DIRECT FLUOROMETRIC DETERMINATION OF DNA IN HUMAN LEUKOCYTE HOMOGENATES

N. A. Fedorov and V. N. Pan'kov

UDC 612.112.1:577.113.3]-088.1

KEY WORDS: DNA; human leukocytes; fluorometric method

It has been shown [3, 5], that bis-benzimide (Hoechst 33258) is superior to other known dyes (ethidium bromide, 4,6-diamidino-2-phenylindole, 3,5-diaminobenzoic acid, etc.) which form fluorescent complexes with DNA, for it does not bind with other components of cells, its fluorescence is not quenched by high concentrations of salts, and it provides a high quantum yield of fluorescence, so that DNA concentrations can be measured with it starting from 20 ng/ml.

In the investigation described below, using human leukocytes, the writers demonstrated that for fluorometric measurement of the intracellular DNA concentration with bisbenzimide it is sufficient to mix an aliquot of the cell homogenate with an equal volume of 5 M NaCl in phosphate buffer.

EXPERIMENTAL METHOD

Leukocytes isolated from healthy human peripheral blood were frozen at −20°C in batches of $(0.5\text{-}1.0)\cdot 10^6$ cells until the time of the investigations. The leukocytes were thawed at 4°C, and treated with 0.5 ml of 0.15 M NaCl in 0.05 M phosphate buffer, pH 7.4, after which the cells were resuspended and homogenized in a glass homogenizer at 0°C until completely disintegrated. The homogenate was mixed with an equal volume of 5 M NaCl in phosphate buffer, allowed to stand for 30 min at 4°C to destroy the deoxyribonucleoprotein, and the DNA concentration was determined fluorometrically with bisbenzimide (excitation wavelength 365 nm, emission wavelength 470 nm) on a J. Y. 30 D spectrofluorometer (Jobin Yvon, France). A 2.5 M solution of NaCl in 0.05 M phosphate buffer, pH 7.3, containing $1.5\cdot 10^{-7}$ M bisbenzimide was used as the control sample. After measurement of background fluorescence, 50-µl doses of saline homogenate of the test leukocytes were added successively to the control sample and each time the intensity of fluorescence was recorded. As the internal standard, 20-µl doses of a standard solution (9.0 µg/ml) of thymus DNA were added successively to this same sample and the intensity of fluorescnece also was measured. A graph was plotted (Fig. 1) and the DNA content in the leukocyte homogenate was calculated by a formula comparing the intensity of fluorescence in the DNA sample for analysis and in the standard:

$$F\mu g/ml = \frac{C.\ D.\ B}{A.\ E.}$$

where A denotes the volume of leukocyte homogenate added to the cuvette (in µl), B the intensity of fluorescence of the homogenate (in relative units), C the volume of the standard DNA solution added to the cuvette (in µl), D the concentration of the standard DNA solution (in µg/ml), E the required DNA concentration in the leukocyte homogenate to be tested (in µg/ml).

EXPERIMENTAL RESULTS

The DNA concentration in the test leukocyte homogenate was calculated by multiplying the DNA concentration in the homogenate, found by the equation, by its volume. The DNA content per single leukocyte was determined by dividing the results thus obtained by the number of cells in the sample taken for analysis, and expressed in picograms per cell. The DNA concentration in the standard samples was determined spectrophotometrically by the method in [1].

The reproducibility of the method was estimated by determining the DNA content (five parallel determinations) in blood leukocytes from three healthy subjects, the results being

Central Research Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 101, No. 4, pp. 500-501, April, 1986. Original article submitted April 20, 1985.

0007-4888/86/0004-0545$12.50

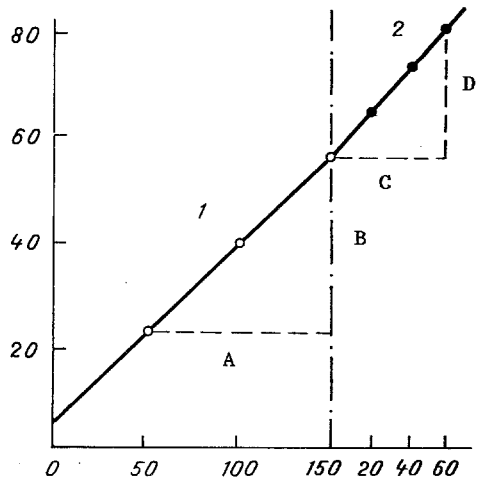

Fig. 1. Dependence of intensity of fluorescence of experimental and standard DNA samples on their volumes. Abscissa, volume (in µl); ordinate, intensity of fluorescence of experimental and standard DNA samples with bisbenzimide (in relative units). 1) Leukocyte homogenate, 2) standard DNA solution. A) 100 µl, B) 34 relative units, c) 60 µl, D) 24 relative units. Concentration of standard DNA solution 9.0 µg/ml.

6.9 ± 0.2, 7.5 ± 0.3, and 7.7 ± 0.2 pg per cell, respectively. Coefficient of variation was 3.2, 3.9, and 2.9%, respectively. The method is fully reproducible. This method described above was used to study the DNA content in peripheral blood leukocytes of 50 healthy persons aged from 23 to 47 years (eight women and seven men). The DNA content was found to be 7.4 ± 0.3 pg per cell. The results are in good agreement with values obtained previously for human leukocytes by other workers: 7.6 ± 0.3 pg [2] and 8.0 ± 0.5 pg [4]. By the method recommended it is possible to determine DNA quantitatively in homogenates of leukocytes and other cells easily and with high sensitivity (0.02 µg/ml). The sensitivity of the method is more than an order of magnitude greater than that of the ethidium bromide method [2], in which the smallest DNA concentration recordable is 0.5 µg/ml. Introduction of an internal standard automatically corrects for the distorting influence of the components of the homogenate.

LITERATURE CITED

1. A. S. Spirin, Biokhimiya, 23, 656 (1958).
2. M. J. Blackburn, T. M. Andrews, and R. W. E. Watt, Anal. Biochem., 51, 1 (1973).
3. T. R. Downs and W. W. Wilfinger, Anal. Biochem., 131, 538 (1983).
4. C. Gautreau, C. Raheul, J.-P. Cartron, and G. Lucotte, Anal. Biochem., 134, 320 (1983).
5. C. Labarca and K. Paigen, Anal. Biochem., 102, 344 (1980).