Biochimica et Biophysica Acta, 1101 (1992) 41–47

BBABIO 43613

Uncoupling activity and physicochemical properties of derivatives of fluazinam

Ulrich Brandt [a], Jürgen Schubert [b], Peter Geck [a] and Gebhard von Jagow [a]

[a] *Universitätsklinikum Frankfurt, Zentrum der Biologischen Chemie, Frankfurt am Main (Germany) and* [b] *BASF AG, Ludwigshafen (Germany)*

(Received 12 September 1991)
(Revised manuscript received 16 January 1992)

Key words: Uncoupler; Fluazinam; Mitochondrion; Solution complexation mechanism

The physico-chemical properties and uncoupling activity of eight derivatives of *N*-phenyl-2-pyridinamines related to the fungicide fluazinam were analyzed using rat liver mitochondria. The uncoupling activity of these compounds relies on the deprotonable secondary amino group. One of the derivatives tested (B-3) was slightly more efficient than fluazinam. By phase-distribution analysis we could show that the *N*-phenyl-2-pyridinamines are chemicals with moderate hydrophobicity. Deprotonation of the compound reduces the water/octanol partition coefficient by about one order of magnitude. The pK_A value of the deprotonable group is affected equally by electron withdrawing substituents of the phenyl- and the pyridinyl-ring, and could be predicted simply from the sum of the Hammett coefficients. The uncoupling efficiency was not dependent on the hydrophobicity of the compound, but appeared to be governed by the pK_A of the deprotonable group. This structure/uncoupling characteristic is different from that of the generally more hydrophobic uncouplers of the salicylanilide-type. The pK_A resulting in the most efficient uncoupling was found to lie in the range of the pH of the reaction medium. A model based on a solution complexation mechanism, which describes this behaviour, is presented. We conclude that the *N*-phenyl-2-pyridinamines uncoupled the mitochondria by a simple protonophoric cycle involving protonation/deprotonation in the bulk phase, and that the kinetics of uncoupling were primarily governed by the total concentration of the limiting uncoupler species.

Introduction

Uncoupling of oxidative phosphorylation has been an important method of testing implications of the chemiosmotic hypothesis [1]; this approach has been used to analyze the generation of protonmotive force by the respiratory chain and its utilization for ATP-synthesis as well as secondary active transport across biological membranes. Weak acids are thought to act as uncouplers if they are hydrophobic enough to cross the membrane in a protonated and deprotonated state [2]. However, it is still a matter of debate whether uncoupling of respiring mitochondria occurs exclusively by diffusion of the uncoupler between the bulk phases of the two compartments; involvement of specific interactions of the uncoupling compound with the respiratory chain complexes [3,4] and of 'localized' protons, which do not equilibrate rapidly with the bulk phase [5,6], are discussed. Furthermore alternate concepts of uncoupler action based on perturbation of the mitochondrial membrane, rather than protonation/deprotonation of the uncoupler, have to be considered [7].

Therefore, it is of interest to analyze the mechanism of uncouplers with respect to their uncoupling efficiency and physico-chemical properties. Guo et al. [8] recently described a new type of uncoupler, fluazinam, which is an *N*-phenyl-2-pyridinamine substituted with several electron-withdrawing substituents. Fluazinam has been shown to uncouple rat liver mitochondria even more efficiently than the most potent uncoupler described in earlier studies, namely SF6847 [9].

Using a similar approach described recently for derivatives of the salicylanilides [10], we studied the uncoupling activity of eight derivatives of fluazinam and correlated it with the pK_A value of the deprotonable group, as well as the partition coefficients of the protonated and deprotonated form of the uncoupler. A

Abbreviations: FCCP, carbonyl-cyanide-*p*-trifluorohydrazon; IC, interfacial complexation; P_{oct}, octanol/water partition coefficient; SC, solution complexation; U_{50}, concentration of uncoupler required for halfmaximal uncoupling of state 4 respiration; $\Sigma\sigma$, sum of Hammett coefficients for the individual substituents.

Correspondence: U. Brandt, Dartmouth Medical School, Department of Biochemistry, 308V, Hanover, NH 03755-3844, USA.

