Simple Screening Test for Qualitative Detection of Increased 
δ-Aminolevulinic Acid in Urine

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A simple qualitative procedure for demonstrating increased δ-aminolevulinic acid (ALA) in urine is needed to identify individuals with an inherited deficiency of the enzyme ALA dehydratase, a newly described porphyria-like syndrome. The present procedure includes two steps, the last of which is carried out only on samples that are positive in the first step. Urine is tested for pyrroles, preformed or formed by heating with acetylacetone, by looking for a pink color after p-dimethylaminobenzaldehyde is added. Samples showing the color are next tested for ALA by adsorbing natural pyrroles onto Dowex-1 in sodium acetate, heating the supernate with acetylacetone, adsorbing the resulting aminoketone pyrroles onto Dowex-1 in water, separating the ALA from other aminoketones by differential extraction, and again looking for a pink color with p-dimethylaminobenzaldehyde. Positive urines, those with concentrations of ALA >10–15 mg/L, yield a pink color in each step. Samples with >30 mg/L ALA show a magenta color. Aminocetone in excess of 3 mg/L also yields a pink color, but such a concentration in urine is unlikely. Many samples can easily be tested concurrently.

Additional Keyphrases: δ-aminolevulinic acid dehydratase deficiency · porphyria · heritable disorders

A recently described entity, inherited deficiency of activity of δ-aminolevulinic acid (ALA) dehydratase [porphobilinogen (PBG) synthase; EC 4.2.1.24], is associated with a syndrome of acute attacks that is indistinguishable from those of the classical acute porphyrias: intermittent acute porphyria, coproporphyria, and variegate porphyria (1, 2). In this syndrome, the virtually complete deficiency of ALA dehydratase, which catalyzes the synthesis of PBG from ALA, results in the excretion of massive quantities of ALA in the urine (>130 mg/day, >25 times normal) (2, 3). A simple qualitative test for detection of such increases in ALA excretion would be valuable in the detection of patients with ALA dehydratase deficiency. By modifying the method of Marver et al. (4) for the quantitative determination of aminoketones we have developed a qualitative test for urinary ALA.

Materials and Methods

Materials

ALA was obtained from Porphyrin Products, Logan, UT 84321; acetylacetone and p-dimethylaminobenzaldehyde from Sigma Chemical Company, St. Louis, MO 63178; and aminocetone hydrochloride from Trans World Chemicals, Inc., Washington, DC 20815. We prepared AG1×8 resin, 100–200 mesh anion-exchange resin in the acetic form (Bio-Rad Laboratories, Richmond, CA 90480) as a slurry in either a 0.5 g/L solution of sodium acetate, pH 4.6, or de-ionized water. Modified Ehrlich’s reagent, prepared freshly each day, consisted of 1 g of p-dimethylaminobenzaldehyde and 9.5 mL of 600 g/L (i.e., 60%) perchloric acid diluted to 50 mL with glacial acetic acid (5).

Step 1: Test for Pyrroles

Mix 1.0 mL of urine with 1.0 mL of 1 mol/L sodium acetate, pH 4.6, and 0.2 mL of acetylacetone in a test tube. Cover the tube, heat for 10 min in a boiling water bath, then remove the tube to room temperature. Add an equal volume of modified Ehrlich’s reagent, mix well, and 15 min later look for a pink color, which indicates the presence of pyrroles.

Step 2: Test for ALA

This further procedure is carried out only on urines for which Step 1 gave positive results. Add 1.0 mL of urine to an equal volume of AG1×8 slurry in sodium acetate. Allow the tube to stand at room temperature for 5 min, with vortex-mixing at least twice for 30 s during this interval. Aspirate the supernatant fluid and place it in a tube containing 1.0 mL of sodium acetate buffer (1 mol/L, pH 4.6) and 0.2 mL of acetylacetone. Cover the tube and heat for 10 min in a boiling water bath, then remove the tube to room temperature. Add 1.0 mL of AG1×8 resin slurry in water. Allow the tube to stand for 5 min, with at least two 30-s vortex-mixings. Aspirate and discard the liquid phase. Add to the resin 2.0 mL of n-butanol containing 0.1 mol of ammonium hydroxide per liter and vortex-mix for 30 s, allow to stand for 2 min, and aspirate. To the resin, add 2.0 mL of methanol:glacial acetic acid (2:1 by vol), agitate the mixture for 30 s, and allow it to settle for 2 min. Mix an aliquot of the supernate with an equal volume of modified Ehrlich’s reagent and examine in 15 min. A pink color here as well as in Step 1 indicates the presence of highly increased concentrations of ALA.

Results

This procedure for qualitative detection of ALA is based on the well-known reactivity of pyrroles with p-dimethylaminobenzaldehyde, the avidity of Dowex-1 resin for pyrroles, and the ability of different solvents to elute different pyrroles from this resin. Thus, in Step 1 both preformed pyrroles (e.g., PBG) and materials that react with acetylacetone to form pyrroles (aminoketones, such as ALA) are detected by their reaction with p-dimethylaminobenzaldehyde. In Step 2, natural pyrroles are separated, by adsorption to resin, from materials that form pyrroles on reaction with acetylacetone (aminoketones). The two major aminoketones, ALA and aminocetone (4), are then separated (as pyrroles) by differential extraction—aminocetone into n-butanol:ammonium hydroxide and ALA into methanol:glacial acetic acid.

