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Asymmetric inhibition by phlorizin of halide movements across the red blood cell membrane

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SUMMARY

Using human red cell ghosts, it was shown that external phlorizin inhibits Cl^- and I^- equilibrium exchange. Internal phlorizin has little effect on I^- exchange and no detectable effect on Cl^- exchange. This asymmetry is similar to that observed with the much more slowly exchanging $SO_4{}^{2-}$ and different from that of sugars like L-arabinose or D-xylose which are inhibited by phlorizin from either surface.

The kinetics of halide and $SO_4{}^{2-}$ permeability show characteristic differences as well as many similarities. The halides penetrate about 10 000–100 000 times faster than $SO_4{}^{2-}$. The permeability constant for the halide exchange at Donnan equilibrium exceeds the permeability constant for net movements under non-equilibrium conditions by about 4 orders of magnitude[1]. In $SO_4{}^{2-}$ permeability, the two constants are of the same order of magnitude (Knauf, Fuhrmann and Rothstein, personal communication; Passow, unpublished observations). The pH dependence of $SO_4{}^{2-}$ and halide exchange is inverse[2,3]. On the other hand, the slowly and the rapidly penetrating anion species show the same unusually high temperature dependence (apparent activation enthalpies of about 32–35 kcal/mole per °K)[2,4]. Moreover, a variety of inhibitors were found to produce similar effects on $SO_4{}^{2-}$ and halide penetration[2,3].

It has recently been reported that the inhibition by phlorizin of $SO_4{}^{2-}$ equilibrium exchange in human red cell ghosts is asymmetric[5]. External phlorizin produces a strong inhibition while internal phlorizin does not. The present work deals with the "sidedness" of the effects of phlorizin on halide permeability. It was undertaken to compare further $SO_4{}^{2-}$ and halide transfer across the red blood cell membrane.

In principle, the experiments were performed as described previously[5]: 1 vol. of a

50% cell suspension in isotonic saline was hemolyzed at 0 °C with 10 vol. of a medium containing 4.0 mmoles/l $MgSO_4$ and 1.5 mmoles/l acetic acid; isotonicity was restored by the addition of sufficient NaCl and Tris–HCl to obtain a final concentration in the hemolysate of 140 and 25 mmoles/l, respectively, and a pH of 7.6. 5 min after reversion to isotonicity, the hemolysate was transferred to 37 °C and incubated at that temperature for 45 min. At the end of this resealing period, the leaky ghosts were separated from the resealed ghosts by centrifugation across a sucrose cushion as described by Bodemann and Passow[6]. The resealed ghosts were then collected on top of the cushion and washed three times at 0 °C. In experiments on $^{36}Cl^-$ equilibrium exchange, the washing medium contained 140 mmoles/l NaCl and 25 mmoles/l Tris–HCl, pH 7.6. In the experiments on I^- exchange, 5.0 mmoles/l of NaCl in this medium were replaced by an equivalent concentration of NaI. After washing, the ghosts were loaded with $^{36}Cl^-$ or $^{131}I^-$, respectively, by incubation for 30 min at 37 °C in radioactive suspension media. Subsequently, the ghosts were centrifuged down and the supernatant was removed. The sediment was cooled to 0 °C and mixed at that temperature with media of the same composition as that of the media in which they were previously loaded with radioactivity. This induced the release of radioactivity into the medium while the system was at Donnan equilibrium with respect to the nonradioactive anion species. The appearance of radioactivity in the medium was followed using the syringe technique of Wieth *et al.*[2] for rapid separation of ghosts and media. Rate constants were calculated by dividing the initial slopes of the curves by the initial radioactivity inside the ghosts.

TABLE I

EFFECTS OF INTERNAL AND EXTERNAL PHLORIZIN ON $^{36}Cl^-$ EQUILIBRIUM EXCHANGE IN RED CELL GHOSTS

In Expts I–III both internal and external media contained 140 mM NaCl, 20 mM Tris–HCl, pH 7.6. In Expts IV and V in place of NaCl, the ghosts contained KCl at a concentration of 140 mmoles/l. The medium had the same NaCl concentration of 140 mmoles/l as in the other experiments. Wherever phlorizin was present, the concentration was 2.0 mM. Temperature 0 °C.