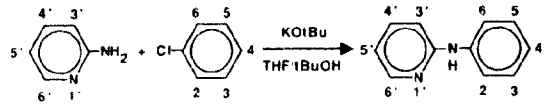

Fig. 1. Synthesis, structural formula and numbering of *N*-phenyl-2-pyridinamine. The numbering of the aromatic rings was adopted from [8]. KOtBu, potassium-*tert*-butylate; THF, tetrahydrofurane; tBuOH, tert-butanol.

mathematical model describing the experimental data is presented.

Materials and Methods

Preparation of N-phenyl-2-pyridinamines (Fig. 1, Table I)

In a typical reaction 0.1 mol of the 2-aminopyridine (-quinoline) and 0.105 mol of the chlorobenzene were dissolved in 300 ml of THF/*tert*-butylalcohol (2:1). To this solution (or suspension) a solution of 0.22 mol sodium-*tert*-butylate in *tert*-butylalcohol was added dropwise at 0°C. After about 30 min the addition was complete and the reaction mixture was allowed to warm up to room temperature by stirring it for another 30 min without cooling.

The deep red or black solution was acidified with acetic acid to pH 4–5, 500 ml water was added and the mixture was extracted with ether (3 × 400 ml). The combined extracts were dried over $MgSO_4$ and evaporated to dryness.

Work up procedures: A: the residue was recrystallised from ethanol. B: the residue was purified by chromatography (silicagel, cyclohexane/ethyl acetate 8:2 to 1:1).

All reagents and substrates (or suitable precursors) are commercially available.

Determination of pK_A values

The pK_A values of the fluazinam derivatives in water:dimethylsulfoxide 1:1 were determined by direct titration of the pure compound and by monitoring the pH-dependent change between the absorption spectra of the protonated and deprotonated form. Spectra were recorded on a Hitachi U3210 dual-beam spectrophotometer. The pK_A was taken either as the inflection point of the titration curve at half neutralisation of the acid or as the pH value at half-maximal absorption change.

The influence of the aromatic substituents (cf. Table I) on the pK_A value was further analyzed by means of the Hammett equation. The Hammett coefficients were taken from [11]. As no steric hindrance was anticipated, *p*-coefficients were also used for the *o*-position. $\Sigma\sigma$ is the overall Hammett coefficient.

Determination of partition coefficients

Partition coefficients between *n*-octanol and water (P_{oct}) were determined by the shaking flask method: approx. 5 ml of a solution of 1 mM uncoupler in *n*-octanol was equilibrated with 100 ml of 5 mM sodium phosphate buffer by vigorous shaking. The pH value of the phosphate buffer was 2–4 for the protonated and 8–12 for the deprotonated form, depending on the pK_A of the deprotonable group. The concentration of uncoupler in octanol was determined spectrophotometrically after appropriate dilution with 0.1 M HCl in ethanol. The aqueous phase was lyophilized and the uncoupler re-dissolved in a defined volume of octanol. The relative amount of compound was determined spectrophotometrically after dilution with 0.1 M HCl in ethanol.

The partition coefficients were calculated according to equation (1) in [10].

Isolation of coupled rat liver mitochondria

Coupled mitochondria were isolated from rat liver according to [12]. The preparation was resuspended in 250 mM sucrose, 20 mM triethanolamine-HCl, 1 mM EDTA, pH 7.2, and stabilized by addition of 50 mM sodium glutamate and 40 mM sodium malate.

TABLE I

Synthesis of fluazinam derivatives

Numbering of the substituents refers to Fig. 1. See methods for details of work up procedures A and B.

Compound	Substituents							m.p. (°C)	Yield (%)	Work-up proc.
	2	3	4	6	3′	5′	6′			
Fluazinam	NO_2	Cl	CF_3	NO_2	Cl	CF_3	H	113	85	B
B-1	NO_2	H	NO_2	Cl	Cl	NO_2	H	134	30	A
B-2	NO_2	H	NO_2	Cl	Cl	CF_3	H	126	60	A
B-3	NO_2	H	NO_2	CF_3	Cl	CF_3	H	90	70	A
B-[8]	NO_2	H	NO_2	CF_3	NO_2	NO_2	H	180 (dec.)	15	A
B-5	NO_2	H	NO_2	CF_3	Cl	NO_2	H	98	45	A
B-6	NO_2	H	NO_2	CF_3	Cl	Cl	H	105	50	A
B-7	NO_2	H	NO_2	CF_3	CN	CN	CH_3	125	40	B
B-8	NO_2	H	NO_2	CF_3	CN	benzo		212 (dec.)	60	B

