MEMBRANE PROTON TRANSPORT MEDIATED BY PHENYLHYDRAZONOPROPANEDINITRILES

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Some fundamental physicochemical characteristics as stability in solutions, solubility in various solvents and association constants describing equilibria with protons and potassium ions in aqueous solutions were determined for phenylhydrazonopropanedinitriles (PHPD). The effect of pH and sodium, potassium, calcium, and magnesium cations on the distribution of PHPD were examined in a two-compartment system 1-octanol–water. The transmembrane transfer of protons by PHPD causing a disturbance of the pH-gradient was verified in vitro using a model three-compartment system water–octanol–water, imitating the in vivo intracristal space – inner mitochondrial membrane – matrix system. Transfer of H^+ ions mediated by PHPD in the system under study was found to be considerably faster when an exchange with K^+ ions (ion-exchanging antiport H^+/K^+) was possible. A model was described indicating the reality of ion-exchanging antiport H^+/Me^+ mediated by PHPD on biomembranes which is in line with the chemiosmotic theory.

The process of substrate oxidation and formation of ATP has been the one of the most investigated phenomenons proceeding in cells. Although a great number of papers aimed at its understanding, still some theories explained it quite differently. At the time being, experimental data are best reflected by the chemiosmotic theory[1,2]. According to it formation of ATP by the synthesis from ADP and phosphate is driven by the gradient of proton electrochemical potential generated at the inner mitochondrial membrane due to oxidation of the appropriate substrate. The process of coupling of oxidation with phosphorylation is noticeably disturbed by many weak acids[3–5], resulting in a so-called uncoupling effect[5–7]. As presumed, its essence is the disturbance of electrochemical pH-gradient, resulting in cessation of the ATP synthesis[9,10].

The mechanism of disturbance of electrochemical potential by PHPD in planar and spherical phospholipide membranes in vitro has already been reported[11–13]. Experimental data served for construction of a simple kinetic model showing the transport of protons through interface by ionized PHPD form. Nevertheless, the

effect of metal cations was not reflected in the model although this phenomenon has been reported[14-16]. Green et al.[14-16] observed the effect of concentration of various cations on the uncoupling capacity of different uncouplers. He considered formation of a complex of uncoupler with a cation in lipidic phase and its passage from one membrane side in the other.

This paper is aimed to investigate the mechanism of pH-gradient disturbance caused by PHPD as a representative protonophoric uncoupler in simple in vitro system, as influenced by metal cations.

EXPERIMENTAL

Phenylhydrazonopropanedinitrile was prepared according to Zsolnai[17]. Spectrophotometer Specord UV VIS (Zeiss, G.D.R.) was employed for spectral measurements. The pH values of aqueous solutions were determined with an OP-208/1 Radelkis (Hungary) pH-meter using a combined Metrohm 9 100 electrode. Temperated vessel provided with a stirrer (25°C, 80 rpm) and a device enabling to withdraw samples from the bottom was employed for the estimation of rate constants of the PHPD transport and partition coefficients in a two-compartment system. Transport phenomena in a three-compartment system were examined in an apparatus schematically illustrated in Fig. 1.

Solubility and spectral properties of PHPD were determined by spectrophotometric measurements of absorption of the substance in solution of various solvents. Saturated PHPD solutions were obtained by a 5 h-stirring of PHPD suspension in the appropriate solvents at 25°C followed by a 10 min-centrifugation at 10 000 *g*. Solubility values for the respective solvents are averages of three measurements.

The transport rate parameters and partition coefficients of PHPD in a two-compartment system were estimated for the solvent system 1-octanol–water by calculation[18] employing Eq.

