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Thermodynamic Analysis of Ion-Pair Partition of Methyl Orange Anion with Alkali Metal Cations in an Octanol–Water System

YUKO YOSHIKAWA and HIROSHI TERADA*

*Faculty of Pharmaceutical Sciences, University of Tokushima,
Shomachi-1, Tokushima 770, Japan*

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The ion-pair partition of methyl orange anion with the alkali metal cations Na^+ , K^+ and Cs^+ in an octanol–water system was studied at various temperatures. The ion-pair partition process could be divided into two processes; ion-pair formation and transfer of the ion-pair. The results showed that the degree of ion-pair formation increased with temperature, while the degree of partition of an ion-pair decreased with temperature for all alkali metal cations. The ion-pair partition of methyl orange anion was shown to be greatest with Cs^+ . The thermodynamic parameters $\Delta G^\ominus$, $\Delta S^\ominus$ and $\Delta H^\ominus$ for these two processes were determined. The values are discussed in relation to the degree of hydration of these alkali metal cations.

Keywords—partition; ion-pair partition; partition coefficient; ion-pair partition coefficient; ion-pair formation constant; ion-pair extraction constant; methyl orange; alkali metal cation; thermodynamic analysis

The partition process of bioactive compounds from water to a water-immisible organic solvent has been regarded as a simple model system of their transfer to biomembranes.¹⁾ However, most studies have been carried out on hydrophobic non-electrolytes and the neutral forms of ionizable compounds.²⁾ Thus, the partition mechanism of ionic compounds is not fully understood.

We recently made detailed studies on the partition process of 2,4-dinitrophenol (DNP), a potent uncoupler of oxidative phosphorylation in mitochondria, from an aqueous to an octanol phase.³⁾ Octanol is regarded as an organic solvent that mimics the hydrophobic nature of biomembranes well.⁴⁾ Most DNP exists as an anionic form under physiological conditions due to its low $\text{p}K_a$ value ($=4.10$), and its transfer from the aqueous to the octanol phase by formation of an ion-pair complex with cations present in the aqueous phase was found to be predominant. Furthermore, we found that this model system is very useful for understanding the mechanism of action of DNP on mitochondrial membranes.³⁾

For further understanding of the action of organic ions on biomembranes, we studied the ion-pair partition process of methyl orange (MO) from water to the octanol phase at various temperatures. This dye has commonly been used as a model of bioactive anions,⁵⁾ because determination of the amount of MO from its visible spectrum is easy and its behavior in solution is well established.⁶⁾ As reported in this paper, as a first step of this study, we examined the process of ion-pair formation of MO with various alkali metal ions, because some of these ions are major components of buffer solutions.

Usually the degree of ion-pair partition is evaluated in terms of the extraction constant E ($=$ ratio of the extracted amount of the solute to the total amount of the solute). However, in this study we regarded the ion-pair partition process as consisting of two processes, ion-pair formation and partition of the ion-pair, and quantitated these two processes separately. This facilitated a precise understanding of the partition process.

Experimental

Materials—MO was obtained from Merck & Co. (Darmstadt). *n*-Octanol was the finest grade product available from Nakarai Chemicals Co., Ltd. (Kyoto) and was saturated with water. The other reagents, including Na^+ , K^+ and Cs^+ salts, were of the highest grade commercially available. Before use in partition experiments, water was passed through ion exchange resins, and then distilled, and its conductivity was less than 10^{-7} Scm^{-1} .

Measurement of Partition Coefficient—The aqueous phase contained $5 \times 10^{-5} \text{ M}$ MO and an alkali metal ion, such as Na^+ , K^+ or Cs^+ . The concentration of the alkali metal ion was always much greater than that of MO, in order to keep the cation concentration in the aqueous phase essentially constant before and after partition equilibrium. The aqueous phase was adjusted to about pH 12 with 0.01 M NaOH for experiments on the effect of Na^+ and Cs^+ , and with 0.0085 M KOH for experiments on the effect of K^+ .

