Semi-Automatic Analysis of Unit Channel Conductance From Voltage Ramp Records

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Abstract. An IBM PC-compatible computer program, RAMP, for evaluation of single-channel recordings acquired using voltage ramp protocols is presented. The program uses semi-automatic procedures to make necessary corrections to a record (e.g. subtraction of baseline shift) and to measure all channel slope conductances as well as reversal potentials. The output is either a hardcopy of graphic display, which includes the calculated parameters, or data in ASCII format for further use (e.g. plots using various graphic software). Originally, the software was developed for the evaluation of voltage ramp records of single channel data from maxi chloride channels in myoblasts of a muscle cell line (Hurňák and Zachar 1992). Records from these membrane patches were also used in this work to demonstrate basic principles of the software and its practical use in evaluating single channel records obtained in response to the application of voltage ramps. The channel conductances calculated from ramp records were compared with those obtained by classical evaluation procedures from voltage step records.

Key words: Software — Microcomputers — C language — Patch clamp — Ion channels — Voltage ramp — Conductance measurement — Chloride channels — Muscle cell line

Introduction

The patch clamp recording technique (Hamill et al. 1981) enabled measuring of currents flowing through single ionic channels in cell membranes, and thus characterizing of their properties and kinship. The measurement of current-voltage (I–V) relationship from single-channel data represents the basic step for the respective channel identification. Two approaches to the measurement of the I–V relationship are possible: a) a series of measurements at different holding potentials or displacements from the holding potential with subsequent construction of the I–V dependence from single points, or b) use of voltage ramp protocol to obtain the I–V characteristic in one run. The ramp clamp method, as probably first used by Yellen

(1982) for measurements of current-voltage relationships in patch-clamp studies, has several advantages over the other method. In addition to a better resolution of reversal potential, it mainly enables quick obtaining of a full continous I–V curve. In many situations this makes it a method of choice; this is, e.g. the case when repeated membrane measurements on the same patch are needed.

The main aim of the present work has been to develop a semi-automatic procedure allowing the construction of I–V relations and calculations of slope conductances from ramp clamp records. A computer program, RAMP, will be presented which can be used to calculate slope conductance from linear regression of single channel data recorded under voltage ramp protocol with the frequently used pClamp (AXON) software package. The output is either a hardcopy from graphic display or data in ASCII format for further use, e.g. construction of plots using various graphic software. The program was tested on patch clamped membranes from rat muscle cell line L6 (Hurňák and Zachar 1992) The results for ramp clamp records were compared with those for fixed potential records from the same membrane patches.

Methods

RAMP program implementation. RAMP is written in C language with some functions in assembler for better performance. The program can be run on any IBM PC compatible computer with at least 640 KB of memory, a hard disk, EGA or VGA graphics adapter, and color display. Epson compatible 9-pin printer is needed for graphics hardcopies. Math coprocessor is not required but recommended. Approximately 310 KB free disk space is needed to install the program.

Biological material. The program was tested using single-channel records from maxi conductance chloride channel in L6 myoblasts as described elsewhere (Hurňák and Zachar 1992).

Input file selection The program can read any Clampex (AXON) data file (see pClamp User's manual 1989) recorded with voltage ramp protocol. It is up to the user to select the input file from the 'Select File' box. The scrollable box displays a list of all files corresponding to a given DOS-like wildcard specification. Actual input is selected by placing highlighted bar over the file to be used. Then, its header is read in and some of its parameters are shown in a pop-up window for the user to confirm the selection.

The first record (or episode in the pClamp jargon, see pClamp manual, 1989) is plotted in current versus command voltage coordinates on the screen (the internal window in Fig. 1A) along with a menu and file identification information (the external window in Fig. 1A). The user can optionally browse through the episodes of the file or proceed with analysis using commands offered by the menu. The upper menu in the external window offers the following possibilities: zooming of the record to fill the full size of the window (Axes); subtraction of baseline shift from the zero ordinate (Level); utility for fitting the slope conductance (Slope); graphics display of results (Browse); measurement with two cursors (Measure), export of data to ASCII file (File); hardcopy of the screen (Hcopy); and restoration of the original file (Restore). Episodes can be selected either with cursors (LEFT, RIGHT) or by jumping to a given number (num or E). The bottom

menu indicates the episode selection (from total number of episodes), channel selection (from all channels recorded), and the path and the file name.

