Available online at www.sciencedirect.com
SCIENCE DIRECT®
J. Biochem. Biophys. Methods 65 (2005) 121–136
JOURNAL OF biochemical and biophysical methods
www.elsevier.com/locate/jbbm

Two analytical methods to study the interaction of AGEs with cell surface proteins

Annett Schmitt [a,*,1], Ina Meiners [a,e,1], Johannes Schmitt [b], Joachim Nöller [b], Christian Ihling [c], Gerald Münch [d], Andrea Sinz [c], Karen Nieber [e]

[a] *Interdisciplinary Centre of Clinical Research (IZKF) at the Faculty of Medicine at the University of Leipzig, Inselstr. 22, 04103 Leipzig, Germany*
[b] *NIMBUS Biotechnologie GmbH, Eilenburger Str. 4, 04317 Leipzig, Germany*
[c] *Biotechnological–Biomedical Center, Faculty of Chemistry and Mineralogy, Linnéstr. 3, 04103 Leipzig, Germany*
[d] *Comparative Genomics Center, James Cook University, Townsville, Australia*
[e] *Institute of Pharmacy, Department of Pharmacology for Natural Sciences, University of Leipzig, Germany*

Received 5 April 2005; received in revised form 31 October 2005; accepted 31 October 2005

Abstract

Advanced glycation end products (AGEs) are sugar-modified proteins that are known to appear in vivo and are suspected to be involved in the pathogenesis of several diseases. Although different cellular responses to AGEs can be measured in cell culture studies, knowledge about the nature of AGE-binding and their cell surface receptors is poor. In the present paper a method for the purification of AGE-binding proteins from membrane fractions derived from different rat organs as well as a method for assaying the binding of fluorescein labelled AGEs to the surface of cells of different cell lines are described. The presence of more than 10 proteins interacting with AGEs could be shown in membrane fractions obtained from rat organs. Additionally, binding of AGE-modified BSA to different cells could be shown using fluorescence-labelled ligands in a flow cytometric approach. The presented methods provide an option to isolate AGE-interacting proteins which is a precondition for the identification of these proteins. Furthermore, the measurement of AGE-binding to cell surfaces bears the potential to gain a deeper

Abbreviations: AGEs, Advanced glycation end products; BSA, Bovine serum albumin; CEA, Chicken egg albumin; DMEM, Dubelco's modified eagle's medium; 2,4-DNPH, 2,4-Dinitrophenyl hydrazine; ESI, Electrospray ionization; HSA, Human serum albumin; FCS, Foetal calf serum; FITC, Fluorescein isothiocyanate; FTICR-MS, Fourier transform ion cyclotron resonance mass spectrometry; RAGE, Receptor for AGEs; RFU, Relative fluorescence units.

* Corresponding author. Tel.: +49 341 9715894; fax: +49 341 9715979.
E-mail address: annett_schmitt@web.de (A. Schmitt).

[1] Both authors contributed equally to this work.

0165-022X/$ - see front matter
doi:10.1016/j.jbbm.2005.10.006

understanding about the nature of AGE-binding to cell surface proteins and might be applied as a preliminary test before performing cell culture studies about AGE effects.

Keywords: AGE-receptors; RAGE; AGE-binding; AGE affinity chromatography; Flow cytometry

1. Introduction

Advanced glycation end products (AGEs) arise from the nonenzymatic reaction of reducing sugars with proteins, namely their amino acid side chains, in a complex multi step reaction. In vivo, numerous proteins, particularly long-lived proteins are found to be AGE-modified. AGEs are signals for cellular activation associated with chemotaxis, oxidative stress and cell proliferation or programmed cell death [1] and are suspected to be involved in the pathogenesis of several diseases such as chronic clinical complications of diabetes [2], renal failure [2] and Alzheimer's disease [3]. Most investigations are dealing with the effects of AGEs mediated by the receptor for AGEs (RAGE) or the binding of AGEs to RAGE. RAGE is a transmembrane protein of the immunoglobuline superfamily with a molecular mass of 45 kDa that was demonstrated to be present in several cell types such as endothelial cells, monocytes, microglia, and neurons [4]. AGE binding to RAGE was described to cause an activation of $p21^{ras}$, MAP kinases and NF-κB [4]. But there are also several other proteins identified that were found to bind AGEs (e.g. [4–6]). The function of AGE-binding to most of those proteins is largely unknown but some of them are likely to mediate cellular effects of AGEs (e.g. [1,7]). Therefore, not only binding to RAGE but also binding to other proteins is likely to be involved in transducing cellular effects of AGEs. Hence, dealing with biochemical effects of AGE is a very complex subject since a large number of different AGE structures are potential ligands for a large number of binding proteins, of which only a small number is identified. Thus, proper cause-effect relations are nearly impossible to follow when investigating biochemical effects of AGEs on cells. Due to the fact that the function of many of the AGE-binding proteins is largely unknown and that there are probably other proteins yet to be identified, researchers are additionally faced with the problem to find appropriate cellular parameters, which are activated in the presence of AGEs. To gain a deeper understanding of AGE-effects on cells it is an essential task to identify the proteins that are able to interact with AGEs and to investigate their cellular function. When dealing with effects of AGEs on cells, it might be advantageous to measure AGE-binding to cell surface proteins before starting to search for their biochemical effects, since cell binding can be assumed to be a precondition for mediating cellular effects.

In the present study we describe a method for the isolation of AGE-binding proteins from crude membrane fractions of different tissues, as well as a flow cytometric approach for the detection of AGE-binding to cell surfaces. To prove the presence of AGE-binding proteins, membrane fractions from different rat organs were isolated and an affinity column loaded with AGE-modified albumin was used to isolate AGE-binding proteins. The presence of several AGE-binding proteins was demonstrated by SDS-PAGE analysis of fractions eluted from the affinity column. Additionally, we established a flow cytometric assay in order to measure the binding of modified proteins to cell surface proteins. Binding experiments were performed with a glucose derived AGE-modified BSA. AGE-BSA was expected to bind to the cell surfaces, since similar AGEs were found to stimulate cellular effects in different experiments