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Conduction and Blocking Properties of a Predominantly Anion-Selective Channel from Human Platelet Surface Membrane Reconstituted into Planar Phospholipid Bilayers

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Summary. We have investigated the basic properties of a predominantly anion-selective channel derived from highly purified human platelet surface membrane. Single channels have been reconstituted into planar phospholipid bilayers by fusion of membrane vesicles and recorded under voltage-clamp conditions.

The channel is found to have the following properties: (i) Channel activity occurs in bursts of openings separated by long closed periods. (ii) The current-voltage relationship is nonlinear. Channel current is seen to rectify, with less current flowing at positive than at negative voltages. Rectification may be due to asymmetric block by HEPES/Tris buffers. In 450 mM KCl, 5 mM HEPES/Tris, pH 7.2, the single channel conductance at −40 mV is ~160 pS and at +40 mV is ~90 pS. (iii) The conductance-concentration relationship follows a simple saturation curve. Half maximal conductance is achieved at a concentration of ~1000 mM KCl, and the curve saturates at a conductance of ~500 pS. (iv) Reversal potentials interpreted in terms of the Goldman-Hodgkin-Katz equation indicate a Cl : K permeability ratio of 4 : 1. (v) The channel accepts all of the halides as well as a number of other anions. The following sequence of relative anion permeabilities (in the presence of K^+) is obtained: $F^- <$ acetate$^-$ $<$ gluconate$^-$ $< Cl^- < Br^- < I^- < NO_3^- < SCN^-$. (vi) Cations as large as TEA^+ are permeant. (vii) Current through the channel is blocked in the presence of DIDS, SITS and ATP, but not by Zn^{2+}.

Key Words platelet surface membrane · lipid bilayer · anion channel · ion selectivity · block

Introduction

It is well established that anion and cation fluxes across platelet membranes play an important role in maintaining homeostasis and in the triggering of metabolic and functional responses initiated by agonists and other platelet activating agents. Despite the obvious importance of these fluxes in platelets, our present understanding of their location, selectivity, rates and magnitudes is somewhat limited. A major reason for this has been the technical difficulties encountered in applying conventional electrophysiological techniques to such small cells (2–4 μm diameter). Microelectrode impalement of platelets has not been reported, and only one paper describing the successful patch clamping of platelets has been published to date (Maruyama, 1987). In this, whole cell currents were measured and some single channel data obtained using the cell-attached variation. A voltage-gated K^+ channel with a conductance of 9 pS was resolved.

Our approach to the study of ion channels in human platelets utilizes a well-characterized method for separating highly purified surface and intracellular membrane vesicles free from cross contamination using continuous flow electrophoresis (Menashi, Weintroub & Crawford, 1981). In this paper we report the reconstitution of a predominantly anion-selective channel from the surface membrane into preformed planar phospholipid bilayers and the basic characterization of its conduction and blocking properties.

The initial identification of this channel was communicated to the Physiological Society (Semba & Williams, 1986).

Materials and Methods

Preparation of Platelet Membranes

The method for preparing highly purified human platelet membranes essentially followed the procedures reported by Menashi et al. (1981). Human platelets were isolated by a differential-centrifugation procedure from fresh buffy-coat residue packs supplied by the Blood Transfusion Service Laboratories, Tooting, London SW17, UK. Laboratory processing commenced within 3–4 hr of blood donation.

The buffy coats were centrifuged at 200 × *g* for 20 min, and the platelet-rich plasma (PRP) was collected and pH adjusted to 6.4 using citric acid. The platelets were then sedimented at 1,200 × *g* for 20 min, resuspended in 10 mM HEPES[1] buffer, pH 6.2, containing 152 mM NaCl, 4.17 mM KCl, 3 mM EDTA, and incubated at 37°C for 5 min. The suspension was then treated with neuraminidase (Sigma Grade X, affinity purified) at a concentration of 0.05 unit/ml for 20 min at 37°C. Treatment with neuraminidase at the whole cell level lowered the electronegativity of the surface membrane by removal of sialic acid groups, thus reducing its electrophoretic mobility. At the end of the incubation period the suspension was rapidly diluted 1:4 with 10 mM HEPES buffer (washing buffer), pH 7.2, containing 152 mM NaCl, 4.17 mM KCl, 3 mM EDTA and centrifuged at 1,200 × *g* for 15 min. The platelets were washed twice by resuspension in the washing buffer and finally resuspended in ice-cold 10 mM HEPES buffer (sonication buffer), pH 7.2, containing 0.3 M sorbitol at a ratio of 4 ml buffer/g wet wt of cells. A protease-inhibitor cocktail containing 5 μg pepstatin A/ml, 10 μg soybean Trypsin inhibitor/ml and 400 μM phenylmethane-sulfonyl fluoride was added to the cell suspension. The sample was then sonicated at setting 6 for 10 sec at 4°C using a Dawe sonicator with a 4 mm diameter probe. The sonicate was centrifuged at 2,000 × *g* for 10 min at 4°C, the supernatant retained on ice and the pellet resuspended in sonication buffer. The sonication and centrifugation steps were carried out three times in all. The resultant supernatants were combined and layered onto a linear sorbitol gradient prepared from 1.0 and 3.5 M sorbitol solutions, pH 7.2, containing 10 mM HEPES and centrifuged at 42,000 × *g* for 90 min at 4°C. A mixed membrane (MM) fraction, containing both surface and intracellular membrane vesicles, was located in the low-density region of the gradient. The fraction was removed and concentrated by centrifugation at 90,000 × *g* for 80 min at 4°C. Membranes were either (i) resuspended in 0.4 M sucrose, 5 mM HEPES, pH 7.2, at a concentration of approximately 1–2 mg protein/ml for subsequent use in reconstitution experiments, or (ii) resuspended in electrophoresis chamber buffer (10 mM triethanolamine, 0.4 M sorbitol, adjusted to pH 6.8 with acetic acid and then to pH 7.2 with NaOH) to a concentration of approximately 3–4 mg protein/ml. The latter MM fraction was applied to the chamber of a Bender Hobein VAP22 continuous-flow electrophoresis unit operating at 160 V/cm, 80 mA with buffer flow conditions as described by Menashi et al. (1981). Two vesicle subpopulations were resolved; the more electronegative fraction represented intracellular membranes (IM), and the least electronegative peak represented surface membrane (SM) vesicles (modified by neuraminidase treatment). Separately pooled IM and SM fractions were centrifuged at 90,000 × *g* for 1 hr at 4°C and resuspended in 0.4 M sucrose, 5 mM HEPES, pH 7.2, to a protein concentration of 1–2 mg/ml.

