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ACTIVITY OF THE ANION EXCHANGE PROTEIN (BAND 3) OF HUMAN ERYTHROCYTES AS AFFECTED BY HYDROXYCHLOROAROMATIC CHEMICALS*†

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Abstract—The membranous segment of the anion transport protein (band 3) of the human erythrocyte membrane has been shown [T. L. Miller and R. J. Smith, *Archs Biochem. Biophys.* **250**, 128 (1986)] to be destabilized by relatively low concentrations of many hydroxychloroaromatic compounds (HO-Cl_x-Ar), including hydroxychlorodiphenyl ethers (HO-Cl_x-DPE), major contaminants of technical grade pentachlorophenol (PCP). In the present study, HO-Cl_x-DPE also caused a concentration-dependent inhibition of the rate of sulfate exchange mediated by band 3 in human erythrocytes. The most active compound studied, 2-HO-Cl_9-DPE, was about nine times more potent in inhibiting sulfate exchange than 2-HO-2′,4,4′-Cl_3-DPE, the least active compound studied. The potency of HO-Cl_x-DPE as inhibitors of anion exchange generally increased with the degree of chlorination. The concentration-dependent decreases in the sulfate exchange rate elicited by 2-HO-Cl_9-DPE and 2-HO-2′,4,4′-Cl_3-DPE paralleled the effects of these compounds on the stability of band 3.

The anion transport protein (band 3) is one of the major membrane-spanning proteins of the erythrocyte membrane. Anion exchange mediated by band 3 is specifically inhibited by a wide variety of compounds [1] including stilbene disulfonates [2], dipyridamole [3], niflumic acid [4] and flufenamate [5]. Many membrane perturbants including local anesthetics [6], phenols [7], alcohols [8], and salicylates [9] are also inhibitors of anion exchange. Structure–activity studies of various derivatives of some of the above compounds have shown a good correlation between inhibition of erythrocyte anion exchange and the hydrophobicity and electron withdrawing power of various substituents [5, 7, 9].

The thermal stability of the membrane-spanning segment of band 3 is altered by a number of specific and nonspecific inhibitors of anion exchange [10–12]. Destabilization of band 3 caused by various chemicals is often accompanied by a corresponding decrease in anion transport activity. For example, the local anesthetic lidocaine causes a commensurate decrease in both the stability of band 3 and the rate of anion transport in erythrocytes [12]. It has been suggested that the destabilization of band 3 is due to the binding of lidocaine to the lipid bilayer, with subsequent disruption of "protein–protein weak interactions" involved in the stabilization of the band 3 protein [12].

Recent results from our laboratory show that relatively low concentrations of hydroxychloroaromatic chemicals (HO-Cl_x-Ar§) destabilize band 3 [13]. The aim of the present work was to determine if HO-Cl_x-Ar are able to inhibit band-3-mediated anion translocation and if such inhibition parallels the observed effects of these compounds on the thermal stability of band 3. We have also studied the relationship between binding affinity of hydroxychlorodiphenyl ethers (HO-Cl_x-DPE) to erythrocytes and effects on band 3.

METHODS

Chemicals. The structures and names of the HO-Cl_x-AR used in this study are shown in Fig. 1. The HO-Cl_x-DPE, hexachlorophene (HCP), and pentachlorophenol (PCP) were obtained as previously described [13]. Other reagents used were: DIDS (Calbiochem Co., San Diego, CA); carrier-free $H_2{}^{35}SO_4$ (I.C.N. Radiochemicals, Irvine, CA); octanol (Fluka Chemical Corp., Ronkonkoma, NY); and Aquasol-2 (New England Nuclear, Boston, MA).

Erythrocyte preparation. Human blood was obtained from healthy donors and stored in acid-citrate-dextrose solution for no more than 3 weeks before use. Erythrocytes were collected by centrifugation and washed three times with isotonic saline containing 10 mM sodium phosphate, pH 7.4;

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§ Abbreviations: HO-Cl_x-Ar, hydroxychloroaromatic compounds; HO-Cl_x-DPE, hydroxychlorodiphenyl ethers; PCP, pentachlorophenol; HCP, hexachlorophene; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; k_o, first-order rate constant for $^{35}SO_4^{2-}$ efflux in the absence of inhibitor; k_i, first-order rate constant for $^{35}SO_4^{2-}$ efflux in the presence of inhibitor; P_{rbc}, erythrocyte/buffer partition coefficient; K_{ow}, octanol/water partition coefficient; n, Hill coefficient; and ΔT_c, change in transition temperature of the membrane-spanning domain of band 3.