Urine with markedly supranormal concentrations of ALA yield a pink color on reaction with modified Ehrlich’s reagent after both steps. An intense pink color is always obtained for urines with ALA concentrations of >15 mg/L.
>30 mg of ALA per liter yields a magenta color. Samples with ALA concentrations between 10 and 14 mg/L usually yield a light-pink color, but occasionally we saw no pink color in this concentration range. Such color was observed in many normal urine samples that we supplemented with ALA, as well as in four samples from two patients with intermittent acute porphyria. In contrast, normal urine always yielded a yellow color, as did normal samples supplemented with ALA to give concentrations <10 mg/L.

The potential of PBG or aminolevulinate to confound the color development from ALA was investigated with normal urine samples supplemented with these materials. The color intensity after Step 2 for a urine with PBG (25 mg/L) as well as ALA (25 mg/L) was no different from that of the urine containing only ALA (25 mg/L). PBG was readily detected in Step 1, yielding the expected deep-pink color, but was completely removed by the first resin in Step 2. Aminolevulinate in concentrations as high as 3 mg/L yielded no pink color in Step 2. Higher concentrations were incompletely extracted and did give some color.

A series of 16 unknown urine samples, including samples from normal individuals, samples from three subjects with intermittent acute porphyria, and normal samples that had been supplemented with ALA (5, 10, 12, 23, 26, and 35 mg/L), was assayed for ALA by the two of us, independently. All samples with highly augmented concentrations of ALA (>20 mg/L) were recognized, and no samples with concentrations of ALA < 12 mg/L were considered to have excessive concentrations of ALA. In addition, we tested urines from 185 inpatients and outpatients that were routinely supplied to the clinical laboratory of this hospital. Tests for ALA were negative for all but one sample, which gave a weakly positive (light-pink) result. Paper electrophoresis of this sample in equal volumes of n-butanol and 1.5 mol/L aqueous ammonia established the identity of the chromogen with authentic ALA.

We checked several of the conditions specified in the assay of Marver et al. (4), on which this test is based, to see if they were optimal or even essential. The intervals we specify for mixing and incubating cannot be shortened, but prolonging any of them, including that for the development of color after the modified Ehrlich's reagent is added, by as much as fourfold does not affect the results. The 10-min heating after acetylation is added is also the minimum time; cooling the solutions to room temperature is not necessary. Washing the resin with 1 mol/L acetic acid between the n-butanol:ammonium hydroxide and the methanol:acetic acid steps as advocated in the original method (4) is not necessary in our modification.

Discussion

This simple qualitative assay is particularly designed to detect large increases in urinary ALA excretion (≥10–15 mg/L; normal concentration <3 mg/L). Its simplicity permits the simultaneous assay of multiple samples. It consists of two sequential steps, the last presupposing a positive result in the first, that would render it useful in the screening of populations. Most individuals would be screened out in Step 1, which can be completed within 30 min. Only urine samples giving a positive test in Step 1 will then be subjected to Step 2, which requires an additional 40 min. Step 2 identifies individuals with markedly increased urinary ALA (≥10–15 mg/L), or those with urinary aminoacetic acid concentrations in excess of 3 mg/L. The urinary excretion of aminoacetate appears to be much lower than this (<1 mg/24 h) under usual circumstances (4, 6), but such data are meager and the final identification of Ehrlich-positive material of Step 2 must be made by more sophisticated, quantitative means.

In our limited studies with unknown urine samples to determine the specificity of this method for ALA, we found that we could consistently distinguish those samples with greatly augmented concentrations (>20 mg/L) from those with lower concentrations (<12 mg/L) of this material. Furthermore, only one of 185 urine samples from the clinical laboratory yielded a positive (light-pink) result, and the chromogen in this sample did indeed appear to be ALA. Thus, we believe this method is highly specific. Moreover, none of the 185 samples yielded a strongly positive (magenta) color, which is the color that develops at urinary ALA concentrations >30 mg/L. In ALA dehydratase deficiency, which this test was developed to detect, urinary ALA concentrations are two to four times higher than this. Thus, one can look for a magenta final color to identify subjects with ALA dehydratase deficiency, or a pink color to identify individuals with lesser increases in urinary ALA concentrations for any reason.

With the aforementioned sensitivity, ease of performance en masse, and low cost (currently estimated to be about $0.55 per test), this test is intended to detect individuals with inherited deficiency of ALA dehydratase (1, 2). These individuals seem to have a clinical syndrome that is indistinguishable from that of intermittent acute porphyria. Like intermittent acute porphyria, the prevalence of this syndrome may be appreciable in some populations, such as patients in psychiatric hospitals (7). Unlike intermittent acute porphyria, affected individuals cannot be detected by screening the urine for PBG (8); their excretion of PBG is only slightly above normal (2). Until now, the prevalence of this syndrome could not be determined, because there was no means for its rapid detection in populations. Thus our test should be of value in furthering our understanding of the nature of this "new" porphyria.

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References