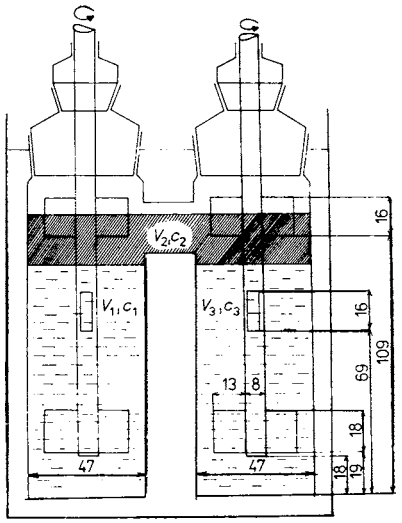


FIG. 1

Schematic sketch of the apparatus for determination of transport rate parameters in the three-compartment system (dimensions in mm)

(*1*)–(*3*):

$$k_1 = k_2 P\,, \tag{1}$$

$$k_2 = -(K/S)/(1/V_2 + P/V_1)\,, \tag{2}$$

$$P = (A_0/A_\infty - 1)\, V_1/V_2\,, \tag{3}$$

where k_1 and k_2 are the rate constants for PHPD transport from the aqueous to the octanol phase and backwards, V_1 the volume of the aqueous phase, V_2 volume of the octanol phase, A_0 the initial absorption of the aqueous solution of the compound, A_∞ the final absorption of the equilibrated aqueous solution, P the partition coefficient, S the surface of the interface, and K the slope of the linear relationship $\ln (A_t - A_\infty)/A_0$ on time. The compound was applied into the aqueous phase.

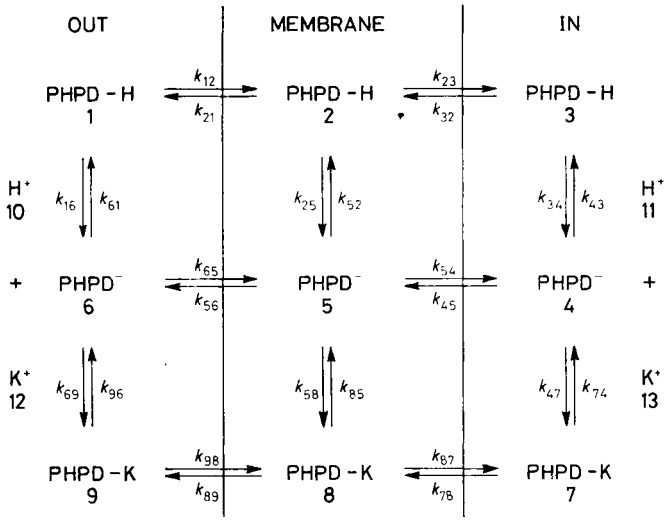
The measuring vessel was filled with the appropriate volume of the aqueous solution of PHPD adjusted at the proper pH and containing various concentrations of sodium, potassium, magnesium or calcium salts. The surface was after temperation to 25°C coated with water-saturated 1-octanol by means of a syringe. At this point time measurement started. The sample of aqueous layer was withdrawn by a syringe ($1{\cdot}5-5{\cdot}0 \,.\, 10^{-3}$ dm^3) in due time for spectrophotometric determination of concentration of the compound after which the sample was returned in the vessel in the same way. The experiment in the vessel was carried out under an argon atmosphere.

Kinetics of transport of PHPD and hydrogen protons in a three-compartment system water–1-octanol–water was examined in an apparatus shown in Fig. 1. Both compartments of the measuring vessel were filled with the aqueous phase (0·180 dm^{-3}) containing various amounts of sodium and potassium salts: 0·001, 0·010, 0·100, and 0·140 mol dm^{-3}. The pH was adjusted either by HCl (pH 3, 4·5), or NaOH (pH 10·3, 11·0). The compound was applied into the aqueous phase of the first compartment as a methanolic solution of PHPD up to the total concentration $1{\cdot}8 \,.\, 10^{-4}$ mol dm^{-3}. The amount of methanol added did not exceed 0·17 vol. %. The aqueous phases were temperated to 25°C and the rotations were kept at 80 min^{-1}. The surface was then coated with water-saturated 1-octanol (0·04 dm^3) from a syringe within a time interval less than 30 s. At this point time measurement started. At predetermined time intervals (0, 0·5, 1, 2, 3, 4, 5, 6, 7, 8, and 23 h) the samples ($1{\cdot}5 \,.\, 10^{-3}$ dm^3) were withdrawn from both compartments by syringes for spectrophotometric determination of PHPD concentration. The samples were returned into the vessel after taking the VIS spectrum and pH measurement. The vessel was saturated by argon preventing thus dissolution of carbon dioxide in aqueous phases and keeping the aqueous compartments at the constant pH value.