Results

Ramp current records represent the sum of membrane leakage current when no channel is open and the current in the presence of an open channel. The opening of the channel during the ramp occurs at random and therefore conditions for optimizing the ramp records from different channels might considerably vary. In the present inquiry of maxi Cl channels in rat muscle cell line myoblasts (see also Hurňák and Zachar 1992), symmetrical ramp pulses from −50 to +50 mV (and vice versa) within 1 s were used every 10 s (sampling rate 1 ms per point).

A typical single-channel record (Fig. 1A) acquired using voltage ramp protocol, displayed as current (ordinate in pA) against command voltage (abscissa in mV) plot, always exhibits several sloping lines. The first one (arrow) corresponds to the leakage membrane conductance which should be subtracted from the record. The remaining slopes represent either transitions of a channel to multiple states with different conductances or openings of more than one channel with a single conductance (or combination of both). Often the baseline of the record is shifted from zero and should be subtracted as well.

Several steps described in following paragraphs have to be taken to analyze such a record with the RAMP program.

Baseline shift subtraction

The very first step of the analysis is the baseline shift subtraction. Two cursors are used to set two edge points of the baseline region (Fig. 1B, "Set region" in the bottom menu). All points inside this region are averaged and the resulting level is subtracted from the record. The result of such a correction can be seen in Fig. 2A with the baseline crossing ordinate at y=0. In addition to this possibility, the baseline can be subtracted from the used region or from a part of the record delineated by cursors (not shown).

Leakage membrane conductance slope subtraction

As a next step after elimination of the off-set the leakage membrane conductance is subtracted. The first estimate is made by the user by positioning two cursors (a circle and a cross in Fig. 2A) over two points somewhere on the slope and entering (as a number from keyboard) the width of a strip to define points which will be used in calculation of the best fit line (Fig. 2B). In color mode the points inside the strip take a different color and are thus clearly distinguished from the surroundings. If the strip contains also points belonging to another conductance
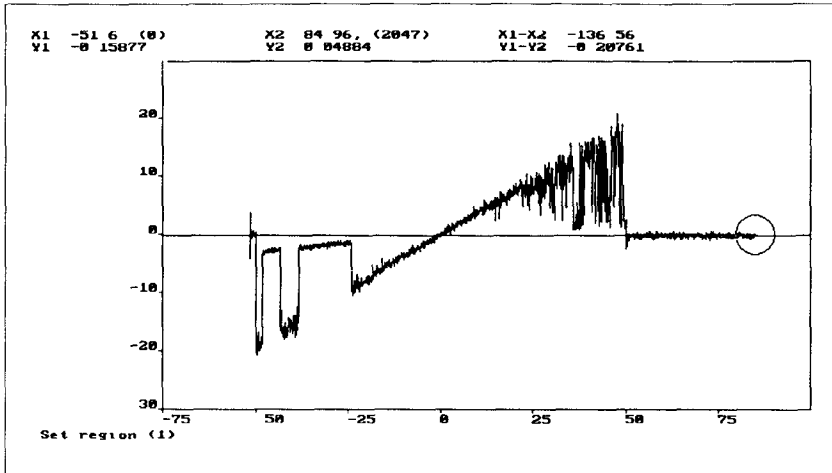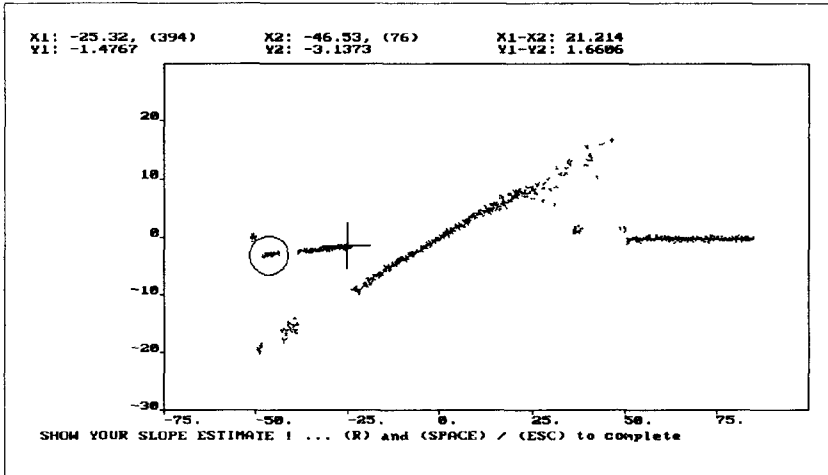
A
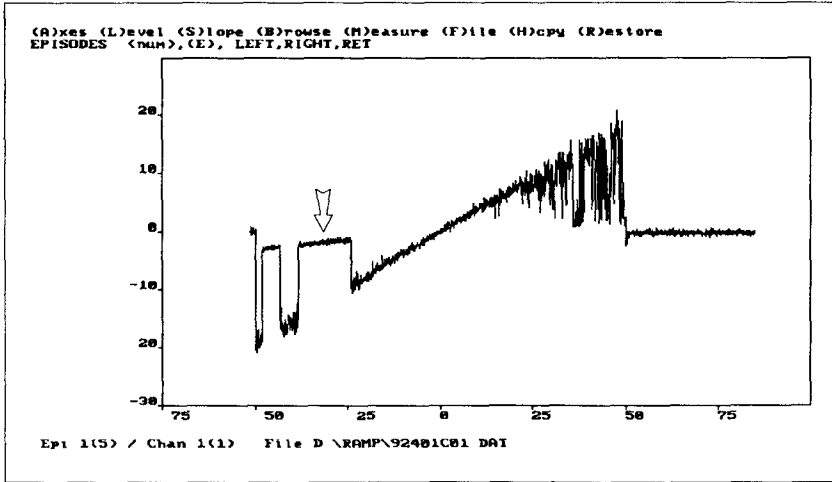
B

