Similar behavior, i.e., formation of the dissymetric substitution product at room temperature, was also observed when **1** was allowed to react with the pentamethylcyclopentadiene analogue $[(C_5Me_5)Mo(CO)_2]_2$.

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Supplementary Material Available: A table of atomic positions and thermal parameters and a table of bond lengths and angles (4 pages). Ordering information is given on any current masthead page.

Electrochemically Activated Binding of Benzo[*a*]pyrene and 6-Methylbenzo[*a*]pyrene to DNA

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Chemically activated and enzymically activated binding of benzo[*a*]pyrene (B[*a*]P) to DNA have been reported by using iodine,[1,3] hydrogen peroxide,[1-3] and horseradish peroxidase.[4] We wish to report a binding of B[*a*]P to DNA activated by electrochemical oxidation of B[*a*]P. Previous activation methods have relied on oxidations in homogeneous solutions by using oxidants of fixed redox potential, while electrochemical activation allows the variation of potential. Also, previous methods have required the oxidant to be in direct contact with the DNA, which is not a requirement for electrochemical activation. These advantages, although not fully exploited in this first report, have motivated this work.

These experiments have been carried out in 50% ethanol solution prepared by mixing equal volumes of 0.6 mM heat-denatured calf thymus DNA (Sigma Chemical Co., St. Louis, MO) in 0.01 M (in Na^+) phosphate buffer (pH 6.8) with a 100% ethanol solution of 0.10 M tetra-*n*-butylammonium perchlorate (TBAP, Southwestern Analytical Chemicals, Austin, TX) and 0.20 mM hydrocarbon. The electrolysis was performed in a three-chamber (working electrode, buffer, and auxiliary electrode chambers) Coulometric cell with 50% ethanol solution containing buffer and TBAP only in the buffer and auxiliary chambers. A potential was applied to a Pt gauze electrode vs. an Ag-wire pseudoreference electrode, and the auxiliary electrode was Pt wire. Standard electrochemical instrumentation which has been described elsewhere was used.[5] The potential was set to approximately 100 mV anodic of the cyclic voltammetric peak potential reported by us in acetonitrile vs. Ag.[6,7] The solution was stirred by using a magnetic stirrer; argon was bubbled continuously, and the entire cell was shielded from room light.

At the end of 2 h, the DNA solution was stored at −15 °C for 1 h to precipitate part of the TBAP and the DNA associated with it, which was then filtered at the same temperature. For a typical solution volume of 30 mL, 10 mL of 0.01 M phosphate buffer was used to suspend the wet crystals, and this slurry was stored at 4 °C for 2 h, at which time 10 mL of chloroform was added to dissolve the TBAP. The whole solution was then stored at 4 °C for an additional hour; the aqueous layer was drawn off and washed until no fluorescent compounds were detected. This was usually accomplished by washing 4 times with buffer-saturated chloroform and then washing 10 times with buffer-saturated ethyl acetate. Finally, UV absorbance spectra were recorded in a Cary 219 spectrophotometer and fluorescence spectra on an Aminco-Bowman spectrophotofluorometer. Excitation spectra were corrected for nonuniform lamp intensity by using power data from a rhodamine B quantum counter measured with an International Light Model 700 radiometer.[8]

A similar experiment was run by using 0.1 mM (in 50° ethanol) 6-methylbenzo[*a*]pyrene kindly furnished by Professor G. H. Daub of our department. A control experiment using 0.1 mM B[*a*]P was run in which the solution was merely stirred at room temperature for 2 h and shielded from light, although not bubbled with argon.

The fluorescence excitation spectrum for DNA which was stirred with B[*a*]P and washed as described above (Figure 1a) exhibits a maximum at 290 nm which corresponds to the absorption maximum for B[*a*]P at 296 nm (ϵ 3922 L mol^{-1} cm^{-1}) and exhibits an emission peak at 420 nm which corresponds to the emission peak at 418 nm obtained in ethanol for B[*a*]P. Thus the spectra are not red shifted appreciably, although the fine structure has been lost. However, the excitation and emission peaks found for the electrolyzed B[*a*]P product at 330 and 435 nm, respectively (Figure 1b), are red shifted, indicating chemical perturbation of the aromatic system. These wavelengths are similar to those obtained in previous fluorescence studies of chemically activated and in vivo binding.[3,9] This experiment was run as a blank to the experiments that follow.

