# Spectrophotometric Determination of DNA Binding Protein, Histone, with 3,4,5,6-Tetrafluoro-2-carboxyphenylfluorone and Manganese(II)[1]

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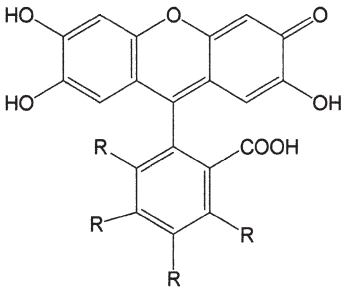
A sensitive spectrophotometric method for the determination of DNA binding protein, histone, is developed. The proposed method is based on color reaction among histone, 3,4,5,6-tetrafluoro-2-carboxyphenylfluorone (TFCPF) and manganese(II). The coloration obeys Beer's law in the concentration range of 1.0–20 $\mu g\,cm^{-3}$ histone. Other proteins such as human serum albumin and $\gamma$-globulin are scarcely produced colored complexes. A detection test of histone on a spot plate was also studied.

Histones[2,3] are a group of small basic proteins and chromosomal proteins (mol wt 12000–20000) possessing an open, unfolded structure, attached to DNA of cell nuclei by ionic linkages. Histones can be classified into four subtypes by composition of amino acids. Classification is based on relative amounts of lysine and arginine: histone I is very rich in lysine and has several subtypes; histone II, moderately rich in lysine with two subtypes; histone III, moderately rich in arginine, contains cysteine; histone IV, very rich in arginine and in glycine. At present, histones are assumed to regulate gene expression by acetylation or deacetylation. So, activity assay of deacetylated histone was reported.[4] Only ELISA assay[5–8] is known as the method for the determination of histone itself, and rapid and a convenient colorimetric determination has not been reported. We have been studying[9,10] the determination of proteins such as human serum albumin using dye-metal complex in micellar media. This paper deals with a new and sensitive procedure for determining histone based on [histone–3,4,5,6-tetrafluoro-2-carboxyphenylfluorone (TFCPF)–manganese(II)] complex system.

TFCPF was newly synthesized by utilization of condensation reaction[11] between 1,2,4-benzenetriol triacetate[12] and tetrafluorophthalic acid. A solution of TFCPF,[13] which had been newly synthesized, was prepared in a $1.0 \times 10^{-3}$ mol $dm^{-3}$ methanol solution containing one drop of hydrochloric acid. Five standard solutions (100 $\mu g\,cm^{-3}$) of histone was prepared by dissolving histone VII-S, II-AS, II-S, III-S, VIII-S (Sigma Chemical Co., from calf thymus) in water. A solution of 1.0% polyethylene glycol mono-*p*-isooctylphenyl ether (Triton X-100) was prepared in water. A manganese(II) solution ($1.0 \times 10^{-3}$ mol $dm^{-3}$) was prepared from a stock solution (Wako Pure Chem. Co. Ltd., 1000 $\mu g\,cm^{-3}$) by dilution with water. A buffer solution (pH 9.0) was made by mixing 0.2 mol $dm^{-3}$ 2-amino-2-hydroxymethyl-1,3-propanediol (Tris) solution and 0.2 mol $dm^{-3}$ hydrochloric acid. Deionized water and purified methanol were used in the preparation of all solutions.

In order to establish a selective and sensitive determination of histone, a combination of a metal ion and dye were studied. The dyes used were TFCPF, 3,4,5,6-tetrachloro-2-carboxyphenylfluorone (TCCPF), 3,4,5,6-tetrabromo-2-carboxyphenylfluorone (TBCPF) and *o*-hydroxyhydroquinonephthalein (QP). These structures are shown in Figure 1. These dyes could be arranged in the following order with respect to the sensitivity: TFCPF > TCCPF > TBCPF, QP.




| Dye | R |
|---|---|
| QP | H |
| TFCPF | F |
| TCCPF | Cl |
| TBCPF | Br |

**Figure 1.** The structure of dyes.

Next, the metal ions were studied by using manganese(II), copper(II), cobalt(II), nickel(II), iron(III), titanium(IV), and molybdenum(VI). Only manganese(II) was effective among various metal ions tested. Maximum and almost constant absorbance was achieved over the pH range 8.5–9.5 by using 2.5 $cm^3$ of the buffer solution in the final volume of 10 $cm^3$. The effect of surfactants (Triton X-100, polyoxyethylene sorbitan monolaurate, polyoxyethylene dodecyl ether, poly-*n*-vinyl alcohol, octylphenoxypolyethoxyethanol, and methyl cellulose) was investigated. Above 0.5 $cm^3$ of 1.0% Triton X-100 solution was most effective. The composition ratio of dye and metal ion was investigated by the continuous variation method. A maximum absorbance was observed when manganese(II) to TFCPF was 1:1. The recommended procedure for the assay of histone is as follows. The following components were mixed in a 10-$cm^3$-volumetric flask: 2.5 $cm^3$ of the buffer solution (pH 9.0), 1.0 $cm^3$ of 1.0% Triton X-100 solution, 0.75 $cm^3$ of $1.0 \times 10^{-3}$ mol $dm^{-3}$ TFCPF solution, 0.5 $cm^3$ of $1.0 \times 10^{-3}$ mol $dm^{-3}$ manganese(II) solution and a solution containing histone. The mixture was diluted to 10 $cm^3$ with water, transferred into a test tube, mixed well and kept at room temperature for 15 min. The absorbance of the resultant solution was measured at 585 nm against a reagent blank without histone. Under the standard conditions, the color development was nearly completed at room temperature for 10 min and the absorbance value remained constant for at least 3 h.