Figure 1. RAMP screen hardcopies A Internal window shows a loaded ramp record Abscissa in mV, ordinate in pA For explanation of the upper and the bottom menus see text B Two- cursor-measurement with coordinates displayed in the upper part of the screen Note the baseline shift in the original record
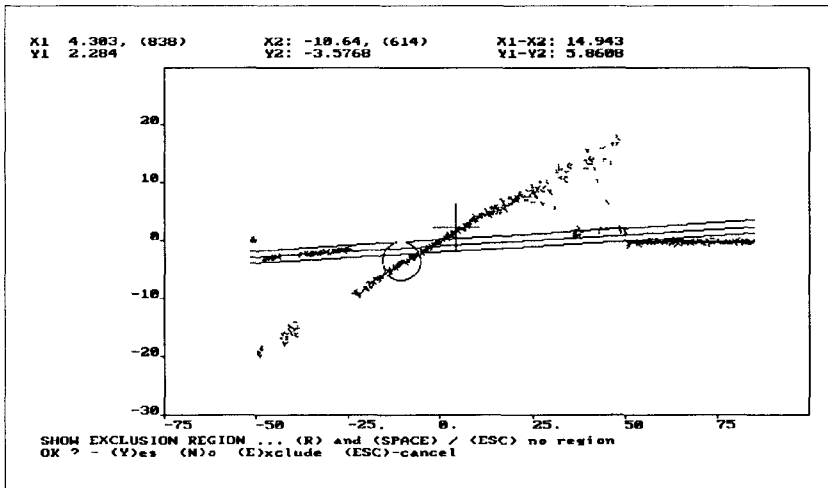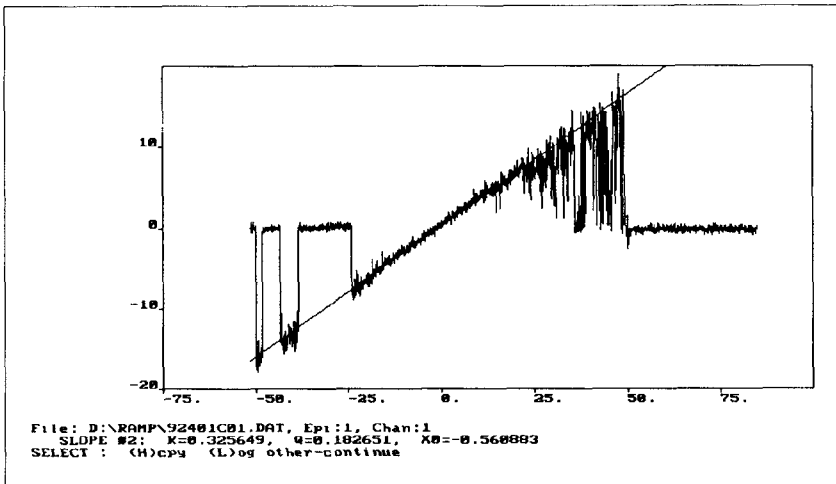
A

