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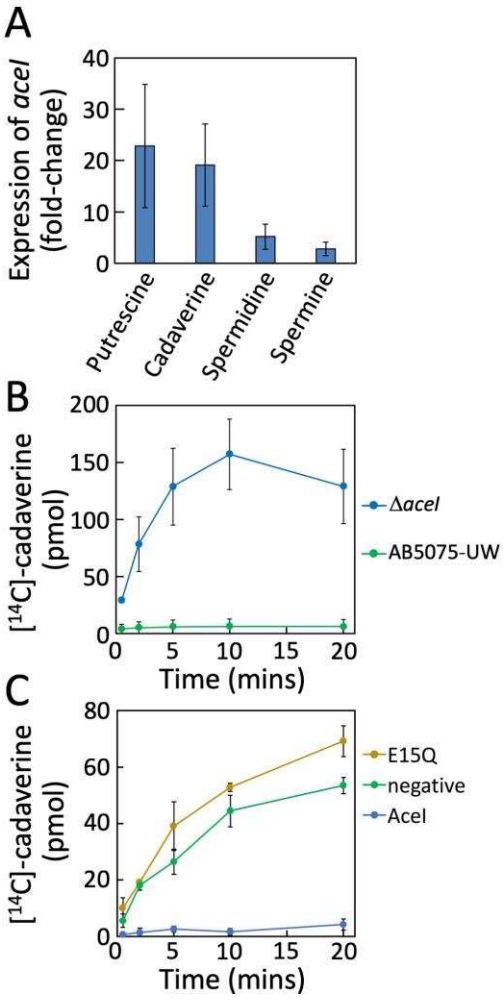

Figure 1. (A) Induction of aceI gene expression by polyamines. Polyamines were added to the A. baumannii AB5075-UW media when the cells were in mid-exponential growth phase (Methods). The bars represent the change in aceI gene expression compared to an untreated control after 30 mins growth in the presence of the polyamines. Error bars show the standard deviations of at least two biological and four technical replicates. (B) Accumulation of [^{14}C]-cadaverine into A. baumannii wild-type (AB5075-UW) or an aceI inactivated mutant (ΔaceI) (13). The cells were exposed to a low concentration of cadaverine for 30 mins to allow aceI expression, then washed and incubated with 50 μM [^{14}C]-cadaverine using 1 % succinate provided as an energy source. Each sample included 100 μL of cells at OD_{600} = 1.0 (Methods). (C) Accumulation of [^{14}C]-cadaverine into E. coli BL21 cells overexpressing the wild-type AceI protein (AceI), the inactive E15Q AceI mutant protein (E15Q) or no additional protein (Negative). In these E. coli cells, expression of aceI or its E15Q variant was induced by IPTG and the harvested, washed cells were incubated with 50 μM [^{14}C]-cadaverine with 1% glucose provided as an energy source. Each sample included 100 μL of cells at OD_{600} = 1.0 (Methods). Error bars show the standard deviation of at least three independent replicate experiments.

