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Review

Differential scanning calorimetry of blood plasma for clinical diagnosis and monitoring

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ABSTRACT

Differential scanning calorimetry (DSC) provides a useful method to study the unfractionated plasma proteome. Plasma from healthy individuals yields a reproducible signature thermogram which results from the weighted sum of the thermal denaturation of the most abundant plasma proteins. Further investigation of the thermogram for healthy individuals showed it to be sensitive to ethnicity and gender. DSC analysis of plasma from diseased individuals revealed significant changes in the thermogram which are suggested to result not from changes in the concentration of the major plasma proteins but from interactions of small molecules or peptides with these proteins. Closer examination of the diseased thermograms showed a thermogram characteristic of each disease. For cervical cancer, the DSC method yields a progressively shifted thermogram as the disease advances from pre-invasive conditions to late stage cancer. Our application of the DSC method has provided a potential tool for the early diagnosis, monitoring and screening of cancer patients.
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Contents

Introduction

Improved early detection and diagnosis of cancer is the "NCI Challenge Goal 2015" with the intent to "… support the development and dissemination of interventions to detect and diagnose early-stage malignancy" (National Cancer Institute). This mission indicates that we are still a long way from successfully applying technologies that can detect and diagnose tumors in their early stages. For some cancers, such as ovarian and pancreatic, no reliable early-stage screening tests are currently available. For others, simple blood tests exist. An example is the PSA test for prostate cancer that detects elevated levels of prostate specific antigen (kallikrein 3). However, the test has a high rate of false-negative results and cannot distinguish other noncancerous prostate conditions (Mayo Clinic Staff). Therefore, there is a need for new methods and technologies to provide reliable early detection of cancers.

Proteomics of blood plasma has been an area of great study for the discovery of disease biomarkers (Adkins et al., 2002; Aebersold et al., 2005; Anderson and Anderson, 1977; Anderson, 2005; Anderson and

Abbreviations: DSC, Differential scanning calorimetry; CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesions; HSA, human serum albumin.

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Anderson, 1991, 2002; Anderson et al., 2004; Ebert et al., 2006; Gygi and Aebersold, 2000; Liotta et al., 2003, 2001; Liotta and Petricoin, 2006; Mor et al., 2005; Omenn et al., 2005; Rosenblatt et al., 2004; Wulfkuhle et al., 2003a,b; Yates, 2000; Zhou et al., 2004). The protein content of plasma is dominated by a relatively small number of proteins with 22 proteins accounting for 99% of the total amount of protein in plasma. These high abundance proteins, such as albumin, immunoglobulins and fibrinogen, serve important roles in homeostasis including maintenance of osmotic pressure, functioning of the immune system and blood coagulation. The remaining 1% is of interest for the identification of disease biomarkers that can be measured in clinical diagnostic assays. Recently, the number of identified proteins in plasma reached 1000 by virtue of ever improving proteomics technologies. However, even though more of the low abundance region of the plasma proteome has been successfully mapped, the number of new disease biomarkers discovered has actually declined (Down, 2005). This is bad news in light of the huge number of potential disease biomarkers thought to be circulating in plasma that are yet to be probed. Furthermore, the technologies and experimental approaches employed in biomarker identification are not amenable to routine clinical tests or validation and the discovery of a new diagnostic technology based on biomarker identification seems a long way off (Down, 2005).

Current clinical laboratory assays monitor levels of the most abundant plasma proteins to provide important information for detecting, diagnosing and monitoring diseases. These assays are based on the technologies of electrophoresis and immunochemistry (O'Connell et al., 2005). Research at the Brown Cancer Center has been focused on developing new diagnostic technologies [in the Chaires' laboratory and at the Center for Regulatory and Environmental Analytical Metabolomics (Lane et al., 2009)]. Our method is based on the biophysical technique of differential scanning calorimetry (DSC) which monitors heat changes in a sample as a function of temperature. Analysis of plasma proteins using DSC is therefore based on an entirely different physical property than those of size, charge and chemical interactions that are utilized by the techniques of electrophoresis, mass spectrometry and immunochemistry, which have been mainstays of plasma protein analysis to-date. This article will discuss the application of a DSC approach as a new diagnostic method for disease detection, diagnosis and monitoring.