RESULTS AND DISCUSSION

The measurements were carried out in the system water–octanol–water imitating the three-compartment system intracristal space – inner mitochondrial membrane – – matrix. The pH value was adjusted with HCl (c = 0·0001 mol dm^{-3}) and NaOH (c = 0·001 mol dm^{-3}). Certain slow transport of protons proceeded under these conditions in the presence of PHPD, but the transport velocity was considerably increased by addition of potassium ions into the third compartment in concentrations up to 0·14 mol dm^{-3} (Fig. 2), what is a physiological concentration of potassium in intact mitochondria[19,20]. This fact, already considered by other authors[14–16] entitled us to propose the scheme of potential PHPD effect (Scheme 1). Constants k_{ij} are the transport rate parameters of the respective PHPD forms in the system under

study, PHPD-H stands for the not ionized form of PHPD, $PHPD^-$ for ionized form and PHPD-K for the associate of $PHPD^-$ with potassium cations characterized by the association constant β_{PHPD-K}.



SCHEME 1

Transport of protons and potassium ions by phenylhydrazonopropanedinitrile (PHPD) through interfaces

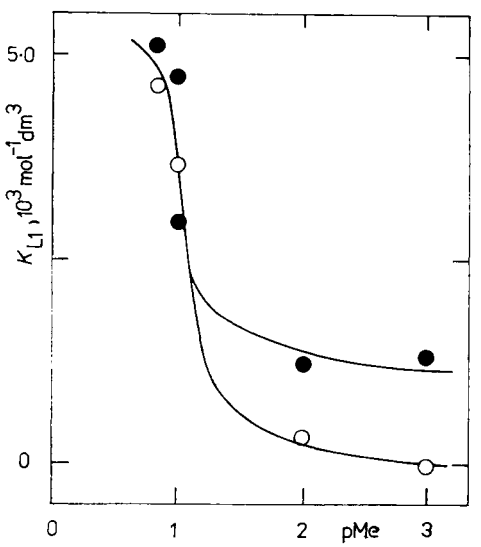


FIG. 2

Concentration dependence of the amount of H^+ ions transported from the first compartment in relation to the total concentration of PHPD in the system and to the maximal number of protons able to pass from the first to the third compartment — K_{L1} on concentration of sodium (○) and potassium (●) ions (Me^+) in the third compartment expressed in a logarithmic form — pMe in the eighth hour of distribution in the system water–octanol–water at 25°C, 80 rpm; $c_{PHPD} = 1{\cdot}8 \cdot 10^{-4}$ mol dm^{-3} and the initial difference $\Delta pH = 5{\cdot}8$

As seen, Scheme 1 includes the one-way proton transport caused by the PHPD itself and leading to an equilibrium of PHPD concentration in compartments, the cyclic transport of PHPD enabled by a backward $PHPD^-$ transport responsible for a one-way transportation of H^+, and also the cyclic PHPD transport enabled by a backward PHPD-K transport associated with the exchange transport of H^+ for K^+. The antiport H^+/K^+ is possible due to a nonzero value of the PHPD-K association constant and the true PHPD-K partition coefficient, as well as to a high concentration of potassium ions and a high pH value in the matrix, a low concentration of potassium and a low pH value in the intracristal space. Transport of protons due to the PHPD transport itself proceeds up to equilibration of PHPD concentrations in the respective compartments, the one-way transport of H^+ up to equilibration of pH in compartments and the antiport H^+/K^+ up to equilibration of K^+ ions concentration in compartments (more precisely up to equilibration of chemical potentials in compartments). An important question is, which of these proton transports and in which time are decisive for the disturbance of pH-gradient. Mathematical description of the suggested model and its confrontation with the experiment require to know several physicochemical constants of PHPD. They were obtained in the following experiments.