In the experiments reported here vesicles from the mixed membrane (MM) (both neuraminidase treated and untreated) and surface membrane (SM) fractions were used. Aliquots were frozen in liquid nitrogen and stored at −80°C.

[1] *Abbreviations:* HEPES, N-2-hydroxyethylpiperasine-N′-2-ethanesulfonic acid; EDTA, ethylenediaminetetra-acetic acid; Tris, tris(hydroxymethyl)aminomethane; TEA, tetraethylammonium; DIDS, 4,4′-diisothiocyanostilbene-2-2′-disulfonic acid; SITS, 4-acetamido-4′-isothiocyanotostilbene-2,2′-disulfonic acid.

Measurement of Single-Channel Activity in Planar Phospholipid Bilayers

Planar bilayers were formed by the 'painting' technique of Mueller and Rudin (1969) using suspensions of 30 mM phospholipid (70% phosphatidylethanolamine, 30% phosphatidylserine, supplied by Avanti Polar Lipids, Birmingham, AL) in decane. A bilayer was formed over a 0.5 mm diameter hole separating two chambers containing aqueous solutions. Electrical connection was made to the chamber solutions via 2% agar glass bridges containing 3 M KCl, and Ag/AgCl electrodes. High ionic strength salt bridges were used to minimize errors in the measurement of reversal potentials under mixed ion conditions (Cukierman, Yellen & Miller, 1985). Platelet membrane vesicles were always added to the chamber, designated *cis*, which could be clamped at a range of holding potentials. The other chamber, *trans*, was held at ground potential. The clamp voltage was provided by a microcomputer linked to a digital-to-analog converter. The current-to-voltage converter for measuring current through the bilayer used a feedback resistor of 10 GΩ (Miller, 1982). The current signal was displayed on an oscilloscope and stored on FM tape for subsequent analysis. The channel traces shown as illustrations (Figs. 1, 2*A*, 7*B*, 8, and 9) were obtained by replaying recorded data onto a hot-pen recorder (Ormed MX216).

Platelet membrane vesicles were incorporated into preformed planar bilayers by fusion. The conditions applied during these experiments were those generally reported by others to promote membrane vesicle fusion (Hanke, 1986), in particular, the presence of negatively charged phospholipids in the bilayer (phosphatidylserine was used throughout), an osmotic gradient across the bilayer and the presence of divalent cations in the *cis* chamber (to which membrane vesicles were added). Fusion conditions were as follows: *cis*—450 mM KCl, 1.0 mM $CaCl_2$, 5 mM HEPES/Tris, pH 7.2; *trans*—150 mM KCl, 5 mM HEPES/Tris, pH 7.2. All solutions used in the experiments reported here were filtered through Millipore membranes (0.45 μm pore size) prior to use. The *cis* chamber solution was mechanically agitated with a magnetic stirrer to increase the rate of fusion of vesicles with the bilayer. 5–10 μl of platelet MM or SM vesicle suspension were added to the *cis* chamber, and fusion usually occurred within 5–10 min. Unfused vesicles were perfused out using a peristaltic pump and ionic conditions in the two chambers altered as required. All experiments were carried out at room temperature (~22°C).

Current-voltage relationships were constructed by applying potentials at 10 mV intervals from +70 to −70 mV. For those experiments carried out in asymmetric solutions reversal potentials (E_{rev}) were measured to the nearest mV, correcting for electrode offset potential (range 1–3 mV) in each case. Anion/cation (P_{Cl}/P_K) selectivity ratios and anion/anion (P_A/P_{Cl}) permeability ratios were calculated according to the Goldman-Hodgkin-Katz (GHK) voltage equation.

Results

Channel Reconstitution into Planar Bilayers

A predominantly anion-selective channel is observed following fusion of vesicles from the mixed membrane fraction (either neuraminidase treated or

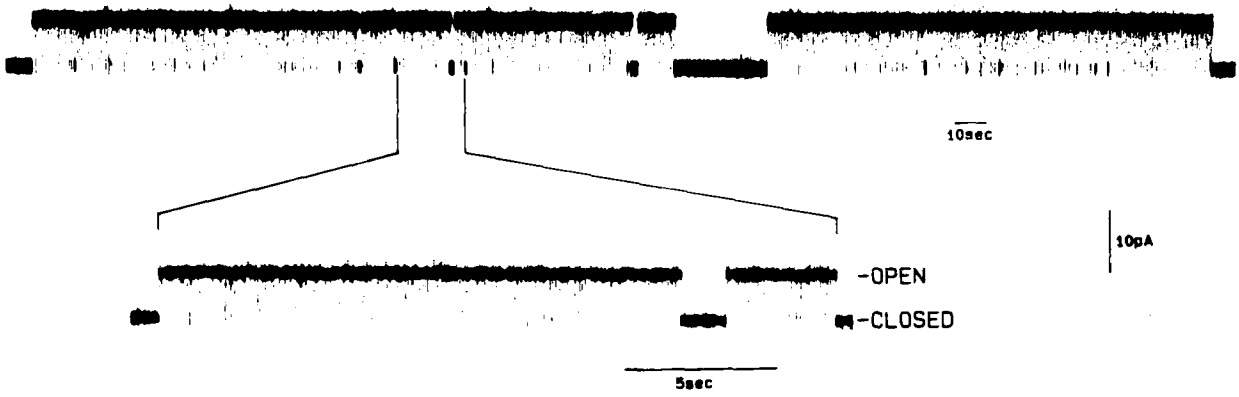

Fig. 1. A single channel from platelet surface membrane reconstituted into a planar phospholipid bilayer as described in Materials and Methods. The recording was made at −40 mV in symmetrical 450 mM KCl, 5 mM HEPES/Tris, pH 7.2, and is shown low-pass filtered at 500 Hz. An upwards deflection represents the channel opening. An expanded time scale trace is displayed (lower trace) to show the rapid closing events in more detail (note the different time calibrations for the two traces)

untreated) and the surface membrane fraction. Fusion of vesicles from the inner membrane fraction does not yield the channel. As far as we can ascertain from our experiments, the channel exhibits both qualitatively and quantitatively comparable characteristics when either MM or SM fractions are used. From this we surmise that (i) the channel protein originates from the platelet surface membrane and (ii) its properties are preserved following neuraminidase treatment and membrane purification by high voltage continuous-flow electrophoresis. The term SM channel is used throughout.

