Application Note

Transporter: Na/amino acid
Sample: Plasma membrane
Origin: CHO cells
Tools: SURFE²R 96SE

Secondary screening assay for amino acid transporter inhibitors on the SURFE²R 96SE

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Background

Na-dependent amino acid transporters play an essential role in the uphill transport of amino acids across the plasma membrane. In this study, a novel transporter target was used for which few specific pharmacological tools are available. The aim of this study was to establish a secondary screening assay using the direct electrophysiological measurement of the transporter to identify inhibitors. Using the SURFE²R 96SE, substrate specificity was determined and inhibitors could be identified. Figure 1 shows typical current responses of the transporter when both Na^+ and an amino acid are present.

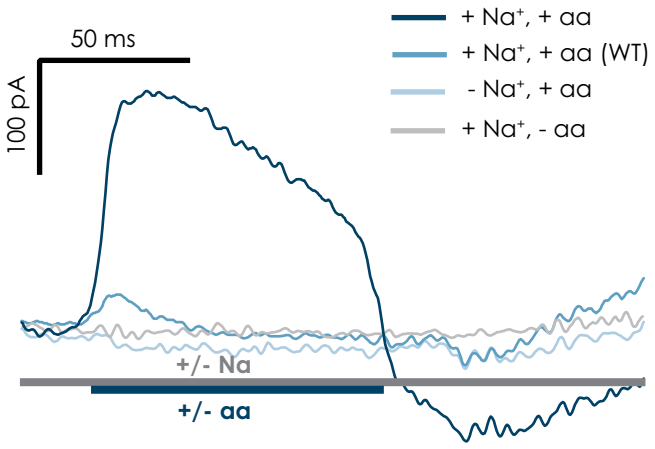


Figure 1: Typical current responses of CHO cell membranes containing the amino acid transporter or WT membranes (no transporter) recorded on the SURFE²R 96SE. Only in the presence of both Na^+ and an amino acid a positive current peak occurs in membranes containing the transporter. In the absence of either Na^+ or the amino acid the transporter is not active. In WT membranes (no transporter) no current is observed in the presence of Na^+ and an amino acid.

Substrate specificity and affinity

To investigate the substrate affinity, a neutral amino acid was added in different concentrations to the sensor containing the membrane sample (Figure 2). A K_M of 1.75 mM was determined. A comparison with other methods (FLIPR, Uptake, TEVC) revealed a high correlation of the K_M. Furthermore, amino acid specificity was investigated, revealing a preference of the transporter for neutral amino acids.

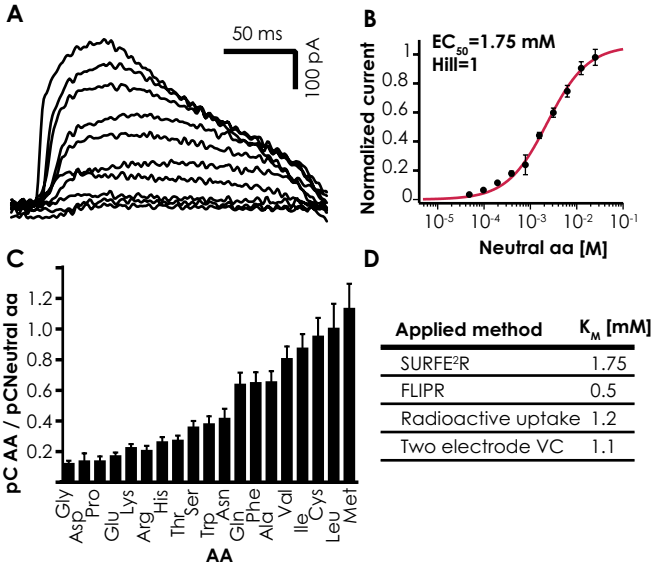
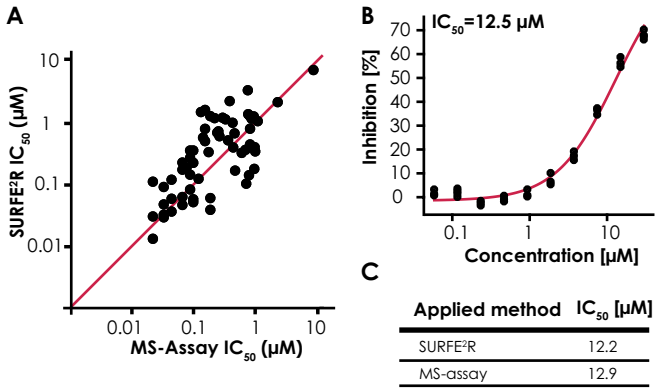


Figure 2: A Raw current traces of an active sensor, showing the increasing current peak with increasing substrate concentration. **B** The concentration response curve fitted with a Hill equation reveals an EC_{50} of 1.75 mM. **C** The current peak height generated by different amino acids normalized to a neutral amino acid reveals that neutral amino acids are preferred as a substrate. **D** The K_M of a neutral amino acid obtained using the SURFE²R 96SE is in good agreement with other methods.