Measurement of uncoupling efficiency

The uncoupling activity was determined as stimulation of state 4 respiration on glutamate/malate of coupled rat liver mitochondria. The rate of respiration was monitored by a Clark-type oxygen micro-electrode. The volume of the cuvette was 700 μl. Buffer: 20 mM triethanolamin-HCl, 250 mM sucrose, 2.5 mM sodium phosphate, pH 7.4. Substrate was added to a final concentration of 7 mM sodium glutamate and 6 mM of sodium malate. Rat liver mitochondria were used at a concentration of 0.5–0.9 g/l.

To compare the uncoupling efficiency of the various fluazinam derivatives a concentration for half-maximal uncoupling (U_{50}) was determined. The concentration required for maximal uncoupling of mitochondria was about 3–4-times higher than U_{50}.

Respiration rate of fully uncoupled mitochondria was approx. 100 nmol O mg^{-1} min^{-1}.

Results

Requirement of the deprotonable secondary amine for uncoupler activity

The fact that all compounds were found to have a single pK_A in the range between 4 and 9 (cf. Table II and below) suggested that they uncoupled mitochondria by acting as protonophores. However, we adressed this important question, namely whether indeed the deprotonable secondary amino group made these compounds act as very potent uncouplers, by testing an *N*-methylated derivative of B-3 (cf. Table I) for uncoupling activity. This tertiary amine had no uncoupling activity (data not shown) and it showed a pH-independent log P_{oct} of 3.50, which is close to that of B-3 (Table II). These results strongly supported a protonophoric mechanism for the group of uncouplers under study. In addition, the absence of a pK_A in the moderate acidic and basic pH range for the *N*-methylated compound confirmed that the pK_A values determined for the other compounds reflected the protonation and deprotonation of the secondary amino group.

TABLE II

pK_A-*values,* P_{oct} *and* U_{50} *values of fluazinam derivatives*

pK_A, $\Sigma\sigma$, P_{oct} and U_{50} values were determined as described in methods. The compounds are listed in the order of increasing pK_A values. $\Sigma\sigma$, sum of Hammett coefficients; P_{oct}, octanol/water partition coefficient; U_{50}, uncoupler concentration required for half-maximal uncoupling.

Compound	pK_A	$\Sigma\sigma$	log P_{oct}		U_{50} (nM)
			protonated	deprotonated	
B-4	4.3	3.62	2.96	2.14	290
B-7	4.6	3.33	2.80	1.65	290
B-5	6.0	3.08	2.80	2.21	11
Fluazinam	6.3	3.17	3.27	2.67	–
B-1	7.1	2.82	2.60	1.54	7
B-8	7.3	–	3.88	2.18	10
B-3	7.4	2.8	3.52	1.90	4
B-6	7.6	2.54	3.47	2.52	7
B-2	8.5	2.54	3.74	2.56	30

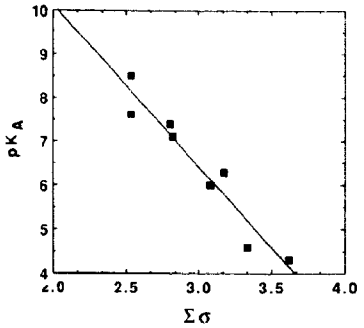

Fig. 2. Hammett plot of the dependence of pK_A on $\Sigma\sigma$ for the fluazinam derivatives. The data were taken from Table II. The line has a slope of 3.8 per $\Sigma\sigma$ and extrapolates to $pK_A = 17.8$ for $\Sigma\sigma = 0$. $\Sigma\sigma$, sum of Hammett coefficients.

