SOLUBILIZATION OF A NEUTRAL PHENAZYL FREE RADICAL BY DNA

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Uncharged aromatic hydrocarbon and heterocylic compounds can be rendered soluble in aqueous solution by association with dissolved nucleic acids (Boyland and Green, 1962, 1964; Liquori et al, 1962; Nagata et al, 1966; Vescia et al, 1968). This fact is of considerable interest relative to the carcinogenic and mutagenic activity of these substances. Although the phenomenon is well established and evidence has accrued that the solubilized species is often aligned relative to the helix axis, it is not completely clear whether the bound molecule exists in a monodispersed form or as microcrystallites, somehow stabilized by the nucleic acid (Giovanella et al, 1964; Van Duuren et al, 1969). This question can be answered for the solubilization of such compounds, if they are paramagnetic, by observing the linewidth of the ESR spectrum of the ligand in the presence and absence of the nucleic acid. If microcrystallites are present, a narrow single line will be observed reflecting electron spin exchange phenomenon characteristic of a solid. If the uncharged free radical is monodispersed, the separation between ligands will be too large for spin exchange and the linewidth will be the envelop of the hyperfine components, smeared out because of association of the paramagnetic species with slowly tumbling biopolymer. Our experiments clearly show the latter to be the case in the solubilization of the N-methylphenazyl-2-nitrile (MPCN·) radical by DNA at pH 10.

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Experimental

MPCN· was prepared according to the method of McIlwain (1937) as black needles, melting point 145.5-148°C. The IR spectrum clearly showed a cyanide Stretching mode at 2200 cm^{-1}, and, by comparison of ESR absorption intensity with that of DPPH, the material is 100% free radical. Salmon sperm DNA (Calbiochem, highly polymerized) was dialyzed three times against 0.001 M NaCl prior to use.

Solutions were prepared by the method of Boyland and Green (1962) by grinding 5 mg of solid MPCN· with 1 ml of 2 mg/ml DNA solution. These solutions were then combined with either additional amounts of buffered DNA solution or with buffer alone to produce aqueous samples of MPCN· in the presence and absence of DNA with ionic strength Na^+ 0.001M and pH 10. This procedure was adopted in order to insure that both samples would be given the same benefit of the buoyancy of the viscous nucleic acid solution during the grinding process. Each solution was then bubbled with N_2 to remove O_2 and then shaked on a mechanical shaker for periods up to twenty hours. After shaking the samples were centrifuged at 39,000 g for 15 min. The supernatant solution was then introduced into an ESR sample tube and the signal detected using previously described procedures. (Ishizu et al, 1966, and 1969).

Results

The ESR spectra of aqueous samples of MPCN· after various treatments are shown in Figure 1. Figure 1a shows the single sharp (ΔH (max.slope) $\sim$ 1.2 gauss) line from MPCN· mixed with buffer at pH 10. A broad component becomes apparent in the spectra of a sample of free radical shaken with DNA but not centrifuged (Figure 1b). Figure 1c shows the spectra of the supernatant solution obtained by centrifugation of the sample used for Figure 1b. Centrifugation of the MPCN·-buffer sample removes completely all radical species from the supernatant. Thus, it is clear that a paramagnetic species is carried into solution by DNA and that the radicals are mono-