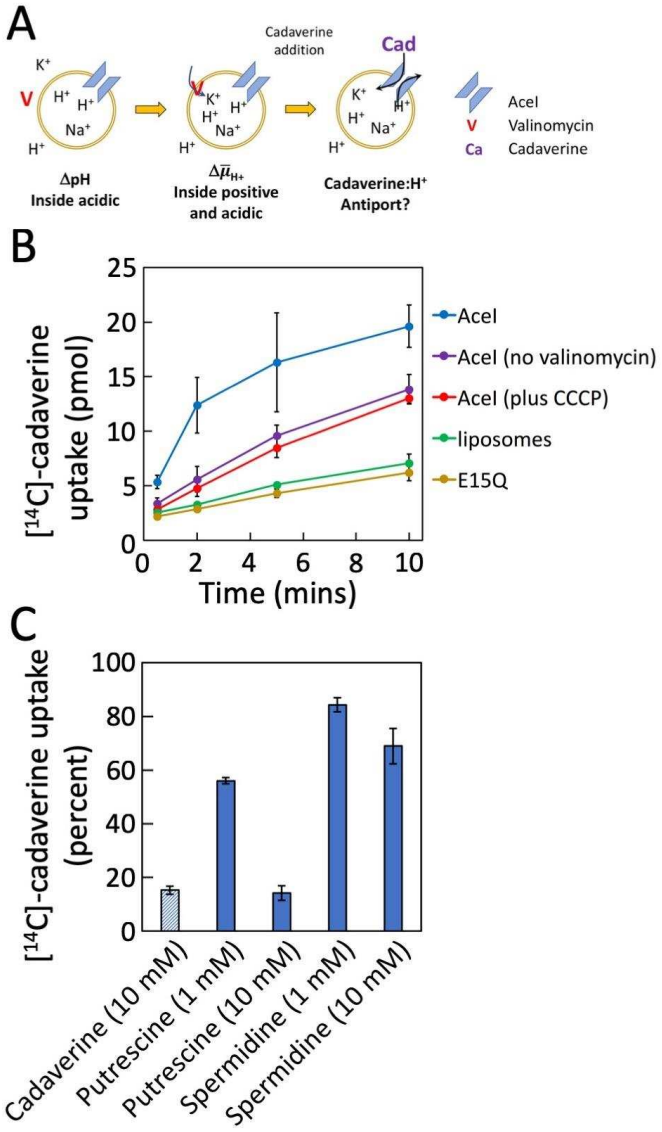

Figure 2. Uptakes of [^{14}C]-cadaverine into (proteo)liposomes. (A) Schematic representation of the strategy used for generating a proton electrochemical gradient ($\Delta\bar{\mu}_{H+}$) across the proteoliposome membrane. (Proteo)liposomes were formed in Na^+-containing buffer at pH 7.0, then diluted into K^+ buffer at pH 8.0 containing the K^+ ionophore valinomycin. (B) Liposomes and proteoliposomes containing AceI or AceI-E15Q were formed (Methods) and treated as shown in panel A. The results show [^{14}C]-cadaverine uptake per fraction. Uptake of [^{14}C]-cadaverine was faster in energised AceI proteoliposomes (blue line) compared to empty liposomes (green line) or proteoliposomes containing the inactive AceI-E15Q protein (tan line). [^{14}C]-cadaverine uptake into AceI proteoliposomes without valinomycin, or with the addition of CCCP occurred at an intermediate rate (purple and red lines, respectively). (C) Inhibition of AceI mediated [^{14}C]-cadaverine transport by alternative AceI substrates. Transport experiments were performed as for the blue trace in panel B, but in the presence of 1 or 10 mM unlabelled cadaverine, putrescine or spermidine. The values shown are the amount of [^{14}C]-cadaverine accumulated after ten minutes as a percentage of the amount accumulated with no inhibitor. Error bars show the standard deviations of at least three independent replicate experiments.

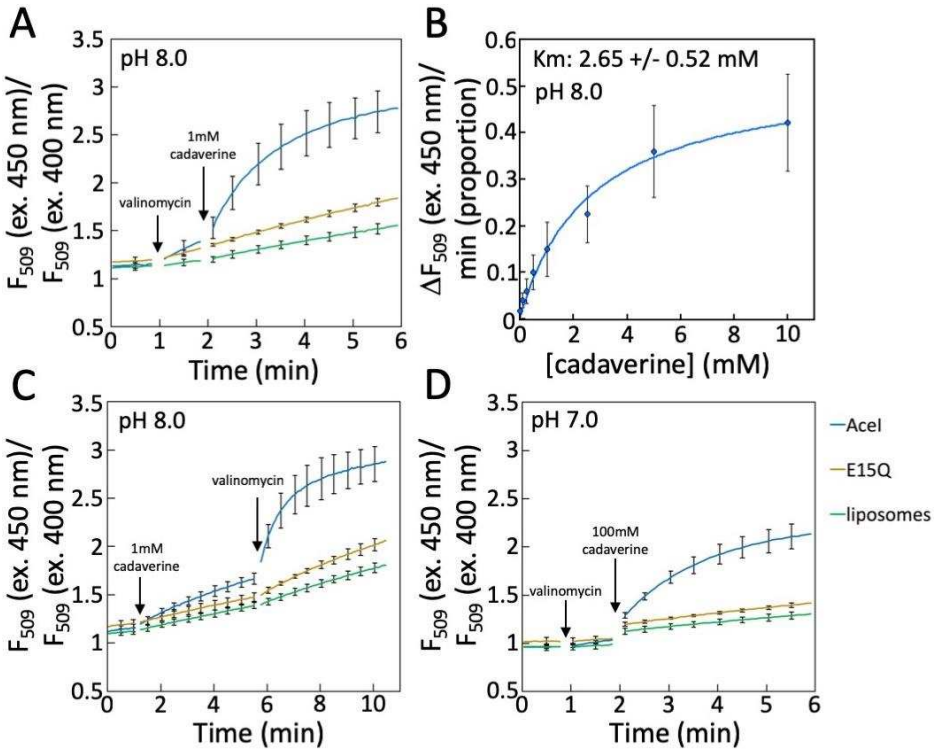

Figure 3. Cadaverine-induced pH changes inside proteoliposomes containing AceI. Proteoliposomes were formed in buffer containing the pH sensitive fluorescent dye pyranine (Methods). The excitation maximum of pyranine shifts from 400 nm to 450 nm with increasing in pH, whereas the emission maximum is stable at approximately 509 nm. Plots A, C and D show the 450:400 nm fluorescence (509 nm) excitation ratio of pyranine in the lumen as follows: control liposomes without added protein (green); proteoliposomes containing the inactive AceI-E15Q mutant protein (tan); and proteoliposomes containing wild-type AceI (blue). An increase in this ratio is indicative of an increase in internal pH. All (proteo)liposomes were formed in Na^+ buffer at pH 7.0 (Methods). (A) (Proteo)liposomes were diluted into K^+ buffer at pH 8.0, 5 nM valinomycin was added at the first arrow and 1 mM cadaverine was added at the second arrow. (B) AceI proteoliposomes were diluted into K^+ buffer at pH 8.0 containing 5 nM valinomycin and the indicated concentrations of [^{14}C]-cadaverine. The initial rates of fluorescence change are plotted relative to the concentration of cadaverine, corrected for the small level of fluorescence change observed in liposomes containing no protein. From a Michaelis-Menten curve fitted to the data points an apparent Km of 2.65±0.52 mM was derived. (C) (Proteo)liposomes were diluted into K^+ buffer at pH 8.0, 1 mM cadaverine was added at the first arrow and 5 nM valinomycin was added at the second arrow. (D) (Proteo)liposomes were diluted into K^+ buffer at pH 7.0, 5 nM valinomycin was added at the first arrow and 100 mM cadaverine was added at the second arrow. The experiments were performed using at least three independent batches of (proteo)liposomes and error bars show the standard deviations.