Measurement in a one-phase system (water, organic solvents) made it possible to determine the dissociation constant ($pK_a = 6{\cdot}55$), molar absorption coefficients and solubilities of PHPD in various solvents (water of different pH, methanol, octanol, decane; see Table I), as well as the association constants of $PHPD^-$ with potassium cation from Eq. (*4*),

$$\frac{\bar{n}}{1 - n} = \beta_{\text{PHPD-K}} c_{\text{K}}^* \quad \text{and} \quad \bar{n} = \frac{c_{\text{K}^+} - c_{\text{K}^+}^*}{c_{\text{PHPD}^-}}, \tag{4}$$

where c_{K^+} is the analytical concentration, $c_{\text{K}^+}^*$ the equilibrium concentration of potassium ions, $\beta_{\text{PHPD-K}}$ the association constant PHPD-K and n the Bjerrum function of complex formation (number of cations per one anion).

The true partition coefficient of PHPD forms (P_{PHPD^+}, $P_{\text{PHPD-K}}$, and P_{PHPDNa}) and their association constants ($\beta_{\text{PHPD-Na}} = 8{\cdot}7\ \text{mol}^{-1}\,\text{dm}^3$ and $\beta_{\text{PHPD-K}} = 59{\cdot}6\ \text{mol}^{-1}\,\text{dm}^3$) were calculated from measurements in a two-compartment system (Table II) employing Eq. (*5*) for the apparent partition coefficient (P_{app}) reduced to a linear form (*6*), (*7*),

$$P_{\text{app}} = \frac{P_{\text{PHPD-H}}\, 10^{pK_a - pH} + P_{\text{PHPD}^-} + P_{\text{PHPD-K}} \beta_{\text{PHPD-K}} c_{\text{K}^+}^*}{10^{pK_a - pH} + 1 + \beta_{\text{PHPD-K}} c_{\text{K}^+}^*}, \tag{5}$$

for $c_{\text{K}^+}^* = 0{\cdot}000\ \text{mol}\,\text{dm}^{-3}$:

$$P_{\text{app}}(10^{pK_a - pH} + 1) = P_{\text{PHPD}}\, 10^{pK_a - pH} + P_{\text{PHPD}^-} \tag{6}$$

$$[(P_{app} + 10^{pK_a - pH}) P_{app} - P_{PHPD-H}(- P_{PHPD^-})]/c^*_{K^+} =$$
$$= -\beta_{PHPD-K} P_{app} + \beta_{PHPD-K} P_{PHPD-K} \cdot \quad (7)$$

The transport rate parameters of PHPD forms (k_1 in direction water–octanol and k_2

TABLE I

Some physicochemical characteristics of phenylhydrazonopropanedinitrile in various solvents; λ the wavelength of the absorption maximum in visible region, ε the molar absorption coefficient, s the solubility in mol dm^{-3} (s_n) and g dm^{-3} (s_m) at 25°C

Solvent	λ nm	$\varepsilon \cdot 10^4$ mol^{-1} dm^3 cm^{-1}	$s_n \cdot 10^3$ mol dm^{-3}	$s_m \cdot 10^1$ g dm^{-3}
HCl ($1 \cdot 10^{-3}$ mol dm^{-3})	262	2·29 ± 0·15	0·44	0·75
NaOH ($1 \cdot 10^{-3}$ mol dm^{-3})	385	2·39 ± 0·13	4·15	7·06
Water	369[a]	2·16 ± 0·13	0·44—4·15	0·75—7·06
Decane	261	1·98 ± 0·15	0·81	1·37
Octanol	263	2·44 ± 0·12	210	357
Methanol	262	2·51 ± 0·14	548	933

[a] The wavelength of isosbestic point.