pK_A values of N-phenyl-2-pyridinamines

For fluazinam and its eight derivatives pK_A values between 4.3 and 8.5 were determined (Table II). A plot of the pK_A values against the $\Sigma\sigma$ values showed a linear dependence of the experimentally determined pK_A values on the electron withdrawing effect of the aromatic substituents, as expected from the Hammett equation (Fig. 2). No $\Sigma\sigma$ value is given for B-8 as this derivative had a quinoline instead of the pyridine moiety. A given substituent had the same effect via the pyridine- as via the phenyl-ring on the pK_A of the amino-group. Only the values for fluazinam revealed some deviation from those of the other compounds, which could be explained by steric hindrance between the subtituents in positions 2, 3 and 4 of the phenyl ring. The extrapolated pK_A for the unsubstituted *N*-phenyl-2-pyridinamine was 17.8, decreasing 3.8 per $\Sigma\sigma$.

For fluazinam we have found a pK_A of 6.3 which is 0.8 units lower than the value given in [8]. We suppose that this deviation can be explained by the fact that we determined the pK_A in 50% dimethylsulfoxide. Unfortunately, in the work of Guo et al. [8] the method and conditions for determining the pK_A of fluazinam were not given.

Partition coefficients of N-phenyl-2-pyridinamines

Table II gives a list of the log P_{oct} values for the nine compounds tested, determined for the protonated form at acidic pH and the deprotonated form at alkaline pH. The log P_{oct} values varied over about one order of magnitude between 2.6 and 3.9 for the protonated and 1.5 and 2.7 for the deprotonated form. The decrease of the partition coefficient induced by dissoci-

ation of the proton was in the range of one order of magnitude.

Thus, compared to the salicylanilides which have log P_{oct} values in the range of 2.9 to 5.5 in the protonated form [10], the *N*-phenyl-2-pyridinamines are markedly less hydrophobic.

Characterisation of rat liver mitochondria

The concentration of total protein of the resuspended rat liver mitochondria was typically about 50 g/l as determined by the biuret method using bovine serum albumin as a standard. The preparations contained approx. 0.2 μmol haem *a*/g protein. The phospholipid content was 0.3 mmol/g protein i.e. 0.2 g phospholipid/g protein.

Typically, complete uncoupling of the mitochondria showed an 8-fold stimulation of state 4 respiration when tested immediately after isolation (not shown). When the mitochondria were stabilized by glutamate/malate, this value decayed to 6-fold stimulation over 6 h.

Uncoupling efficiency of N-phenyl-2-pyridinamines

The uncoupling efficiencies (U_{50}) of the derivatives tested varied between 4 nM for B-3 and 290 nM for B-4 and B-7. To compare our results with those from earlier studies we also determined U_{50} for FCCP (carbonyl-cyanide-*p*-trifluorohydrazone), which was found to have a U_{50} of 25 nM. This means that the fluazinam derivative B-3 was 6-times more efficient in uncoupling rat liver mitochondria than FCCP, and that five of the eight derivatives were at least 2-times more efficient.

For fluazinam we observed the same inactivation phenomenon as reported by Guo et al. [8]. No U_{50} value for this compound is given because 2,4-dinitrochlorobenzene was not used to prevent degradation. However, as our result for FCCP is in good agreement with those obtained by other authors [2], we could deduce that B-3 has an efficiency similar to or slightly better than fluazinam.

Correlation between physico-chemical parameters and uncoupling efficiency

Despite their moderate hydrophobicity the *N*-phenyl-2-pyridinamines are very potent uncouplers. A comparison of B-1 and B-6, which were found to have pK_A values comparably close to the physiological pH value of 7.4, revealed that even a difference of one order of magnitude for the partition coefficients of the protonated as well as for the deprotonated form was not reflected in the uncoupling efficiency (Table II). Note that even a log P_{oct} as low as 1.5 for the deprotonated form (B-1) had no limiting effect on the protonophoric activity of the uncoupler.

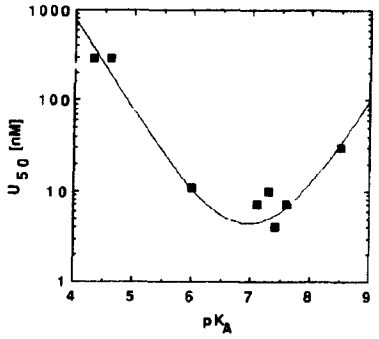

Fig. 3. Dependence of the uncoupling efficiency U_{50} on the pK_A of the deprotonable group. Data were taken from Table II. Experimental conditions are as described in Materials and Methods. The line is calculated according to equation (10) using a = 0.3 nmol/l and b = 8.