B

Figure 2. *A*: The slope estimate of the closed state membrane conductance with two cursors. *B*: Points to be used in the fit are selected by delineating the strip width. Points to be excluded are marked with cursors set to the edge points of the "exclusion region".
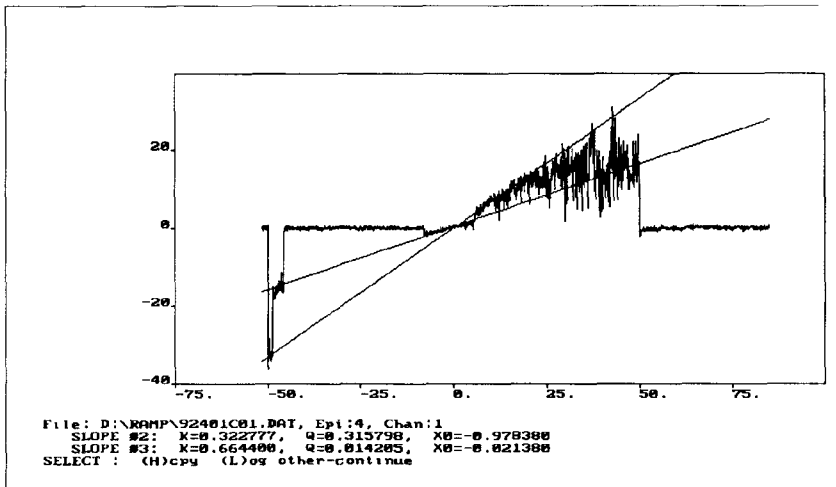
A


```
                                                 10_

                                                  0_

                                                -10_

                                                -20_
                             -75.     -50.    -25.      0.      25.     50.     75.
              File: D:\RAMP\92401C01.DAT, Ep1:1, Chan:1
                  SLOPE #2:   K=0.325649,   Q=0.182651,   X0=-0.568883
              SELECT :   (H)cpy  (L)og other-continue
```

B


```
                                                 20_

                                                  0_

                                                -20_

                                                -40_
                             -75.     -50.    -25.      0.      25.     50.     75.
              File: D:\RAMP\92401C01.DAT, Ep1:4, Chan:1
                  SLOPE #2:   K=0.322777,   Q=0.315798,   X0=-0.978380
                  SLOPE #3:   K=0.664400,   Q=0.014205,   X0=-0.021380
              SELECT :   (H)cpy  (L)og other-continue
```

Figure 3. *A*: Shape of the record with the leakage membrane conductance subtracted. The best fit line $(y = Kx + Q)$ through the open channel levels during the ramp was drawn by the program. Note the displayed parameters of the channel. *B*: Evaluation of a two channel record from a different patch.

slope, as in the example illustrated, they can be excluded by setting the cursors to the beginning and the end of the overlap region, where the strip and the other slope cross (Fig. 2B). The program then fits, by least squares procedure, a linear function to all points selected inside the strip. The result can be subtracted from the record; note the zeroed baseline level in Fig. 3A.
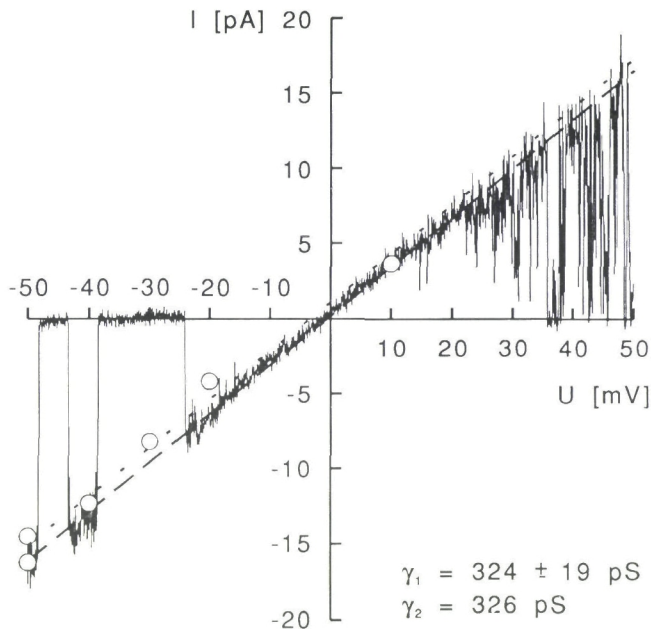

Figure 4. An overlay of a ramp record with the best fit line of the channel slope conductance determined by RAMP and of the regression line through the experimental points (circles) determined by variation of the membrane potential. The same membrane patch from a myoblast membrane was used for both measurements. The RAMP record was exported in ASCII format to the regression line plot.