Figures 1 and 2*A* show representative recordings following incorporation of the SM channel into a planar phospholipid bilayer. Channels are bathed in symmetrical 450 mM KCl, 5 mM HEPES/Tris, pH 7.2, and recorded at the holding potentials indicated. The lower trace in Fig. 1 shows channel fluctuations in more detail at higher time resolution. The channel shows characteristic bursting behavior in which comparatively long 'silent' periods alternate with 'active' periods.

Figure 2*B* shows the relationship between single-channel current and holding potential over the range ± 70 mV in symmetrical 150 and 450 mM KCl solutions (buffered with 5 mM HEPES/Tris to pH 7.2). The current-voltage relationship is nonlinear. Channel current is seen to rectify with less current flowing at positive than negative holding potentials. In symmetrical 450 mM KCl a conductance of 159.4 ± 20 pS (mean ± SD, $n = 12$) is obtained at −40 mV and a conductance of 92.6 ± 19.7 pS (mean ± SD, $n = 12$) at +40 mV.

The dependence of the single channel conductance on the salt concentration is shown in Fig. 3. Each point represents the mean conductance (± SD, $n = 4$ to 12 determinations) at −40 mV at a given KCl concentration. The data could be fitted with a simple Michaelis-Menten type saturation curve. Half-maximal conductance is achieved at a concentration of 1008 mM KCl, and the curve saturates at a conductance of 503 pS.

Single Channel Rectification

The effect of the buffering compounds (HEPES and Tris) on the degree of single channel rectification was investigated. Figure 4*A* shows the current-voltage relationship for the SM channel exposed to symmetrical 450 mM KCl buffered with either 5 mM HEPES/Tris, pH 7.2 ('standard' conditions), 0.5 mM HEPES/KOH, pH 7.2 ('reduced HEPES') or 5 mM HEPES/KOH, pH 7.2 ('no Tris'). In the absence of Tris, rectification appears slightly less than that obtained in 'standard' conditions (the HEPES concentration being the same in both cases). However, when the concentration of HEPES buffer is reduced tenfold, from 5 to 0.5 mM, current at positive holding potentials increases significantly and rectification is considerably reduced. In Fig. 4*B*, the mean conductance (G) measured at each holding potential under 'reduced HEPES' conditions is taken as $G_{O_{app}}$. The ratio $G/G_{O_{app}}$ is plotted with G values from either 'no Tris' conditions (▽) or 'standard' conditions (△). In both of these conditions the HEPES concentration is the same (5 mM), and in both cases blocking of single channel conductance is apparent. The degree of block is seen to increase as the holding potential is made more positive. With

