We are deeply grateful to the postmenopausal women who participated in this study. We are very grateful to Eva Molero, Antonio González, and Rosío Pascual for their collaboration during this work. The authors F.J. Morón, M.E. Saez, and A. Ruiz, have declared that conflicts of interest exist. Some of the work described here is subject to patent filings for diagnostics purposes. Neocodex has been partially funded by the Ministerio de Educación y Ciencia of Spain (PTQ2003-0546, PTQ2003-0783) and the European Commission (Gendisrupt project:QLK4-CT-2002-02403).

References


4. Dixit H, Rao LK, Padmalatha VV, Kanakavalli M, Deenadayal M, Gupta N, et al. Missense mutations in the BMP15 gene are associated with menopausal women who participated in this study. We are very grateful to Eva Molero, Antonio González, and Rosío Pascual for their collaboration during this work. The authors F.J. Morón, M.E. Saez, and A. Ruiz, have declared that conflicts of interest exist. Some of the work described here is subject to patent filings for diagnostics purposes. Neocodex has been partially funded by the Ministerio de Educación y Ciencia of Spain (PTQ2003-0546, PTQ2003-0783) and the European Commission (Gendisrupt project:QLK4-CT-2002-02403).

Grant/funding support: None declared. Financial disclosures: None declared.

Determination of ITPase Activity by Capillary Electrophoresis

To the Editor:

Shipkova et al. (1) recently reported in Clinical Chemistry that liquid chromatography can be used to determine the activity of inosine triphosphate pyrophosphohydrolase (ITPase) in erythrocytes. We find that ITPase can also be measured by capillary electrophoresis.

We performed capillary electrophoresis on a Beckman P/ACE 5510 with a diode array detector. Electrophoretic separations were carried out in an uncoated silica capillary (20 cm effective/27 cm total length, 50 μm internal diameter; Polymicro CE and CEC Technologies) at a constant voltage of ~30 kV (1111 V/cm). We set the data rate of the detector at 16 Hz. Samples were loaded by a low-pressure injection (0.5 psi, 6 s). Ultra-violet detection was performed at 250 nm.

We prepared buffer containing citric acid (40 mmol/L) and cetyltrimethylammonium bromide (0.8 mmol/L), adjusted to pH 4.4 with γ-aminobutyric acid (2), filtered and sonicated for 0.5 min before use. At the beginning of each working day, the capillary was washed for 2 min with water and separation buffer, and also washed between runs for 1 min with separation buffer. We prepared a 100 μmol/L aqueous mixture of inosine monophosphosphate (IMP) and inosine triphosphate (ITP). The compounds could be separated within 0.8 min with separation efficiency up to 1 200 000 theoretical plates/m in a mixture and 300 000 theoretical plates/m in biological samples (Fig. 1).

We collected blood samples in EDTA tubes from healthy blood donors and patients undergoing azathioprine therapy (who gave informed consent). Erythrocytes were separated by centrifugation (1200 g, 5 min) and washed twice with 3 volumes of NaCl, 9 g/L. We lysed 200 μL of erythrocytes with 1 mL of ice-cold distilled water, centrifuged the lysates at 5000 g for 10 min, and mixed 25 μL of supernatant with 100 mmol/L Tris buffer (150 mmol/L dithiothreitol (10 μmol/L), and 1 mol/L MgCl2 (10 μL). The mixture was preincubated for 5 min at 37 °C, after which 40 mmol/L ITP (10 μL) was added and incubated for 15 min at 37 °C (2). The samples were deproteinized with 20 μL of trichloroacetic acid, 1 mol/L, sonicated (30 s), and centrifuged at 5000 g for 1 min. The supernatant was injected into the capillary or stored at ~50 °C. We measured hemoglobin (Hb) in the lysate with a Radiometer ABL 725 (Diamond Diagnostics).

