Communications to the Editor

Chem. Pharm. Bull.
34(2) 944—947 (1986)

AFFINITY OF AROMATIC COMPOUNDS HAVING THREE FUSED RINGS TO COPPER PHTHALOCYANINE TRISULFONATE

Hikoya Hayatsu,* Hiroshi Kobayashi, Akiko Michi-ue and Sakae Arimoto
Faculty of Pharmaceutical Sciences, Okayama University,
Tsushima, Okayama 700, Japan

Affinity of 9-aminoacridine, 3-amino-1-methyl-5H-pyrido[4,3-b]-indole, quinacrine, 2-aminofluorene and 2-acetylaminofluorene to copper phthalocyanine trisulfonate in sodium phosphate buffer at pH 7.4 was studied. Use of copper phthalocyanine trisulfonate covalently bound to cellulose has allowed the study to be done quantitatively; the known aggregation of copper phthalocyanine sulfonates in water, which would have made the analysis impossible, is considered to be absent in this system. The results show that these five compounds form one-to-one complexes with the copper phthalocyanine trisulfonate on the cellulose.

KEYWORDS——copper phthalocyanine trisulfonate; 9-aminoacridine; 3-amino-1-methyl-5H-pyrido[4,3-b]indole; quinacrine; 2-aminofluorene; 2-acetylaminofluorene; complex formation

Copper phthalocyanine is a widely used blue pigment.[1)] We have recently found that absorbent cotton bearing covalently bound copper phthalocyanine trisulfonate (blue cotton, Fig. 1) is capable of adsorbing aromatic compounds having three or more fused rings.[2)] Blue cotton has been used as an adsorbent to isolate mutagens of this type of structure from crude environmental materials such as foods,[3)] river water,[2)] and human excreta.[4)] The underlying mechanism for this adsorption has been considered to be a complex formation between the multi-ringed compound and the copper phthalocyanine trisulfonate (Cu-pc-SO_3^-).[2)] There appears to be no report on the affinity of aromatic compounds to copper phthalocyanines except for those on the adsorption of gaseous benzene derivatives to the surface of crystalline copper phthalocyanine.[5)] This lack of information is perhaps due to the fact that copper phthalocyanine sulfonates form the dimers and higher aggregates reversibly in aqueous solutions.[6)] Any attempt to study quantitatively a complex formation between Cu-pc-SO_3^- and another substance would have been hampered by this complicated situation.

In blue cotton Cu-pc-SO_3^- is fixed on a solid support; hence no aggregation of Cu-pc-SO_3^- is expected to occur. It should be noted that the method for preparing blue cotton involves washing the material with dimethylsulfoxide and methanol.[2)] This would irreversibly dissociate any Cu-pc-SO_3^--aggregates present in this material.[6)] On blue cotton a complex already formed between a Cu-pc-SO_3^- moiety and a given compound would not interfere with other Cu-pc-SO_3^- moieties making complexes.

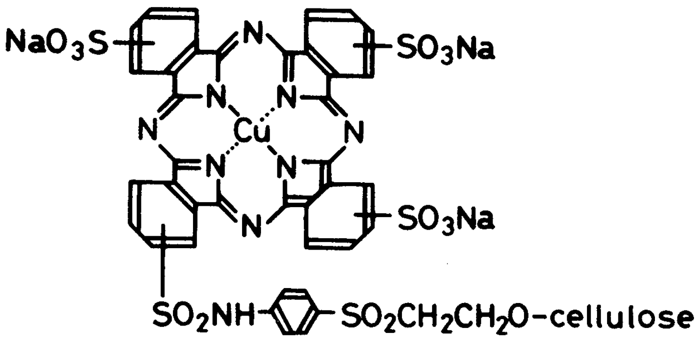

Fig. 1. Structure of Copper Phthalocyanine Trisulfonate Bound to Cellulose (Blue Cotton)

Therefore, it is hoped that this complex formation can be quantitatively analyzed using blue cotton and appropriate compounds having strong affinity to it. Among the multi-ringed compounds already reported to have strong affinities to blue cotton,2) we chose five compounds with three-ringed aromatic structures, having UV absorptions strong enough to allow effective quantitation. Here we report that these compounds form one-to-one complexes with Cu-pc-SO_3^-.