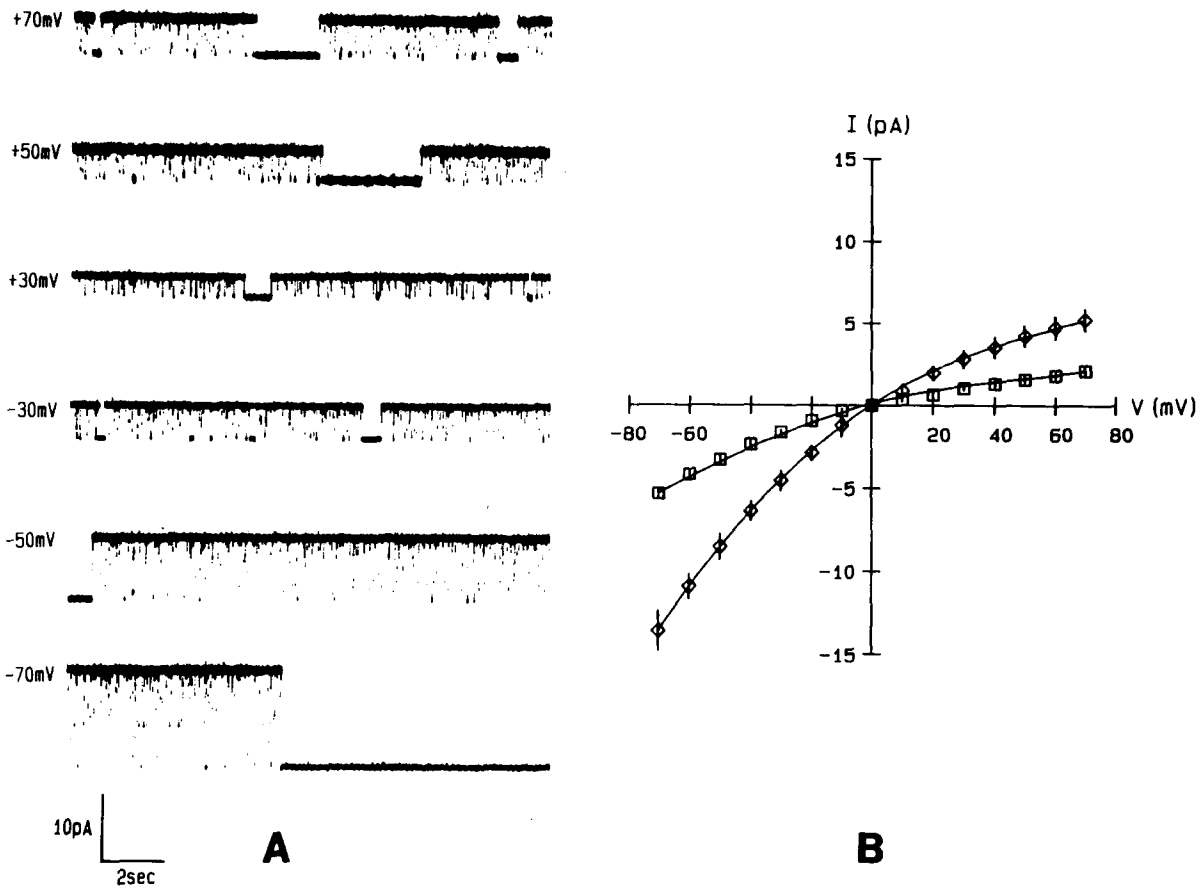

Fig. 2. (*A*) Platelet SM channel fluctuations at the holding potentials indicated on the left. 450 mM KCl, 5 mM HEPES/Tris, pH 7.2, *cis* and *trans*. The recording was low-pass filtered at 250 Hz. Traces at negative holding potentials have been inverted so that all channel openings are shown as upwards deflections. (*B*) Single-channel current-voltage relationship in symmetrical solutions of (□) 150 mM and (◇) 450 mM KCl, 5 mM HEPES/Tris, pH 7.2. Each point represents the mean ± SD of 12 separate determinations. Curves were drawn by eye

the added presence of Tris buffer ('standard' conditions), the apparent block is increased.

Anion *vs.* Cation Selectivity

Reversal potential measurements determined in the presence of asymmetric KCl solutions show that although the channel is preferentially selective for Cl^-, K^+ is also permeant.

Figure 5 (△) shows the single-channel current flow over a range of holding potentials with a 3 : 1 KCl gradient across the bilayer (450 mM KCl, 5 mM HEPES/Tris, pH 7.2 *cis* : 150 mM KCl, 5 mM HEPES/Tris, pH 7.2 *trans*). The measured reversal potential, +16 mV, deviates from that calculated from the Nernst equation for an ideally anion-selective channel under these conditions (i.e., +28 mV). The deviation from the Nernst potential does not vary significantly in size upon reversing the gradient across the bilayer. Figure 5 (▽) shows the single-channel current-voltage relationship with a 1 : 3 KCl gradient *cis* : *trans* (150 mM KCl *cis* : 450 mM KCl *trans*). The channel appears to show no significant asymmetry in cation/anion discrimination. Figure 5 (□) shows the current-voltage relationship for experiments carried out with an increased KCl gradient of 5 : 1 *cis* : *trans* (750 mM KCl *cis* : 150 mM KCl *trans*). There is a shift in the measured reversal potential to +22 mV. Under these conditions, the Nernst equation predicts a reversal potential of +41 mV for an ideally selective Cl^- channel. The results of all three reversal potential measurements are consistent with an anion/cation selectivity ratio of 4 : 1, as calculated from an appropriate version of the GHK voltage equation (*see* figure legend). The current measured in these experiments is therefore the resultant of the movement of both Cl^- and K^+ through the channel.

Relative Anion Permeabilities and Conductances

The relative permeabilities of various anions compared to Cl^- were estimated from reversal potential

measurements with 450 mM KCl in the *trans* chamber and either 450 mM KF, KBr, KI, KNO_3, KSCN, K-acetate or K-gluconate in the *cis* chamber. All solutions were buffered to pH 7.2 with 5 mM HEPES/Tris. Figure 6 shows three of the current-voltage curves obtained from these experiments. The measured reversal potential for F^- is negative (−20 mV), indicating that F^- is less permeant than Cl^-. The reversal potentials for Br^- and SCN^- are positive (+10 and +20 mV, respectively), indicating that they are accepted by the channel in preference to Cl^-.

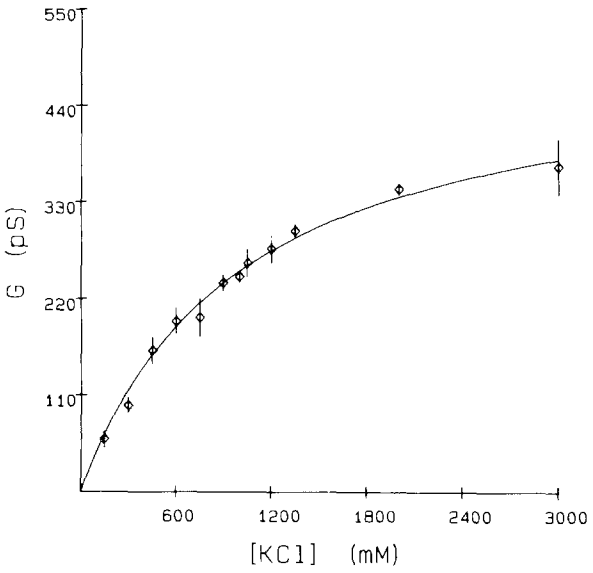

Fig. 3. Dependence of single channel conductance (G) on the KCl concentration. All values were measured at −40 mV and points represent the mean ± SD of 4 to 12 separate determinations at each concentration. The curve was drawn according to the equation $G/G_{max} = aK(aK + K_d)$, where G_{max} = 503 pS and K_d = 1008 mM

The Table summarizes the data from the anion permeability experiments in terms of permeability ratios. The sequence of relative anion permeabilities (in the presence of K^+) is as follows: $F^- <$ acetate$^-$ < gluconate$^-$ < $Cl^- < Br^- < I^- < NO_3^- < SCN^-$.

The conductance at −40 mV of the above anions (except acetate) was determined in a separate series of experiments. The mean conductance values (± SD) are shown in the Table. Channels were bathed in symmetrical 450 mM KA (where A is the test anion), 5 mM HEPES/Tris, pH 7.2. Among the monovalent anions, nitrate has the highest conductance (198 ± 10 pS, n = 4) and gluconate the lowest (47 ± 4 pS, n = 3).