Mixtures of 5.0 ml of MO solution containing various amounts of alkali metal cation and 5.0 ml of *n*-octanol saturated with water were gently shaken in a water bath at a constant temperature for about 6 h, and then allowed to stand for 12 h at the same temperature. Then, the concentration of MO in the aqueous phase was determined spectrophotometrically in a Shimadzu spectrophotometer, model UV-180, at 464 nm. The concentration of MO in the *n*-octanol phase (C_o) was determined from the difference between the initial concentration of MO in the aqueous phase (C_i) and the concentration after equilibration (C_w) according to Eq. 1, where V represents the volume of the aqueous (subscript w) or organic (subscript o) phase.

$$C_o = (C_i - C_w)(V_w/V_o) \quad (1)$$

Possible Mechanism of the Ion-Pair Partition Process—Figure 1 shows the possible mode of partition of MO between the aqueous phase (w) and the octanol phase (o), where MH and M^- represent the neutral molecular species and the ionic form of MO, respectively. The ion-pair complex of M^- with X^+ , $\text{M}^- \text{X}^+$, is represented as MX. X^+ was added to the aqueous phase in the form of $\text{X}^+ \text{Y}^-$ (Y^- was Cl^- in this study). In Fig. 1, all possible partitions of MO in the presence of X^+ are taken into consideration. However, in this study the partition of M^- and X^+ as well as $\text{X}^+ \text{Y}^-$ was ignored because of the very small partitions of these species under the present experimental conditions.³⁾ The partition coefficients P for MH (P_{MH}) and $\text{M}^- \text{X}^+$ (P_{MX}) are expressed by Eqs. 2 and 3, where square brackets represent concentrations.

$$P_{\text{MH}} = [\text{MH}]_o / [\text{MH}]_w \quad (2)$$

$$P_{\text{MX}} = [\text{MX}]_o / [\text{MX}]_w \quad (3)$$

The acid dissociation constants of MH in the aqueous and octanol phases, referred to as K_a and K'_a , respectively, are expressed by Eqs. 4 and 5, respectively, where the activity coefficient of H^+ is assumed to be 1. Furthermore, the ion-pair formation constants of M^- with X^+ in the aqueous (K_{MX}) and octanol (K'_{MX}) phases are expressed by Eqs. 6 and 7, respectively.

$$K_a = [\text{M}^-]_w [\text{H}^+]_w / [\text{MH}]_w \quad (4)$$

$$K'_a = [\text{M}^-]_o [\text{H}^+]_o / [\text{MH}]_o \quad (5)$$

$$K_{\text{MX}} = [\text{MX}]_w / [\text{M}^-]_w [\text{X}^+]_w \quad (6)$$

$$K'_{\text{MX}} = [\text{MX}]_o / [\text{M}^-]_o [\text{X}^+]_o \quad (7)$$

Thus, from the above relations, the apparent partition coefficient (P') of MO expressed by Eq. 8, can be represented by Eq. 9.

$$P' = C_o / C_w = \frac{[\text{MH}]_o + [\text{M}^-]_o + [\text{MX}]_o}{[\text{MH}]_w + [\text{M}^-]_w + [\text{MX}]_w} \quad (8)$$

Fig. 1. Possible Mode of Partition of MO between Aqueous (w) and Octanol (o) Phases

$$= \frac{P_{MH}[H^+]_w + P_{MH}K'_a[H^+]_w/[H^+]_o + P_{MX}K_{MX}K_a[X^+]_w}{[H^+] + K_a + K_aK_{MX}[X^+]_w} \quad (9)$$

Under conditions where the alkali metal cation X^+ is absent ($[X^+]_w = 0$), Eq. 9 becomes Eq. 10.

$$P' = \frac{P_{MH}[H^+]_w + P_{MH}K'_a[H^+]_w/[H^+]_o}{K_a + [H^+]_w} = P'_o \quad (10)$$

Thus, from Eq. 10, Eq. 9 is expressed by Eq. 11.

$$P' = \frac{P'_o(K_a + [H^+]_w) + P_{MX}K_{MX}K_a[X^+]_w}{[H^+]_w + K_a + K_aK_{MX}[X^+]_w} \quad (11)$$

When $pH \gg pK_a$, Eq. 11 becomes Eq. 12.

$$P' = \frac{P'_o + P_{MX}K_{MX}[X^+]_w}{1 + K_{MX}[X^+]_w} \quad (12)$$

Equation 12 indicates that with increase in the concentration of the cation X^+ added to the aqueous phase, P' should first increase almost linearly, then more gradually, and finally reach a constant level. The intercept of the plot of P' vs. $[X^+]_w$ equals P'_o according to Eq. 12. P'_o can also be expressed by Eq. 13 from the relations of Eqs. 5 and 7.

$$P'_o = \frac{P_{MH}[H^+]_w}{[H^+]_w + K_a} \quad (13)$$

Thus, the partition coefficient P_{MH} for the neutral form of MO can be determined from Eq. 13. The ion-pair complex formation constant K_{MX} and the ion-pair partition coefficient P_{MX} can be determined by use of Eq. 14.

