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Partition and Ion-pair Partition of 2,4-Dinitrophenol, an Uncoupler of Oxidative Phosphorylation

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The partition of 2,4-dinitrophenol (DNP) between *n*-octanol and water was studied under various conditions. It was found that DNP is transferred to the organic phase in three forms: in the neutral form, in the form of an ion-pair complex with cations in the aqueous phase, and directly in the anionic form. The values of the ion-pair partition coefficient, the ion-pair complex formation constants of the DNP anion with K^+ and the *n*-butyltrimethylammonium ion, and the partition coefficients of the neutral and anionic forms of DNP, indicated that upon partition at pH 7, most DNP is present in *n*-octanol in the form of the ion-pair complex with the quaternary ammonium ion, and the relative amount of the neutral form of DNP in *n*-octanol is about the same as that of the ion-pair with K^+. Above pH 8, direct transfer of the DNP anion to *n*-octanol became significant, while the transfer of the neutral form of DNP became very small. These results are discussed in relation to the uncoupling activity of DNP in mitochondria.

Keywords——uncoupler (of oxidative phosphorylation); 2,4-dinitrophenol; partition; ion-pair partition; partition coefficient; ion-pair partition coefficient; ion-pair formation constant; extraction constant

Hydrophobic phenols, such as 2,4-dinitrophenol(DNP), pentachlorophenol and 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalononitrile(SF 6847), are known to be potent uncouplers of oxidative phosphorylation in mitochondria.[1)] Since the innermitochondrial membrane is regarded as the site of action of these uncouplers, the transfer of uncoupler molecules to the membrane is directly related to the exhibition of uncoupling activity. The binding of uncouplers to the mitochondrial membrane is reported to increase linearly with the uncoupler concentration, just as in the case of their partition between a lipid and aqueous phase,[2)] and the activity is dependent on the amount of their binding to mitochondria.[3)] Since the neutral and anionic forms of some hydrophobic phenols are stable in organic solvents and in the mitochondrial membrane,[2b,4)] the mechanism of this partition is regarded as complex. However, information on this partition mechanism under various conditions is very important for understanding the mechanism of action of uncouplers.

An organic solvent that is immiscible with water has been considered as the simplest model of bio-membranes.[5)] Results on the partition coefficients of bio-active compounds between an organic solvent and water have been found to correlate well with the ability of these compounds to permeate through bio-membranes, and with their biological activities in cases where bio-membranes are the site of action.[6)] Though there have been many determinations of the partition coefficients of bio-active compounds, various factors that may influence the partition process have been considered in only a few cases.[7,8)]

The present study was undertaken to examine the process of transfer of the most commonly used uncoupler, DNP, from an aqueous to a hydrophobic phase by measuring its partition coefficients between *n*-octanol and water under various conditions. *n*-Octanol is thought to be the organic solvent most nearly reflecting the properties of bio-membranes.[6)]

Experimental

Materials——2,4-Dinitrophenol was purchased from Wako Pure Chemical Industries, Ltd., Osaka. It appeared pure on thin–layer chromatography and was used without further purification. *n*-Octanol was the finest grade product of Nakarai Chemical Co. Ltd., Kyoto, and was distilled 3 times before use. An aqueous solution of *n*-butyltrimethylammonium hydroxide was prepared from *n*-butyltrimethylammonium iodide as follows: Excess silver oxide was added to an aqueous solution of *n*-butyltrimethylammonium iodide, and the solution was stirred for a few hrs, and then filtered to remove silver iodide and silver oxide. During the reaction, the solution was protected from light. The other reagents used were standard commercial products.