Determination of channel conductances and reversal potentials

After these corrections has been performed, the remaining slopes can be measured and the channel slope conductance determined. The procedure is performed in the same way as described in the preceding paragraph. The line through the open channel level in Fig. 3A was arrived at by the same procedure as shown above, i.e. by positioning the cursors in the most discernible region and by delineating a

strip along the region   No delineation of "exclusion region" was required in this particular case

The RAMP program can calculate and store several slopes   Each slope can be subtracted from the record or its part(s)   Subsequently the intercept of each slope with the zero current line, i e   the reversal potential, can be calculated and displayed along with the slope parameters (Fig 3A)   The channel record in the indicated file episode showed the following parameters   slope conductance $K = 0\ 325$ nS, reversal potential $X0 = -0\ 56$ mV and current at zero voltage $Q = 0\ 182$ pA   One or more slopes can be plotted overlaid on the original data record with calculated results (Fig 3B)   The screen can be optionally hardcopied and the results can be saved to an ASCII log file and transferred to other programs (Fig 4)

Ramp-clamp and fixed-potential conductances

Fig 4 shows an overlay of maxi channel I–V relation obtained by measurements in a fixed potential mode (circles) using an I–V record obtained by the ramp pulse procedure   It is evident that both methods yield very close results, $\gamma_1 = 324 \pm 19$ and $\gamma_2 = 326$ respectively   The measurements were made in symmetrical 150 NaCl on the same excised patch from a myoblast of the rat muscle cell line L6

Discussion

The RAMP program was developed for semi-automatic analysis of single-channel data acquired by the patch-clamp technique for maxi Cl channels from rat muscle cell line L6 myoblasts (see Hurňák and Zachar 1992)   The ramp clamp records from this preparation resemble those recorded from other preparations using different ramp voltage protocols (Schwarze and Kolb 1984, Kolb et al 1985)

Most of our typical records could be analyzed successfully   However, some of the slopes were not linear, which is the case that currently cannot be handled by the program properly   The solution would require a modification of the procedure for the first estimate of (nonlinear) slope parameters and the possibility to fit a nonlinear function to the selected part of a record   No attempt has been made in this directon so far

Sometimes situations have been encountered with a strip along the first estimate of a slope crossing the other slopes in more than two regions   In spite of the fact that RAMP does not allow setting of more than one "exclusion region", we found that this does not represent a serious problem, it could be solved by simply selecting another estimate and using a proper width of the strip

A major advantage of using ramp stimuli to determine open-channel current-voltage relationship is the quickness of the procedure, which enables to measure the required parameters, channel conductance and reversal potential substantially

faster than by using the fixed membrane procedure. The method has occasionally been used for this reason, especially in situations, of imminent breakdown of the membrane patch or of the risk of time related deterioration. Also, the ramp method is the method of choice when the patch currents records represent a sum of several membrane channels of the same kind and the number of channels is to be determined (Carl and Sanders 1990).

According to Yellen (1984) there are three sources of artifacts that must be kept in mind and eliminated when determining the I–V curves from ramp data (see also Eisenman et al. 1986). The first one is the capacitive current-associated artifact. This may play a significant role in ramp records from planar lipid bilayers, where the membrane area is large. This artifact could hardly have played a significant role in our recordings from patch membranes of much smaller membrane area. The second source of artifacts is channel flickering, which might introduce a bias into results if averaging techniques are used to determine the closed and open channel levels as practiced in planar lipid bilayers. The selection of flicker-free regions by the RAMP program techniques made this effect negligible. The third problem which is encountered when the ramp data are evaluated by summation techniques is due to the heterogeneity among individual channels of the same general type.

There are also claims (Grissmer et al., 1992) that the ramp procedure results in small, depolarizing shift in the apparent voltage dependence for the voltage-gated K^+ channels, relative to that determined by voltage steps; the effect was, however, absent in voltage-independent Ca-activated K^+ channels. We therefore determined the unit channel conductances and the reversal of potentials of maxi Cl channels by both methods (Fig. 4). The results obtained by the methods compared were not significantly different. The possible interference must be, however, considered, when the method is to be used for another voltage-dependent channel species.

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