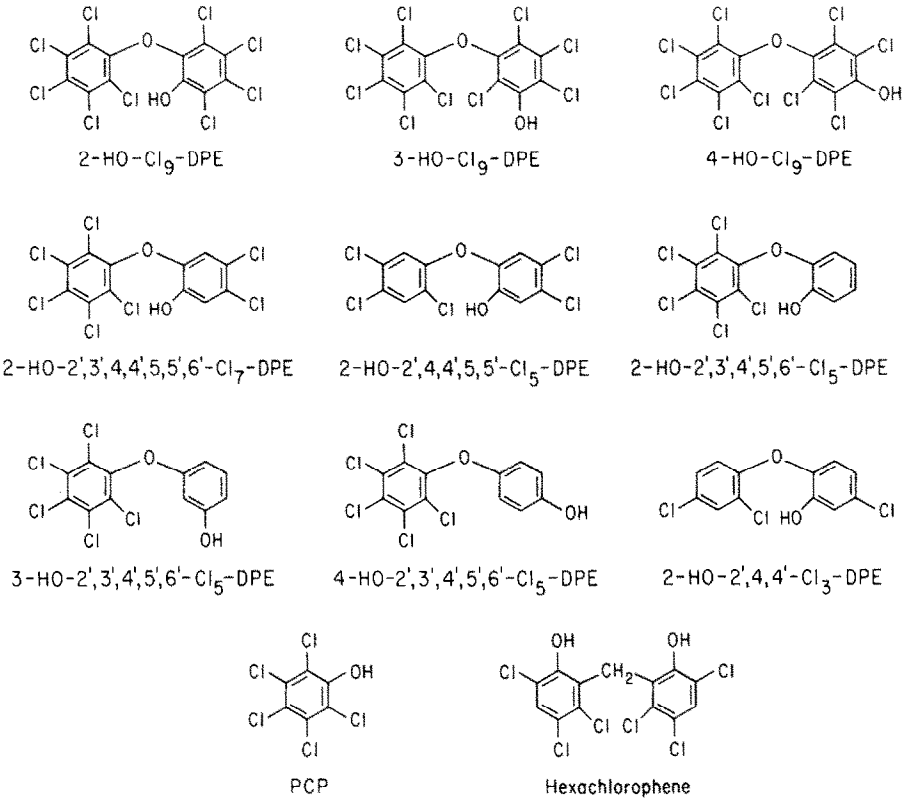

Fig. 1. Names and structures of hydroxychloroaromatic compounds.

care was taken to remove the buffy coat completely. The concentration of erythrocytes was determined from direct Coulter counting of each batch of blood and from the absorbance at 540 nm of lysed erythrocyte suspensions [by use of an extinction coefficient of 0.90 $(mg/ml)^{-1}$ for hemoglobin].

Self-exchange of sulfate. The rate of SO_4^{2-} self-exchange across the erythrocyte membrane was determined by a procedure similar to that described by Davio and Low [12]. Erythrocytes were washed three times in the assay buffer (5 mM Na_2SO_4, 134 mM NaCl, 10 mM sodium phosphate, pH 7.4) and then incubated at 37° for 30 min at 10% hematocrit. Cells thus equilibrated with SO_4^{2-} were then loaded with $^{35}SO_4^{2-}$ by incubation (at 20% hematocrit) in assay buffer, containing tracer amounts of $H_2^{35}SO_4$, for 1.5 hr at 37°. To remove $^{35}SO_4^{2-}$ from outside the cells, erythrocytes were washed five times with buffer at 0°; finally, the cells were resuspended to a 7% hematocrit.

Efflux experiments were conducted as follows: Erythrocytes were added to assay buffer at 37°, to a final cell concentration of 2.9×10^7 cells/ml. Immediately, the resulting suspension was vortexed and an aliquot of a methanolic stock solution of HO-Cl_x-Ar was added slowly. Aliquots of the erythrocyte suspension were taken at zero time and at 5- to 8-min intervals and immediately centrifuged for 20 sec in a Beckman (Palo Alto, CA) Microfuge B. An aliquot (0.2 ml) of the supernatant fraction was added to 2 ml of Aquasol for determination of $^{35}SO_4^{2-}$. The first-order rate constant, k, was calculated from the data according to the equation

