Efficient and Selective Enzymatic Synthesis of *N*-Acetyl-Lactosamine in Ionic Liquid: a Rational Explanation

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Supporting Information

Experimental Section

SPR studies: Assays were carried out at 25ºC and 5 μL/min on a Biacore 3000 (GE Healthcare, Uppsala, Sweden) using commercial CM-5 sensor chip (GE Healthcare, Uppsala, Sweden) over Fc-2 (enzyme coated surface) and Fc-1 (control empty surface) 10 μL of TTP0042 were immobilized at pH 4.50 with sodium acetate buffer according to preconcentration study, using amine coupling kit (GE Healthcare, Uppsala, Sweden) and following manufacturer's protocol until 6749 RU (relative response units) were reached. The interaction between TTP0042 his_6tag enzyme and (slightly) soluble IL's was studied using 4.18, 8.36, 16.7, 25.1 and 33.5 mM solutions of [Bmim][PF_6] and 1.19, 2.38, 3.56, 4.75, 5.94 and 7.13 mM solutions of [Omim][PF_6], IL's were dissolved in sodium phosphate buffer pH 6.0, 50 mM. Using these values steady state affinity plots were performed for both IL's. Maximum solubility of IL in buffer reached in this study were made after shaken the buffer-IL mixture over 10 minutes at 25ºC in a sonic-bath and then mixed in a vortex. The maximun solubility reached was 33.5 mM for [Bmim][PF_6], and 7.13 mM for [Omim][PF_6], no solubility over this value was reached, high concentrated solutions tested in our study correspond to saturated solutions.

Results and Discussion

SPR studies: The sensograms for the interaction with [Bmim][PF_6] and [Omim][PF_6] are shown in Figure 1a and 1b respectively. The initial portion of these curves represents buffer flowing past the sensor surface. The second and rising portions of the curve correspond to the response of the sensor surface as a sample flows past the immobilized β-galactosidase. The final portion of the curves corresponds to the dissociation of bound IL after the sample volume has finished and the buffer flows past the sensor surface again. As shown in Figure 1, the relative response (RU) is directly proportional to the mass close to the surface. An increase of this response means that the injected compound bound to the surface or interacted with the compound immobilized on that surface, for that reason both IL's bound to enzyme and the kinetics were very rapid. First the kinetic parameters were determined by simultaneous global analysis of the association and dissociation phases for a complete concentration series using a 1:1 model (results not shown). However, for both IL's the rate constants could not be reliably estimated because the affinity was weaker than the acceptable range for quantifications.

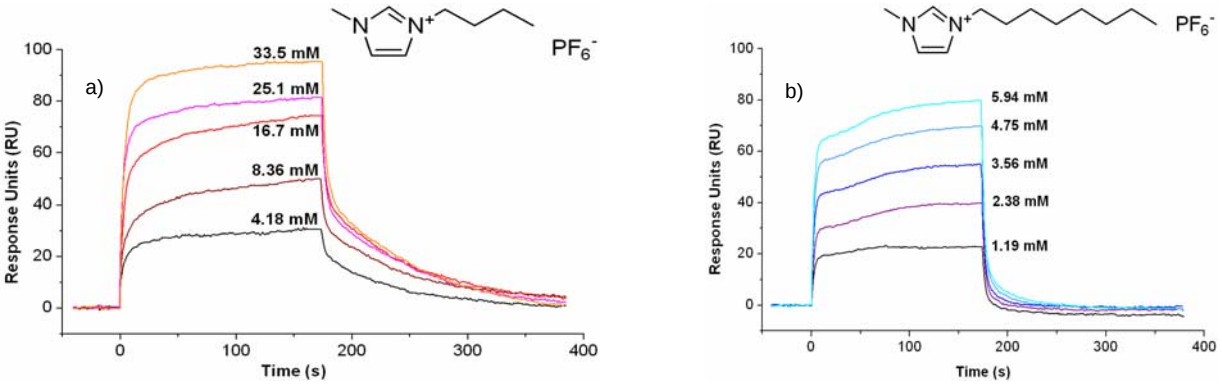

Fig. 1 Sensograms of IL-TTP0042 interactions: a) [Bmim][PF_6] and b) [Omim][PF_6].

Due to the difficulties in the determination of kinetic rate constants, the concentration dependence of steady-state values (Req) was used to estimate apparent affinity (KD values). The apparent KD value obtained for [Bmim][PF_6] and [Omim][PF_6] was 16.5 mM and 8.73 mM respectively (Figure 2). These experiments were carried out at different concentrations than the enzymatic reaction due to solubility problems.

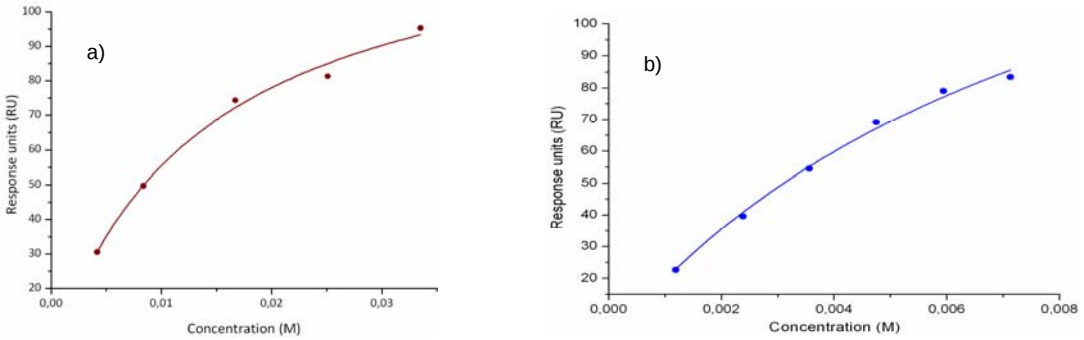

Fig. 2 Steady state affinity constant for enzyme-IL: a) [Bmim][PF_6] and b) [Omim][PF_6].