Blue cotton was prepared as described.2) The copper content of the cotton, as determined by the atomic absorption, was 8.54 ± 0.14 (sd) μmol/g (six determinations). This was taken as representing the Cu-pc-SO_3^- content. No detectable amount of the blue pigment was released from this material during the process of the adsorption experiments described below. As a control for non-specific adsorption to cotton, a sample of absorbent cotton was used which had undergone the treatment with sodium carbonate necessary for preparing blue cotton.2) The buffer used for the adsorption was 0.05 M sodium phosphate, pH 7.4, containing 0.2 M sodium chloride. The compounds used and their molar absorption coefficients in this buffer were as follows: 3-amino-1-methyl-5H-pyrido[4,3-b]indole, 5.63×10^4 at λ_{max} 264 nm; 9-aminoacridine, 7.79×10^4 at λ_{max} 260 nm; quinacrine, 4.88×10^4 at λ_{max} 280 nm; 2-aminofluorene, 2.38×10^4 at λ_{max} 282 nm; and 2-acetylaminofluorene, 2.17×10^4 at λ_{max} 277 nm. The adsorption to blue cotton was performed at 25°C during the horizontal shaking of a 50 ml-solution in a 100 ml Erlenmyer flask, for 30 min at 110 rounds/min. By measuring the UV absorption spectrum of the solution, the quantity of compound adsorbed to the cotton was determined. No change resulted from an additional 30 min shaking. The temperature was not critical: there was no change in adsorption between 20-30°C. These adsorptions are reversible because the original compounds can be quantitatively recovered by eluting the treated blue cotton with methanol-concentrated ammonia (50 : 1) or with methanol-trifluoroacetic acid (100 : 1, used for 2-acetylaminofluorene). Nonspecific adsorptions found with the control cotton were generally lower than 1/100 of the values found with blue cotton, and these were subtracted to obtain the net adsorptions to Cu-pc-SO_3^-.

First, blue cotton (50 mg) was titrated with 9-aminoacridine. Up to a 6-

Table I. Analysis of Complex Formation between Aromatic Compounds and Copper Phthalocyanine Trisulfonate Covalently Linked to Cellulose

Compound	Dissociation constant ($k_d \times 10^6$, M)	Molar ratio at saturation (compd/Cu-pc-SO_3^-: 1/*n*)	Correlation coefficient for linearity (*r*)	Initial concn. of compound (M x 10^5)
9-Aminoacridine	5.90 (6.24, 5.56)	1.10 (1.04, 1.16)	0.997 (0.998, 0.996)	1.10
3-Amino-1-methyl-5H-pyrido[4,3-*b*]indole	2.90 (2.98, 2.82)	1.25 (1.22, 1.28)	0.994 (0.997, 0.991)	1.11
Quiancrine	4.83 (5.00, 4.66)	1.03 (0.98, 1.08)	0.998 (0.999, 0.997)	1.03
2-Aminofluorene	73.1 (75.2, 71.0)	1.00 (0.93, 1.07)	0.994 (0.990, 0.997)	2.39
2-Acetylamino-fluorene	18.5 (18.9, 17.3)	0.96 (0.80, 1.30)	0.973 (0.986, 0.958)	2.12

Averages of results in two independent experiments, shown in parentheses,are given. k_d was calculated on the basis *n* = 1.

molar excess of 9-aminoacridine was used, and the saturating amount of 9-aminoacridine was 1.15 mol/mol Cu-pc-SO_3^-. Similar titration showed that quinacrine saturated the blue cotton at 1.2 mol/mol Cu-pc-SO_3^-. These results suggest that the compounds form one-to-one complexes with Cu-pc-SO_3^-.

