Application Forum

Cell-free Protein Expression of Membrane Proteins Using Nanolipoprotein Particles

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INTRODUCTION

Membrane proteins provide critical roles in cell-to-cell contact, surface recognition, cytoskeleton contact, signaling, enzymatic activity, or transporting substances across the membrane. However, membrane proteins are challenging because their expression can be toxic to cells or cause inclusion bodies, which limit protein yield, and current methods require tedious optimization making purification difficult and time consuming. We report a cell-free approach for expressing and inserting integral membrane proteins into planar phospholipid membrane bilayer surrounded by a scaffold protein (also called a nanolipoprotein particle or NLP; brand name MembraneMax). Proteins are inserted into these particles, circumventing the need of extracting and reconstituting the product into membrane vesicles. Moreover, the planar nature of the membrane support makes the protein freely accessible from both sides of the lipid bilayer.

MATERIALS AND METHODS

As described in J. Proteome Res. <http://pubs.acs.org/cgi-bin/abstract.cgi/jprobs/asap/abs/pr800265f.html>.

RESULTS AND DISCUSSION

We studied the in vitro expression and solubility of a host of membrane proteins of different topologies, sizes, origins, and proposed roles. Sixty-four percent of the proteins analyzed expressed at a level >0.1 mg/mL of the reaction, and, without exception, the proteins significantly increased their solubility in the presence of the MembraneMax reagent (Table 1 and Figure 1). Our method for the expression of integral membrane proteins is unlike conventional membrane protein methods in that: (i) the method generates sufficient and soluble protein yields for functional and structural studies; (ii) the process is high throughput—amenable; and is (iii) compatible with a variety of cell-free lysates; (iv) native membrane protein may be expressed; and (v) the final complex is freely accessible from both sides of the lipid bilayer.

Figure 1. Soluble membrane protein expression achieved using the MembraneMax™ HN kit. The in vitro expression and solubility of membrane proteins of different topologies, sizes, origins, and proposed roles were analyzed. Proteins were expressed in the presence (blue) or absence (red) of MembraneMax™ HN reagent. For the analyzed data set, the overall solubility increased from $17.3 \pm 2.2\%$ (in the absence of NPLs) to $78.8 \pm 3.4\%$ (in the presence of NPLs). GPCRs exhibited a remarkable increase in solubility in the presence of NPLs. TMS = transmembrane segments.

Table 1: Shown below is a small subset of proteins that we expressed using the NLP (MembraneMax™ reagent) with a cell-free protein expression approach. Over 64% of the proteins expressed at >0.1 mg/ml of reaction ($n = 32$).

Membrane Protein (GenBank Accession Number)	Yield (mg/ml)
Glycophorin (NM_002102.2)	0.41
Bacteriorhodopsin (J02755.1)	0.33
CRHR1 (NM_004382)	0.30
MGST2 (NM_002413.3)	0.30
Glycophorin E (NM_002102.2)	0.30
MGST2 (NM_004528.2)	0.30
Epiregulin (NM_001432.1)	0.30
b2 adrenergic receptor (NM_000024.3)	0.30
CKLF (NM_81640.1)	0.30
UNQ1887 (BC025781.1)	0.30
SPPL2B (BC001788.1)	0.30
CHRM2 (NM_000739.2)	0.30
CHRM1 (NM_000738.2)	0.27
PPAPDC1B (NM_032483.2)	0.20