TABLE II

The transport rate parameters and partition coeficient values of phenylhydrazonopropanedinitrile in the system water–octanol at 25°C and $c_{PHPD} = 3 \cdot 10^{-5}$ mol dm^{-3}

Ion	c, mol dm^{-3}	pH	log P_{app}	log k_1	log k_2
H^+	—	5·3	2·69	−4·50	−7·19
Na^+	0·100	5·6	2·63	−4·60	−7·23
K^+	0·100	5·3	2·69	−4·49	−7·18
Mg^{2+}	0·100	5·3	2·67	−4·49	−7·16
Ca^{2+}	0·100	5·4	2·70	−4·51	−7·20
Na^+	0·001	10·04	−0·23	−5·90	−5·70
Na^+	0·001	11·69	−1·74	−7·35	−5·60
Na^+	0·010	11·91	−1·00	−6·49	−5·50
Na^+	0·100	11·84	−0·21	−5·66	−5·45
K^+	0·100	11·76	−0·13	−6·30	−6·17
Ca^{2+}	0·100	11·23	−0·39	−6·09	−5·70
Mg^{2+}	0·010	9·79	−0·76	−7·31	−6·55

backwards) were determined according to Eqs (*8*) and (*9*), ref.[21].

$$\log k_1 = \log P - \log(BP + 1) + C \qquad (8)$$

$$\log k_2 = -\log(BP + 1) + C\,. \qquad (9)$$

The values of constants B and C are not influenced by the structure of transported substances, they only depend on the hydrodynamics of the model system. Therefore, values $B = 0{\cdot}261$ and $C = -5{\cdot}600$, designed for transport of 2-furylethylene derivatives in the same system[22] could be employed. The final values of rate constants are summarized in Table III. Values in Table II show that the influence of ions on the apparent partition coefficient and transport parameters rate of PHPD in the acid range is minimal, whereas in alkaline range a one-order difference was observed depending on the pH of the medium, concentration and type of the cation (sodium, potassium, magnesium, or calcium). The greatest influence on the values of constant exerts potassium. The effect of sodium is only a little smaller. Even though the magnesium ions do substantially not influence the partition coefficient, still they slow down the kinetics of transport. The graphic plot of Eq. (*5*), after introducing the calculated values of true partition coefficients for various pH and concentration of sodium ions shows that this effect of ions on the apparent partition coefficient becomes evident in alkaline range (Fig. 3).

The transport of phenylhydrazonopropanedinitrile ions through the inner mitochondrial membrane was modelled in a three-compartment system water–octanol–water, imitating the system intracristal space – mitochondrial membrane – matrix. The protonmotive force about 350 mV resulted from the pH gradient (pH_{out} 4·5, pH_{in} 10·3), what represented twice the value generated in intact mitochondria[19]. Concentration of potassium ions in the third compartment varied within 0·001

TABLE III

True partition coefficient and transport rate parameter values in the system water–octanol at 25°C for various forms of phenylhydrazonopropanedinitrile (not ionized, ionized, and associates)

Form	P	$\log k_1$	$\log k_2$
PHPD-H[a]	490	−5·02	−7·71
$PHPD^-$	0·003	−8·12	−5·60
PHPD-K	0·870	−5·75	−5·69
PHPD-Na	1·27	—	—

[a] Measured values.

and 0·140 mol dm^{-3}, what is the concentration of K^+ in a mitochondrial matrix[19,20]. The time course of PHPD concentration and pH in the first and third compartments were registered during the interface distribution of PHPD in the system water–octanol–water (Fig. 4). The amount of transported protons was calculated from the time courses in relation with the magnitude of proton gradient and concentration of counterions; this number does not depend on the pH of the first compartment. Some dependence appeared when investigating the effect of pH of the third compartment on the proton transport. More interesting was found to be the effect of ion concentrations at a constant pH (Fig. 2) on the amount of protons transported from the first compartment in relation to the total PHPD concentration in the system and the number of protons able to pass from the first into the third compartment (K_{L1}). At low concentrations of ions (0·01 – 0·001 mol dm^{-3}) the trasnport of protons is also low, but, at concentration of the salt above 0·1 mol dm^{-3} the proton transport increases several times. In general, proton transport proceeds better in the presence of potassium ions in the third compartment.