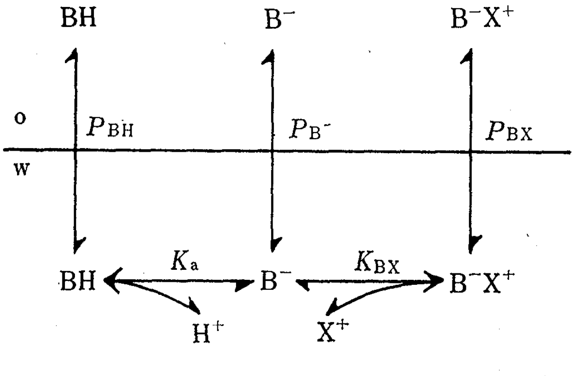

Fig. 1. Possible Partition Mechanism of a Weak Acid BH between Organic (o) and Aqueous (w) Phases

Measurement of Partition Coefficient——Aqueous solutions of 2×10^{-4} to 10^{-3} M DNP at a fixed pH in test tubes were gently shaken with *n*-octanol in a water bath at 25±0.1° for about 8 hr, and then the tubes were allowed to stand for 12 hr at the same temperature. The concentration of DNP in the aqueous phase was determined spectrophotometrically in a Shimadzu spectrophotometer, model UV-180, at the $\lambda_{\max}$ of DNP. The concentration of DNP in the *n*-octanol phase (C_0) was determined from the difference between the initial concentration of DNP in the aqueous phase (C_i) and the concentration after equilibriation (C_w) according to Eq. (1), where V represents the volume of the aqueous (subscript, w) or organic (subscript, o) phase.

$$C_o = (C_i - C_w)(V_w/V_o) \tag{1}$$

V_w/V_o was changed with pH so that the condition $1 \leq (C_i - C_w)/C_w \leq 10$ was maintained.[8] Each phase of the partition system was equilibrated with the other before the partition.

Possible Mechanism of Partition of a Weak Acid——When there is no intermolecular association of a weak acid BH, its partition between an organic and aqueous phase can be expressed as shown in Fig. 1, where the neutral molecular species BH, the ionic form B^- and the ion-pair complex B^-X^+, formed between B^- and a certain cation X^+, are transferred to the organic phase from the aqueous phase. The partition coefficient P due to each molecular species is expressed by Eqs. (2) to (4) (ref. 9).

$$P_{BH} = [BH]_o/[BH]_w \tag{2}$$

$$P_{B^-} = [B^-]_o/[B^-]_w \tag{3}$$

$$P_{BX} = [B^-X^+]_o/[B^-X^+]_w \tag{4}$$

The acid dissociation constant K_a and the ion-pair complex formation constant K_{BX} are expressed by Eqs. (5) and (6), respectively.

$$K_a = [B^-]_w(H^+)/[BH]_w \tag{5}$$

$$K_{BX} = [B^-X^+]_w/[B^-]_w \cdot [X^+]_w \tag{6}$$

Thus, from the above relations the apparent partition coefficient P', expressed by Eq. (7), can be represented by Eq. (8).

$$P' = C_o/C_w \tag{7}$$

$$= \frac{[BH]_o + [B^-]_o + [B^-X^+]_o}{[BH]_w + [B^-]_w + [B^-X^+]_w}$$

$$= \frac{P_{BH}(H^+) + P_{B^-} \cdot K_a + P_{BX} \cdot K_a \cdot K_{BX}[X^+]_w}{(H^+) + K_a + K_a \cdot K_{BX}[X^+]_w} \tag{8}$$

Eq. (8) is fundamental for the partition of a weak acid.

Under conditions where the contribution of the ion-pair complex to the partition can be ignored, Eq. (8) becomes Eq. (9).

$$P' = \frac{P_{BH}(H^+) + P_{B^-} \cdot K_a}{(H^+) + K_a} \tag{9}$$

Generally, it is considered that the anionic form B^- cannot be transferred to the hydrophobic region, although some anions were reported to be transferred to the organic phase.[10] In the former case, the relation in Eq. (9) can be expressed by Eq. (10).

$$P' = P_{BH} \cdot \frac{(H^+)}{(H^+) + K_a} \tag{10}$$

According to Eq. (10), P' is pH-dependent, and the following relations hold over a wide pH range.

