

Bovine β -lactoglobulin receptors on transformed mammalian cells (hybridomas MARK-3): characterization by flow cytometry

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Abstract

Flow cytometry was used to demonstrate the presence of β -lactoglobulin (β LG) receptors on living murine hybridoma MARK-3 cells using a fluorescein isothiocyanate- β LG conjugate (FITC- β LG; molar ratio of 5:1). A site occupation curve was produced using a shift in the mean channel fluorescence at various concentrations of FITC- β LG. The binding of labelled ligand was concentration dependent and was inhibited by unlabelled β LG. The on-rate constant was $3.2 \times 10^2 \text{ M}^{-1} \text{ min}^{-1}$ and the off-rate constant was 0.002 min^{-1} . Scatchard plot analysis gave a dissociation constant (K_d) of $44 \pm 21 \times 10^{-7}$ and $39 \pm 24 \times 10^{-5} \text{ M}$ ($n=3$). Flow cytometry indicated that at least 15% of the FITC- β LG were internalized for 5 min and that internalization was temperature- and time-dependent. The internalization was confirmed by 3-D fluorescence microscopy (CELLScan™ system).

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