Cation Conductance

An inert, nonpermeant cation was sought to allow the measurement of anion current alone. An obvious candidate, tetraethylammonium, TEA^+, was

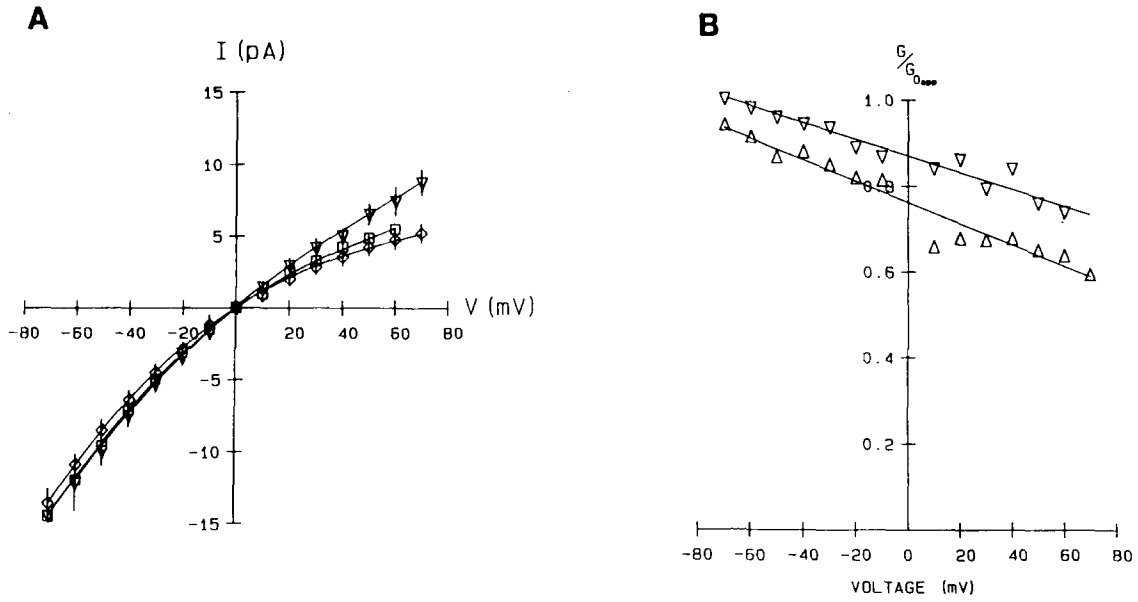

Fig. 4. The effect of HEPES/Tris buffers on single channel current. (*A*) Current-voltage relationships for single channels bathed in symmetrical 450 mM KCl and buffered as follows: (▽) 0.5 mM HEPES/KOH, pH 7.2 ('reduced HEPES'); (□) 5 mM HEPES/KOH, pH 7.2 ('no Tris'), or (◇) 5 mM HEPES/Tris, pH 7.2 ('standard' conditions, as for Fig. 2*B*). Each point represents the mean ± SD for four to 12 separate determinations. Curves were drawn by eye. (*B*) Relationship between $G/G_{\infty app}$ and voltage for the experiments shown in *A*. Experimental points are the ratios between mean conductances measured in the presence of (▽) 450 mM KCl, 5 mM HEPES/KOH, pH 7.2 ('no Tris'), or (△) 450 mM KCl, 5 mM HEPES/Tris, pH 7.2 ('standard' conditions). In both cases $G_{\infty app}$ values were measured in the presence of 450 mM KCl, 0.5 mM HEPES/KOH, pH 7.2 ('reduced HEPES'). Lines were drawn by eye

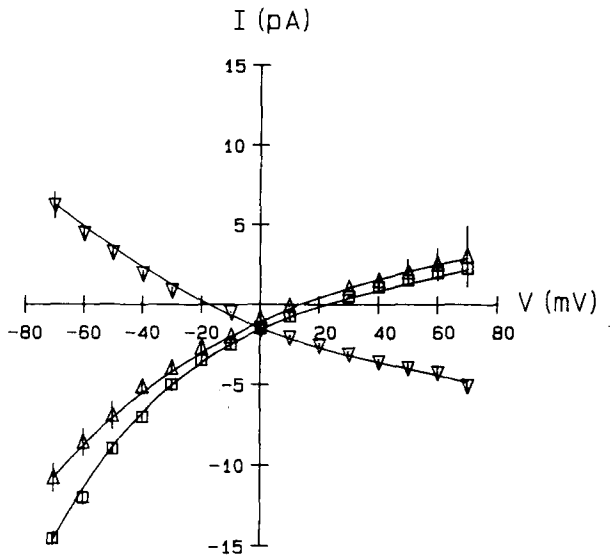

Fig. 5. Test for anion/cation selectivity. Single channel current-voltage relationships under the following conditions: [KCl] *cis*: [KCl] *trans*—(△) 450:150 mM; (▽) 150:450 mM; (□) 750:150 mM. All solutions were buffered with 5 mM HEPES/Tris to pH 7.2. Each point represents the mean ± SD for three to 10 separate determinations under each experimental condition. Curves were drawn by eye. The reversal potential, E_{rev} for a 3:1 KCl gradient (△) is measured as +16 mV and for a 5:1 KCl gradient (□) as +22 mV. The anion/cation selectivity ratio is calculated from $P_{Cl}/P_K = [(n.\exp(E_{rev}/k) - 1)/(n - \exp(E_{rev}/k))]$, where $k = RT/F$ and n is the *cis*:*trans* concentration ratio. P_{Cl}/P_K for these experiments is 4

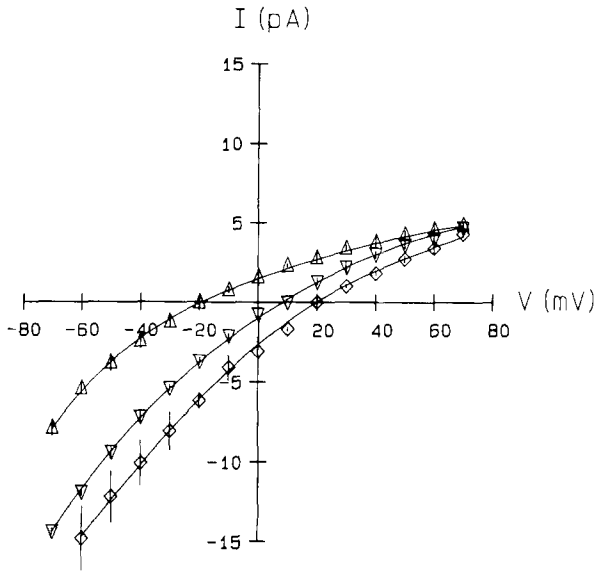

Fig. 6. Comparison of anion permeabilities. Single channel current-voltage relationships with 450 mM KCl in the *trans* chamber, and either (△) 450 mM KF, (▽) 450 mM KBr or (◇) 450 mM KSCN in the *cis* chamber. All solutions were buffered with 5 mM HEPES/Tris to pH 7.2. Each point represents the mean ± SD for five to eight separate determinations under each experimental condition. Curves were drawn by eye