When $\mathrm{pH} \ll \mathrm{p}K_a$,

$$\log P' = \log P_{BH} \tag{11}$$

and when $\mathrm{pH} \gg \mathrm{p}K_a$,

$$\log P' = \log P_{BH} + \mathrm{p}K_a - \mathrm{pH} \tag{12}$$

Thus, P' in the region of $\mathrm{pH} \ll \mathrm{p}K_a$ is P_{BH} itself, and in the region of $\mathrm{pH} \gg \mathrm{p}K_a$, $\log P'$ should decrease linearly with a slope of -1 as the pH increases. From Eqs. (11) and (12), the values of P_{BH} and $\mathrm{p}K_a$ can easily be determined under the conditions where Eq. (10) holds. Most results on the partition of weak acids have been interpreted according to Eq. (10) (refs. 7 and 11), *i.e.*, on the basis that only the neutral form is responsible for the partition process.

In some cases, such as when $\mathrm{pH} \gg \mathrm{p}K_a$, ion-pair partition should be significant. In this case, Eq. (8) becomes Eq. (13).

$$P' = \frac{\{P_{BH}\cdot(\mathrm{H}^+)/K_a + P_{B^-}\} + P_{BX}\cdot K_{BX}[\mathrm{X}^+]_w}{1 + K_{BX}[\mathrm{X}^+]_w} \tag{13}$$

Eq. (13) indicates that, when (H^+) remains constant, upon increase in the concentration of the cation X^+ added to the aqueous phase, the partition of the weak acid P' first increases almost linearly, then increases more gradually, and finally reaches a constant value. The extrapolated intercept on the ordinate in the plot of P' *vs.* $[\mathrm{X}^+]_w$ is $\{P_{BH}\cdot(\mathrm{H}^+)/K_a + P_{B^-}\}(=Q)$ according to Eq. (13). The ion-pair complex formation constant K_{BX}, and the ion-pair partition coefficient P_{BX} can be determined by means of Eq. (14).

$$\frac{(P'-Q)}{P'}\cdot\frac{1}{[\mathrm{X}^+]_w} = P_{BX}\cdot K_{BX}\cdot\frac{1}{P'} - K_{BX} \tag{14}$$

When there are two cations X^+ and Y^+, which can both form ion-pair complexes with B^-, the apparent partition coefficient P' is expressed by Eq. (15) in the region of $\mathrm{pH} \gg \mathrm{p}K_a$.

$$P' = \frac{\{P_{BH}(\mathrm{H}^+)/K_a + P_{B^-} + P_{BX}\cdot K_{BX}[\mathrm{X}^+]_w\} + P_{BY}\cdot K_{BY}[\mathrm{Y}^+]_w}{\{1 + K_{BX}[\mathrm{X}^+]_w\} + K_{BY}[\mathrm{Y}^+]_w} \tag{15}$$

Denoting $\{1 + K_{BH}[\mathrm{X}^+]_w\}$ as R, and $\{P_{BH}(\mathrm{H}^+)/K_a + P_{B^-} + P_{BX}\cdot K_{BX}[\mathrm{X}^+]_w\}$ as S, Eq. (15) is simplified to Eq. (16).

$$P' = \frac{S + P_{BY}\cdot K_{BY}[\mathrm{Y}^+]_w}{R + K_{BY}[\mathrm{Y}^+]_w} \tag{16}$$

Thus, under conditions where (H^+) and $[\mathrm{X}^+]_w$ are kept constant in the presence of various amounts of Y^+, P_{BY} and K_{BY} can be determined from the linear relation between $(P'-S/R)/P'\cdot[\mathrm{Y}^+]_w$ and $1/P'$ as shown in Eq. (17), if the constants which characterize the ion-pair partition with regard to X^+ are known.

$$\frac{P'-(S/R)}{P'}\cdot\frac{1}{[\mathrm{Y}^+]_w} = \frac{P_{BY}\cdot K_{BY}}{R}\cdot\frac{1}{P'} - \frac{K_{BY}}{R} \tag{17}$$

The ion-pair extraction constant E_{BX} can be expressed in terms of P_{BX} and K_{BX} as shown in Eq. (18).

$$E_{BX} = \frac{[\mathrm{B}^-\cdot\mathrm{X}^+]_o}{[\mathrm{B}^-]_w\cdot[\mathrm{X}^+]_w} = P_{BX}\cdot K_{BX} \tag{18}$$