$$\frac{P' - P'_o}{P'} \cdot \frac{1}{[X^+]_w} = P_{MX}K_{MX} \cdot \frac{1}{P'} - \frac{1}{K_{MX}} \quad (14)$$

The ion-pair extraction constant $E_{MX} (= [MX]_o/[M^-]_w[X^+]_w)$ is expressed by Eq. 15.

$$E_{MX} = [MX]_o/[M^-]_w[X^+]_w = P_{MX}K_{MX} \quad (15)$$

When there are two alkali metal cations X^+ and Z^+ , as in the experiments on the effect of Cs^+ ($X^+ = Cs^+$) on the partition of MO, in which the pH of the aqueous phase was adjusted with NaOH ($Z^+ = Na^+$), the apparent partition coefficient P' is expressed by Eq. 16.

$$P' = \frac{(P'_o + P_{MX}K_{MX}[X^+]_w) + P_{MZ}K_{MZ}[Z^+]_w}{(1 + K_{MX}[X^+]_w) + K_{MZ}[Z^+]_w} \quad (16)$$

Denoting $(1 + K_{MX}[X^+]_w)$ as R and $(P'_o + P_{MX}K_{MX}[X^+]_w)$ as S , Eq. 16 is simplified to Eq. 17.

$$\frac{P' - (S/R)}{P'} \cdot \frac{1}{[Z^+]_w} = \frac{P_{MZ}K_{MZ}}{R} \cdot \frac{1}{P'} - \frac{K_{MZ}}{R} \quad (17)$$

Thus, a plot of $(P' - (S/R))/P'[Z^+]_w$ against $1/P'$ should give a straight line with a slope of $P_{MZ}K_{MZ}/R$ and an intercept of K_{MZ}/R .

When the initial concentration of $[X^+]_w$ or $[X^+]_w$ plus $[Z^+]_w$ is much larger than that of MO, it can be regarded as the same after partition equilibrium. Thus, measurement of the concentration of X^+ or Z^+ in the aqueous phase at partition equilibrium is not necessary.

Results

We determined the dependence of the apparent partition coefficient P' of methyl orange MO on the concentration of alkali metal cation X^+ in the aqueous phase at alkaline pH, where almost all the MO was in the anionic form MO^- .

Figure 2 shows the results with Na^+ at various temperatures. In all cases, P' increased with increase in the concentration of Na^+ in the aqueous phase $[Na^+]_w$; the increase was at first almost linear, and then became gradual. This increase was due to the formation of an ion-

Fig. 2. Apparent Partition Coefficient P' of MO in the Presence of Na^+ at Various Temperatures

Fig. 3. Linear Relation between $(P' - P'_0)/P'[\text{Na}^+]_w$ and $1/P'$ in the Ion-Pair Partition of MO Anion with Na^+

Fig. 4. Relationship between $(P' - (S/R))/P'[\text{Cs}^+]_w$ and $1/P'$ in the Ion-Pair Partition of MO Anion with Cs^+ at 25°C

Fig. 5. Dependence of Apparent Partition Coefficient P' of MO on Alkali Metal Cations X^+ at 25°C

pair complex between MO^- and Na^+ as predicted by Eq. 12. From the linear extrapolation of P' to $[\text{Na}^+]_w = 0$, P'_0 was determined to be 0.08 at 10°C , 0.06 at 15°C and 0.05 at 25°C . These values are very small, but are non-zero (see Eq. 10). Furthermore, P' increased with decrease in temperature. The partition of MO in the presence of K^+ and Cs^+ showed similar tendencies to those in the presence of Na^+ (data not shown).

According to Eq. 14, the ion-pair formation constant K_{MX} and ion-pair partition coefficient P_{MX} can be determined from the results in Fig. 2.

The linear relationships between $(P' - P'_0)/P'[\text{Na}^+]_w$ and $1/P'$ are shown in Fig. 3, and P_{MNa} and K_{MNa} were determined from the slope and the intercept on the Y-axis. Similarly, the values of P_{MK} and K_{MK} were determined. In the case of Cs^+ , where the pH of the aqueous phase was adjusted with NaOH, the partition behavior of MO was similar to that of Na^+ shown in Fig. 2. In this case, K_{MCs} and P_{MCs} can be determined from the linear relation between $(P' - (S/R))/P'[\text{Na}^+]_w$ and $1/P'$ according to Eq. 17, instead of Eq. 14. This linear relation is depicted in Fig. 4 for the case at 25°C . Similar linear relations were obtained at other temperatures (data not shown).