Table. Reversal potential shifts, E_{rev}, are expressed with respect to control KCl solution[a]

Test anion	E_{rev} (mV)	$P_A/P_{Cl}(n)$	G (pS)	(n)
Fluoride	−20	0.32 (8)	77 ± 12	(4)
Acetate	−17	0.35 (5)	—	
Gluconate	−16	—[b] (10)	47 ± 4	(3)
Chloride	0	1.00 (−)	159 ± 20	(12)
Bromide	+10	1.58 (7)	175 ± 6	(4)
Iodide	+13	1.76 (4)	154 ± 20	(3)
Nitrate	+14	2.23 (8)	198 ± 10	(4)
Thiocyanate	+20	2.48 (5)	189 ± 12	(4)

[a] In all experiments the *trans* chamber contained the control 450 mM KCl solution; the *cis* chamber contained the K salt of the test anion, A, at the same concentration. E_{rev} represents mean values obtained from four to 10 experiments in each case. Permeability ratios are calculated from (Franciolini & Nonner, 1987); $E_{rev} = RT/F.\ln[(P_K a_K + P_{Cl} a_{Cl})]$, where a_K, a_A and a_{Cl} are the activities of K^+, A^- and Cl^-, respectively (taken from Robinson & Stokes, 1959). P_K/P_{Cl} is taken as 0.25 (*see* Fig. 5) and is considered to be independent of the test anion.

[b] No activity value was available for gluconate.

Single-channel conductance, G, at −40 mV for channels bathed in symmetrical 450 mM KA, 5 mM HEPES/Tris, pH 7.2, where A denotes the test anion. Mean conductance values (from 3–12 experiments) and standard deviations are shown.

tested but found to be permeant. Figure 7*A* shows the current-voltage relationships with a 3:1 KBr and a 3:1 TEABr gradient across the bilayer (*cis*, 450 mM KBr: *trans*, 150 mM KBr or *cis*, 450 mM TEABr: *trans*, 150 mM TEABr). From the reversal potential measurement in asymmetric KBr (+13 mV), the K:Br ratio is calculated as 1:3. In asymmetric TEABr, there is less apparent rectification and the current reverses at 0 mV, indicating that TEA^+ and Br^- are equally permeant. The single channel records shown in Fig. 7*B* illustrate an alteration in gating observed in asymmetric TEABr solutions compared with that observed in KBr. In TEABr the channel remains predominantly in the open state with only occasional brief closures.

Channel Block by DIDS, SITS and ATP

Agents which have been used as inhibitors of Cl^- transport proteins were applied to the SM channel; namely, DIDS and SITS (Cabantchik & Rothstein, 1972, 1974; Russell & Brodwick, 1979; Miller & White, 1984; Nelson, Tang & Palmer, 1984; Hanrahan, Alles & Lewis, 1985) and zinc (Woodbury & Miles, 1973; Franciolini & Nonner, 1987). Figure 8 shows current fluctuations from channels at holding potentials of −40 and +40 mV, recorded after symmetrical application of 100 μM DIDS or SITS.

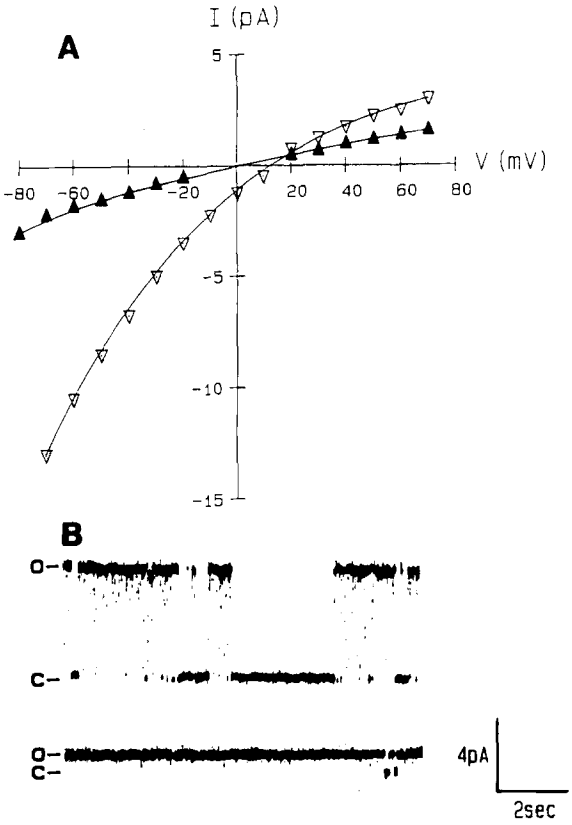

Fig. 7. (*A*) Single channel current-voltage relationships under the following conditions: *cis* : *trans*—(▽), 450 : 150 mM KBr; (▲) 450 : 150 mM TEABr. All solutions were buffered with 5 mM HEPES/Tris to pH 7.2. Each point represents the mean of three separate determinations. Sample standard deviations are contained within the symbols. Curves were drawn by eye. (*B*) Single-channel records from the above experiments at −40 mV in asymmetric KBr (upper trace) and TEABr (lower trace). Recordings are low-pass filtered at 250 Hz. An upwards deflection represents channel opening