Furthermore, from the relations of Eqs. (2) to (8), the relative amount of each molecular species in the organic phase, $[\mathrm{BH}]_o/C_o$, $[\mathrm{B}^-]_o/C_o$ and $[\mathrm{B}^-\mathrm{X}^+]_o/C_o$, can be determined by means of the following equations.

$$\frac{[\mathrm{BH}]_o}{C_o} = \frac{P_{BH}(\mathrm{H}^+)}{Z} \tag{19}$$

$$\frac{[\mathrm{B}^-]_o}{C_o} = \frac{P_{B^-}\cdot K_a}{Z} \tag{20}$$

$$\frac{[\mathrm{B}^-\mathrm{X}^+]_o}{C_o} = \frac{P_{BX}\cdot K_a\cdot K_{BX}[\mathrm{X}^+]_w}{Z} \tag{21}$$

In these cases,

$$Z = P_{BH}(\mathrm{H}^+) + P_B\cdot K_a + P_{BX}\cdot K_a\cdot K_{BX}[\mathrm{X}^+]_w \tag{22}$$

Results

pH-Dependent Partition Coefficient

At various pH values of the aqueous phase, the apparent partition coefficient P' of DNP (the symbols DH and D^- (or D) are used hereafter to express the neutral form of DNP and the DNP anion, respectively) between *n*-octanol and water was determined with initial concentrations of DNP of 2×10^{-4} to 10^{-3} M at 25°. Under these conditions, P' was independent of the initial concentration at all pH values, but was dependent on the pH, as shown by closed circles in Fig. 2. At below pH 2, log P' had a constant value of 1.55 ($P'=35.5$), which is log P_{DH} itself according to Eq. (11), and this coincides well with the value of 1.50 reported by Fujita.[12] Log P' decreased with increase of pH from pH 4.5. However, at high pH values, the observed values for P' did not fit the curve (solid line in Fig. 2), which was drawn according to Eq. (10) by taking the pK_a of DNP as 4.10 (determined spectrophotometrically) and P_{DH} as 35.5. Values of P_{DH} (◐ in Fig. 2), calculated according to Eq. (10) based on P' at a given pH, deviated from the value of 35.5 (dotted line in Fig. 2), and this deviation became greater with increase in pH. Thus the parttion process of DNP is not as simple as is generally thought, *i.e.*, it cannot be explained simply in terms of transfer of the neutral form to the organic phase.

Partition Due to an Ion-pair Complex

It is expected from the results in Fig. 2 that some portion of the DNP anions, D^-, moves to the *n*-octanol phase by forming an ion-pair complex with a cation, X^+, which is a constituent of the aqueous phase. The most probable cation is the potassium ion, because the aqueous phase contains a large amount of potassium ions as a buffer component. Thus, the partition coefficient of DNP was measured in the presence of various amounts of K^+ at about pH 12, where almost all DNP is present as the anion. In this case, the pH of the aqueous was kept constant with 0.0085 N KOH and various amounts of KCl were added to maintain the concentration of K^+ constant under the condition $[K^+]_w \gg [DNP]_w$. The P' of DNP took a constant value with different initial concentrations of DNP when the concentration of K^+ was kept constant, indicating that there is no association of DNP molecules in the two phases.

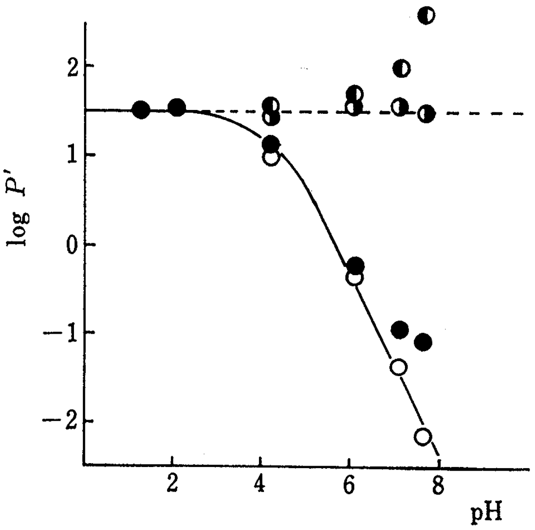

Fig. 2. pH-Dependent Partition of DNP between *n*-Octanol and Water

●; P' determined experimentally.
○; P' determined by calculation to eliminate the effects of direct and ion-pair partitions of DNP anion (P'_{DH}).
◐; P_{DH} determined from ●.
◑; P_{DH} determined from ○.

