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Characterisation of the Adenosine 2a Receptor Solubilised by Styrene Maleic Acid Lipid Particles (SMALPs)

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Membrane proteins are normally solubilised with detergents; however, these molecules are likely to strip away endogenous lipids which can perturb the properties of the protein. Consequently, there is interest in finding alternative surfactants for protein purification, but all new molecules to date have required the use of detergent for the initial solubilisation step (1). It has recently been reported that styrene maleic acid (SMA) co-polymers spontaneously encapsulate membrane proteins, removing the protein with its associated lipids in a nanoparticle, an SMA lipid particle (SMALP) (2). Here we use this co-polymer to purify the adenosine 2a receptor (A2aR) and to characterise it pharmacologically and biophysically.

Expression in *Pichia pastoris* of an adenosine 2a receptor engineered with an N-terminal His_{10}-tag and C-terminally truncated was as described previously (3). Membranes (40 mg/ml wet weight) were incubated with SMA co-polymer (2.5 % w/v final concentration) for 20 h at 25^{o} C with gentle stirring, followed by 100,000 x g centrifugation for 1 h at 4 ^{o}C. The supernatant was recirculated through a 1 ml HisTrap HP Ni^{2+}-NTA Sepharose column (GE Healthcare) overnight at 4 ^{o}C. The column was washed with 20 column volumes (cv) of 50mM Tris-HCl, pH 8.0, 500 mM NaCl, 10 % glycerol, 20-60 mM imidazole, complete EDTA-free protease inhibitor. The A2aR was eluted with 250 mM imidazole (10 cv) collected in 0.5 ml fractions. Eluted fractions containing A2aR were pooled, dialysed overnight against buffer (50 mM Tris-HCl, 150 mM NaCl, 10 % glycerol, then concentrated using a centrifugal concentrator (Vivaspin 20, 30 kDa cut-off, Sigma). Radioligand binding was performed with spin columns as described previously (3).

Analysis of the 250mM eluate from the HisTrap column showed a single band on a silver-stained gel with a molecular weight of 40kDa, consistent with the size for A2aR. Furthermore, mass-spectrometry yielded peptides corresponding to residues 112-120, 210-220 and 305-315 of the A2aR. The receptor bound [^{3}H]-ZM241385 (a radiolabelled A2aR inverse agonist) with a pKd of 7.79 ± 0.14, compared to 7.95 ± 0.45 in yeast membranes (n=3). Thus SMALPs allow the solubilisation and purification of the A2aR in a form that retains high affinity ligand binding. To further investigate the properties of the receptor, tryptophan fluorescence was measured. The unliganded receptor had a broad peak with a maximum of 330nm, consistent with a protein with a number of tryptophans in a hydrophobic environment. On addition of ZM241385, the spectrum contained 3 peaks at 321, 335 and 350nm. These all decreased in intensity, with pEC_{50}'s of 6.93 ± 0.14, 7.00 ± 0.10 and 7.01 ± 0.10 (n=3), indicating that each was responding to the same ligand binding event. There are 6 tryptophans in our A2aR construct, two in the transmembrane region, two in the extracellular loops and two in the intracellular loops and it is possible that ZM241385 binding exposes these three classes to different environments.

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