	Rate constant (min^{-1}); expt No.				
	I	*II*	*III*	*IV*	*V*
No phlorizin	1.5	1.38	1.01	1.09	1.11
External phlorizin removed by washing prior to measurement*	1.56	1.38	1.10	1.10	1.14
Internal phlorizin	1.43	1.54	0.96	1.10	1.18
External phlorizin	0.15	0.09	0.097	0.048	0.055

*To show the reversibility of phlorizin binding to the outer cell surface.

TABLE II

EFFECTS OF INTERNAL AND EXTERNAL PHLORIZIN ON $^{131}I^-$ EQUILIBRIUM EXCHANGE IN RED CELL GHOSTS

In all experiments both internal and external media contained 140 mM NaCl and 20 mM Tris–HCl. In Expts I and II, 5.0 mM NaI was also present on both sides of the membrane. In Expt III, 5.0 mM NaI was only present inside the ghosts but not in the external medium. This latter experiment shows that the Cl^-/I^- exchange is affected by phlorizin similar to the I^-/I^- exchange. Wherever phlorizin was present, the concentration was 2.0 mM. Temperature 4 °C.

	Rate constant (min^{-1}); expt No.		
	I	*II*	*III*
No phlorizin	0.075	0.113	0.098
External phlorizin removed by washing prior to measurement	0.080	0.111	0.093
Internal phlorizin	0.071	0.080	0.071
External phlorizin	0.016	0.019	0.017
pH	7.35	7.70	7.35

Tables I and II show our results. External phlorizin at a concentration of 2.0 mmoles/l reduces the rate constant for Cl^- and I^- equilibrium exchange to about 10% or less of the original value. This inhibition is completely reversible. This is demonstrated in experiments in which, at the end of the resealing period, sufficient solid phlorizin was added to the hemolysate to establish a phlorizin concentration of 2.0 mmoles/l. After subsequent washing in phlorizin-free media, the rate constant assumed the control value observed in ghosts which had never been exposed to phlorizin. Incorporated phlorizin had no detectable effect on $^{36}Cl^-$ exchange and a small inhibitory effect on $^{131}I^-$ exchange. Control experiments confirmed the earlier finding that internal phlorizin blocks sugar transport (Table III). These control experiments demonstrate again that the incorporated phlorizin has free access to the internal surface of the ghost membrane. Previous analytical determinations of the incorporated phlorizin showed that the incorporated agent is trapped inside the ghosts at the same concentration at which it was present in the hemolysate, *i.e.* at a concentration of 2.0 mmoles/l (ref. 5).

Our findings indicate that inspite of certain characteristic differences of the kinetics of $SO_4{}^{2-}$ and halide equilibrium exchange, the asymmetry of inhibition by phlorizin can be demonstrated for halides as well as for $SO_4{}^{2-}$ (ref. 5). Together with the observations described in the introduction this may be taken as further evidence for the assumption that $SO_4{}^{2-}$ and the halides share at least parts of the same penetration pathway.

It is not possible to provide an unequivocal explanation for the asymmetry of the

TABLE III

EFFECTS OF INTERNAL AND EXTERNAL PHLORIZIN (2.0 mM) ON EQUILIBRIUM EXCHANGE OF ^{14}C-LABELED HEXOSES IN HUMAN RED CELL GHOSTS

The sugars were present at a concentration of 10 mM on both sides of the membrane. Otherwise the experimental conditions were identical with those under which the Cl^- equilibrium exchange was measured. The experiments with arabinose and xylose were performed at 10 and 0 °C, respectively.

	Rate constant (min^{-1} x 10^{-2}); sugar	
	L-Arabinose	*D-Xylose*
No phlorizin	2.70	2.2
External phlorizin removed by washing prior to measurement	–	2.05
Internal phlorizin	0.41	0.40
External phlorizin	0.56	0.65

action of phlorizin. The sidedness of the effect strongly suggests that the agent does not exert its action by direct combination with a mobile anion carrier. However, it cannot be excluded that a mobile carrier has different affinities for phlorizin in the two membrane surfaces and hence may be able to combine with phlorizin on the outer surface but not on the inner surface. Nevertheless, it seems much more likely that phlorizin produces its effect by combination with immobile binding sites in the outer membrane surface. No ambiguous conclusions can be drawn about the mechanism by which phlorizin binds to such immobile binding sites in the outer membrane surface inhibiting anion exchange. The combination with such binding sites may either alter the access of the diffusible anions to an anion channel or affect the rate of loading or unloading of a phlorizin-insensitive anion carrier. Moreover, it remains open whether or not the fixed phlorizin-binding sites play a role in anion exchange in the absence of phlorizin.

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