$$\ln \frac{y_\infty - y_t}{y_\infty - y_o} = -kt$$

where y_o, y_t, and y_∞ represent the radioactivity in the supernatant fraction at the start of the efflux experiments, at time t, and at infinite time respectively. y_∞ was computed from the total amount of entrapped $^{35}SO_4^{2-}$, as determined from the amount of radioactivity in the supernatant fraction from cells lysed in the presence of 10% trichloroacetic acid. Results are expressed as the ratio of the first-order rate constant for $^{35}SO_4^{2-}$ efflux in the presence of HO-Cl_x-Ar to that of control (i.e. k_i/k_o) to correct for the small variability in k_o for blood from different donors and of differing ages. Values of k_o varied from 0.030 to 0.035 min^{-1}. In some experiments, 5 μM DIDS was added to facilitate determination (by difference) of the rate of sulfate self-exchange specifically due to band 3.

Binding of HO-Cl_x-Ar to erythrocytes. Erythrocytes (2.9×10^7 cells/ml in 144 mM NaCl/10 mM sodium phosphate, pH 7.4; in glass centrifuge tubes) were treated with specific HO-Cl_x-Ar and then incubated at 25° for 30 min. Subsequently, aliquots were taken for determination of the total amount of compound in the erythrocyte suspension. The suspension was then centrifuged at 12,000 g for 20 min, and aliquots of the supernatant fraction were taken for determination of the concentration of unbound HO-Cl_x-Ar (i.e. not bound to erythrocytes). HO-Cl_x-Ar were extracted and quantified as described pre-

viously [13]. Standard solutions of HO-Cl_x-Ar were prepared by addition of a known amount of compound to erythrocytes or buffer, followed by extraction and methylation of the compound as previously described [13]. Efficiency of extraction of the HO-Cl_x-Ar was in the range of 94–100%. The amount of HO-Cl_x-Ar bound to erythrocytes was calculated by subtraction of the concentration of compound in the supernatant fraction from its total concentration in the erythrocyte suspension.

Molal erythrocyte/buffer partition coefficients (log P_{rbc}) for HO-Cl_x-Ar, at pH 7.4, were calculated, as previously described [14], from the above binding data. The mass of an erythrocyte membrane was taken to be 13×10^{-13} g [15]. Control studies, employing lysed erythrocyte suspensions, showed that the presence of hemoglobin or other cytoplasmic proteins had a negligible effect (i.e. $< \pm 0.1$ log unit) on log P_{rbc}. Octanol/buffer (pH 7.4) partition coefficients (K_{ow}) were determined essentially as described by Chiou *et al.* [16, 17].

RESULTS

Inhibition of sulfate efflux by HO-Cl_x-Ar. The observed concentration-dependence of the inhibition of erythrocyte anion exchange (as measured by $^{35}SO_4^{2-}$ efflux) by 2-HO-Cl_9-DPE and 2-HO-2',4,4'-Cl_3-DPE is presented in Fig. 2. Anion exchange was inhibited by relatively low concentrations of compound, such that the sulfate exchange rate decreased by 50% with only 1.1 μM 2-HO-Cl_9-DPE and 10 μM 2-HO-2',4,4'-Cl_3-DPE. The rate of $^{35}SO_4^{2-}$ efflux decreased with increasing concentration of 2-HO-Cl_9-DPE over the range studied (0.16 to 3 μM); at 3 μM 2-HO-Cl_9-DPE, only about 10% of the initial anion transport activity remained. For 2-HO-2',4,4'-Cl_3-DPE over the range of 1.4 to 24 μM, the concentration-dependent decrease in sulfate exchange rate essentially paralleled that observed for 2-HO-Cl_9-DPE. At the highest concentration of each compound studied, there was a marked amount of nonspecific leakage of sulfate (as determined in the presence of DIDS, a specific inhibitor of anion transport) which, for 3 μM 2-HO-Cl_9-DPE and 24 μM 2-HO-2',4,4'-Cl_3-DPE, accounted for about 50% of the total efflux of $^{35}SO_4^{2-}$. Data of Fig. 2 were fitted to a Hill equation, as derived for inhibition studies [18], to yield a Hill coefficient of $n = 1.52$ for 2-HO-Cl_9-DPE and $n = 2.23$ for 2-HO-2',4,4'-Cl_3-DPE.