Application Note

Secondary screening for inhibitors

Finally, the SURFE²R 96SE was used in a secondary screening assay to characterize and confirm compound hits generated in a primary screen. In order to first validate the assay a known compound was used. A time dependence of inhibition intensity was observed. The longer the sensor was incubated with the compound, the higher the percentage of inhibition. Using a workflow with a 30 min incubation step resulted in an IC_{50} of 12.9 µM, which correlates with the IC_{50} generated using a mass spectrometry assay (MS-Assay; 12.2 µM). Using this secondary screening assay it was possible to measure 12 concentration response curves with IC_{50} values per day (n = 4) using 6 SURFE²R 96SE sensors. The assay was further validated by comparing the IC_{50}s of 62 selected compounds determined with the SURFE²R assay with those generated using an MS-Assay. The correlation curve is shown in Figure 3 with a correlation factor of 0.84 which confirms the validity of the SURFE²R 96 SE assay.



Applied method	IC_{50} [µM]
SURFE²R	12.2
MS-assay	12.9

Figure 3: A Correlation curve between IC_{50}s obtained using the SURFE²R 96SE and MS-Assay. The correlation factor of 0.84 indicates excellent correlation between the two assays. **B** Concentration response curve reveals an IC_{50} = 12.9 µM. **C** Comparison of the IC_{50} values show good agreement between the two methods.

Success rate and stability

An investigation into reproducibility revealed that in a typical experiment 98% of the sensors in a 96 well plate met the quality criteria and generated current signals between 200 and 500 pA. Using 10 µM of the known inhibitor led to 40-50% inhibition of the signal in 94% of wells and over a series of 5 plates the overall success rate was approximately 90% in terms of baseline current, maximum current, signal to noise ratio and stability of inhibition (Figure 4). The SURFE²R 96SE is, therefore, an excellent tool for secondary screening of compounds on transporters.

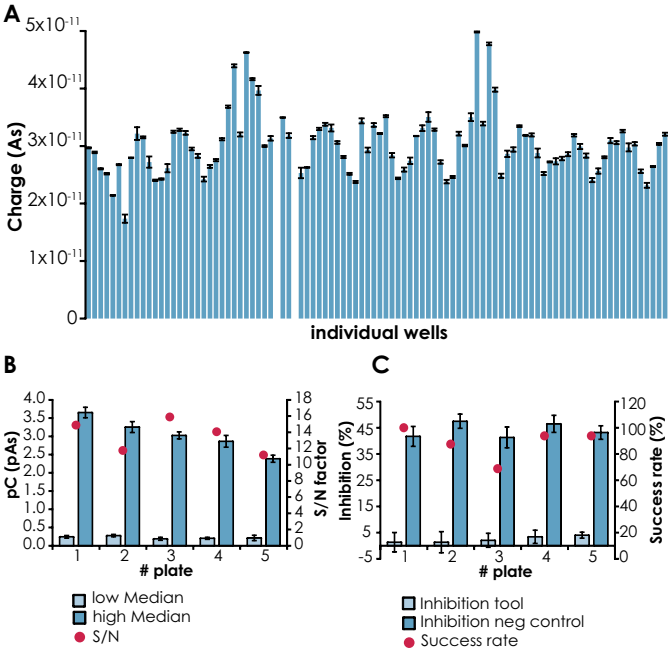


Figure 4: Stability of current signal and inhibition. **A** Transported charge per well compared within a 96 well plate. Total success rate 98%. **B** Current response (charge) compared between different 96 well plates. Values compared: Maximum signal, baseline current and signal to noise ratio. **C** Total success rate and reproducibility of inhibition compared between 5 plates.

Methods

Experimental setup

The target protein was stably expressed in CHO cells and the cell membrane was isolated and frozen in aliquots. On the day of the experiment the membrane was added to a SURFE²R 96SE sensor plate. To evoke protein specific current activity a suitable amino acid was added to the sensor in the presence of a Na^+-containing assay buffer. The co-transport of the amino acid together with Na^+ generated a positive current signal.

Acknowledgement

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