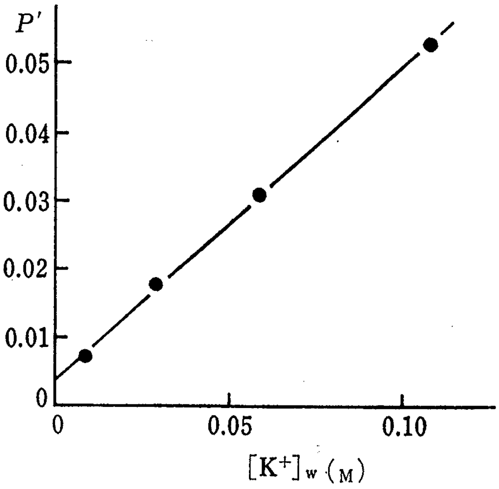

Fig. 3. Ion-pair Partition of the DNP Anion with K+

Figure 3 shows the relation between the P' of DNP and the concentration of K^+ in the aqueous phase. As shown in the figure, P' increased with increase in the concentration of K^+ in the aqueous phase, suggesting that at this pH the anionic form of DNP is transferred to the *n*-octanol phase *via* ion-pair complex formation with K^+. The increase in P' with $[K^+]_w$ is almost linear, as predicted by the relation in Eq. (13) at low concentrations of K^+, and the intercept on the ordinate ($=0.0035$) should give the contribution of the partition of DNP anion itself and of the neutral form of DNP; $\{P_{DH}(H^+)/K_a + P_{D^-}\}$. At this pH the partition due to the neutral form of DNP ($P_{DH} \cdot (H^+)/K_a$) was calculated to be negligibly small ($=8.13 \times 10^{-7}$), and thus the partition coefficient of the anionic form of DNP, P_{D^-}, was determined as 0.0035 ($\log P_{D^-} = -2.46$). It should be noted that in the strong alkali used in this experiment, partition due to the DNP anion is greater than that due to the neutral form, though the value of P_{DH} is much greater than that of P_{D^-}.

According to Eq. (14), the ion-pair partition coefficient P_{DK} and the ion-pair formation constant K_{DK} can be determined from the linear relation between $(P'-Q)/P'[K^+]_w$ and $1/P'$ by using the results in Fig. 3. This relation is shown in Fig. 4, and from it the values of P_{DK} and K_{DK} were determined to be 0.41 and 1.20, respectively.

Since the values of P_{D^-}, P_{DK} and K_{DK} were determined as described above, the contribution of the neutral molecular form of DNP to the partition at various pH values could be calculated from the values of P' shown in Fig. 2. The apparent partition coefficients thus calculated (denoted as P'_{DH}), shown by open circles in Fig. 2, fit the curve drawn based on Eq. (10), which is derived on the assumption that only the neutral form is responsible for the partition process. The true partition coefficient P_{DH}, corrected for the effects of direct partition of the DNP anion and the ion-pair partition of the DNP anion, was found to be 35.5. This value is the same as that determined in the region of $\mathrm{pH} \ll \mathrm{p}K_a$, over a wide range of pH values (◑ in Fig. 2).

Next, ion-pair partition experiments with the DNP anion and the hydrophobic cation *n*-butyltrimethylammonium hydroxide (BTMA; abbreviated as N^+ or N hereafter) were carried out at alkaline pH: DNP solution containing various amounts of BTMA under the condition $[BTMA^+]_w \gg [DNP]_w$ was equilibrated with *n*-octanol. The pH was kept constant at about pH 12 with 0.05 N KOH. With a fixed amount of BTMA, the apparent partition coefficient took a constant value at various concentrations of DNP, indicating that under the experimental conditions used, there was no intermolecular association of DNP in either phase. Values of P' increased linearly as the concentration of BTMA increased, as shown in Fig. 5. In the figure, the P' value in the absence of BTMA is 0.025 ($=S/R$ in Eq. (16)), due to the partitions of the neutral and anionic forms of DNP and the ion-pair partition with K^+ (*cf.* Eq. (15)).