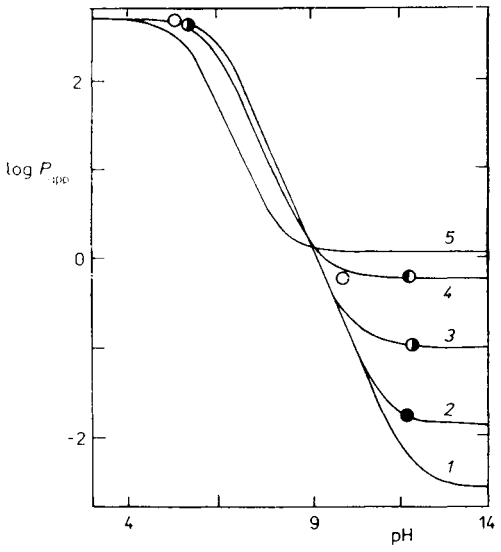


FIG. 3

Relationship of the logarithm of apparent PHPD partition coefficient on the pH of medium at 25°C as calculated from experimental points; concentration of sodium ions, mol dm^{-3}: *1* 0·000 (○); *2* 0·001 (●); *3* 0·010 (◑); *4* 0·100 (◐); *5* 1·00

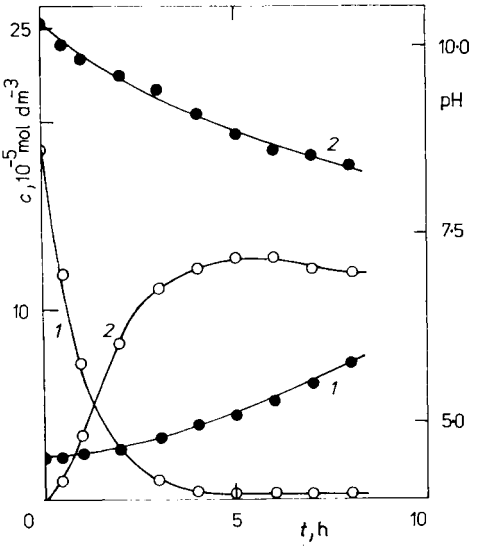


FIG. 4

Time courses of PHPD concentration (○) and pH (●) in the first (*1*) and third compartments (*2*) during an interface PHPD distribution in the system water–octanol–water at 25°C and 80 rpm; $c_{PHPD} = 1{\cdot}85 \cdot 10^{-4}$ mol dm^{-3}, $\Delta pH = 5{\cdot}8$, $c_{K^+} = 0{\cdot}14$ mol dm^{-3}

The proposed Scheme 1, outlining the general view on the manner of PHPD action, is too complex from the viewpoint of an exact mathematical solution: It represents a system of thirteen differential equations. A relative simple solution of the system is possible when respecting the following simplifying presumptions: *1*) concentrations 10 and 13 are high and virtually constant during measurement; *2*) concentrations 11 and 12 equal aproximately zero; *3*) equilibria in the lipidic phase can be neglected; *4*) equilibria in aqueous phases virtually occured immediately and could be described by equilibrium constants K_a and β; *5*) concentrations 3, 6, and 9 are negligible as a result of high or low pH in compartments; *6*) rate constants k_{21}, k_{54}, and k_{87} could be neglected in the first time intervals. For such a simplified scheme following differential equations hold:

$$-\frac{dc_1}{dt} = \frac{Sk_{12}}{V_1} c_1 - \frac{Sk_{56}}{V_1} c_5 - \frac{Sk_{89}}{V_1} c_8 \qquad (10)$$

$$-\frac{dc_2}{dt} = \frac{Sk_{23}}{V_2} c_2 - \frac{Sk_{12}}{V_2} c_1 \qquad (11)$$

$$-\frac{dc_3}{dt} = \left(\frac{dc_3}{dt}\right)_{aq.} - \frac{Sk_{23}}{V_1} c_2 = 0 \qquad (12)$$

