Multivalent ligands for diagnosis and therapeutics

Shubh D. Sharma[a], Victor J. Hruby[a], Mac E. Hadley[b], Michael E. Granberry[c] and Stanley P.L. Leong[c]

Departments of [a]Chemistry, [b]Anatomy and [c]Surgery, University of Arizona, Tucson, AZ 85721, U.S.A.

Introduction

Multivalent ligands have been shown to exhibit extraordinary properties such as: a) enhanced affinity towards interacting cellular binding sites, b) increased potency, c) stability towards proteolytic enzymes, and d) longer duration of activity [1,2]. Such properties result from simultaneous multiple interactions (cooperative affinity) between the ligand and the acceptor. We are developing a new class of multivalent peptide hormone (neurotransmitter) - macromolecular composites which may serve as powerful diagnostic, imaging, and therapeutic tools. Composites have been synthesized in which multiple copies of a biospecific ligand were covalently attached to a biologically compatible but inert polyfunctional macromolecule. In addition, the conjugation of multiple copies of a fluorophore directly to the macromolecule provided an enhanced visual means of detection of ligand-macromolecular composites bound to target cells in in vitro binding assays. A fluorescent melanotropin (MSH) - macromolecular composite has been synthesized and used to demonstrate the presence of specific melanotropin receptors on various human melanoma cell lines.

Results and Discussion

The composition of various composites synthesized in this study are described in Table 1. Poly-lysine or Poly-vinyl alcohol used as a substrate was derivatized either with 4-(*p*-maleimidophenyl)butyric acid-*N*-hydroxysuccinimide ester, or with 3-(2-pyridyldithio)-propionic acid-*N*-hydroxysuccinimide ester for the synthesis of composites in which the MSH molecules are attached to the polymer via a thioether or a disulfide linkage, respectively. The successive treatment of the derivatized polymers with fluorescein isothiocyanate and a sulfhydryl-containing ligand (thioethanol, an MSH analog or a dynorphin analog) provided the desired composites. The polymer substrate was dialyzed extensively after each reaction step and the degree of substitution calculated spectrophotometrically. The peptide derivatives used for conjugation (Table 1) were synthesized by SPPS and purified by HPLC.

The binding of the fluorescent conjugates with a variety of cultured melanoma cells and other malignant and normal cell types as studied by fluorescence microscopy (Table 1) demonstrated that: a) the MSH-PVA conjugates bound

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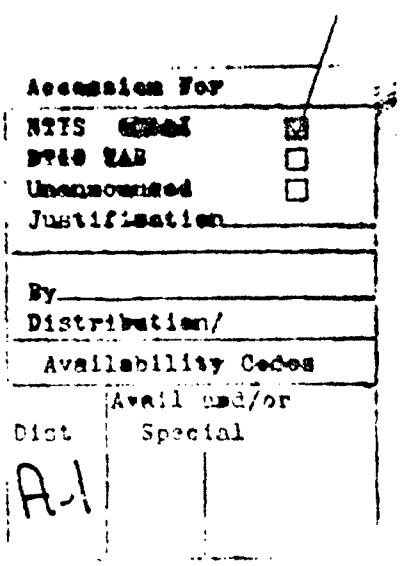

Table 1 *Results demonstrating specific fluorescence labeling of melanotropin receptors on various melanoma cell types by fluorescent MSH-macromolecular composites*

Conjugate[d]	Malignant cell types									Normal cell types	
	Mouse melanoma		Human							Mouse	
			Melanoma						Breast cancer		
	B-16	B-16[a]	JH[b]	LR 1649	LR 1650	LR 1714[b]	WC[b]	MA[c]	MCF-7	Spleen	Liver
FITC-PVA	-	-	-	-	-	-	-	-	-	-	-
FITC-PVA-S-MSH	+	+	+	+	+	+	+	+	-	-	-
FITC-PVA-S-S-MSH	+			+	+	+					
FITC-PVA-S-S-MSH + DTT	-			-	-	-					
FITC-PVA-S-MSH + DTT	+			+	+	+					
FITC-PVA-TE	-		-	-	-	-			-		
FITC-PVA-S-TE	-			-	-	-					
FITC-PVA-S-DYN	-	-									

(+) Indicates fluorescence labeling.
(-) Indicates no fluorescence labeling.
[a] Cells prefixed in 1% formalin.
[b] Amelanotic cell lines.
[c] Similar positive responses were obtained with all other human melanoma cells that were assayed.
[d] Abbreviations:
DTT = Dithiothreitol.
FITC = Fluorescein.
PVA = Poly vinyl alcohol.
S-DYN = Dynorphin analog; [Lys(β-thiopropionyl)^{1,3}]Dynorphin(1-13)-NH_2.
S-MSH = MSH analog; N$^{\alpha}$-des acetyl-N$^{\alpha}$-thiopropionyl[Nle^4, *D*-Phe^7]-α-MSH.
TE = Thioethanol.

to all the melanoma cell lines but not to MCF-7 and to several normal cell types used as controls; b) conjugates lacking the MSH analog, or instead containing a dynorphin analog or thioethanol moieties did not bind melanoma cells; c) the treatment of FITC-PVA-S-S-MSH with DTT prior to its use in fluorescence labeling experiment abolished its ability to bind the melanoma cells. All these observations strongly supported the specificity of the binding between fluorescent MSH-PVA conjugates and melanoma cells.

Acknowledgements

This work was supported in part by a research grant from the U.S. Public Health Service (DK-17420 to VJH).

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