Fig. 3 shows a pK_A/efficiency profile for the tested *N*-phenyl-2-pyridinamines. The minimum U_{50} value was obtained for B-3, which had a pK_A of 7.4. B-1 (pK_A = 7.1) and B-6 (pK_A = 7.6) were slightly less efficient (Table II). For both more alkaline and more acidic pK_A values, higher U_{50} values were determined. The same correlation is observable from the data of Guo et al. [8] as the stable *i*-propoxy analog tested by these authors was reported to have a higher pK_A and lower uncoupling efficiency than fluazinam.

The only exception to this rule was the compound B-8 (pK_A = 7.3), which was found to have a U_{50} 2.5-times higher than B-3 (pK_A = 7.4). However, this cannot be due to different concentrations in the membrane, as both compounds have comparable partition coefficients. Remarkably, B-8 is the only of the compounds tested which contains an unsubstituted quinolinyl- instead of the substituted pyridinyl-residue.

Discussion

The *N*-phenyl-2-pyridinamines, which are derived from the structure of fluazinam, appear to be the most potent uncouplers known so far [2,8]. One of the derivatives tested (B-3) was even slightly more efficient than fluazinam. The uncoupling activity relies on the deprotonable secondary amino group (Fig. 3). This could be demonstrated by the fact that an *N*-methylation of B-3 abolished its uncoupling activity completely. By phase-distribution analysis we could show that the *N*-phenyl-2-pyridinamines are chemicals of moderate hydrophobicity. Deprotonation of the compound shifts the partition coefficient only by about one order of magnitude. The pK_A value of the deprotonable group is affected equally by electron-withdrawing substituents of the phenyl- and the pyridine-ring, and could be predicted simply from the sum of the Hammett coefficients.

The uncoupling efficiency was not correlated to the hydrophobicity of the compound, but appeared to be governed by the pK_A of the deprotonable group (Fig. 3). This is a behaviour different from that of the generally more hydrophobic salicylanilides. The pK_A value as determined in 50% dimethylsulfoxide that gave the lowest U_{50} value was found to be 7.4 (B-3). Although these values are not identical to those in aqueous solution, optimal uncoupling seemed to require a pK_A in the range of the pH of the reaction medium.

Thus, uncoupling by the *N*-phenyl-2-pyridinamines not only required the deprotonable secondary amino group, but its efficiency was strictly dependent on the pK_A of this function. Remarkably, this correlation was not affected by variations of the other relevant physico-chemical parameter, namely P_{oct}, over more than one order of magnitude. This strongly supports a protonophoric mechanism of uncoupling for the 2-*N*-phenyl-pyridinamines and makes any type of proton conductive action based on perturbation of the mitochondrial membrane unlikely.

Variation of the substituents in all positions of the two aromatic rings appeared to have no influence on the uncoupling efficiency other than inducing a change of the pK_A as predicted by their Hammett coefficients. This makes it difficult to visualize any significant involvement of binding of the uncoupler to specific mitochondrial proteins in a protonophoric cycle. However, we cannot completely exclude this possibilty based on the physico-chemical approach presented in this work.

We concluded that the mechanism by which the 2-*N*-phenylpyridinamines uncouple mitochondria is a protonophoric cycle, which is most efficient near the pK_A, i.e. when the concentrations of both the protonated and the deprotonated form are nearly equal, and that it is not significantly affected by hydrophobic partitioning or specific binding to the mitochondrial membrane.

If our conclusion is correct, it should be possible to describe our results quantitatively using a mathematical model based purely on protonophoric uncoupling over a membrane.

