The normal oligonucleotide melts from the sickle DNA at 57°C but remains hybridized to pβF5 DNA until 63–64°C. Similarly, the sickle oligonucleotide dissociates from pβF5 DNA at 57°C, but does not melt from pH85 DNA until 64°C.

A luminescent detection system for non-radioactively labelled DNA probes

HERMIA FIGUEIREDO and ALAN D. B. MALCOLM
Department of Biochemistry, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF, U.K.

The application of luminescent assays in biological systems is becoming more popular. Recently the luciferin-enhanced oxidation of luminol has been used to quantify horseradish peroxidase in enzyme immunoassays (Thorpe et al., 1984). We have used a similar approach to detect enzyme-labelled DNA.

DNA probes were chemically constructed by cross-linking modified horseradish peroxidase to DNA using glutaraldehyde (Renz & Kurz, 1984). The β-globin probe (β15) consisted of a 1.9 kb fragment spanning the 3' end of the human β-globin gene inserted into the BamHI site of pAT153 (Moschonas et al., 1982). Free peroxidase was removed from the cross-linked material by precipitating the probe with polyethylene glycol.

The sensitivity of detection of the peroxidase-labelled DNA in solution was first investigated. Assay conditions used were H2O2 (2.7 mM), luminol (1.25 mM), luciferin (36 μM) in 0.1 M-sodium phosphate, pH 8.0. The DNA probe was used to initiate the reaction and the light emitted was integrated over 60 s on the LKB 1251. As little as 1.25 amol of DNA could be detected.

A comparison of the sensitivity of detection after immobilization of the DNA probe on cellulose nitrate was made. Dilutions of the enzyme-labelled DNA probe were spotted on to cellulose nitrate and placed on Whatman 3 MM filters in a radioautograph cassette. The Whatman 3 MM filters were soaked in substrate: H2O2 (1.35 mM), luminol (0.625 mM), luciferin (18 μM) in 0.1 M-sodium phosphate, pH 8.0. The nitrocellulose filter was exposed to pre-flashed X-ray film (Kodak XAR5) for 20 min. This method allowed the detection of as little as 5.4 amol of the DNA probe. It was found that the reduced substrate concentrations used for assays on nitrocellulose did not affect the sensitivity of the reaction, but helped reduce the background.

The chemically modified DNA probe was hybridized to complementary sequences blotted on nitrocellulose (Renz & Kurz, 1984). Under optimal conditions as little as 13 amol of plasmid DNA could be detected.

DNA probes were also constructed by the incorporation of biotin-11-dUTP in nick translation (Leary et al., 1983) or random priming reactions. These probes were hybridized to their complementary sequences and detected with a streptavidin–peroxidase-labelled complex. The sensitivity of detection was found to be 270 amol with the luminescent assay.

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Use of the glucose clamp technique to assess the roles of insulin and glucose in the regulation of glycogen synthase

YOLANTA T. KRUSZYNSKA and PHILIP D. HOME
Department of Medicine, Medical School, Framlington Place, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, U.K.

The regulation of skeletal muscle and liver glycogen synthase has not been investigated in vivo at physiological blood glucose concentrations. Insulin and blood glucose concentrations in rats were therefore adjusted independently by means of feedback glucose infusions (glucose clamps; DeFronzo et al., 1979) to investigate the effects of these variables without confounding metabolic interactions. To avoid endogenous insulin secretion during the hyperglycaemic clamps, studies were performed on normoglycaemic short-term streptozotocin (0.15 g/kg) diabetic rats. Before study animals were treated with soluble insulin (3 units/day) from subcutaneously implanted Alzet miniosmotic pumps to achieve blood glucose concentrations between 3.5 and 7.0 mmol/l.

Five days after induction of diabetes venous cannulae were implanted under ether anaesthesia 24 h before study. Mini–osmotic pumps were removed and insulin in Haemaccel was infused intravenously overnight to maintain blood glucose between 3.5 and 4.0 mmol/l. Normal rats received Haemaccel alone (0.04 ml/h). Rats were fasted for 18 h before study. All blood samples were taken from unaesthetized, unrestrained rats. After a basal blood sample at 09:00 h the insulin infusion rate was increased to 85 munits/h. Blood glucose was clamped at 4.0, 6.0 or 10.0 mmol/l in three separate studies (n = 6 in each) by means of frequent blood glucose estimations and adjustment of the infusion rate of 500 g of glucose/l in water. At +180 min animals were anaesthetized with ether and liver and quadriceps muscle freeze-clamped for determination of glycogen synthase activity (Golden

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