The excitation spectrum for the electrolyzed 6-MeBa[*a*]P (figure 1c) shows a strong blue shift to 250 nm from the ethanol-solution absorption peak at 300 nm. The emission maximum at 410 nm is also blue shifted from its solution value of 423 nm. The blue shift found for the excitation peak could have arisen from an inaccuracy in the spectrum correction procedure or could indicate a condition of electron withdrawal from the aromatic system upon binding. We might also point out that possible excitation peaks near 400 nm might have been lost due to the intense scattering of the instrument used.

It may be noticed that the signal level for the 6-MeB[*a*]P–DNA emission peak is of the same order of magnitude as that for the DNA control. However, the amount of DNA recovered, as assayed by absorbance at 261 nm, was much less with the 6-MeB[*a*]P experiment. One way of correcting for DNA recovery, which also provides a rough estimate of the extent of binding, is to assume that the fluorescence quantum yield for hydrocarbon is unchanged after incorporation into DNA, an assumption which is not unreasonable, at least for 6-MeB[*a*]P.[4] This assumption allows us to calculate a minimum level of binding (MLB) in numbers of hydrocarbons bound per nucleotide unit

$$\mathrm{MLB} = I_{em}F\epsilon/A_{261} \qquad (1)$$

where I_{em} is the fluorescence emission intensity measured in μV (arbitrary units) when excited at the excitation maximum (uncorrected), F is a conversion factor relating concentration of hydrocarbon to emission intensity under identical spectrophotometric conditions (mol L^{-1} μV^{-1}), ϵ is molar absorptivity for DNA at 261 nm, and A_{261} is absorbance of DNA. MLB values for the three experiments are listed in Table I; 1/MLB values are also listed.

The level of enhancement of fluorescence signal over simple physical binding is then better than a factor of 10 in these experiments and is comparable to levels achieved by other workers using I_2 activation.[2] For the sake of comparison, we ran an I_2-activated experiment by using the same reagent concentrations listed above plus 5 mM (in I) I_2 and achieved a MLB value of 9-μmol B[*a*]P per mol of nucleotide (using phenol washing and subtracting phenol-washed blank) as compared to about 3000 for calf thymus DNA quoted by Hoffmann et al.[2] However, they

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Table I

	MLB × 10⁶	1/MLB	maximum yield of DNA, %	charge passed in 2 h in C	maximum current efficiency, %
B[a]P control	149	6.71 × 10³	100.0		
B[a]P electrolysis	3031	3.30 × 10²	40.6	51.2	3.5[a]
6-MeB[a]P electrolysis	1584	6.31 × 10²	9.4	28.3	2.2[b]
DNA electrolysis			0.9	79.3	
I_2 activation[c]	9	1.09 × 10⁵			

[a] Based on a 6-electron process according to ref 7. [b] Based on a 2-electron process according to ref 6. [c] This sample was washed with distilled phenol according to ref 11.