The number of cycles of these moderately lipophilic uncouplers is significantly higher than 1000 s^{-1} as given in [2] for unhindered Brownian motion. This might be illustrated by the following approximation for B1: at half-maximal stimulated respiration (50 nmol O mg^{-1} min^{-1}), 500 nmol H^+ mg^{-1} min^{-1} are extruded by the respiratory chain and transported back into the mitochondria by the uncoupler, if one assumes transport of 10 nmol H^+ per nmol O reduced to H_2O. For the concentration of uncoupler in the mitochondrial membrane an upper limit can be given if one assumes that all uncoupler is accumulated in the membrane. If one refers proton translocation via the uncoupler to this value a lower limit for the number of cycles results: U_{50} of 7 nmol/l and a protein content of 500 mg/l gives a ratio of uncoupler to protein of $1.4 \cdot 10^{-2}$ nmol/mg. Referring proton translocation of 500 nmol $H^+ \cdot mg^{-1} \cdot min^{-1}$ to this value results in an lower limit for the number of cycles of 600 s^{-1}, which would be in agreement with the upper limit as given in [2]. However, given that there is no indication for specific binding to any component of the mitochondrial membrane for these moderately lipophilic uncouplers, the true concentration in the membrane is expected to be much lower. From a phospholipid/protein ratio of 0.2 and using P_{oct} of 400 (Table II) as a fair estimate of the uncoupler distribution ratio between phospholipid phase and aqueous phase, 7 nmol/l give an uncoupler content in the mitochondria of $5.6 \cdot 10^{-4}$ nmol/mg protein resulting in a number of cycles of $1.5 \cdot 10^4$ s^{-1}, which is one order of magnitude higher than the upper limit given by Terada [2].

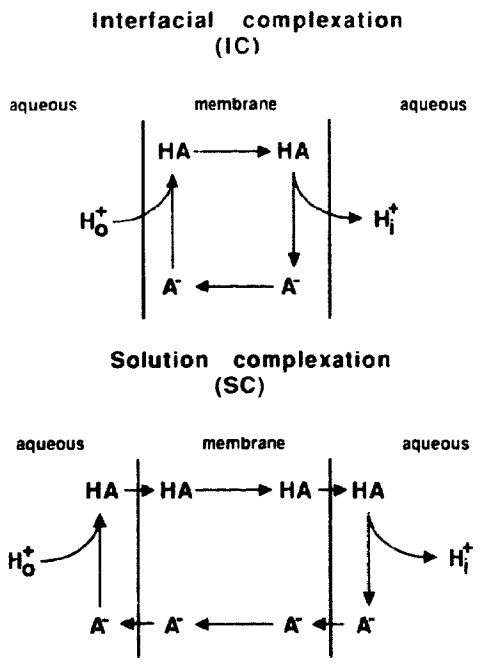

Fig. 4. Generalized scheme for possible mechanisms of uncoupler action. See discussion for details. HA, protonated uncoupler; A^-, deprotonated uncoupler; H_o^+, H_i^+, protons in the outer and inner bulk phase, respectively.

Two basically different mechanisms can be visualized for uncoupling by weak acids, which according to the nomenclature of Benz et al. [13] are called interfacial complexation (IC) and solution complexation mechanism (SC) (Fig. 4). In the IC mechanism protonation and deprotonation occurs while the uncoupler remains in the membrane, whereas the SC mechanism requires that it partitions into the bulk phase. The

observation that uncoupling is independent of the partition coefficient generally can be explained by both models. The IC mechanism would be expected to be operative for uncouplers with a lipophilicity high enough to make the uncoupler concentration in the phospholipid phase independent of gradual differences in distribution ratio. For the SC mechanism it was predicted that diffusion through the unstirred layers could become rate limiting if the membrane permeabilities for the anion and the undissociated acid are high enough. In this case steady-state conductance is expected to attain a maximum value at a pH close to the pK_A of the dissociating group [13–15]. As the fluazinam derivatives tested were found to be of moderate lipophilicity and showed maximal uncoupling efficiency at a pK_A near the pH of the experiment, we conclude that these compounds uncouple predominantly by a SC mechanism. A mathematical description of this situation is formulated in Appendix I. The formulation describes the pK-dependence of U_{50} as given in Fig. 3. Curve fitting gives an estimate of the kinetic constants k_{AH} and k_A for the transfer of protonated and deprotonated uncoupler from the aqueous outer phase across the phospholipid phase to the aqueous inner phase. From these kinetic constants, permeability coefficients p_{AH} and p_A have been calculated: p_{AH} approx. 50 cm/s and $p_{AH}/p_A = 10^3$–10^4. The values determined appear reasonable when compared to those obtained for other uncouplers [15–17] (for details see Appendix II).

