existence of a known or as yet unknown naturally occurring degradation product may be of interest with respect to the etiology of neural tube defects or the homocysteine that is associated with this variant. At a more practical level, the comparative evaluation of the two assays reported here indicates that folate concentrations are not being measured accurately by one of the assays in the 5% to 15% of people who are homozygous for the thermolabile variant.

References

Simplified Simultaneous Assay of Total Plasma Homocysteine and Methionine by HPLC and Pulsed Integrated Amperometry

To the Editor:

Measurement of total plasma homocysteine (tHcy) can be a useful adjunct in the diagnosis of cobalamin or folate deficiency and is emerging as an independent predictor in many vaso-occlusive diseases [1]. As clinical interest in this metabolite grows, the demand for simple and efficient methods of determination has increased. In some situations, a methionine-loading test may be conducted to evaluate homocysteine catabolism, but methionine is rarely measured concomitantly, because it usually requires a different assay methodology altogether. In homocystinuria caused by cystathionine beta-synthase deficiency, circulating methionine is often increased, whereas homocystinuria resulting from a relative deficiency of the remethylation pathway is characterized by hypomethioninemia [2].

In our previously reported serum assay for tHcy [3], we used the DX-500 Ion Chromatograph ( Dionex Canada), outfitted with two pumps (in parallel), valves, and two columns (a 4 × 50 mm OmnifC PCX-500 precolumn and a 4 × 250 mm OmnifC PCX-500 analytical column) plumbed in series to permit “heart-cut” trapping of tHcy [4]. However, with the ED40 electrochemical detector set for pulsed integrated amperometry (PIA) mode, any compound with a reduced sulfur atom, including methionine, will generate a signal proportional to concentration [5].

In our initial procedure, the disulfide reduction procedure with sodium borohydride (NaBH₄) [6] was the most labor-intensive step and constituted a substantial source of assay error. Here, we report a simplified protocol for the tHcy assay that permits accurate simultaneous quantification of methionine.

As suggested by Gilfix et al. [7], we used tris(2-carboxyethyl)phosphine (TCEP) as a reductant instead of NaBH₄. To 300 μL of plasma we added 30 μL of 100 g/L TCEP (Pierce Chemical Co.) and gently mixed with a rotating stirrer at room temperature for 30 min. Then, we added 1170 μL of mobile phase (150 mmol/L NaClO₄, 100 mmol/L HClO₄, and 50 mL/L CH₃CN) and centrifuged the mix at 10 000g for 5 min. The supernatant was passed through a C₁₈ solid-phase extraction cartridge, as described before [3], and 50 μL of filtrate was injected directly. Altering the valve-switch times to 1 min and 2 min generated a larger “heart-cut” of the eluting peaks, with homocysteine eluting at 7.9 min and methionine at 11.3 min.

With our plasma control, we found that TCEP reduction is complete within a minute or so at room temperature (Fig. 1A). Reduction of Hcy by borohydride at the same temperature was still incomplete at 30 min, and even at 50 °C required at least 15 min to approach completion. Moreover, use of the TCEP reductant significantly decreased between-run variation (CV = 3.1%, n = 10). Omission of the urea denaturant resulted in a 4% increase of our target tHcy value for the control sample, but the chromatographic profile without urea was less noisy and the assay variation (within-run CV) was correspondingly decreased from 4.8% (n = 16) to 3.8% (n = 16).

Assay of 58 patients’ samples with
a wide range of homocysteine values (Fig. 1B) showed excellent correlation ($r^2 = 0.96, S_{y|x} = 0.80$), and the line of best fit was not significantly different from the line of identity: slope 0.947 (95% confidence interval 0.91–1.02); $y$-intercept 0.23 (0.52–0.97). Analysis of residuals by a runs test and examination of the Bland–Altman plot [8] revealed no significant nonlinear trends (Fig. 1B, inset).