Figure 2 shows the absorption spectra of TFCPF–manganese(II) and TFCPF–manganese(II)–histone solutions. A maximum absorbance was observed at 585 nm when histone was added to a TFCPF–manganese(II) solution. The reproducibility is good because the relative standard deviation (histone 10 $\mu g\,cm^{-3}$) is 0.65% ($n = 7$).

The color reactions of various proteins containing histone with TFCPF–manganese(II) complex were examined. The re-

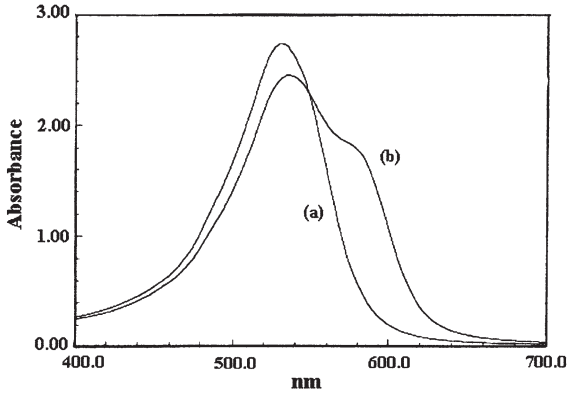



**Figure 2.** Absorption spectra of TFCPF–manganese(II) (a) and TFCPF–manganese(II) in presence of histone (20 μg $cm^{-3}$) solutions (b).

sults are shown in Table 1. Several proteins tested, such as human serum albumin (HSA) and γ-globulin, scarcely produce colored complexes, even in large amounts. On the other hand, basic proteins containing large amounts of basic amino acids easily produced any colored complexes with TFCPF–manganese(II). The experimental results imply that the difference of reactivities of proteins is correlated with each isoelectric point (pI) and molecular weight (MW).

Five types of histones (Sigma Chemical Co.) were investigated; Type VII-S, II-AS, II-S, III-S, VIII-S. Lysine-rich type histones (Type III-S, VII-S) were more sensitive than other types. The differences of reactivity by types of histones were considered to be influenced by amount of basic amino acids in histones. And, an acetylated histone was not investigated in this paper.

The influence of diverse substances (copper(II), calcium(II), iron(III), zinc(II), hydrogen peroxide, sodium dihydrogenphosphate, sodium chloride, sodium carbonate, sodium oxalate, potassium nitrate, glucose, fructose, uric acid, urea, creatinine, glycine, histidine, arginine, glutamic acid, and so on) on determination of histone was investigated under the standard conditions. Iron(III) and copper(II) gave positive errors at a concentration of $1.0 \times 10^{-7}$ mol $cm^{-3}$. Most substances tested did not interfere ($100–10 \times 10^{-7}$ mol $cm^{-3}$).

**Table 1.** Reaction between several proteins and TFCPF–manganese(II) complex

| Protein[a] | Absorbance at 585 nm/%[b] | | pI | MW[c] /kDa |
|---|---|---|---|---|
| Histone | 0.634 | (100.0) | 10.5 | 21.5 |
| Myelin basic protein | 0.272 | ( 42.9) | 12.0–13.0 | 18.4 |
| Lysozyme | 0.227 | ( 35.8) | 10.5–11.0 | 14.5 |
| Cytochrome C | 0.182 | ( 28.7) | 10.1 | 12.4 |
| Trypsin | 0.061 | ( 9.6) | 10.1–10.5 | 24.0 |
| Lactoferrin | 0.050 | ( 7.9) | 8.2–9.2 | 75–80 |
| HSA | 0.021 | ( 3.3) | 4.7 | 60.4 |
| Protease (*S. griseus*) | 0.016 | ( 2.5) | 8.5–9.2 | 19.0 |
| γ-Globulin | 0.009 | ( 1.6) | 6.9 | 155–160 |

[a] Protein taken, 10 μg $cm^{-3}$.
[b] Percent with respect to histone.
[c] Molecular weight.

A detection test of histone on a spot plate using the TFCPF–manganese(II) complex was also investigated. On an addition of a solution containing histone to the TFCPF–manganese(II) solution, the color of the solution immediately changed from red to reddish-violet. Therefore, after about 5 min at room temperature, the color development was compared with those of the histone run done in parallel. Detection limit on a spot plate, under these conditions, was 1 μg/0.05 $cm^3$ of histone when 0.05 $cm^3$ of a sample solution was used.

In conclusion, the proposed method was sensitive and selective. Though further investigations are necessary, especially concerning the elucidation of the reaction mechanism, it should be useful and convenient for the assay of histone in biological samples.

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**References and Notes**

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