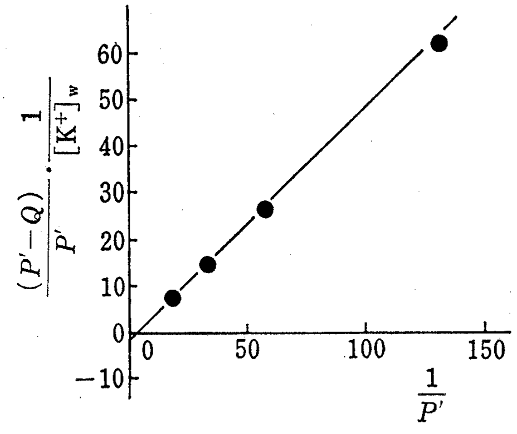

Fig. 4. Linear Relation between $(P'-Q)/P'[K^+]_w$ and $1/P'$ in the Ion-pair Partition of the DNP Anion with K^+

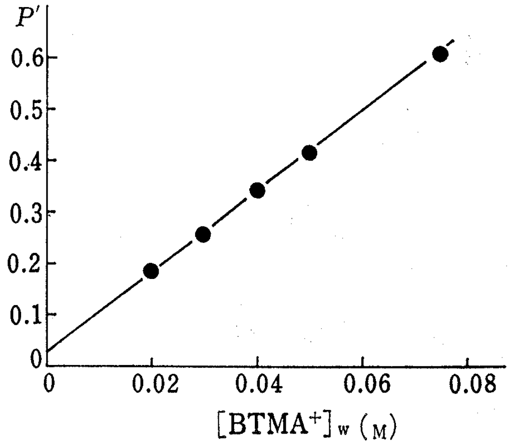

Fig. 5. Ion-pair Partition of the DNP Anion and $BTMA^+$

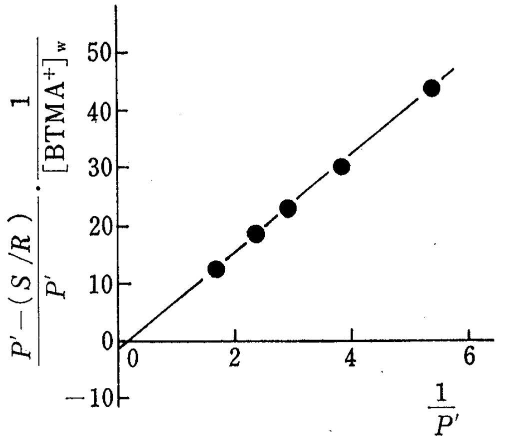

Fig. 6. Relationship between $P'-(S/R)/P'[\mathrm{BTMA}^+]_{\mathrm{w}}$ and $1/P'$ in the Ion-pair Partition of the DNP Anion with BTMA$^+$

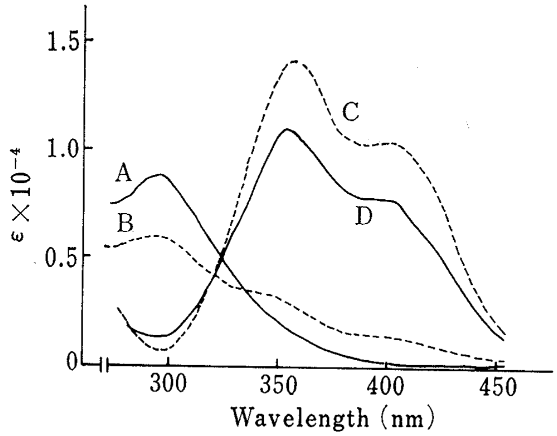

Fig. 7. Absorption Spectra of DNP under Various Conditions

A; in H_2O at pH 1 (Concentration, 10^{-4} M).
B; in *n*-octanol equilibrated with 0.085 N KOH (Concentration, 1.67×10^{-3} M).
C; in H_2O at pH 12 (Concentration, 10^{-4} M).
D; in *n*-octanol after ion-pair partition with K^+ at pH 12 (concentration, 3.67×10^{-4} M).