Inhibition of anion exchange was studied further by comparing the effects of various HO-Cl_x-Ar at a single total concentration of 3 μM (Table 1). Each of the compounds decreased the ability of band 3 to transport $^{35}SO_4^{2-}$, although they differed considerably in potency. Among the HO-Cl_x-DPE, the most highly chlorinated compounds were generally the more active inhibitors of anion exchange activity. The order of potency (Table 1) of the HO-Cl_x-Ar (3 μM) at inhibiting the exchange of SO_4^{2-} was as follows:

3-HO-Cl_9-DPE $\simeq$ 2-HO-Cl_9-DPE
$\simeq$ 4-HO-Cl_9-DPE > HCP
> 2-HO-2',4,4',5,5'-Cl_5-DPE
> 3-HO-2',3',4',5',6'-Cl_5-DPE
$\simeq$ 2-HO-2',3',4,4',5,5',6'-Cl_7-DPE
> 2-HO-2',3',4',5',6'-Cl_5-DPE
$\simeq$ 4-HO-2',3',4',5',6'-Cl_5-DPE > PCP
> 2-HO-2',4,4'-Cl_3-DPE.

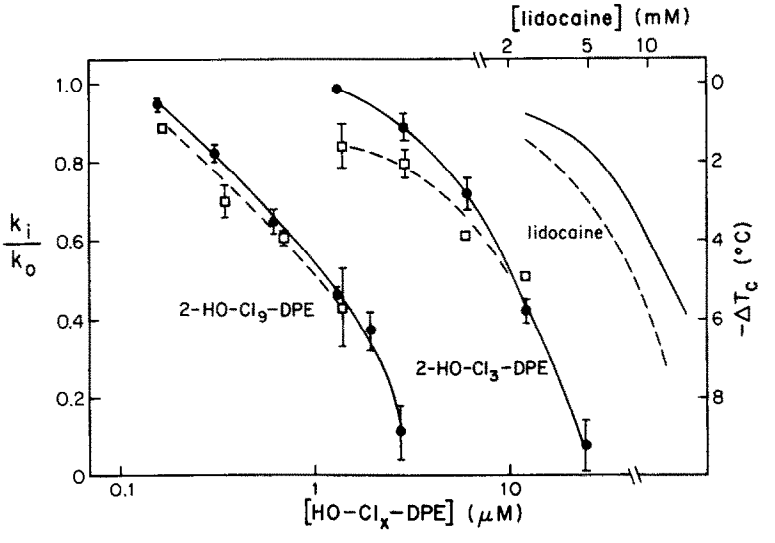

Fig. 2. Concentration-dependent effect of 2-HO-Cl_9-DPE, 2-HO-2',4,4'-Cl_3-DPE, and lidocaine on both the rate of $^{35}SO_4^{2-}$ efflux (k_i/k_o) from erythrocytes (●—●) and the stability of the membrane-spanning domain of band 3 (□---□). The values of ΔT_c for 2-HO-Cl_9-DPE and 2-HO-2',4,4'-Cl_3-DPE are taken from Miller and Smith [13], and the values for lidocaine were obtained from Fig. 2 of Davio and Low [12]. At the highest concentrations of compound employed, data are corrected for nonspecific sulfate efflux (i.e. DIDS insensitive efflux). The total concentration of HO-Cl_x-DPE in the erythrocyte suspensions was determined as described in Methods. Erythrocytes were employed at 2.9×10^7 cells/ml.

Table 1. Effects of hydroxychloroaromatic compounds (at 3 μM) on sulfate efflux from erythrocytes and on the thermal stability of the anion transport protein; binding of HO-Cl_x-Ar to erythrocytes*

