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Review Article

With or without you — Proteomics with or without major plasma/serum proteins

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ABSTRACT

The first sections of this review compile and discuss strategies and protocols for managing plasma/serum as a source of biomarkers relevant to human disease. In many such cases, depletion of abundant protein(s) is a crucial preliminary step to the procedure; specific conceptual and technical approaches, however, make it possible to effectively use to this purpose whole plasma/serum. The final sections focus instead on the complexity associated with each of the major serum/plasma proteins in terms of both, multiple molecular structures (existence of a number of protein species) and of multiple molecular functions (behavior as multifunctional/multitasking/moonlighting proteins). Reviewing evidence in these and some related fields (regulation of the synthetic pattern by proteins and non-protein compounds and its connection with health and disease) prompts the suggestion/recommendation that information on the abundant components of plasma/serum proteome is routinely obtained and processed/mined as a valuable contribution to the characterization of any non-physiological condition and to the understanding of its mechanisms and of its implications/sequels.

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1. Forewords: powers of 10

There are some questions the seniors of us have answered tens of times when advising younger researchers at their first experiences with electrophoresis and proteomics. One of them is *how* to remove albumin from plasma/serum and other biological fluids before analysis. We challenge that this question is in fact the most appropriate. We maintain, first, that all major proteins in those samples are at issue; then, that a proper question should be, instead, *when* to remove them and *when* do not. In the following, we are going to present our point of view — which we know is not shared by the majority of the scientific community. Most of the information will focus on human specimens although similar concepts and procedures apply to plasma/serum from laboratory and farm animals as well.

Clinical biochemistry has long recognized that the concentration in blood of tissue components increases as a result of tissue damage. The recognition of tissue-specific isoforms, either as sequence variants or as heteromultimeric assembly variants, has been the basis for discriminating tissue origin. The difference in subcellular derivation (plasma membrane, cytoplasm, mitochondria) has been associated with nature/severity/duration of the noxa. The typical intracellular proteins currently quantitated in blood by the clinical biochemistry laboratory are medium-abundance enzymes, assayed through their catalytic activity, and high-abundance structural proteins, identified through their immunological reactivity.

Either innovative or more effective disease markers should be as sharply as possible both *tissue- and disease-specific*. Proteomics studies have demonstrated that some major biological limitations exist in these directions. The thorough investigation about the tissue distribution of gene products in different organs and cell types carried out under 'The Human Protein Atlas' project — which participates in the overall 'The Human Proteome' endeavor — has demonstrated that *differences in proteome composition among cell types are more of a quantitative than of a qualitative nature*. Absolute tissue specificity has been demonstrated only for a small percentage of proteins, the most obvious of which had been known for decades. Even more disturbing for its practical, and more intriguing for its biological implications, is the finding that, under conditions of cellular stress, the *changes in proteome composition hardly depend on the nature of the stress*.

How does the circulating concentration of current and perspective disease markers compare with that of other proteins in plasma/serum? Fig. 1 — redrawn from [1] — provides an overview on the quantitative relationships among the various classes of proteins within plasma/serum proteome. The overall dynamic range for their concentrations exceeds 10^{10}-fold. The range associated with proteins secreted continuously, if at a varying rate, in blood (*e.g.* binding/transport proteins, protease inhibitors, coagulation factors, immunoglobulins) is wider than 10^5-fold, that for proteins discontinuously secreted in blood (as long-range extracellular effectors e.g. hormones and cytokines) is narrower than 10^2-fold. The remaining $>10^3$-fold corresponds to the concentration range for proteins not targeted for secretion but leaking from tissues either as intact molecules or as proteolytic fragments.

Proteins leaking from tissues would become major components of a sample only after the removal of hepatocyte and plasma cell secretion products, which are 10^{-3}–10^{-6} times more abundant. The subtraction of albumin alone results in the removal of 50% of the total proteins: while outstanding *per se*, such an achievement is still inadequate to meet the requirement for trace component enrichment. A suitable protocol using the depletion approach demands a much more extensive cut of (all) major proteins. One way to meet this requirement is through the use of immunoaffinity resins; some are marketed, which are able to bind high- or medium-abundance plasma/serum components, resulting in the depletion of up to 99% of the total proteins.

2. Without major plasma/serum proteins

2.1. How to remove major plasma/serum proteins

2.1.1. Removing albumin

From the above, removing albumin alone from plasma/serum, and from all biological fluids that derive from plasma/serum (*e.g.* urine and CSF), definitely falls short of significantly enriching the samples in low-abundance proteins. Still, some specific indications exist for this procedure. The M_r of albumin is close to that of many other plasma/serum components so the already low resolution by mass afforded by 1DE electrophoresis (SDS-PAGE) is further limited by the presence of albumin. The p*I* of albumin differs significantly from that of other plasma/

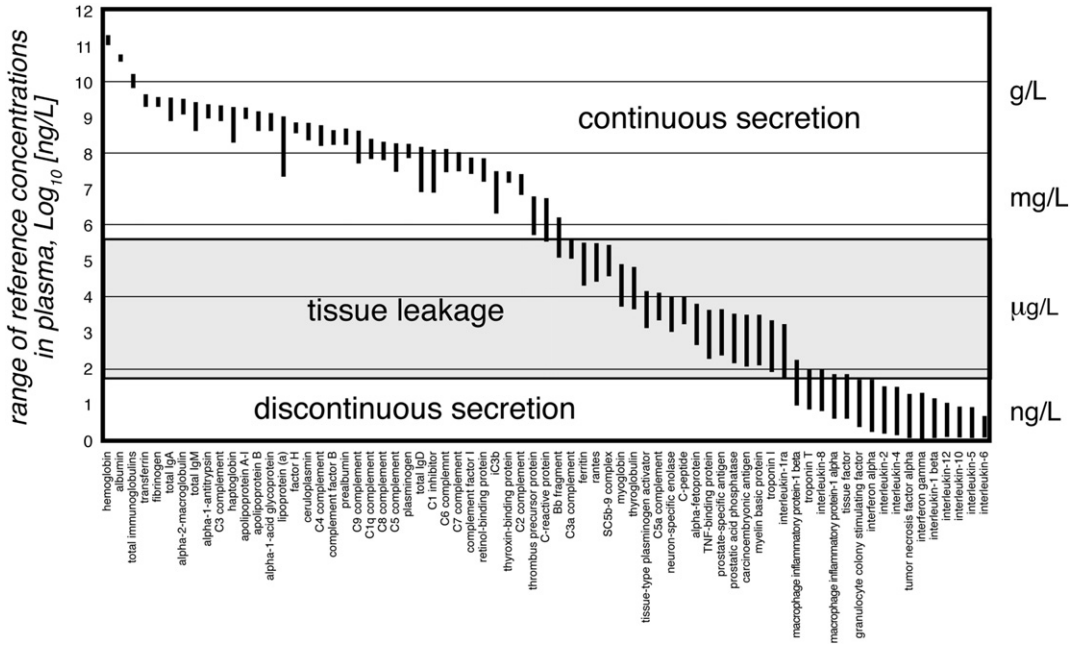

Fig. 1. A chart of the circulating concentrations of different classes of proteins found in plasma/serum. (Redrawn from [1]).