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FLOW MICROFLUOROMETRIC AND SPECTROPHOTOFLUOROMETRIC ANALYSIS OF DNA STAINING IN MAMMALIAN CELLS. H. A. Crissman, A. Stevenson, and R. J. Kissane, Biophysics and Instrumentation Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87545 U.S.A.

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The effects of pH, ionic strength, stain concentration, and magnesium concentration on DNA staining with the antibiotics mithramycin, chromomycin A3, and olivomycin were examined with DNA in solution and in mammalian cells. Ethanol-fixed Chinese hamster cells (line CHO) stained with mithramycin solution in the pH range 4.0 to 10.0 and analyzed by flow microfluorometry (FMF) showed only a slight increase in fluorescence intensity from pH 4.0 to 8.0. Above this range, there was a more dramatic increase in intensity of stained cells, and at pH 10.0 the flouorescence intensity was 1-1/2 times greater than cells stained at pH 4.5. The resolution in DNA distribution patterns also improved as a function of increasing pH, corresponding with a marked decrease in the coefficient of variation (CV). However, the distribution of cells in various phases of the cell cycle remained essentially the same over the pH ranges tested.

The fluorescence intensity of cells stained in mithramycin solutions containing 0 to 1.75 NaCl increased gradually up to about 1.0 M NaCl. The intensity of cells stained in 1.0 M NaCl was 1-1/2 times greater than cells stained in the absence of NaCl. However, the CV of the G1 peaks changed only slightly from 0.15 to 1.00 M NaCl. Cells stained in 1.75 M NaCl yielded poor DNA distributions, with a large CV compared to cells stained in 0.15 M NaCl.

Cells stained with increasing concentrations of mithramycin up to 250 µg/ml showed a sharp increase in fluorescence intensity, judged by FMF, up to 50 µg/ml and then a more gradual increase up to 100 µg/ml. The relative intensity of stained cells was changed very little at dye concentrations above 0 µg/ml. DNA distributions for cells stained at all concentrations yielded actually the same percent of cells in the various phases of the cell cycle.

Cells stained with mithramycin solutions containing 0 to 70 mM $MgCl_2$ showed a gradual increase in fluorescence intensity as a function of increasing $MgCl_2$ concentration. At a concentration of 70 mM $MgCl_2$, cells exhibited a 23% increase in intensity above cells stained in 15 mM $MgCl_2$; however, no improvement in resolution of the DNA distribution patterns was noted. Cell staining in the absence of $MgCl_2$ was extremely poor. Other metal ions tested included the chlorides (15 mM) of cadmium, calcium, cobalt, copper, lead, manganese, mercury, and zinc. None of these divalent metal ions proved superior to magnesium, and only cells stained in the presence of zinc, lead, and cadmium yielded bimodal DNA distributions. Of these, only zinc could be recommended as an alternative for magnesium; however, cells stained in 15 mM $ZnCl_2$ yielded only about half the intensity of cells stained in 15 mM $MgCl_2$.

Spectrophotofluorometric analysis of mithramycin, chromomycin A3, and olivomycin yielded spectral patterns quite similar but not identical in shape. Addition of magnesium to the solutions induced a spectral shift in the emission peaks toward the longer wavelengths by about 25 nm and also produced a three-fold increase in the fluorescence intensity of the dye. Addition of DNA in solution to the mithramycin-magnesium complex quenched the fluorescence by about one-third but did not produce any significant spectral shift. Increasing the ionic strength of the mithramycin solution did not change the spectral patterns of either the mithramycin-magnesium or mithramycin-magnesium-DNA complex, nor did it produce a change in fluorescence intensity of these complexes. These results and those obtained by FMF analysis indicate that the increase in fluorescence intensity of stained cells as a function of increasing ionic strength is due to changes in chromatin structure, providing a greater number of binding sites for the dye complex. (This work was supported by the U. S. Energy Research and Development Administration and the National Cancer Institute.)