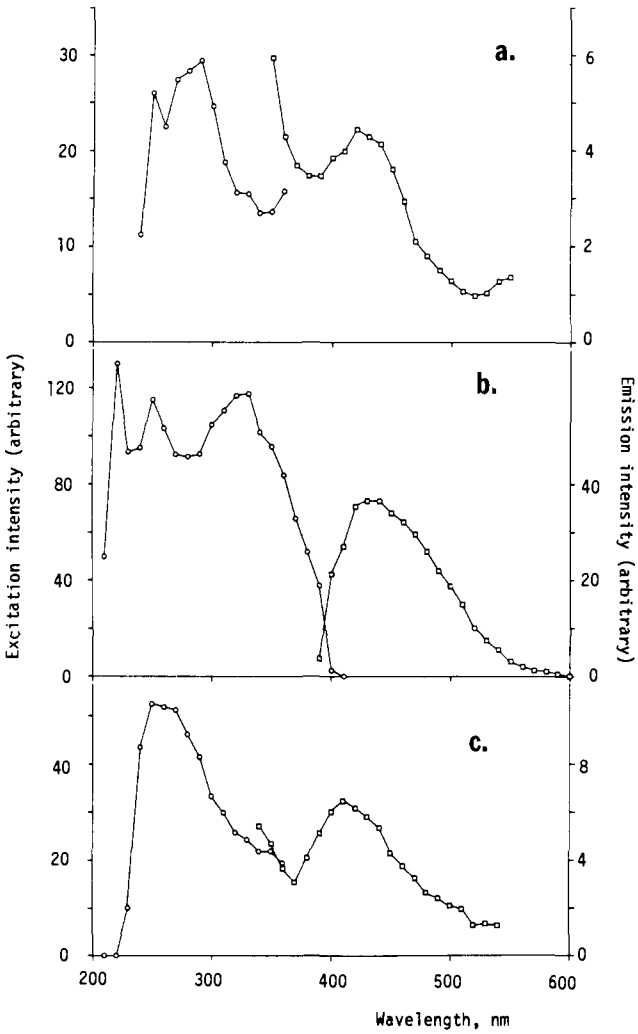

Figure 1. Corrected fluorescence excitation and emission spectra for DNA in 50% ethanol solutions which are initially 0.3 mM heat-denatured DNA, 0.005 M phosphate buffer, 0.015 M TBAP, and 0.1 mM hydrocarbon. (a) B[a]P stirred with DNA for 2 h; (b) B[a]P electrolyzed at +1.15 V vs. Ag reference for 2 h; (c) 6-MeB[a]P electrolyzed at +1.03 V vs. Ag reference for 2 h. The emission spectra were excited at 300, 360, and 300 nm for (a), (b), and (c), respectively, while the excitation spectra were monitored at 410, 425, and 410 nm, respectively.

replenished B[a]P several times during the course of the reaction. Also, differences in washing procedures, low quantum yield, or the presence of TBAP could account for this difference. In any case, it seems that electrochemically activated binding is also more efficient than I_2-activated binding by about 2 orders of magnitude.

Another aspect of these experiments is the nature of the electrolytic process. As seen in Table I, the maximum current efficiencies (assuming total consumption of hydrocarbon) are extremely low. The bulk of the current seems to be channeled into another process, possibly oxidation of ethanol and/or DNA. The detailed mechanism for these processes are being studied.

It is highly unexpected that the total charge passed should be actually less with hydrocarbon than with an electrolysis experiment in which hydrocarbon was absent. This result is also supported by cyclic voltammograms in which the anodic current is suppressed upon addition of B[a]P. This suppression could be accounted for by the tendency of B[a]P and B[a]P cation radical to adsorb onto Pt.[7]

The fact that the recovery of DNA was the highest with B[a]P electrolysis points toward an unexplained process in which the destruction of DNA, possibly by reactive products of ethanol oxidation or by the electrode itself, is decelerated by this adsorption. One reason for the difference is protective ability of B[a]P and 6-MeB[a]P could be that B[a]P, with its demonstrated tendency to catalytically regenerate itself during oxidation,[7,11] together with the greater number of electrons required for its consumption, remains in solution for a longer time and can exert its protective effect longer.

One could draw a final conclusion in the light of this argument about the relative MLB values of B[a]P and 6-MeB[a]P. Since they are of the same order of magnitude and since we would expect that B[a]P would have much more opportunity to form cation radicals, 6-MeB[a]P binding may not result from a cation radical but rather from the more stable benzylic carbonium ion. The carbonium ion would form as a result of loss of a proton and an electron from the cation radical and has been postulated as a reactive intermediate in the reaction of 7-methylbenz[a]anthracene cation radical with pyridine.[12,13]

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Acyl-Transfer Reactions in the Gas Phase. The Question of Tetrahedral Intermediates

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Esterification and hydrolysis reactions of carboxylic acids and derivatives commonly proceed by mechanisms in which an acyl group is transferred to the attacking nucleophile by way of tetrahedral intermediates. Evidence supporting addition–elimination mechanisms refers almost entirely to solution-phase reactions catalyzed by acids, bases, or enzymes.[1] Although related gas-phase reactions have been observed in the ion–molecule chemistry of acyl compounds by using ion cyclotron resonance (ICR) techniques,[2-4] certain features of these reactions are inconsistent

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