A CONTINUOUS BIOLUMINESCENT ASSAY OF GLYCOGEN PHOSPHORYLASE

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Recent results have demonstrated a high sensitivity of glycogen phosphorylase (EC 2.4.1.1) (GP) for diagnosing and monitoring acute myocardial infarction during its early phase. For that reason an immunoenzymometric assay for human GP isoenzyme BB has been developed, which is able to detect the low enzyme concentrations increased in plasma samples above the upper reference limit (7 ng/ml) within 4h of onset of chest pain (range of phosphorylase peak concentrations: 15-1210 ng/ml) (1,2).

A simple one-step bioluminescent method for the determination of GP activity was developed. The method utilizes a multienzyme system containing phosphoglucomutase (PGM), glucose-6-phosphate dehydrogenase (G6PDH), NAD(P): FMN oxidoreductase and bacterial luciferase and it is based on continuous NADPH monitoring in the reaction using a BioOrbit-Galaxy Luminometer. The concentrations of enzymes, substrates and cofactors in the final reaction system were optimized as follows: PGM, 56 mU/ml; G6PDH, 10 mU/ml; NADP, 0.1 mM, FMN, 5 μM; decanal, 10 μM and bacterial luciferase with contaminating oxidoreductase 0.14 mg/ml.

The assay is highly sensitive, easily detecting 1 pmol of rabbit muscle glycogen phosphorylase b. With some modifications this procedure may be suitable for the repeated measurement of serum GP in the realm of clinical diagnosis.

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