Figure 5 compares the effects of the alkali metal cations Na^+ , K^+ and Cs^+ on the partition of MO at 25 °C. The order of P 's was $\text{Na}^+ < \text{K}^+ < \text{Cs}^+$, showing that P became greater with increase in the ionic radius of the alkali metal cation. Table I summarizes the values of K_{MX} , P_{MX} and E_{MX} .

In Table I, values of P_{MX} and E_{MX} became greater in the order Na^+ , K^+ and Cs^+ . With increase in temperature, K_{MX} increased, but P_{MX} decreased.

From the values of K_{MX} , P_{MX} and E_{MX} at various temperatures, the thermodynamic parameters $\Delta G^\ominus$, $\Delta S^\ominus$ and $\Delta H^\ominus$ were determined by applying the relations in Eqs. 18, 19 and 20.

$$\Delta G^\ominus = -RT \ln K \quad (18)$$

$$\left(\frac{\partial \ln K}{\partial (1/T)} \right)_P = - \frac{\Delta H^\ominus}{R} \quad (19)$$

$$\left(\frac{\partial (-RT \ln K)}{\partial T} \right)_P = -\Delta S^\ominus \quad (20)$$

These values with regard to the processes of ion-pair formation (K_{MX}), ion-pair partition (P_{MX}) and extraction (E_{MX}) are summarized in Tables II, III and IV, respectively, taking those

TABLE I. Values of the Ion-Pair Partition Coefficient (P_{MX}), Ion-Pair Formation Constant (K_{MX}) and Ion-Pair Extraction Constant (E_{MX}) of MO Anion (M^-) with Alkali Metal Cations (X^+) at Various Temperatures

X^+	Temp. (°C)	K_{MX} (M^{-1})	P_{MX}	E_{MX} (M^{-1})
Na^+	10	2.55	7.99	20.37
	15	2.64	6.47	17.09
	25	3.16	4.81	15.20
K^+	10	2.48	9.44	23.42
	25	3.09	5.35	16.54
	35	3.53	4.05	14.31
Cs^+	10	2.86	13.46	38.50
	15	3.24	9.59	31.07
	25	4.23	6.05	25.60
	30	5.11	4.34	22.18

TABLE II. Thermodynamic Parameters of the Ion-Pair Formation Constant (K_{MX}) of MO Anion with Alkali Metal Cations at 25 °C

X^+	$\Delta H^\ominus$ (kJ mol^{-1})	$\Delta S^\ominus$ ($\text{JK}^{-1} \text{mol}^{-1}$)	$\Delta G^\ominus$ (kJ mol^{-1})
Na^+	10.20	43.79	-2.85
K^+	10.15	43.42	-2.79
Cs^+	20.18	79.70	-3.57

TABLE III. Thermodynamic Parameters of the Ion-Pair Partition Coefficient (P_{MX}) of MO Anion with Alkali Metal Cations at 25 °C

X^+	$\Delta H^\ominus$ (kJ mol^{-1})	$\Delta S^\ominus$ ($\text{JK}^{-1} \text{mol}^{-1}$)	$\Delta G^\ominus$ (kJ mol^{-1})
Na^+	-23.51	-65.84	-3.89
K^+	-24.50	-68.29	-4.15
Cs^+	-38.71	-114.93	-4.46

TABLE IV. Thermodynamic Parameters of the Ion-Pair Extraction Constant (E_{MX}) of MO Anion with Alkali Metal Cations at 25 °C

X^+	$\Delta H^\ominus$ (kJ mol ⁻¹)	$\Delta S^\ominus$ (JK ⁻¹ mol ⁻¹)	$\Delta G^\ominus$ (kJ mol ⁻¹)
Na ⁺	-12.91	-20.70	-6.74
K ⁺	-14.35	-24.83	-6.95
Cs ⁺	-18.53	-36.44	-7.67

at 25 °C as standard. With K_{MX} , $\Delta G^\ominus$ for all alkali metal cations took negative values, while the signs of $\Delta H^\ominus$ and $\Delta S^\ominus$ were always positive. In the case of P_{MX} , all the thermodynamic parameters took negative values. The very large negative values of $\Delta H^\ominus$ and $\Delta S^\ominus$ for Cs⁺ were especially noteworthy. The large negative values of $\Delta H^\ominus$ and $\Delta S^\ominus$ for all alkali metal cations with P_{MX} lead to negative values for E_{MX} .