$$-\frac{dc_4}{dt} = \frac{Sk_{45}}{V_3} c_4 - \frac{Sk_{23}}{V_3} c_2 \qquad (13)$$

$$-\frac{dc_5}{dt} = \frac{Sk_{56}}{V_2} c_5 - \frac{Sk_{45}}{V_2} c_4 \qquad (14)$$

$$-\frac{dc_6}{dt} = \left(\frac{dc_6}{dt}\right)_{aq.} - \frac{Sk_{56}}{V_1} c_5 = 0 \qquad (15)$$

$$-\frac{dc_7}{dt} = \frac{Sk_{78}}{V_3} c_7 - \frac{Sk_{23}}{V_3} c_2(1 - \alpha) \qquad (16)$$

$$-\frac{dc_8}{dt} = \frac{Sk_{89}}{V_2} c_8 - \frac{Sk_{78}}{V_2} c_7 \qquad (17)$$

$$-\frac{dc_9}{dt} = \left(\frac{dc_9}{dt}\right)_{aq.} - \frac{Sk_{89}}{V_1} c_8 = 0\,, \qquad (18)$$

where $(dc_i/dt)_{aq.}$ stands for the change associated with dissociation (association) in aqueous medium, $\alpha = 1/(1 - \beta c^*_{K+})$, S is the interface surface, V_i the volume of individual compartments and t time. The rate constant values of transport of PHPD

forms in the system water–octanol–water are listed in Table III. Equations (*10*)–(*18*) were integrated by the Runge and Kutta first order method (the Euler method[23]) for the initial conditions $c_1(0) = c_1$, $c_2(0) = c_3(0) = \ldots c_9(0) = 0$, $pK_a = 6{\cdot}55$, $\beta_{PHPD-K} = 59{\cdot}6\ mol^{-1}\ dm^3$, $S = 1{\cdot}735 \cdot 10^{-3}\ m^2$, $c_3(t) = c_6(t) = c_9(t) = 0$, and transport rate parameters of PHPD forms:

$$k_{12} = k_{32} = k_{1,PHPD-K}, \quad k_{21} = k_{23} = k_{2,PHPD-K},$$

$$k_{65} = k_{45} = k_{1,PHPD^-}, \quad k_{56} = k_{54} = k_{2,PHPD^-},$$

$$k_{98} = k_{78} = k_{1,PHPD-K}, \quad k_{89} = k_{87} = k_{2,PHPD-K} \quad \text{(Table III)}.$$

Fig. 5 shows the time course of the measured and calculated values of PHPD concentrations during an interface distribution in the first and third compartments at optimal transport conditions. The not ideal agreement between calculated and measured values follows from the fact that already after five hours a considerable decrease of pH in the third compartment took place and it was necessary to calculate with a non-zero concentration of PHPD-H in this compartment. The calculated

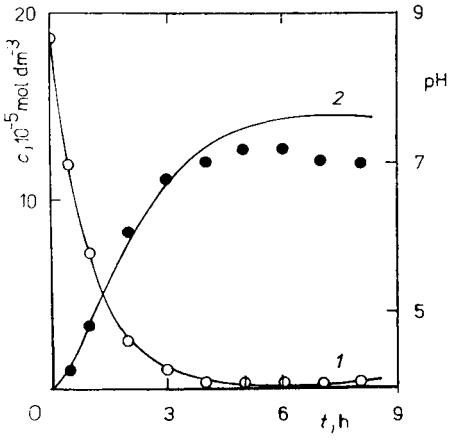


FIG. 5

Time dependence of the measured values of PHPD concentration in the first (○) and third (●) compartments in the system water–octanol–water at 25°C, 80 rpm; ΔpH = 5·8; $c_{PHPD} = 1{\cdot}85 \cdot 10^{-4}$ mol dm^{-3}, $c_{K^+} = 0{\cdot}14$ mol dm^{-3} together with values modelled *1* c_1, *2* c_3

