COMMUNICATION

[BINDING OF BENZO[a]PYRENE-SEMIQUINONE RADICALS WITH DNA AND POLYNUCLEOTIDES]

We have previously found that 6-oxybenzo[a]pyrene (6-oxy-B[a]P) radical, which was deemed to be one of the active metabolites of benzo[a]pyrene (B[a]P), was bound covalently to DNA or polynucleotides. This fact prompted us to investigate the binding between the benzo[a]pyrene (B[a]P)-semiquinone radical and DNA or polynucleotides, because 6-oxy-B[a]P radical has been proved to be an obligatory intermediate in the oxidation process from 6-hydroxy-B[a]P to the B[a]P-quinones.

A mixture of B[a]P-3,6-, -1,6-, and -6,12-quinones was obtained by oxidation of B[a]P with chromic acid and each quinone was separated by partially deactivated alumina column following the method of Masuda and Kuratsune. B[a]P-quinones were reduced by adding dropwise an aqueous solution of sodium borohydride (1mg/0.5ml) to the quinone solution (1mg/10ml dimethyl sulfoxide), with immediate development of violet (3,6-quinone) or blue (1,6- and 6,12-quinone) color. The reduced solutions gave strong electron spin resonance (ESR) signals which were identified as those of respective B[a]P-semiquinone anion radicals by comparing with the signals reported before.

Aqueous solution of calf thymus DNA (10mg/10ml) was added to the solutions of semiquinone radicals, which were incubated at 37°C for 18 hr. Then, DNA was precipitated with ethanol after addition of sodium perchlorate (1.2 g), and it was dissolved in 15 ml of 1% SDS and extracted three times with 80% phenol and twice with ether. After DNA was precipitated with ethanol, it was washed extensively with ethanol and ether, and dried for the ESR measurement. The binding experiments with polynucleotides were also done under similar conditions.

The ESR signals in Fig. 1 show the amount of covalently bound complex between the B[a]P-semiquinone radicals and DNA. It is seen that binding of 3,6-semiquinone radical was largest and 1,6-semiquinone radical binds half as much as the former radical. It was remarkable that no binding could be detected when 6,12-semiquinone radical was interacted with DNA. This might be ascribed partly to instability of the B[a]P-6,12-semiquinone radical, because its half-lifetime was far shorter than those of 3,6- and 1,6-semiquinone radicals. However, some other factors might also be considered, for not a small amount of semiquinone radical could be detected in the reaction mixture under the condition of binding experiment.

Base specificity in the binding was investigated by using poly(G), poly(A), poly(C), and poly(U). Among them, the ESR signal due to the bound complex was extremely...
large for poly(G), and other polynucleotides gave only a small signal which was less than 1/20 of the signal for poly(G). This shows that guanine residue is the site of binding as in the case of 6-oxy-B[a]P radical.²)

The g-value and linewidth, which are the indices for the characteristic of the structure of the free radical, were investigated. Thus, the g-value of the ESR signal for the bound complex between the B[a]P-3,6- or -1,6-semiquinone radical and poly(G) was 2.004, which is identical with that of the free semiquinone radical. Half-line width for the bound state was 7.2 gauss which was a little broader than that of the free semiquinone radical, i.e., 5.3 gauss. This strongly suggests that semiquinone radicals are active species in binding to the base moiety.

Whether the active species involved in the binding between 6-oxy-B[a]P radical and poly(G) or DNA is the same as the B[a]P-semiquinone radical remains to be determined and is under study in our laboratory.

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REFERENCES