Plots of $\{P'-(S/R)\}/P'\cdot[N^+]_{\mathrm{w}}$ *versus* $1/P'$ are shown in Fig. 6. The values of P_{DN} and K_{DN} were determined to be 8.15 and 1.12, respectively, from the relation in Eq. (17), taking the values of S and R as 0.027 and 1.06 (*cf.* $K_{\mathrm{DK}}=1.20$ and $[K^+]_{\mathrm{w}}=0.05$ M), respectively. Note that K_{DN} is very similar to K_{DK}, while P_{DN} is about 20 times greater than P_{DK} due to the high hydrophobicity of BTMA.

Spectral Properties of DNP

From the results of the partition of DNP, it was concluded that in alkali the DNP anion mainly moves to the *n*-octanol phase by forming ion-pair complexes with cations, such as K^+ and BTMA. To confirm the above conclusion, the absorption spectra of DNP under various conditions were recorded.

As shown in Fig. 7, the absorption spectrum of the neutral form of DNP has a $\lambda_{\max}$ at about 300 nm, measured at pH 1 (curve A), and that of the anionic form, measured at pH 12, is at 358 nm with a shoulder at about 400 nm (curve C). From the pH-dependent absorption change, the pK_{a} of DNP was determined to be 4.10. The absorption spectrum of DNP in *n*-octanol was just the same as that of the neutral form of DNP in water, indicating that in *n*-octanol DNP is present in the neutral form. However, the spectrum of DNP in *n*-octanol which had been equilibrated with KOH shows that some DNP is present in the anionic form (curve B in Fig. 7), probably because *n*-octanol solubilizes rather a large amount of water. (The solubility of H_2O in *n*-octanol is 2.3 M.[13])

Next, the absorption spectrum of DNP in *n*-octanol after partition equilibrium was measured. It was found that below pH 7, the spectrum of DNP in *n*-octanol was the same as that of the neutral form of DNP, whereas at an alkaline pH the spectrum was very similar to that of the DNP anion, with the same absorption maximum but smaller absorption intensity. (One of the spectra is shown as D in Fig. 7.) Thus, it is concluded that the electronic nature of DNP in *n*-octanol after partition at alkaline pH is similar to that of the DNP anion. It is probably in the form of an ion-pair complex, although at present we cannot distinguish the ion-pair and anionic forms clearly from the absorption spectra alone.

Discussion

This study showed that both the neutral molecular species of DNP and the anionic form, either directly or in the form of an ion-pair complex, are transferred to an organic phase.

Table I summarizes the various constants which characterize the partition of DNP. The hydrophobicity of DNP in the neutral molecular form is about 90 times that of its ion-pair complex with K^+, and about 10^4 times that of its anionic form. The affinity of the DNP anion for H^+ is about 10^4 times greater than that for K^+. Thus the neutral molecular form of DNP tends to be transferred to the *n*-octanol phase about 9×10^5 times more readily than $DNP^- - K^+$ (*cf.* E_{DX} value in Table I). However, according to Eq. (10), transfer of the neutral form of DNP to the organic phase decreases 10-fold with an increase of one pH unit, due to a decrease in (H^+) in the region of $pH \gg pK_a$. Thus, at alkaline pH values, DNP mainly enters the organic phase in the form of an ion-pair complex. Fig. 8 shows the pH-dependent transfers of DNP as neutral and anionic forms and as an ionpair with K^+, when 0.1 M K^+ is present in the aqueous phase. The values are expressed as relative concentrations of these molecular species in the organic phase determined by means of Eqs. (19)—(21) from the values in Table I.

Table I. Values Characterizing the Partition of the 2,4-Dinitrophenol (DNP) Anion in Combination with the Cation X^+

X^+	P_{DX}	($\log P_{DX}$)	K_{DX}[a]	($\log K_{DX}$)	E_{DX}	($\log E_{DX}$)
H^+	35.5	(1.55)	1.26×10^4	(4.10)	4.48×10^5	(5.65)
K^+	0.41	(−0.39)	1.20	(0.08)	0.49	(−0.31)
$BTMA^+$	8.15	(0.91)	1.12	(0.05)	9.12	(0.96)
DNP anion	3.5×10^{-3}	(−2.46)	—		—	

[a] The value of K_{DH} is taken as the reciprocal of K_a.