Differential scanning calorimetry (DSC)

DSC is a thermoanalytical technique which monitors small heat changes (as low as 0.1 μcal) between a sample and reference as a function of temperature (Biltonen and Freire, 1978; Brandts and Lin, 1990; Bruylants et al., 2005; Freire, 1995; Sanchez-Ruiz, 1995). For biological samples, a dilute aqueous solution of a biomolecule is loaded into a sample chamber and a matched reference buffer loaded into a reference chamber. Both chambers are heated at an identical, precisely-controlled rate by the main instrument heaters. As the temperature increases, thermally-induced processes occurring in the sample cell result in heat either being absorbed or released. This creates a thermal imbalance between the sample and reference chambers which is compensated for by electrically-powered feedback heaters. This electrical power output is directly proportional to the apparent heat capacity of the sample and reference solutions and is the raw data recorded during a DSC experiment.

After appropriate baseline subtraction and normalization of the data, the result of a DSC experiment of the denaturation of a pure single domain protein is a unimodal melting curve from which the mid-point temperature and enthalpy of the transition can be directly determined. The melting curve, or thermogram, is a unique signature for a given protein under given solution conditions. Depending on the structural nature of the protein, denaturation might reflect the independent melting of individual domains within the tertiary structure of the protein resulting in a complex thermogram with multiple transitions. A primary DSC thermogram is an extensive property of a protein solution and is therefore directly proportional to the mass of protein in solution. For plasma, assuming there are no significant interactions between the plasma proteins, the DSC thermogram will reflect the melting of a complex mixture of proteins with the observed thermogram representing the sum of the individual protein thermograms weighted according to their mass in solution. This illustrates a significant benefit of applying DSC to the analysis of plasma thermograms, the ability to deconvolute a plasma thermogram into the characteristic melting curves of the individual protein components and their relative solution concentrations.

The high sensitivity of DSC towards binding interactions presents a further intrinsic advantage of this method. If a ligand were to bind to a native protein it might stabilize the protein structure so that the protein becomes more resistant to thermal denaturation. If this were so then an elevated melting temperature would be observed for this protein on a DSC thermogram. However, if the ligand preferentially bound to the denatured form of a protein, a lower melting temperature would be observed (Le Chatelier–Braun principle). This has important implications for the study of the plasma proteome. If low molecular weight protein or peptide biomarkers, circulating in plasma as a result of a specific disease, were to form complexes with more abundant plasma proteins then these interactions would be observed in the plasma thermograms for these diseases. The high sensitivity of DSC towards binding interactions means that dramatic shifts in DSC thermograms can result from binding interactions. This represents an intrinsic advantage of the DSC method over other methods applied to the study of the plasma protein, such as electrophoresis and mass spectrometry. These methods are based on the properties of mass and charge of the plasma proteins, properties which will change very little as a result of the binding of small peptide fragments to a much larger protein receptor. The implication of this is that DSC might offer a useful alternative method to study plasma biomarkers.

We have observed promising early results from the application of a DSC approach to the study of the plasma proteome. In light of the difficulties of biomarker discovery with the current thrust of plasma proteome research, a completely new experimental approach based on a different physical basis might be invaluable. The DSC approach is extremely sensitive to both changes in protein composition and protein interactions. Although the method neither directly detects nor identifies disease biomarkers, the method does report the

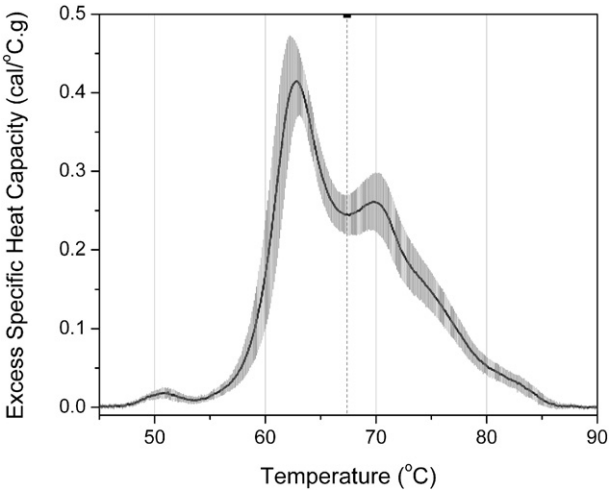

Fig. 1. DSC thermogram of plasma from healthy individuals. The solid line is the average of 15 thermograms of plasma samples from 15 healthy individuals (9 male, 6 female; ages 22–50). The shaded area is the standard deviation at each temperature. The vertical dashed line is the first moment of the thermogram at 67.4 °C. Reproduced with permission from Garbett, N. C., et al., 2008. Biophys. J. 94, 1377–1383.

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