author wishes to thank Dan Toews fore reading and commenting on the manuscript. Supported by DFG Gr 456 and Po 278/1-1.

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