Based on the SC mechanism, the equations presented here seem to be able to describe the physicochemical behaviour of the *N*-phenyl-2-pyridinamines in an applicable way.

Appendix I

The transfer of protons by the uncoupler from the outer aqueous phase (c) to the inner aqueous phase (mitochondrial matrix, m) is described by the following flux equation:

$$J_H = J_{AH} = p_{AH} \cdot \{[AH]_c - [AH]_m\} \quad (1)$$

For the flux ofdeprotonated carrier the following equation holds *:

$$J_A = p_A \cdot \{[A^-]_c - [A^-]_m \cdot \exp(-F\Psi/RT)\} \quad (2)$$

For a closed system in the steady state net movement of uncoupler is zero:

$$J_{AH} + J_A = 0 \quad (3)$$

The uncoupler in the aqueous phases is in acid/base equilibrium:

$$[A^-]_c \cdot [H^+]_c / [AH]_c = [A^-]_m \cdot [H^+]_m / [AH]_m = K \quad (4)$$

Heterophasic acid–base reactions between protons in the aqueous phases and deprotonated uncoupler in the membrane are neglected.

Combining Eqns. 1–4 one arrives at:

$$J_{AH} = \{p_{AH} \cdot [A_{ges}]_c \cdot (1-q)\} / \{(1+K') \cdot (1+r \cdot q/K')\} \quad (5)$$

with:

ratio of electrochemical activities of protons:

$$q = \{[H^+]_m / [H^+]_c\} / \exp(-F\Psi/RT)\} \quad (6)$$

relative dissociation constant of the uncoupler:

$$K' = K/[H^+]_c \quad (7)$$

and ratio of permeabilities:

$$r = p_{AH}/p_A \quad (8)$$

Rearranging the equation for $[A_{ges}]_c = U_{50}$ and $J_{AH} = J_{AH,50}$ gives:

$$U_{50} = \{J_{AH,50}/[p_{AH} \cdot (1-q)]\} \cdot (1+K') \cdot (1+r \cdot q/K') \quad (9)$$

Therefore, the data are described by an equation of the following structure ($y = U_{50}$; $x = K'$):

$$y = a \cdot (1+x) \cdot (1+b/x) \quad (10)$$

with $a = J_{AH,50}/[p_{AH} \cdot (1-q)]$ and $b = r \cdot q$.

Appendix II

Nonlinear regression of ln y to x gives the following estimates for a and b:

$$a = 0.3 \pm 0.1 \text{ nmol/l}, \qquad b = 8 \pm 2$$

Assuming a pmf of approx. 200 mV one gets an estimate of approx. 10^{-3} for $q = \{[H^+]_m/[H^+]_c\}/\exp(-F\Psi/RT)$.

Assuming that the uncoupler-mediated transport proceeds across the phospholipid component of the membrane one can refer the flux to phospholipid surface area.

Assuming 0.5 nm^2 surface area/phospholipid molecule [18], 1 cm^2 surface of a phospholipid bilayer is equivalent to approx. $6.7 \cdot 10^{-10}$ mol phospholipid. With 0.3 μmol phospholipid/mg protein one can refer flux to phospholipid surface area:

$$J_{AH} = \{500 \text{ nmol}/(\text{mg}_{\text{prot}} \cdot \text{min})\} \cdot \{6.7 \cdot 10^{-10} \text{ mol}_{\text{pl}}/\text{cm}^2\}$$

$$/\{0.3 \ \mu\text{mol}_{\text{pl}}/\text{mg}_{\text{prot}}\} = 0.5 \text{ nmol} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$$

* p_A may depend on membrane potential [19]; from $(\delta J_A/\delta\Psi) \geq 0$ results $1 \geq (RT/F) \cdot (\delta \ln p_A/\delta\Psi) > 0$.

With $a = 3 \cdot 10^{-4}$ nmol/cm^3 one gets:

$P_{AH} = 50$ cm/s

From $b = 8$ and $q = 10^{-3}$ one gets:

$r = (P_{AH}/P_A) = 8 \cdot 10^3$.