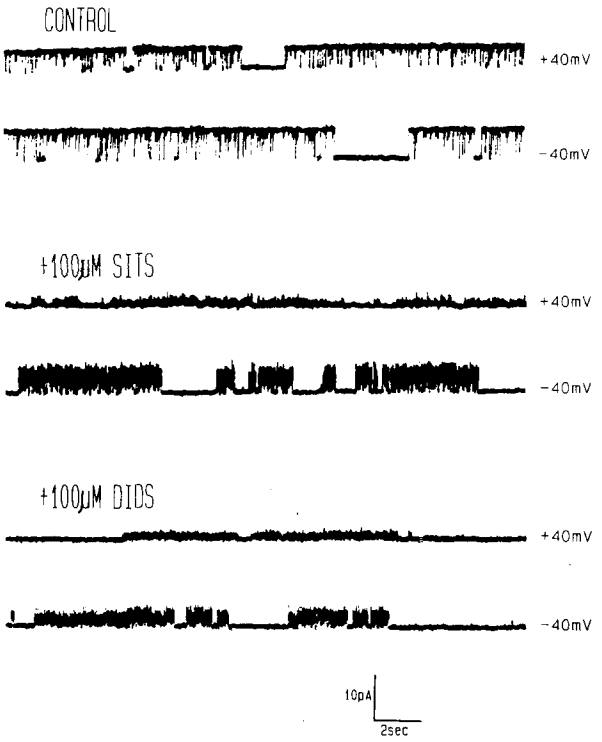

Fig. 8. The effect of DIDS and SITS on the platelet SM channel. All recordings were made in symmetrical 450 mM KCl, 5 mM HEPES/Tris, pH 7.2, with 100 μM SITS or 100 μM DIDS added symmetrically as shown. Current traces are shown low-pass filtered at 150 Hz at +40 and −40 mV. Traces at negative holding potentials have been inverted so that all channel openings are shown as upwards deflections

Openings at positive voltages are poorly resolved, and at negative voltages openings are noisy and burst durations appear to be shortened. At both positive and negative holding potentials the single channel conductance is reduced. The addition of up to 2 mM zinc symmetrically has no effect on the activity of the SM channel (*data not shown*).

The action of ATP on the SM channel was also investigated. Figure 9 shows the effect of 1, 3, and 5 mM ATP added to both *cis* and *trans* faces of a SM channel. In this case the rapid flickering block is seen only at negative holding potentials (−10 to −70 mV) and appears to increase as the ATP concentration is raised. No effect is seen at positive potentials (+10 to +70 mV). Thus ATP appears to be effective only from the *cis* face of the channel. The ATP blocking effect is reversed when ATP is removed by perfusion (*data not shown*).

Discussion

We report the basic characterization of a predominantly anion-selective channel found in the surface membrane of human platelets. The majority of fusion events incorporate a single active channel into the bilayer. Using the criterion of the direction of rectification of the current-voltage relationship in symmetrical KCl solutions, it is found that approximately 98% of insertions result in the channel being orientated in the same direction in the bilayer. No information is available concerning the initial orientation of vesicles formed following membrane separation and purification.

Channel activity occurs in characteristic bursts of openings (Fig. 1) often lasting several tens of seconds. Bursts are separated by long closed periods, and closed intervals between bursts lasting >5 min have been recorded. No subconductance states have been observed. A detailed analysis of the gating kinetics of this channel will be presented in a subsequent paper.

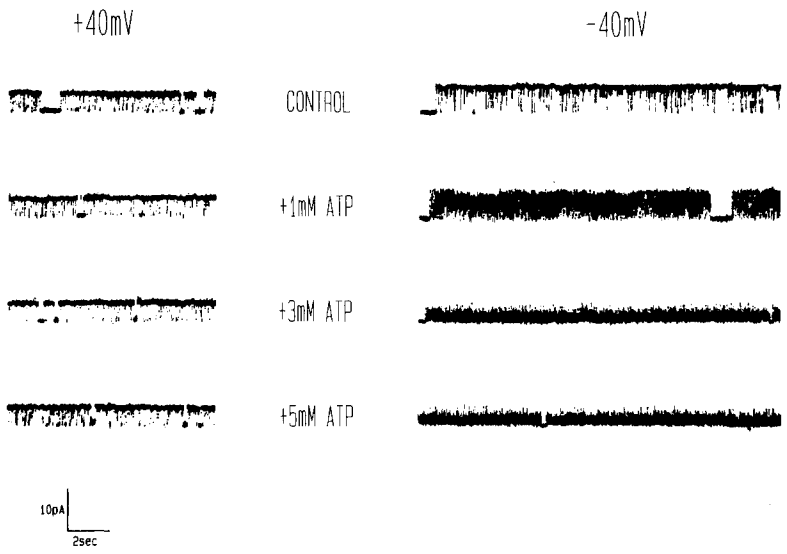

Fig. 9. The effect of ATP on the platelet SM channel. Current traces shown are from a single experiment in which ATP was added to both *cis* and *trans* faces of a channel bathed in symmetrical 450 mM KCl, 5 mM HEPES/Tris, pH 7.2, to give the final ATP concentrations indicated. Recordings are shown low-pass filtered at 150 Hz at +40 and −40 mV. Traces at −40 mV have been inverted so that all channel openings are shown as upwards deflections

Single Channel Rectification

We observe single channel current rectification under all ionic conditions applied to the channel, with more current flowing at negative holding potentials. This behavior was investigated by examining whether the presence of HEPES or Tris in our solutions was related to the degree of rectification (Fig. 4). It has been demonstrated by others that some anionic buffering compounds (including HEPES) may block chloride channels when applied in mM concentrations, e.g. in cultured *Drosophila* neurones (Yamamoto & Suzuki, 1987) and smooth muscle cells (Soejima & Kokubun, 1988). Tris is also known to compete for cationic binding sites in *Aplysia* neurones (Ascher, Marty & Neild, 1978).

From the results in Fig. 4, we surmise that the rectification observed in our experiments is caused, for the most part, by the asymmetric blocking of current flow. HEPES blocks in a voltage-dependent manner, mainly from the *trans* face of the channel (i.e., at positive voltages) with Tris buffer also blocking, though to a lesser degree and presumably mainly from the *cis* face.

