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Heavy Hitting: Using Water to Label Humans

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Abstract

Monitoring protein dynamics, compared to measuring static protein expression profiles taken with snapshot evaluations, have recently been the focus of proteomics studies examining tissue or blood samples where time course changes occur. Using deuterium oxide (²H₂O) to label amino acids is a useful method to monitor protein turnover rates. The synthesis rate for individual proteins is calculated from the rate of ²H incorporation into specific proteins analyzed by high resolution mass spectrometry. In this issue, Wang and colleagues measured the plasma protein turnover dynamics in healthy humans by *in vivo* ²H₂O labeling [Wang et al *Proteomics* 2014]. The authors developed and validated a safe and accessible ²H₂O administration protocol to record the turnover dynamics of 542 plasma proteins using mass spectrometry. Their study demonstrates a promising new way to evaluate plasma protein dynamics in clinical trials where such knowledge could help for prognosis and evaluating treatment efficacy.

Keywords

deuterium oxide; mass spectrometry; matrix metalloproteinase; protein turnover; proteomics

Protein turnover in an organism is controlled by both synthesis and degradation rates. A balance in protein turnover is critical for normal organismal development and maintenance of homeostasis. Imbalanced protein turnover is both a key initiator and fundamental response in multiple human pathologies [1, 2]. Therefore, examining protein turnover dynamics can be used both to identify novel biomarkers and provide insights into the molecular mechanisms of disease development and progression. As such, this tool would be useful in clinical trials where prognostic information and indicators of response to treatment are needed.

Traditional proteomics methodologies provide information on static protein identification and expression profiles, as snapshots taken at particular times. The conventional approach to

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assess *in vivo* protein biosynthesis is based on the delivery of amino acids labeled with radioactive (^3H , ^{14}C) or stable (^{13}C , ^2H , ^{15}N) isotopes [3]. However, amino acid labeling artificially enhances amino acid levels and perturbs normal protein metabolism, which complicates data interpretation. In addition, challenges for *in vivo* evaluation of proteome dynamics include label delivery and tolerance, precursor enrichment determination, and data interpretation [4]. Deuterium oxide ($^2\text{H}_2\text{O}$), heavy water, has a density that is 11% greater than water. This attribute means that deuterium atoms can generate ^2H -labeled amino acids that incorporate into newly synthesized proteins and shift the distribution of the protein. This shift can be detected by mass spectrometry (MS) to examine turnover rates of target proteins.

In this issue, Wang and colleagues evaluated the plasma protein turnover dynamics in healthy humans by analyzing *in vivo* $^2\text{H}_2\text{O}$ incorporation [5]. Briefly, subjects received $^2\text{H}_2\text{O}$ for 14 consecutive days, and plasma protein turnover rates at different time points were measured using high resolution MS. The authors designed and optimized the $^2\text{H}_2\text{O}$ intake protocol by adjusting $^2\text{H}_2\text{O}$ dosages. They validated that 1.5% to 2.0% $^2\text{H}_2\text{O}$ is an optimal concentration for proteomics evaluations. Using this workflow, they monitored turnover dynamics of 542 plasma proteins in 10 human subjects. All 542 proteins were structurally and functionally diverse, and 119 proteins were associated with cardiovascular diseases. The protein turnover rates were independent of protein abundance and were reproducible among diverse subjects regardless of demographics.

Labeling of $^2\text{H}_2\text{O}$ is superior to labeled amino acids for assessing protein biosynthesis rates [6]. For one, continuously administering labeled amino acids for a long period of time (e.g. weeks) is not feasible due to safety, efficacy, and cost issues; in contrast, labeling amino acids in the presence of $^2\text{H}_2\text{O}$ can be accomplished without perturbing normal protein metabolism. For another, calculating rates of protein synthesis for short-term periods (e.g. hours) requires utilizing a precursor/product labeling ratio, which is problematic when using labeled amino acids because it is difficult to accurately quantify the labeled precursor; However, it is possible to reliably identify and measure the labeled precursor when using $^2\text{H}_2\text{O}$ [6].

In conjunction with MS and bioinformatics computational tools, $^2\text{H}_2\text{O}$ metabolic labeling has been used to assess *in vivo* protein turnover dynamics in skin, heart, plasma, and liver in humans and rodents [7–10]. For example, Emson and colleagues demonstrated that deuterium-labeled proteins from lesional psoriatic skin showed up earlier than from non-lesional skin, which opened a new pathway to non-invasive studies of protein kinetics in psoriasis or other skin-related conditions [7]. It has been demonstrated that $^2\text{H}_2\text{O}$ administration leads to stable enrichment of the cardiac precursor amino acid pool, and protein synthesis is slower in mitochondria located among the myofibrils than in the subsarcolemmal region in rats [8]. The turnover rates of 458 proteins in mouse cardiac and hepatic mitochondria were characterized using deuterium labeling [11]. The Hellerstein laboratory measured dynamics of about 100 human plasma proteins using metabolic labeling with $^2\text{H}_2\text{O}$ [9]. The half-life of identified proteins varied from 0.4 to 40.0 days, indicative of widespread range of application for $^2\text{H}_2\text{O}$ labeling. Li and colleagues applied $^2\text{H}_2\text{O}$ labeling to determine synthesis rates of plasma lipoproteins and acute phase response proteins in rats

[12]. Their findings revealed that fasting increased ApoB100 synthesis, but decreased formation of albumin and fibrinogen [12]. Gardner and colleagues used *in vivo* labeling with $^2\text{H}_2\text{O}$ to evaluate collagen synthesis in murine models of carbon tetrachloride induced hepatic cirrhosis [10]. Fluctuations in $^2\text{H}_2\text{O}$ measured collagen synthesis were more sensitive and reproducible than conventional trichrome staining for fibrosis.

The Ping laboratory recently developed a work flow protocol integrating $^2\text{H}_2\text{O}$ labeling, MS, and bioinformatics computational approaches to systematically measure *in vivo* protein turnover [4]. Utilizing this workflow, they monitored turnover kinetics of 2964 proteins in a mouse model of isoproterenol-induced cardiac remodeling. These proteins regulated calcium signaling, metabolism, proteostasis, and mitochondrial dynamics during cardiac remodeling. By translating this workflow, turnover rates of 496 plasma proteins from 4 healthy subjects were examined to set the groundwork for the current study. The procedure for *in vivo* $^2\text{H}_2\text{O}$ labeling and MS analysis illustrates the potential for using this approach to unravel the plasma proteome dynamics in human pathologies.

Before translating findings to widespread use in clinic, the following issues need to be further addressed. First, the current study was limited in sample size, since only ten subjects were recruited for this proof of concept evaluation. Large scale clinical trials are warranted to confirm the findings shown above and determine the usefulness of this approach for prognostic evaluations. This will also allow the assignment of a normal range for individual protein turnover rates over a wide range of subjects. Lastly, the link between individual protein turnover rates and extent of disease needs to be determined using bioinformatics tools, before in depth mechanistic studies can be undertaken. This approach would be useful for situations where turnover rates are high, such as during post-myocardial infarction scar formation [13]. Further, evaluating the effects of enzymes (e.g. matrix metalloproteinases) that regulate scar formation on protein turnover rates, could be performed using this method [14].

In conclusion, the Ping laboratory has introduced a novel approach of using heavy water labeling to assess protein turnover in humans, which will allow evaluations of disease progression and treatment efficacy. This first step evaluation opens the door for using a similar approach in a wide variety of clinical trial settings.

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