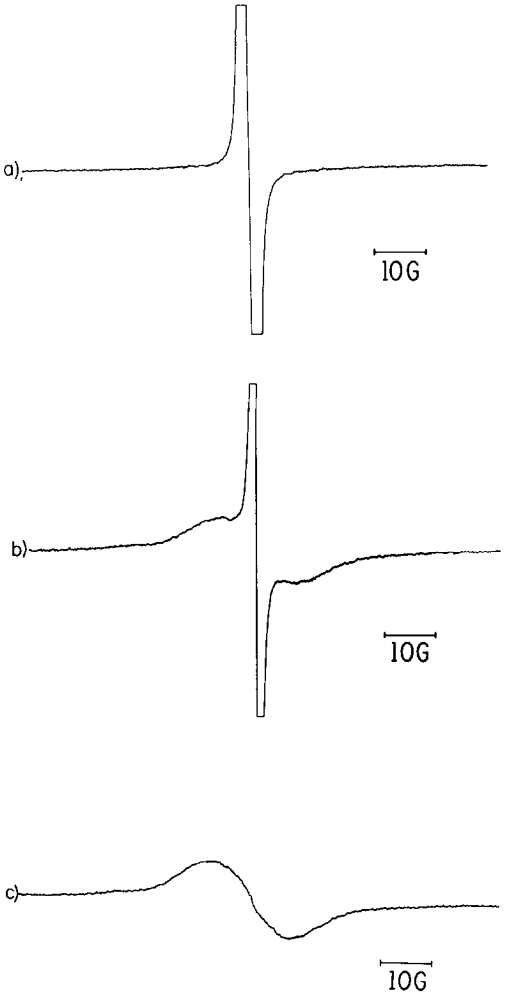

Fig. 1. Room temperature ESR spectra of aqueous samples of N-methylphenazyl-2-nitrile at pH 10.
a) Solid MPCN· mixed with buffer alone.
b) Solid MPCN· mixed with DNA solution.
c) Supernatant from centrifugation of b).

dispersed. Our evidence that the "dissolved" species is MPCN· comes from a study of the pH dependence of the equilibria involving various phenazyl species in mixed solvents which will be published elsewhere. The primary finding of interest here is that the pK_a of the conjugate, water-soluble, phenazinium cation-radical, $MPCNH^{+}$· is 4.7 and, consequently, is present in negligible concentration at pH 10. We have studied the binding of $MPCNH^{+}$· to DNA at lower

pH (Akasaka and Dearman, to be published) and found qualitative and quantitative differences in the ESR spectra of that complex.

Although MPCN· is insoluble in aqueous solution at pH 10, it is slightly soluble in a mixed solvent of acetone-water (1:9) at this pH and, for example, can be extracted into an immiscible third solvent, such as benzene. Of further interest is the fact that the complex between DNA and MPCN· can be studied in the acetone-water mixture without introduction of solid free radical. Under the same experimental conditions the ESR line width of the complex in the mixed solvent is 15.7 gauss in good agreement with the value 15.4 gauss in Figure 1c. Both these widths are different from 18.5 gauss found for MPCN· in the acetone-water solvent without DNA, indicating that some narrowing occurs upon association. However, when the radical in mixed solvent is frozen to 77°K, a linewidth of $\sim$ 15 gauss is again obtained showing that the room temperature narrowing caused by association with DNA is not of the spin exchange type. Electrophoresis of MPCN· in mixed solvent at pH 10 showed no movement of the spot under conditions for which the parent cation, N-methyl phenazinium, (MP^+), moves to the cathode. Optical absorption by acetone prevented our determination of the state of the DNA in the mixed solvent by the usual criterion of absorbance at 260 nm. However both by sedimentation velocity experiments and by ORD at 350 nm, we could detect no difference between DNA in the acetone-water solvent at pH 10 and DNA in aqueous solution at pH 10, whereas large differences in these parameters were easily detected for heat denatured DNA. For example in the mixed solvent we obtained the value, 565°± 10 for $[\alpha]_{350}$, which can be compared with the value 576° calculated from the Drude constants for native salmon sperm DNA reported by Samejima and Yang (1964).

We can obtain an estimate of the number of radicals bound per nucleotide, n, by comparing the intensity of the absorption in Figure 1c with that of a known concentration of MPH^+· (Zaugg, 1964; Ishizu et al, 1969) and obtain the value n = 0.05 ± 0.01. The same value is obtained by comparison of the

optical absorption of the sample in Figure 1c with that of the MPCN·-DNA complex in acetone-water mixed solvent. This is a somewhat lower value than is characteristic of the "tight-binding" sites on DNA for heterocyclic cations such as proflavine (Blake and Peacocke, 1968) or ethidium (Waring, 1965) but is approximately the order of magnitude for aromatic hydrocarbons (Ball et al, 1965). If we can associate the site here with the intercalated "tight-binding" site of the acridine cations, a statistical distribution of MPCN· places the radicals some 35Å apart. At these separations no contribution from the exchange interaction to the line shape is expected. In this context it is of interest to note that a broad ESR line is again found for the solid complex of MPH^{+}· and poly U showing that also in this case, presumably comparable to the DNA-acridine complex, no exchange narrowing is observed.

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