EVANESCENT FIELD FLUORESCENCE DETECTION OF SPECIFIC AND NON-SPECIFIC BINDING OF INSULIN TO RECEPTOR-CONTAINING PLANAR BILAYERS SUPPORTED ON SUBSTRATES

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Binding of insulin to the receptor leads to a conformational change of the later which is accompanied by a change in lipid/protein interaction. This effect is expected to lead to a change in surface potential of the supported bilayer which could be exploited for biosensors.

The purpose of the present work is to present firstly the method of the reconstitution of receptors in the planar bilayers supported on the sub-

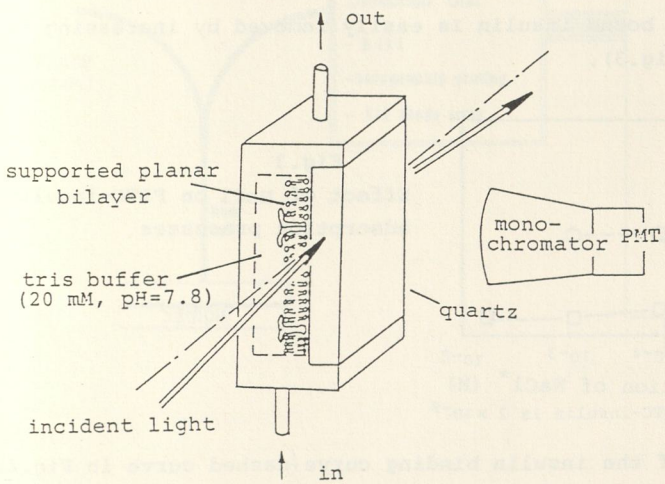

Fig.1
Schemtic view of experimental technique

strates. Secondly a sensitive technique for the distinction of specific and non-specific hormone binding.

The insulin-receptors isolated from the placenta are reconstituted into DMPC vesicles by dialysis. The receptor-containing planar bilayers are prepared by fusing these vesicles on a fresh quartz surface(cf.poster of H.Gaub). The method of measurement is based on the evanescent field fluorescence technique(Fig.1) by using fluorescence labelled(Fluorescien isothioeyanate) insulin. Fig.2 shows typical binding curves. It is clearly seen that insulin binds only to receptor-containing planar bilayer at the bulk concentration of insulin smaller than 6×10^{-10} M.

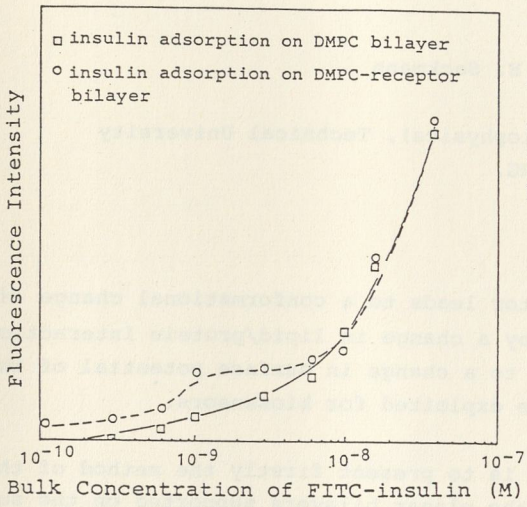

Fig.2
Variation of FITC-insulin adsorption processes on DMPC and DMPC-receptor supported planar bilayers

The non-specifically bound insulin is easily removed by increasing the salt concentration(Fig.3).

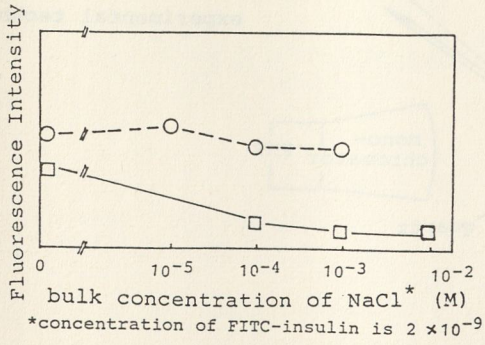

Fig.3
Effect of NaCl on FITC-insulin adsorption processes

The two step shape of the insulin binding curve(dashed curve in Fig.2) implies that there may be two binding sites for insulin-receptor.