Compound	k_i/k_o†	$-\Delta T_c$‡	Percent HO-Cl_x-Ar bound	Log P_{rbc}§	Log K_{ow}¶
2-HO-Cl_9-DPE	0.11 ± 0.07	>10	91 ± 1	5.44 ± 0.07	5.76 ± 0.25
3-HO-Cl_9-DPE	0.08 ± 0.01	5.8 ± 0.4	95 ± 1	5.70 ± 0.10	5.89 ± 0.05
4-HO-Cl_9-DPE	0.10 ± 0.01	6.4	96 ± 1	5.83 ± 0.14	5.79 ± 0.15
2-HO-2',3',4,4',5,5',6'-Cl_7-DPE	0.49 ± 0.05	4.8 ± 0.2	94 ± 1	5.59 ± 0.06	5.76 ± 0.12
2-HO-2',4,4',5,5'-Cl_5-DPE	0.32 ± 0.02	4.8	70 ± 2	4.80 ± 0.04	5.61 ± 0.20
2-HO-2',3',4',5',6'-Cl_5-DPE	0.55 ± 0.04	3.1	68 ± 4	4.74 ± 0.08	5.63 ± 0.08
3-HO-2',3',4',5',6'-Cl_5-DPE	0.47 ± 0.02	4.4 ± 0.1	ND	ND	5.63 ± 0.12
4-HO-2',3',4',5',6'-Cl_5-DPE	0.56 ± 0.02	4.5 ± 0.5	87 ± 3	5.23 ± 0.11	5.58 ± 0.08
2-HO-2',4,4'-Cl_3-DPE	0.89 ± 0.03	2.0 ± 0.3	27 ± 5	4.00 ± 0.10	4.82 ± 0.05
Hexachlorophene	0.16 ± 0.01	2.2	88 ± 2	5.30 ± 0.07	ND
Pentachlorophenol	0.66 ± 0.02	−0.3	12 ± 11	3.60 ± 0.81	ND

* All values are reported as the mean ± SD of at least three determinations (except where no standard deviation is shown, in which case the values are the average of two independent determinations). ND = not determined.

† k_i/k_o equals the ratio of first-order rate constant in the presence, to that in the absence, of inhibitor.

‡ ΔT_c is the difference in C transition temperature in the presence of inhibitor and control; data from Miller and Smith [13].

§ P_{rbc} is the erythrocyte/buffer partition coefficient for HO-Cl_x-Ar at pH 7.4.

¶ K_{ow} is the octanol/buffer partition coefficient at pH 7.4.

The position of hydroxyl substitution for HO-Cl_9-DPE and HO-2',3',4',5',6'-Cl_5-DPE did not strongly influence the degree of inhibition of band 3 activity.

Binding of compounds to erythrocytes. A study of the binding of HO-Cl_x-Ar to erythrocytes was undertaken to determine whether differences in their abilities to inhibit anion exchange might be related in part to the affinity of individual compounds for the plasma membrane. All of the HO-Cl_x-Ar (at 3 μM total concentration), except PCP and 2-HO-2',4,4'-Cl_3-DPE, had a high affinity for erythrocytes, as shown (Table 1) by the values obtained for percent HO-Cl_x-Ar bound and log P_{rbc}. The more highly chlorinated HO-Cl_x-DPE generally had a greater affinity for erythrocytes than the lower chlorinated congeners, although there were significant differences in log P_{rbc} even for HO-Cl_x-DPE of the same degree of chlorination. Examination of the data in Table 1 indicates that the effects of HO-Cl_x-Ar on the anion exchange protein were poorly correlated with log P_{rbc} and log K_{ow}.

DISCUSSION

Studies have shown that many amphiphilic compounds are able to inhibit the anion exchange activity of erythrocyte band 3 [1] and decrease the thermal stability of the membrane-spanning domain of this integral membrane protein [10–12]. Previous results from our laboratory have shown that HO-Cl_x-Ar also decrease the stability of the membranous domain of band 3 [13]. To further characterize their interaction with erythrocyte membrane proteins, we determined the effects of HO-Cl_x-Ar on anion translocation mediated by band 3. In addition, we present here a comparison of the effects of these compounds on the anion transport activity of band 3 with effects on its thermotropic properties.

Results obtained here show that the HO-Cl_x-Ar strongly inhibited band-3-mediated anion transport, with a potency comparable with other compounds which have been classified as potent, reversible inhibitors of anion transport [1, 4, 5, 7]. HO-Cl_x-Ar, like many of the more potent inhibitors of anion transport, are anionic, amphiphilic molecules. As such, they possess the physicochemical properties which give them the ability to interact with the region of band 3 protein where transported anions and various inhibitors bind, which is thought to involve cationic amino acid residues and a hydrophobic domain(s) [1, 5, 19, 20].