In evaluating our methionine assay, we found near-quantitative recovery (98.4% ± 3.1%, n = 6) of 6.0 μmol/L reagent-grade L-methionine added to a sample with a nominal methionine concentration of 12.1 μmol/L. For 31 samples (Fig. 1C), the correlation between our method and conventional amino acid chromatography with ninhydrin detection (Beckman 7300 Amino Acid Analyzer) [9] was excellent ($r^2 = 0.96, S_{y|x} = 0.755$). By linear regression analysis, the line of best fit ($y = 0.97x - 0.89$) passed through the origin (95% confidence interval for $y$-intercept: −0.71 to 2.5). Runs test analysis of residuals and Bland–Altman plot (Fig. 1C, inset) revealed no significant deviation from linearity. The within-run CV was 3.4% (n = 8) and the between-run CV was 4.3% (n = 6). Although identical control sample aliquots were stored at −74 °C and used only once, the measured methionine concentration showed a noticeable downward drift, equivalent to a decrease of 1.6% per week. Similar changes were observed with the calibrators. The susceptibility of methionine to oxidation (forming methionine sulfoxide) is well-described [10] and should be kept in mind when interpreting plasma methionine data [11].

For our group, we found a mean ± SD plasma methionine concentration of 21.9 ± 2.4 μmol/L (range 18.3–26.5 μmol/L), which is within 3.5% of, and intermediate between, the means reported by Guttormsen et al. (22.7 ± 3.5 μmol/L, n = 12) [12] and Potgieter et al. (21.3 ± 2.1 μmol/L, n = 127) [11], who used phthalic aldehyde derivatization and fluorescence HPLC. Our results were also within 3.5% of the values obtained with the ninhydrin-based amino acid chromatography method (22.7 ± 0.35 μmol/L, n = 10) [13]. Our method simplifies the assay of...
tHcy and reduces assay time and cost. It may also enhance the assessment of methionine loading as a tool for the investigation of hyperhomocystinemia and the potential role of methionine as an antioxidant [14]. It offers a rapid and simple alternative to the separate assay of methionine by conventional amino acid chromatography or tandem mass spectrometry [15] and is an attractive alternative to the simultaneous assay of homocysteine and methionine by GC-MS [16].

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References

David E.C. Cole* Denis C. Lehotay Jovan Evrovski
* Rm. 415, 100 College St. Toronto, ON M5G 1L5 Canada

* Author and address for correspondence. Fax (416) 978-5650; e-mail davidec.cole@utoronto.ca.

The Sia Euglobulin Precipitation Test Revisited
To the Editor:
The Sia test (Sia euglobulin precipitation test) was first described more than three-quarters of a century ago for evaluation of the euglobulin fraction in certain infectious diseases [1]. In this test, serum is diluted with water so that the ionic strength of the serum is decreased, resulting in the precipitation of euglobulins and macroglobulin [2]. Later, positivity of the Sia test was demonstrated in conjunction with clonal gammapathies [3–6]. A more high-tech version of this simple test has been encountered with use of the Paramax™ chemistry analyzers [7].

When we tested a sample on our Dade Paramax 720 ZX analyzer and obtained hemolysis/lipemia warnings on all tests, even though the plasma was not visibly hemolytic/lipemic, we became suspicious of a protein dyscrasia. We mimicked the dilution of the sample blank cuvette by adding 2.0 mL of H2O to 50 mL of serum [2]. When the mixture was visibly cloudy, cases were referred for serum protein electrophoresis.

Four cases of monoclonal gammopathy detected by this technique were described in a Paramax technical bulletin [7]. Over ~2 years, a total of 61 cases of this kind have been observed in our laboratory. Of these, 41 revealed monoclonal or biclonal gammopathies; the other 20 were polyclonal. Thirty-three of these clonal proteins were further characterized by immunoelectrophoresis (18 IgM kappa, 5 IgM lambda, 8 IgG kappa, and 2 IgG lambda).

Although clinical follow-up has been incomplete, four cases of lymphoproliferative disease have been reported (one case each of multiple myeloma, Waldenstrom macroglobulinemia, chronic lymphocytic leukemia, and malignant lymphoma).

Although the Sia test is now largely thought of as being of only historical interest, these cases reveal the utility of understanding such “obsolete” tests and how they may be applied to the modern era of automated chemistry.

References

Gary C. Mockli
New Hampshire Med. Labs
2456 Brown Ave.
Manchester, NH 03103