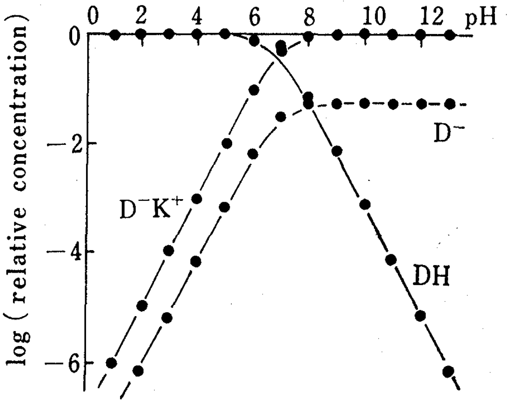

Fig. 8. Relative Concentrations of Various Molecular Species of DNP in *n*-Octanol after Partition Equilibrium ($[DH]_0/C_0$, $[D^-]_0/C_0$ and $[D^-K^+]_0/C_0$, when 0.1 M K^+ is present in the Aqueous Phase

It is clear from the figure that below pH 5, DNP moves to the organic phase almost entirely as the neutral form, while at pH 6 about 10% of the DNP in the organic phase is present as the ion-pair complex with K^+, and at pH 7 the concentration of the neutral form is about the same as that of the ion-pair complex. At alkaline pH values, the proportion of the ion-pair complex is always greatest and above pH 8, direct transfer of the DNP anion exceeds that of neutral DNP.

The action of weakly acidic uncouplers, such as DNP, is generally considered to be due to their proton-conducting activity.[1] That is, the uncoupler anion binds to H^+ in the reaction medium to give the neutral form, which then moves to the inner side of mitochondria, where the uncoupler molecule changes to the anionic form again by release of H^+. Thus the uncoupler dissipates the H^+-gradient across the mitochondrial membrane.[1] However, according to the present results, DNP could play a role as a K^+-carrier as well as a H^+-carrier under physiological conditions (at about pH 7). Furthermore, the results in Table I show that the DNP anion forms an ion-pair complex with $BTMA^+$ to the same degree as with K^+, and that the hydrophobicity of $DNP^- - BTMA^+$ is about 20 times that of $DNP^- - K^+$. In view of these findings it seems likely that most DNP in biological membrane exists in the form of an ion-pair complex with the cationic head group of phospholipids. In this case, the efficiency of DNP as a H^+– or K^+– carrier will be very low. The finding that the uncoupling activity of DNP is almost independent of the amount of mitochondria in the reaction medium, while the activities of other uncouplers are dependent on the amount of mitochondria,[2,3] may be related to the preferential interaction of DNP with quaternary ammonium cations. Information on the ion-pair partition of various types of uncouplers with alkali metal cations and quaternary ammonium ions should be very useful for understanding the mechanism of action of uncouplers.

In the quantitative structure-activity relationships developed by Hansch and Fujita,[14] the hydrophobicity of bio-active compounds has been represented by the partition coefficient P_{BH} between n-octanol and water. Due to experimental limitations, P_{BH} is sometimes calculated on the basis of Eq. (10) from P' at a certain pH where ionic and neutral molecular species co-exist.[8] With some acids, such as N-phenylanthranilates,[15] sulfonamide, [7a] and tetracycline,[7b] the value of P_{BH} calculated from P' according to Eq. (10) is almost the same as that determined experimentally under conditions where Eq. (11) holds. However, as shown in Fig. 2, P_{BH} cannot always be calculated from P'. Generally, at least the effect of the partition due to an ion-pair should be taken into account in the determination of P_{BH} from P'. Furthermore, the results in Fig. 7 indicate that, if the acid is not adsorbed appreciably on the surface of the vessel containing the sample solution, C_0 should be determined as the difference between the concentrations of acid in the aqueous phase before and after partition equilibrium, C_i–C_w, unless the partition is carried out in the region of pH $\ll$pK_a.

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