For a detailed analysis of the adsorption, 50-ml solutions of each compound at a fixed concentration (with an absorbance of 0.4-0.9) were treated with different amounts of blue cotton (20, 25, 30, 45, 55 and 80 mg). The results were analyzed by the Langmuir equation:[7)]

$$[C]_{total}/[C \cdot \text{Cu-pc-}SO_3^-] = (\underline{n}k_d/[\text{Cu-pc-}SO_3^-]) + 1/\underline{n},$$

where $[C]_{total}$ represents the concentration of compound before addition of blue cotton, [C·Cu-pc-SO_3^-] represents the concentration of the compound·Cu-pc-SO_3^- complex at the equilibrium (assuming that the complex is in solution) which is identical with the concentration of compound adsorbed, and [Cu-pc-SO_3^-] represents the concentration of Cu-pc-SO_3^- at the equilibrium (again assuming that these residues are in solution). k_d is the dissociation constant for the equilibrium, i.e., [C] x [Cu-pc-SO_3^-]/[C·Cu-pc-SO_3^-], where [C] is the concentration of the compound that remained in the solution after the equilibration. *n* is the number of sites, i.e., the molar ratio in the complex between Cu-pc-SO_3^- and the compound. With 1/[Cu-pc-SO_3^-] on the abscissa and $[C]_{total}$/[C·Cu-pc-SO_3^-] on the ordinate, the observed values should give a linear relationship, the slope of the line representing $\underline{n}k_d$.

The experimental results were consistent with this equation. The k_d, the correlation coefficient (*r*) for the linearity, and the intercept of the ordinate (1/*n*) were determined (Table I). All five compounds formed one-to-one complexes with Cu-pc-SO_3^-, and the dissociation constants were in the range 10^{-5} - 10^{-6} M.

The complex formation is probably due to hydrophobic interactions between Cu-pc-SO_3^- and the three-ringed aromatic compound. Ionic attractions do not appear to be a major factor in the complexing: 3-amino-1-methyl-5H-pyrido[4,3-b]indole and quinacrine are cations but the others are uncharged under the conditions used. As reported before,[2)] 19 three-ring compounds, 1-nitropyrene, and benzo[a]pyrene all showed strong affinity to blue cotton, whereas two-ring, one-ring or no-ring compounds showed weak or no affinity. This suggests that in the complex the two planar molecules stack face to face.

The fact that a copper phthalocyanine derivative can form a complex with aromatic compounds having three rings (and probably those with four or more rings) has revealed a new property of this class of compounds.

ACKNOWLEDGMENT This work was supported by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare and those from the Ministry of Education, Science and Culture, Japan. We thank Dr. M. Nagao of the National Cancer Center Research Institute, Tokyo, for a gift of 3-amino-1-methyl-5H-pyrido[4,3-b]-indole.

REFERENCES

1) F.H. Moser, and A.L. Thomas, "The Phthalocyanines," CRS Press: Boca Raton, Florida, 1983.
2) H. Hayatsu, T. Oka, A. Wakata, Y. Ohara, T. Hayatsu, H. Kobayashi, and S. Arimoto, Mutation Res., 119, 233 (1983).
3) H. Hayatsu, Y. Matsui, Y. Ohara, T. Oka, and T. Hayatsu, Gann, 74, 472 (1983); M. Takahashi, K. Wakabayashi, M. Nagao, M. Yamamoto, T. Masui, T. Goto, N. Kinae, I. Tomita, and T. Sugimura, Carcinogenesis, 6, 1195 (1985).
4) H. Kobayashi, and H. Hayatsu, Gann, 75, 489 (1984); H. Hayatsu, T. Hayatsu, Y. Wataya, and H.F. Mower, Mutation Res., 143, 207 (1985); H. Hayatsu, T. Hayatsu, and Y. Ohara, Jpn. J. Cancer Res. (Gann), 76, 445 (1985).
5) C.R.S. Dean, R.R. Mather, and K.S.W. Sing, Thermochimica Acta, 24, 399 (1978); J. Schröder, Farbe Lack, 88, 100 (1982).
6) R.J. Blagrove, and L.C. Gruen, Aust. J. Chem., 25, 2553 (1972); R.J. Blagrove, L.C. Gruen, Aust. J. Chem., 26, 225 (1973).
7) I.J. Langmuir, J. Am. Chem. Soc., 37, 1139 (1915)

(Received December 4, 1985)