Discussion

We examined the effects of alkali metal cations, X⁺, such as Na⁺, K⁺ and Cs⁺, at various temperatures on the apparent partition coefficients P' of MO in the alkaline region where almost all MO is present in the anionic form M⁻. In all cases, P' increased with increase in the concentration of X⁺ due to the ion-pair partition of M⁻ with X⁺. According to our present model, the ion-pair partition of MO consists of two processes. The first process is the formation of an ion-pair complex of M⁻ with X⁺ in the aqueous phase, and the degree of ion-pair formation is expressed by K_{MX} . The second process is the transfer of the ion-pair complex into the organic phase from the aqueous phase. The partition coefficient is expressed by P_{MX} and the extraction constant, $E_{MX}(=K_{MX}P_{MX})$, represents the total degree of transfer of M⁻ into the organic phase.

The thermodynamic parameters of E_{MX} summarized in Table IV show that the values of $\Delta G^\ominus$, $\Delta H^\ominus$ and $\Delta S^\ominus$ were negative in all cases. The negative values were the greatest with Cs⁺, and the smallest with Na⁺, indicating that ion-pair partition of M⁻ is greatest with Cs⁺.

Since we divided the partition into two processes, the process of ion-pair partition could be clarified precisely. First, we considered the process of ion-pair formation of M⁻ with an alkali metal cation, X⁺. The polar water molecules in the vicinity of an ion are aligned in the electrical field of the ion. Thus, the entropy of hydration of an ion takes a negative value.⁷⁾ In this study, the entropies $\Delta S^\ominus$ of K_{MX} were found to have positive values for all X⁺'s (cf. Table II). This could be due to dehydration of MO⁻ and X⁺ when the ion-pair complex is formed.

Figure 6 shows two possible models of the ion-pair formation process, contact ion-pair (A) and solvent separated ion-pair (B).⁸⁾ In the case of the contact ion-pair, dehydration of ions (process 1 in Fig. 6A) is followed by formation of an ion-pair complex. Then, hydration of the ion-pair takes place (process 2 in Fig. 6A). The first dehydration process could govern the values of $\Delta H^\ominus$ and $\Delta S^\ominus$. However, in the case of the solvent separated ion-pair, the ion-pair is formed between hydrated M⁻ and X⁺. During ion-pair formation, a few water molecules that bind to these two ions are liberated. Thus, due to the partial dehydration, the heat necessary to release hydrated water $\Delta H^\ominus$ and the accompanying loss of entropy, $\Delta S^\ominus$, should be smaller than those in the case of contact ion-pair formation.

The degree of hydration is inversely dependent on the atomic radii of alkali metal ions (Na⁺ > K⁺ > Cs⁺). In the case of a strongly hydrated ion, such as Na⁺, a solvent separated ion-pair can be formed, but in the case of a weakly hydrated ion, such as Cs⁺, a contact ion-pair tends to be formed. Thus, $\Delta H^\ominus$ and $\Delta S^\ominus$ with Cs⁺ should be greater than those with Na⁺. The results with Na⁺ and Cs⁺ in Table II well represent these two ion-pair formation

Fig. 6. Two Models of the Ion-Pair Formation Process; Contact Ion-Pair (A) and Solvent Separated Ion-Pair (B) Complexes

Dotted lines round M⁺ and X⁺, and small dotted circles indicate hydrated shell and liberated water molecules, respectively. X⁺, alkali metal cation; M⁻, methyl orange anion.

processes. It is not clear which type of ion-pair is formed with K⁺. Probably, the type of ion-pair depends on the conditions. However, the similar values of $\Delta H^\ominus$ and $\Delta S^\ominus$ with K⁺ to those with Na⁺ suggest the formation of a solvent separated ion-pair.

The process of the partition is complex, consisting of various steps, dehydration in the aqueous phase, crossing of the water-octanol interface, formation of a "hole" in the octanol phase and solvation in the octanol phase, and it is difficult to separate each step. The results in Table III show that the partition process is exothermic, and is driven by enthalpy. The negative value of $\Delta S^\ominus$ suggests the important role of the arrangement of the octanol molecules for making "holes" for the entrance of the ion-pair and the accompanying solvation. The large negative $\Delta S^\ominus$ with Cs⁺ supports this possibility. With Na⁺ and K⁺, large loss of entropy by dehydration on crossing the interface is possibly cancelled by the greater gain of entropy by solvation in the octanol phase.

The present study supports the validity of our ion-pair partition mechanism consisting of ion-pair formation and partition of the ion-pair complex. With the aid of this model system, the mechanism of the ion-pair partition process, and the mechanism of the transfer of ionic drugs to biomembranes can be analyzed in more detail, as will be reported in subsequent papers.

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