The signal-to-noise ratio was >6 at 2.0 nkat/g Hb [7.2 μmol of IMP/(g Hb · h)]. The calibration curve was linear from 0.01 to 10 mmol/L (y = 11.3x – 0.6 nkat/g Hb; r = 0.9974). We evaluated recovery and imprecision by assaying erythrocytes with the added mixture of IMP. Recoveries were 85%, 83%, 76%, 75%, and 80% for 0.06, 0.25, 0.54, 1.80, and 3.00

Francisco J. Morón1
Nicolás Mendoza2
Francisco Quereda3
Francisco Vázquez3
Reposo Ramírez-Lorca4
Juan Velasco1
Jose L. Gallo2
Ana Salinas1
Txantón Martínez-Astorquiza5
Rafael Sánchez-Borrego5
María E. Sáez4
Agustín Ruiz4*

1 Departamento de Genómica Estructural Neocodex
Sevilla, Spain
2 Servicio de Ginecología y Obstetricia
Hospital Universitario Virgen de las Nieves
Granada, Spain
3 Servicio de Ginecología y Obstetricia
Hospital San Juan de Alicante
Alicante, Spain
4 CEGA
Clínica de Ginecología
Lugo, Spain
5 Servicio de Ginecología
Clínica Sanatorio Bilbaíno
Bilbao, Spain
6 Clínica Diatros Gavá
Barcelona, Spain

*Address correspondence to this author at: Departamento de Genómica Estructural. Neocodex. C/ Charles Darwin no.6, Acc. A. Parque Tecnológico Isla de la Cartuja, 41092-Sevilla, Spain. Fax 34-955-047325; e-mail aruiz@neocodex.es.

DOI: 10.1373/clinchem.2006.081307
mmol/L added IMP (n = 6), respectively. The lower recoveries reflect coprecipitation of IMP with proteins, which agrees with previously published findings (1). Imprecision values (as CV, n = 10) were 2.1%, 1.2%, and 1.0% (within-day CV) and 4.2%, 3.2%, and 2.4% (between-day CV) for 0.06, 0.54, and 3.00 mmol/L additions of IMP, respectively. The reproducibility values (CV) of migration times for 10 samples from healthy volunteers were 0.92%, 2.8%, and 2.5% for run-to-run, sample-to-sample, and between-day measurements (n = 10), respectively. Because of the use of acidic separation medium, we observed no interference during the analysis of samples from 80 healthy blood donors and 20 patients undergoing azathioprine therapy.

With this simple capillary electrophoresis method, we estimated a reference interval (n = 80, 38 males and 42 females) for healthy white individuals of 13.3–112.2 nkat/g Hb (5%–95%), with a median of 57.8 nkat/g Hb, which agrees with the previously published data (3).

Availability of alternative methods is important because available analytical equipment varies among laboratories. Measurements of ITPase activity are important because ITPase deficiency may alter 6-mercaptopurine (azathioprine) metabolism, leading to adverse reactions (3), and the deficiency affects >10% of the population (4).

Grant/funding support: This study was supported by Grant MSM 6198959205 from the Ministry of Education, Youth and Sports (Czech Republic).

Financial disclosures: None declared.

References

David Friedecky1*
Jana Tomkova2
Tomas Adam1

1 Laboratory for Inherited Metabolic Disorders
Department of Clinical Biochemistry
University Hospital and
Palacky University Olomouc
Olomouc, Czech Republic

2 Department of Analytical Chemistry
Palacky University Olomouc
Olomouc, Czech Republic

*Address correspondence to this author at: Laboratory for Inherited Metabolic Disorders, University Hospital, I. P. Pavlova 6, 775 20 Olomouc, Czech Republic. Fax 420-5-88442509; e-mail david.friedecky@gmail.com.

DOI: 10.1373/clinchem.2007.086058

Novel Mutation (c.G1124A) in Exon 9 of the APOB Gene Causes Aberrant Splicing and Familial Hypobetalipoproteinemia

To the Editor:

Familial hypobetalipoproteinemia (FHBL) is commonly caused by mutations in the apolipoprotein B gene (APOB). The APOB gene encodes 2 proteins, apolipoprotein (apo) B-48 and apo B-100. Apo B-48 is formed in the intestine and is essential for the formation and recognition of dietary derived chylomicrons, and apo B-100 is found in VLDLs and LDLs of hepatic origin and is involved in the endogenous transport of triglycerides, cholesterol, and fat-soluble vitamins. A number of abnormally truncated apo B proteins have been described, and by convention are referred to by a centile system reflect-