List of symbols

- $[A^-]_c$, concentration of deprotonated uncoupler in the cytosol
- $[A^-]_m$, concentration of deprotonated uncoupler in the matrix
- $[AH]_c$, concentration of protonated uncoupler in the cytosol
- $[AH]_m$, concentration of protonated uncoupler in the matrix
- $[A_{tot}]_c$, total concentration of uncoupler in the cytosol
- $[H^+]_c$, proton concentration in the cytosol
- $[H^+]_m$, proton concentration in the matrix
- a, constant
- b, constant
- F, Faraday's constant
- J_A, flux of deprotonated uncoupler
- J_{AH}, flux of protonated uncoupler
- $J_{AH,50}$, proton flux at halfmaximal uncoupling
- J_H, proton flux via uncoupler
- K, dissociation constant of uncoupler
- K', relative dissociation constant of uncoupler
- k_A, transport coefficient of deprotonated uncoupler [$l \cdot g_{prot}^{-1} \cdot min^{-1}$]
- k_{AH}, transport coefficient of protonated uncoupler [$l \cdot g_{prot}^{-1} \cdot min^{-1}$]
- p_A, permeability coefficient of deprotonated uncoupler [cm/s]
- p_{AH}, permeability coefficient of protonated uncoupler [cm/s]
- q, ratio of electrochemical activities of protons
- R, gas constant
- r, ratio of permeabilities
- T, absolute temperature
- U_{50}, uncoupler concentration at halfmaximal uncoupling
- Ψ, membrane potential (matrix - cytosol)

Acknowldgements

This work was supported by the Deutsche Forschungsgemeinschaft SFB169/A12 to G.v.J.. We thank F.J. Streb for skilful technical assistance.

References

1 Mitchell, P. (1966) Biol. Rev. 41, 445–502.
2 Terada, H. (1990) Env. Health Persp. 87, 213–218.
3 Rottenberg, H. and Hashimoto, K. (1986) Biochemistry 25, 1747–1755.
4 Rottenberg, H. (1990) Biochim. Biophys. Acta 1018, 1–17.
5 Luvisetto, S., Pietrobon, D. and Azzone, G.F. (1987) Biochemistry 26, 7332–7338.
6 Pietrobon, D., Luvisetto, S. and Azzone, G.F. (1987) Biochemistry 26, 7339–7347.
7 Terada, H., Shima, O., Yoshida, K. and Shinohara, Y. (1990) J. Biol. Chem. 265, 7837–7842.
8 Guo, Z., Miyoshi, H., Komyoji, T., Haga, T. and Fujita, T. (1991) Biochim. Biophys. Acta 1056, 89–92.
9 Muraoka, S. and Terada, H. (1972) Biochim. Biophys. Acta 275, 271–275.
10 Terada, H., Goto, S., Yamamoto, K., Takeuchi, I., Hamada, Y. and Miyake, K. (1988) Biochim. Biophys. Acta 936, 504–512.
11 March, J. (1968) Advanced Organic Chemistry. Reactions, Mechanisms, and Structure, McGraw-Hill Kogakusha Ltd., Tokyo.
12 Johnson, D. and Lardy, H. (1967) Methods Enzymol. X, 94–96.
13 Benz, R., Gisin, B.F., Ting-Beall, H.P., Tosteson, D.C. and Läuger, P. (1976) Biochim. Biophys. Acta 455, 665–684.
14 McLaughlin, S. and Dilger, J.P. (1980) Phys. Rev. 60, 825–863.
15 LeBlanc Jr., O.H. (1971) J. Membr. Biol. 4, 227–251.
16 Borisova, M.P., Ermishkin, L.N., Liberman, E.A., Silberstein, A.Y. and Trofimov, E.M. (1974) J. Membr. Biol. 18, 243–261.
17 Dilger, J. and McLaughlin, S. (1979) J. Membr. Biol. 46, 359–384.
18 Watts, A., Harlos, K. and Marsh, D. (1981) Biochim. Biophys. Acta 645, 91–96.
19 Geck, P. and Heinz, E. (1976) Biochim. Biophys. Acta 443, 49–53.