Many studies have shown that the ability of a given compound to function as a potent inhibitor of anion transport is strongly correlated with the lipophilicity and electrophilicity of its substituents [1]. Since chloro-substituents are hydrophobic and electron-withdrawing, the observed increase in inhibitory activity of the HO-Cl_x-DPE with increasing degree of chlorination can probably be accounted for on the basis of unique hydrophobic and electronic characteristics of each of these compounds. These unique properties would be expected to depend on the number and position of chloro-substituents. This interpretation is consistent with the results of Motais *et al.* [7] which show that the ability of simple chlorinated phenols to inhibit band-3-mediated anion exchange is highly correlated with the increased lipophilicity, and the greater number of electron-withdrawing substituents, which accompanies the addition of successive chloro-substituents. A similar correlation between the hydrophobicity and electronegativity of substituents of various substituted benzene sulfonic acids and stilbene disulfonic acids and the ability to inhibit anion transport has also been demonstrated [2].

The nonspecific leakage of $^{35}SO_4^{2-}$ induced by higher concentrations of 2-HO-Cl_9-DPE and 2-HO-2',4,4'-Cl_3-DPE can be explained on the basis of

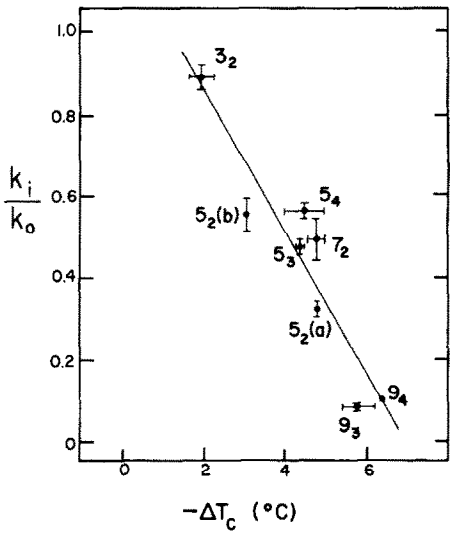

Fig. 3. Relationship between the rate of sulfate efflux from erythrocytes and the stability [13] of the membrane-spanning domain of band 3 in the presence of various HO-Cl_x-DPE at 3 μM. The error bars are the standard deviation of at least three measurements. Abbreviations are as follows: 9_3, 3-HO-Cl_9-DPE; 9_4, 4-HO-Cl_9-DPE; 7_2, 2-HO-2',3',4,4',5,5',6'-Cl_7-DPE; 5_2(a), 2-HO-2',4,4',5,5'-Cl_5-DPE; 5_2(b), 2-HO-2',3',4',5',6'-Cl_5-DPE; 5_3, 3-HO-2',3',4',5',6'-Cl_5-DPE; 5_4, 4-HO-2',3',4',5',6'-Cl_5-DPE; and 3_2, 2-HO-2',4,4'-Cl_3-DPE.

known effects of HO-Cl_x-Ar on passive membrane permeability. Thus, it is known that various HO-Cl_x-Ar interact directly with the erythrocyte plasma membrane and alter its passive permeability to monovalent cations, subsequently leading to colloid-osmotic hemolysis [21–23]. Similar changes in passive permeability may account for the observed non-specific efflux of $^{35}SO_4^{2-}$.

Hill coefficients obtained for 2-HO-Cl_9-DPE (n = 1.52) and 2-HO-2',4,4'-Cl_3-DPE (n = 2.23), which are higher than the value of 1.0 expected for interaction of a compound with a single, saturable site [18], suggest that inhibition may result from the binding of more than one molecule of HO-Cl_x-DPE/band 3. Thus, inhibition by HO-Cl_x-DPE appears to be different than for many inhibitors, e.g. 4,4'-dinitrostilbene-2,2'-disulfonate [24] and flufenamate [25], where it has been shown that inhibition results from interaction at a single site.

The work of Brandts and co-workers [10] and Davio and Low [12] suggests a relationship between the abilities of a compound to destabilize and to inactivate the anion exchange protein of erythrocytes. It is of interest to contrast the abilities of HO-Cl_x-Ar to inhibit band-3-mediated anion transport with the effects of these compounds on the calorimetric properties of band 3 [13]. For this purpose, the effects of HO-Cl_x-DPE at 3 μM on the rate of anion exchange in erythrocytes and destabilization of the membranous domain of band 3 are compared in Table 1 and plotted in Fig. 3. Examination of the plot of k_i/k_o versus the band 3 transition temperature (ΔT_c) (Fig. 3) for the various HO-Cl_x-DPE studied (each at 3 μM) reveals that there was generally a good correlation between inhibition and destabilization of band 3. The concentration-dependence of 2-HO-Cl_9-DPE and 2-HO-2',4,4'-Cl_3-DPE on both the rates of anion exchange and the destabilization of the membranous segment of band 3 are compared in Fig. 2. For both compounds the decrease in the observed rate of erythrocyte anion exchange, with increasing concentration of HO-Cl_x-Ar, generally paralleled the decrease in stability of the membrane-spanning domain of band 3.

