BIOC2300: Membrane protein anchorage

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1 Introduction

Two peripheral membrane proteins, alkaline phosphatase and aminopeptidase N, are anchored to the brush-border membrane of pig kidney cells. Determination of the method of anchorage (peptide anchor or glycosyl-phosphatidylinositol (GPI) anchor), was carried out by attempting to solublize the proteins with a variety of detergents, trypsin, or bacterial phosphatidylinositol-specific phospholipase C (PI-PLC).

The results were assayed for enzyme activity; in addition, native PAGE was performed and the mobility of alkaline phosphatase after solublization attempts was observed.

2 Solublization

Samples of brush-border membranes were solublized with the detergents octyl glucoside and Triton X-114, and the enzymes PI-PLC and trypsin. In addition, two controls were used at different temperatures: the temperatures used were those used elsewhere for optimum activity of the enzymes (37°C) and detergents (4°C) respectively. After solublization attempts, the result was centrifuged at 13500rpm for 5 minutes, and the pellet resuspended.

Each sample was assayed for alkaline phosphatase and aminopeptidase N activity. After centrifugation, the pellet contains the membrane and the supernatant contains any solublized proteins.

The amount of solublization can be seen from figures 1 and 2 by comparing the heights of pairs of adjacent bars. The two controls, in which no solublizing agents were added, show that most of the active protein is in the pellet, as expected.

PI-PLC is extremely efficient at solublizing alkaline phosphatase but shows little effect on aminopeptidase, whereas trypsin, a protease, effciently solublizes aminopeptidase but has little effect on alkaline phosphatase. This would seem to indicate that aminopeptidase has a peptide anchor and alkaline phosphatase has a GPI anchor.

The results with detergents were much less clear. Triton X-114 is marginally better at solublizing alkaline phosphatase than aminopeptidase, which is unexpected if alkaline phosphatase is indeed GPI-anchored. Octyl glycoside seems uniformly efficient at solublizing regardless of anchor type.

The error bars seem too wide for a quantitative interpretation of the data to be meaningful. In addition, the linear region of the plate reader stops at an absorbance of about 1, and many data points are significantly in excess of that. Nonetheless, see table 1 for an attempt.

| | Relative activity in supernatant (%) | |
|-------------------|--------------------------------------|------------------|
| Solublizing agent | Alkaline phosphatase | Aminopeptidase N |
| Octyl glucoside | 84 | 78 |
| Triton X-114 | 70 | 59 |
| PI-PLC | 92 | 22 |
| Trypsin | 13 | 77 |
| 4°C control | 18 | 28 |
| 37°C control | 11 | 15 |

Table 1: Relative activity in the supernatant compared to the pellet after solublization. High values indicate more efficient solublization.



Figure 1: Solublization of aminopeptidase N, as measured by hydrolysis of Ala-4-nitroanilide.

Figure 2: Solublization of alkaline phosphatase, as measured by hydrolysis of 4-nitrophenyl phosphate.



Figure 3: Alkaline phosphatase activity after native PAGE. Lanes from left to right: commercial alkaline phosphatase preparation, unsolublized membrane, membrane solublized with PI-PLC, membrane solublized with trypsin, membrane solublized with octyl glucoside, membrane solublized with Triton X-114, unsolublized membrane, commercial alkaline phosphatase preparation.

3 Native PAGE

Membranes solublized with the same methods (prior to the centrifugation step) were run on a non-denaturing (native) polyacrylamide gel. A commercial alkaline phosphatase preparation was also included The gel was stained with a histochemical stain (nitro-blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP)) to show alkaline phosphatase activity. The results are shown in figure 3.

The commercial alkaline phosphatase preparation moved a substantial distance on the gel, indicating its mobility in solution. The only other lane to show significant mobility is the one containing PI-PLC, which is consistent with alkaline phosphatase being GPI-anchored. The low mobility of all the other lanes indicates that the protein is still membrane-bound. In particular, treatment with octyl glucoside was ineffective at solublization, contradicting previous results.

The bands on the gel are particularly broad, in contrast to the sharp resolution usually obtained with SDS-PAGE.

4 Discussion

As noted before, the data point towards alkaline phosphatase having a GPI anchor and aminopeptidase N having a peptide anchor due to their solublization by PI-PLC and trypsin respectively.

GPI is a complex glycophospholipid that is attached to the C-terminus of the polypeptide chain post-translationally. PI-PLC specifically cleaves GPI anchors at the diacylglycerol-phosphate bond, as shown in figure 4. The sugar linkage is still attached to the solublized protein after hydrolysis with PI-PLC.

Trypsin is an endoprotease and cleaves proteins at the carboxyl side of Lys-Arg residue pairs (with exceptions for adjacent proline residues which disturb the stereochemistry at the reaction site). That aminopeptidase N is solublized by trypsin without losing activity is lucky: the cleavage point(s) must lie between the transmembrane region (the membrane-bound domain is unlikely to be easily accessible to the enzyme) and the catalytic domain. Note that Lys and Arg are both hydrophilic, positively charged residues that are unlikely to be found in the polypeptide anchor region.



Figure 4: Schematic of a GPI anchor, with the PI-PLC cleavage site marked.

Globular proteins, such as the commercial alkaline phosphatase preparation, have a hydrophilic exterior and are easily mobile in solution. Transmembrane proteins are amphipathic: they have both hydrophobic and hydrophilic regions. Hydrophobic regions (either peptide or a post-translational modification such as GPI) are stabilized by van der Waals interaction with the hydrophobic alkyl chains in the lipid bilayer, whereas the hydrophilic region is exposed to solution where it can undergo hydrogen bonding with water. The hydrophobic region is the anchor: it is the point of attachment to the membrane. Because of the relative sizes of typical membrane proteins and the lipid bilayer to which they are attached, mobility of membrane-bound proteins is very limited compared to proteins in solution. Thus native PAGE is a good test of solublization, as membrane-bound proteins are almost immobile in the gel.

5 Conclusions

Alkaline phosphatase has a GPI anchor. Aminopeptidase N has a peptide anchor. Enzymes that can specifically cleave the anchor are extremely effective at solublization, and at determining the anchor type. Aminopeptidase N has a trypsin cleavage point (Lys-Arg), but not within its catalytic domain. Detergents are much less effective at determining anchor type than enzymes are, but at least octyl glucoside may show promise at solublizing membrane proteins regardless of their anchorage while maintaining activity.

Native PAGE is messy.