Conductance and Selectivity

The SM channel has a relatively high conductance: approx. 160 pS in 450 mM KCl at −40 mV. With increasing KCl concentration, the single channel conductance increases to a saturating value of approx. 500 pS. The conductance-concentration relationship follows a simple rectangular hyperbola expected for Michaelis-Menten kinetics (Fig. 3). There is no deviation from this relationship at a conductance of three times the half-maximal conductance (approx. 1000 mM). Behavior of this type is typically, but not exclusively, found in channels that can be occupied by a single ion at a time (Läuger, 1973) and suggests the presence of a saturatable binding site within the channel. This is unlike the situation in VDAC, a large conductance nonselective channel in the mitochondrial outer membrane, where no saturation of conductance occurs at up to 4 M KCl (Colombini, 1986) and ions move through the channel under electrostatic influences rather than via ion-binding sites.

Reversal potential measurements in asymmetric KCl solutions indicate that both K^+ and Cl^- permeate the channel (Fig. 5). Several other 'anion' channels have been shown to have similar significant cation permeabilities. Blatz and Magleby (1985) found a small conductance anion channel in myotubules with a Cl : K permeability ratio of 5 : 1. Franciolini and Nonner (1987) measured a Cl : Na permeability ratio of 5 : 1 for a channel in hippocampal neurons. Woll et al. (1987) report an estimated Cl : Na ratio of 3.5 : 1 for a high conductance channel in skeletal muscle. The mechanism of ion permeation through channels of this type may be complex. Franciolini and Nonner (1987) have presented the only attempt to describe a possible mechanism. They postulate that anions and cations interact while passing through the hippocampal neuronal channel, and that cations will pass only when a permeant anion is present. This mechanism was tested in their experiments by replacing Cl^- with SO_4^{2-}, which is impermeant, and observing no cation current through the channel. We have no evidence for this sort of mechanism occurring in the platelet SM channel since all anions tested so far have been permeant, including SO_4^{2-} (*results not shown*). Cations as large as TEA^+ also pass through the SM channel

(Fig. 7), implying that the channel has a very large cross-sectional area, at least 37 Å^2 (Coronado & Miller, 1982). TEA^+ is found to be as permeant as Br^- from experiments in asymmetric TEABr (Fig. 7*A*), although the single channel conductance is considerably lower than in the presence of the same asymmetric concentrations of KBr. In TEABr the duration of channel openings is markedly increased (Fig. 7*B*). Similar low conductance and prolonged openings have been reported for Cs^+ conductance in the skeletal muscle sarcoplasmic reticulum K^+ channel (Cukierman et al., 1985).

Reversal potential measurements indicate that the relative permeability sequence for halide anions in the SM channel (*see* Table) corresponds to Eisenman's (1965) first halide anion sequence. The same sequence has been observed in Cl^- channels from the Schwann cell (Gray, Bevan & Ritchie, 1984), vascular smooth muscle (Soejima & Kokubun, 1988) and pulmonary epithelial cells (Schneider et al., 1985). The relative permeability sequence for all anions tested is approximately within the range of sequences described as lyotrophic (*see* Dani, Sanchez & Hille, 1983).

Channel Block by DIDS, SITS, and ATP

In addition to asymmetric block by HEPES acting from the *trans* face of the channel, we have observed block in SM channels by a number of other agents previously reported to block anion channels. The reactive disulfonic acid stilbenes (DIDS and SITS) are found to block from both sides of the channel when applied symmetrically (Fig. 8). These agents are known to block some Cl^- channels including the low conductance channels of *Torpedo* electroplax and rabbit urinary bladder (Miller & White, 1984; Hanrahan et al., 1985) and the large conductance channels of A6 epithelial cells and urinary bladder (Nelson et al., 1984; Hanrahan et al., 1985). Block in the SM channel is characterized by a series of small noisy openings at positive voltages and shortened bursts containing rapid flickering events at negative voltages. This behavior is unlike the reported action of these agents on other anion channels; for example, DIDS is seen to eliminate all channel openings in the *Torpedo* electroplax and rabbit urinary bladder channels (Miller & White, 1984; Hanrahan et al., 1985).

Zinc has been shown to block Cl^- currents in other preparations (Woodbury & Miles, 1973; Franciolini & Nonner, 1987). However, the SM channel is unaffected by the presence of up to 2 mM zinc applied to both *cis* and *trans* solutions.

The effect of ATP on the channel was investigated since Maruyama (1987) applied mM ATP to the pipette solution while patch clamping whole platelets to improve seal formation. No reference is made in the Maruyama paper to a Cl^- channel, and we considered the possibility that it may have been blocked in his experiments. ATP has recently been reported to induce a flickery channel block in a nonselective channel in neonatal rat central neurones (Ashford et al., 1988). The effect of ATP on our channel is shown in Fig. 9. Very fast flickering events are seen at negative voltages, though no effect is observed at positive voltages. ATP appears to be blocking from the *cis* face of the channel with the degree of block increasing as the ATP concentration is increased. A similar blocking effect is observed with ADP applied symmetrically to the channel (*results not shown*). This suggests that ATP and ADP may be acting as large impermeant anions, but only from one face of the channel. The presence of 3 mM ATP, together with the lower anion concentrations applied by Maruyama (1987) may explain the absence of the channel reported here from his patch recordings. If this is the case, it would imply that the *trans* side of our channel would face the platelet cytosol.

It should be noted that the blocking effects of SITS, DIDS and ATP were not observed in all applications to reconstituted SM anion channels. In some cases no effect on channel activity was found (*results not shown*), despite increasing the concentration of agent up to 10 mM. The blocking effect of SITS was observed in five out of eight applications; DIDS, three out of six and ATP, seven out of 10 applications. These observations may reflect some heterogeneity in the SM channel population, though in all other conduction properties studied (e.g., conductance and selectivity) channels that were sensitive or insensitive to these agents were indistinguishable.

The platelet surface membrane anion channel described here would appear to belong to an increasing class of 'anion' channels that also show a degree of cation permeability. As with other members of this class, the physiological role of the platelet SM channel remains to be established.

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