A similar relationship (see Fig. 2) between destabilization of band 3 and inhibition of anion exchange has been observed [12] in detailed studies with the anesthetic lidocaine. These qualitative similarities in the effects of 2-HO-Cl_9-DPE and 2-HO-2',4,4'-Cl_3-DPE and lidocaine on band 3 activity and stability are rather interesting, considering the structural differences between these compounds and the observation that HO-Cl_x-Ar were as much as 10^3 to 10^4 times more active than lidocaine. The HO-Cl_x-DPE and lidocaine are amphiphilic compounds that are able to bind to biological membranes. From our data for the binding of HO-Cl_x-DPE, and data of Davio and Low [12] and Seeman [26] for lidocaine, one can calculate the concentration of each of these amphiphilic compounds in the erythrocyte membrane, and the number of molecules of compounds bound/band 3, under conditions for 50% inhibition. The values obtained

Table 2. Binding of 2-HO-Cl_9-DPE, 2-HO-2',4,4'-Cl_3-DPE, and lidocaine to erythrocytes and inhibition of band 3 activity

Compound	I_{50}* (μM)	Conc in membrane†	Molecules‡ Band 3	Log P_{rbc}	Log K_{ow}
2-HO-Cl_9-DPE	1.1	33	22	5.44	5.76
2-HO-2',4,4'-Cl_3-DPE	10.0	80	54	4.00	4.82
Lidocaine	13.000§	220§	150	1.18‖	1.65¶

* Concentration of compound for 50% inhibition of band 3 activity.

† Units of concentration = molality $\times 10^{-3}$. Values were calculated as described by Seeman *et al.* [14]. Erythrocytes were exposed to a bulk concentration of compound equivalent to the I_{50}.

‡ Molecules of compound bound to the erythrocyte membrane per molecule of band 3, assuming 1×10^6 molecules of band 3/erythrocyte [1].

§ Calculated from data of Davio and Low [12].

‖ Data of Seeman [26], at pH 7.0.

¶ Data of McKenzie and Foye [27].

are summarized in Table 2, along with a comparison of I_{50}, log P_{rbc}, and log K_{ow}. Examination of the data in Table 2 indicates that not only are higher bulk solution concentrations of the less active inhibitors needed for inhibition, but evidently a higher membrane concentration is also required. This suggests that the most active inhibitors interact in a more effective way with band 3, thus being more disruptive to its function and stability than the less active compounds.

In addition to the quantitative differences between HO-Cl_x-DPE and lidocaine mentioned above, there are other distinct differences in the effects on band 3. For example, the HO-Cl_x-DPE cause a considerable broadening, and a decrease in calorimetric enthalpy, of the thermal transition of band 3 [13]; lidocaine, on the other hand, causes an increase in enthalpy and has only a small effect on the breadth of the transition [12]. The extent to which these differences in effects on calorimetric properties of band 3 may be related to inhibition of anion exchange is not known.

Previous studies from our laboratory have shown that HO-Cl_x-Ar have a number of other direct effects on the erythrocyte membrane. Thus, HO-Cl_x-DPE [21, 28], HCP [23, 29], and hydroxychlorobiphenyls [22], at low concentration, cause a dose-dependent efflux of K^+ from erythrocytes (with resultant colloid-osmotic hemolysis), induce gross changes in erythrocyte morphology, and inhibit the activity of erythrocyte (Na^+,K^+)-activated Mg^{2+}-dependent adenosine triphosphatase. Results presented herein, together with those of studies on the thermotropic properties of band 3 [13], indicate that HO-Cl_x-Ar are capable of altering the structural and functional integrity of an integral membrane protein, namely the anion transport protein. The extent to which the effects of HO-Cl_x-Ar on band 3, and on other erythrocyte membrane properties, are mediated by specific effects on membrane proteins, as compared with phospholipid, is unknown. Concerning their potential for interaction with phospholipid, we have observed previously [13] that HO-Cl_x-Ar modify the phase transition properties of phospholipid vesicles devoid of membrane proteins. An understanding of the role of membrane proteins as contrasted with phospholipid in the membrane-effects of HO-Cl_x-Ar awaits the results of further investigation of this interesting class of membrane perturbants.

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