The Use of PicoGreen and Ethidium Bromide in Determination of Degree of DNA Damage in Calf Thymus DNA

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A method of detection of DNA damage was developed using two intercalating dyes : PicoGreen (PG) and ethidium bromide (EtBr). PG is a cyanine dye with a central methine bridge between two ring structures. In solution the molecule can rotate about this bridge allowing for non-radiative deactivation. When intercalated between the basepairs of DNA this pathway is cut off and fluorescence is enhanced. In double-stranded DNA (dsDNA) the rings of the dye are held firmly between basepairs, while in single-stranded DNA (ssDNA) the connection is looser allowing for more non-radiative deactivation. This allows for clear distinction of dsDNA versus ssDNA. In the case of EtBr, the dye is firmly held in both dsDNA and ssDNA such that there is little to no distinction between them in their fluorescence making it a good reference.

This method made use of steady-state fluorescence and a solution of the two dyes in TRIS buffer (pH=7.4) such that the ratio of PG to EtBr was 1:9. At this ratio the two dyes had approximately the same absorbance at 503nm which was then used as the excitation wavelength. When combined in a DNA :dye ratio of 25 :1 with Calf Thymus DNA of known proportion of ssDNA to dsDNA (0%dsDNA, 25%, 50%, 75%, and 100%), fluorescence was measured at 525nm and 610nm. When the ratio of fluorescence intensity at 525nm to 610nm was plotted against percent dsDNA a strong linear correlation was seen.

Therefore the method was successful in determining the ratio of ssDNA to dsDNA which can in turn be used to assess the degree of DNA damage. The method, in comparison to previous methods such as the comet assay has the advantages of being quantitative, cost effective, and simple. One of its many applications is the detection of irradiated foods.