The Effect of Cupric Ions on the Indole Reaction for the Determination of Deoxyribonucleic Acid*

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SUMMARY

The presence of cupric ions or ferric ions was found to enhance the development of color in the indole reaction for the colorimetric determination of deoxyribonucleic acid. The addition of calcium, cobaltous, magnesium, manganous, nickel, or zinc ions did not produce this effect. In the absence of added cupric ions, the indole reaction gave variable results, and the color developed to less than half of the optimum level. It is recommended that the reaction mixture for this assay include cupric ions at a concentration of 15 \(\mu\)M. Under these conditions, the molar extinction coefficient (based on the molarity of DNA phosphorus) of the colored product at 490 nm is equal to 15,800 \(M^{-1}cm^{-1}\). The assay conforms to Beer's law until the concentration of DNA exceeds 25 \(\mu\)g per ml (\(A_{490nm} = 1.25\)) in the reaction vessel.

EXPERIMENTAL PROCEDURE

Materials—Salmon sperm DNA (sodium salt, highly polymerized) was purchased from Calbiochem and was purified further by the procedure of Marmur (2). The water used to prepare the reagents for the indole reaction was either from a general laboratory distilled water supply or it was distilled, deionized, filtered through charcoal, and redistilled from a glass vessel (double distilled water). The other chemicals were reagent grade.

Methods—All measurements of absorbance were with a Beckman spectrophotometer, model DU. The concentration of DNA in the stock solution was determined by measuring the phosphorus content (3). The absorbance of this solution was measured at 260 nm, and the extinction coefficient, based on the molarity of DNA phosphorus, was found to be 7060 \(M^{-1}cm^{-1}\). Thereafter, the concentration of DNA was routinely determined by measuring the absorbance at 260 nm. The indole reaction for the assay of DNA was performed as described by Ceriotti (1); the final volume of the reaction mixture was 2.0 ml. The concentration of cupric ions in the water was determined with diethyldithiocarbamate (4). The concentration of ferric plus ferrous ions in the water was determined with 1,10-phenanthroline (5).

RESULTS

When distilled water from one general laboratory supply was used in the indole reaction for DNA, the extinction coefficient (based on the molarity of DNA phosphorus) of the colored product at 490 nm was found to be equal to 14,600 \(M^{-1}cm^{-1}\); this is 6% higher than the value of 13,800 \(M^{-1}cm^{-1}\) reported by Ceriotti (1). Reagents prepared with double distilled water, on the other hand, yielded a colored product with an extinction coefficient less than half this value, and the level of color development was quite variable with different preparations of reagents and samples. The results of an experiment in which color development was compared for reagents made with varying proportions of water from the two sources are shown in Fig. 1.
These results suggest that a substance which enhances color development was present in the water from the general laboratory supply, and they do not suggest that a substance which inhibits color development was present in the double distilled water. In support of this hypothesis is the fact that replacing only 10% of the total volume of the reaction mixture with water from the general laboratory supply was sufficient to enhance color development 2-fold; the dilution of any inhibitor in the double distilled water by this amount of single distilled water would have been too small to produce a change of this magnitude. Further support comes from the fact that when the data are plotted as shown, the shape of the curve is convex as would be expected in the case of an activator in the laboratory water supply rather than concave as would be expected in the case of an inhibitor in the double distilled water.

A sample of water from the general laboratory supply (2 liters) was passed through a column of Dowex 50 ion exchange resin, hydrogen form, 1.1 x 10 cm. When the effluent solution was used to prepare the indole reagents, the extent of color development in the reaction with DNA was of a low level comparable to that obtained with double distilled water. A greenish band at the top of the used ion exchange column suggested that cupric ions might be present in the laboratory water supply. The concentration of cupric ions was found to be 10.4 μM; their presence in the double distilled water was not detectable with diethyldithiocarbamate (4). No ferric ions were detectable with 1,10-phenanthroline (5) in either sample of water.

Cupric, ferric, calcium, cobaltous, magnesium, manganous, nickel, and zinc ions were tested at levels of 25 and 250 μM for their effect on the intensity of color produced by the indole reaction in the presence of 6 μg per ml of DNA in the reaction mixture. Only cupric and ferric ions had a detectable effect.

Fig. 2 shows the effect of concentration of added cupric and ferric ions on the intensity of color produced by the indole reaction. The optimum concentration of cupric ions in the reaction mixture is 15 μM; the optimum concentration of ferric ions is 40 μM. When the indole reaction is carried out in a reaction mixture that is 15 μM with respect to cupric ions, the molar extinction coefficient (based on molarity of DNA phosphorus) of the colored product at 490 μm is equal to 15,800 M⁻¹ cm⁻¹. The assay conforms to Beer's law until the concentration of DNA exceeds 25 μg per ml (A₄₉₀ cm⁻¹ = 1.25) in the reaction vessel.

**DISCUSSION**

Ceriotti (1) indicated that cupric ions do not enhance the development of color in the indole reaction for the determination of DNA. Following an observation that the reaction of indole with DNA gave a higher absorbance at 490 μm with one source of distilled water than with another, we have discovered that the reaction is enhanced by either ferric or cupric ions. The extinction coefficient of the indole reaction was only 7,000 M⁻¹ cm⁻¹ (based on DNA phosphorus) when all reagents were prepared with water from which cupric ions had been removed, and it was 15,800 M⁻¹ cm⁻¹ in the presence of the optimum concentration of cupric ions.

Since the extinction coefficient calculated from Ceriotti's work (15,800 M⁻¹ cm⁻¹) corresponds closely to our maximum value, it is likely that the water used by him contained nearly optimum concentration of cupric or ferric ions. Keck (6) has developed an ultramicro method for the determination of DNA based on a modification of the Ceriotti method. He obtained an extinction coefficient of 10,600 M⁻¹ cm⁻¹ when the reaction was run as described by Ceriotti and an extinction coefficient of 7,300 M⁻¹ cm⁻¹ for his modified method. It appears that the water or reagents that he used also contained appreciable, but not optimum, amounts of cupric ions.

We have elected to use cupric ions in the indole reaction be-
cause a higher extinction coefficient and more reproducible results are obtained. The poorer reproducibility of results in the absence of these ions is probably due to the effect of uncontrolled low levels of ions in the reagents, water, and samples. Cupric ions may be included in the reaction mixture by making the stock indole solution (1) 60 μM with respect to cupric sulfate. This reagent solution is stable when stored in the refrigerator.

When ferric chloride is added to the reaction mixture to the optimum final concentration of 40 μM, the solution has a distinct brownish tint. In addition, when the indole reaction is carried out in the presence of ferric ions, the relationship between the absorbance and the concentration of DNA is not linear. For these reasons, the use of this ion is not recommended.

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REFERENCES
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