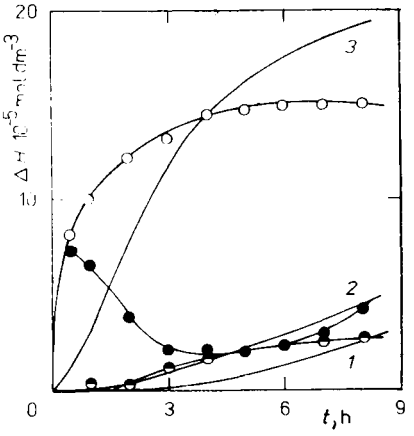


FIG. 6

The amount of transported protons from the first compartment ΔH_1 (◓) into the third compartment ΔH_3 (○) and with taking off of dissociation PHPD-H ΔH_{3c} (●) in the system water–octanol–water at 25°C, 80 rpm, ΔpH = 5·8; $c_{PHPD} = 1{\cdot}85 \cdot 10^{-4}$ mol dm^{-3}, $c_{K^+} = 0{\cdot}14$ mol dm^{-3} together with values modelled *1* ΔH_1, *2* ΔH_{3c}, *3* ΔH_3

numbers of transported protons ΔH_1, ΔH_3, and ΔH_{3c} in the investigated system together with the modelled curves from the measured pH vlaues and PHPD concentrations in the first and third compartments are plotted in Fig. 6. The ΔH_1 stands for the amount of transported H^+ from the first to the second compartment, ΔH_3 from the second to the third compartment and ΔH_{3c} the amount of transported H^+ by means of a cyclic transportation of PHPD from the second to the third compartment. The cyclic transport of PHPD could be divided into cyclic transport os $PHPD^-$ and PHPD-K. Analysis of the model indicated that transport of protont through a cyclic transportation of the associate is up to 10^4-times faster than thaf of ionized form of $PHPD^-$. Although a full agreement between the measured and calculated values was not attained, following conclusions could be deduced from the model: *1*) the transport of PHPD itself participates in the disturbance of pH gradient by approximately 75%, the ion-exchanging transport of H^+/K^+ by 25% (transport through PHPD-K) and the cyclic transport of H^+ by 0·0025% (transport by means of $PHPD^-$). The proportion of ion-exchanging transport on the total proton transport will raise with time; *2*) decrease of the pH gradient to the values attained in mitochondria shall result in a decrease of the proportion of PHPD transport itself on the total distrubance (due to dissociation equilibria in the third compartment); *3*) thinning the lipidic phase thickness towards physiological values will cause a many-order increase of transport rate. After the equilibrium was reached, PHPD dissolved in the lipidic double-layer will be acting as an ion-exchanging H^+/K^+ transmitter. The rate of ion-exchanging transport under the given conditions and in the presence of only pH gradient as a component of the protonmotive force is by a 10^4-times higher than that of cyclic transport of H^+; *4*) the substantial component of the protonmotive force in mitochondria is the transmembrane difference of electric potential. Introduction of it into the system under study will result in a noticeable increase of the rate of cyclic transport of H^+. This one-way motion of charged particles trough membrane is probably responsible for the uncoupling effect of PHPD. Nevertheless, it is necessary to investigate the presence of an undissociated form of PHPD-Me (the associate of $PHPD^-$ with a metal cation) in the membrane, where its concentration can be considerably high in comparison with PHPD-H; consequently, the intensity of uncoupling effect of PHPD derivatives can be influenced, namely at pH over 7·0 and concentration of cations more than $1 \cdot 10^{-1}$ mol dm^{-3} (values encountered in both cells and mitochondria[19,20]).

In conclusion one can say that disturbance of the proton gradient by PHPD in a three-compartment system water–octanol–water can proceed according to Scheme 1 as an ion-exchanging antiport H^+/K^+, which first of all depends on the concentration of univalent cations in the third compartment, but also on pH values of both components determining the association-dissociation equilibria in aqueous solutions and thereby determining the disturbance effect of the pH-gradient. Concentration of univalent ions has to be of a 10^{-1} mol dm^{-3} order and pH value above 7·0.

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