Carrier Detection for Sanfilippo A Syndrome

R. MATALON, M. DEANCHING, R. MARBACK and K. MICHALS
University of Illinois at Chicago, Department of Pediatrics, Division of Genetics and Metabolism, and Department of Nutrition, 840 South Wood Street, Suite 1311N, Chicago, Illinois 60612, USA

Summary: In Sanfillipo A families, sulphamidase activities in leukocytes and cultured fibroblasts determined at 55°C distinguish between heterozygote-carriers, normal individuals and the homozygotes.

Sanfilippo A syndrome, mucopolysaccharidosis IIIA (McKusick 25290), is an autosomal recessive disorder of heparan sulphate degradation. Patients with Sanfilippo A syndrome are severely retarded and have mild skeletal features of dysostosis multiplex. They accumulate in tissues, and excrete in urine, excessive amounts of heparan sulphate (Matalon, 1983). Sulphamidase (EC 3.10.1.1) deficiency has been identified as the defect responsible for Sanfilippo A syndrome (Kresse, 1973; Matalon and Dorfman, 1973, 1974). Cultured skin fibroblasts have been the primary specimen used for the enzyme assay to determine sulphamidase deficiency. Attempts at carrier detection for Sanfilippo A syndrome have generally been unsuccessful (Whiteman and Young, 1977). In this report we have utilized the thermal characteristics of sulphamidase for carrier detection using both leukocytes and cultured skin fibroblasts. The data presented indicate that carriers can be reliably identified using the method described.

MATERIAL AND METHODS

Heparinized blood, 8–10 ml, was centrifuged for 10 min at 600 g and the top layer containing the leukocytes was removed. The top layer was then centrifuged at 800 g for 10 min and the plasma was removed; the remaining white cell precipitate was then collected. Any contaminating red cells were lysed with 7 ml of ice-cold water for 45 s, then 7 ml of 1.8% (w/v) sodium chloride was added to restore isotonicity. This procedure was repeated and the leukocyte pellet was suspended on 0.8–1.0 ml of 0.1 mol L⁻¹ acetate in 0.15 mol L⁻¹ sodium chloride buffer, pH 5.0. Skin fibroblasts were cultured according to the method of Matalon and Dorfman (Matalon and Dorfman, 1966). The cultured skin fibroblasts were washed with 0.1 mol L⁻¹ acetate in 0.15 mol L⁻¹ sodium chloride buffer, pH 5.0. The fibroblasts were scraped and suspended in 1.0–2.0 ml of the same buffer. Leukocytes and fibroblasts were placed in an ice bath and sonicated with a Heat Systems Sonicator
Model W 185 F, using three bursts, 10 s each, at a setting of 3 with the meter reading ranging from 60–70 watts. The leukocyte sonicate was used for the sulphamidase assay. The fibroblast sonicate was centrifuged at 10000 g for 10 min to remove cell debris and the supernatant was used for enzyme assay. Protein content of the leukocyte and fibroblast preparations was determined by the method of Lowry et al. (1951).

Heparin, \( [^{35}S] \)-N-sulphated 12.7 mCi g\(^{-1} \), was purchased from Amersham/Searle Corp., Arlington Heights, IL. Scintillation cocktail 3A070B was obtained from Research Products International Corp., Mt. Prospect, IL. The ECTEOLA (Cellex E) was obtained from Bio-Rad Laboratories, Richmond, CA.

### Enzyme assay

The sulphamidase assay was a modification of the methods of Matalon and Dorfman (1974) and Hall et al. (1978). The assay contained 50 \( \mu l \) leukocyte (300 \( \mu g \) protein per assay) or fibroblast (150 \( \mu g \) protein per assay) extract, 10 \( \mu l \) of 1.8% (w/v) Triton X-100 and 15 \( \mu l \) acetate buffer, 0.5 mol L\(^{-1} \), pH 5.0. The substrate, 50000 cpm of \( [^{35}S] \)-N-sulphated heparin in 20 \( \mu l \), was added together with 10 \( \mu l \) (20 \( \mu g \)) non-radioactive heparin. The enzyme reaction was carried out at 55°C, for 18 h for the leukocytes and 30 min for the fibroblasts. The samples were placed in an ice bath and 10 \( \mu l \) of 0.1 mol L\(^{-1} \) sodium sulphate was added. After 15 min 1 ml of 0.1 mol L\(^{-1} \) sodium formate was added. The reaction mixture was applied to the ECTEOLA columns (0.4 ml bed resin). The ECTEOLA columns were eluted with 4 ml of 0.1 mol L\(^{-1} \) sodium formate and the total eluates (5 ml) were counted for radioactive sulphate with 15 ml 3A70B scintillation cocktail in a Beckman LS-9800 scintillation counter.

### RESULTS AND DISCUSSION

Fifty-four normal individuals, 15 patients with Sanfilippo A and 16 obligate carriers were assayed for sulphamidase at 55°C. The results of the leukocyte extracts are presented in Table 1. In one carrier the activity overlapped with the deficiency range. This overlap was resolved by the use of cultured skin fibroblasts. The optimal time for identification of carriers using cultured skin fibroblasts was found to be 30 min. Incubation time for sulphamidase activity in cultured fibroblast

\[ J. \text{Inher. Metab. Dis. 11 (1988)} \]