MASTER

POLYCYCLIC AROMATIC HYDROCARBON

-PROTEIN INTERACTIONS

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Abstract

Interacting with bovine serum albumin (BSA), both the very weak carcinogenic hydrocarbon benzo(e)pyrene (BeP) and the powerful carcinogen benzo(a)pyrene (BaP) form pyrene-type compounds, indicating chemical modification at the bay region of the molecules. In contrast to the BaP-BSA reaction apparently similar to the metabolic activation to the bay region oxidation product, the BeP-BSA reaction differs from the known metabolic change of BeP which occurs at the K-region. While the BaP-BSA reaction also produces a BaP radical as well as other UV-fluorescent species, no BeP radical is formed in interaction with BSA and two sharp UV fluorescences at about 330 and 350 nm probably come from the higher excited states of BeP. Furthermore, from fluorescence and excitation spectral studies particularly at low temperature, it is suggested that the UV fluorescences at 320-380 nm of the BaP- BSA complex originate from a few distinct species. A new UV fluorescence at 330 nm (preferentially excited at 295 nm), as well as a new excitation peak at 325 nm for the longer wavelength UV fluorescences at 357 and 378 nm, has been found. The extract from the aqueous BaP-BSA solution also emits phosphorescence at 400-440 nm (excited at 310 nm) in EPA solution.

1. Benzo(e)pyrene (BeP) - bovine serum albumin (BSA) Interactions

BaP

BeP

The powerful carcinogenic hydrocarbon benzo(a)pyrene (BaP) is known to be activated to the bay region BaP-7, 8-diol-9, 10-epoxide, which is a pyrene-type product. While BaP contains only one bay region, BeP has two bay regions. Nevertheless, BeP is a very weak carcinogen. This very weak carcinogenicity of BeP was attributed to the lack of metabolic formation of the reactive bay region BeP-9, 10-diol-11, 12-epoxide. The main metabolic product was the K-region BeP-4,5-dihydro-diol. The former is a pyrene-type product; whereas the latter is a triphenylene type product. Thus, there has been no evidence for pyrene-type product from BeP-enzyme interactions.

In a previous study we found that BaP, when mixed with BSA in aqueous solution at 4°C in the dark for several days, produced a BaP radical, a pyrene-like product and other BaP derivatives. The formation of the radical species was detected by electron spin resonance spectrum, while that of the pyrene-like product was observed by both fluorescence and excitation spectra characteristic of a pyrene compound. BeP was treated in the same way with aqueous BSA solution, centrifuged and extracted with benzene to remove the excess hydrocarbon. When the mixture was excited at 335 nm, the normal fluorescence of BeP was observed at 380, 390, 400 and 410 nm. The excitation spectrum for the 390 nm fluorescence showed peaks at 326 and 337 nm, corresponding to the absorption spectrum of BeP. When excited at 345 nm, however, the mixture was found to emit at 377, 398 and 420 nm. The excitation spectrum for the 377 nm fluorescence showed two clear peaks at 329 and 344 nm. These fluorescence and excitation spectra indicate the formation of a pyrene-type molecule. Since this emission occurs from the aqueous protein solution which was treated with benzene, the molecule responsible for the emission must be bound to the protein and/or of more polar hydrocarbon-type. These results show that both BeP and BaP interact with BSA to form pyrene-like products. In contrast to BaP, however, BeP-BSA interactions did not produce any free radical.

Excitation at 335 nm of the BSA-BeP solution at 77°K exhibited a structural phosphorescence in the region of 530-610 nm, having a maximum at 540 nm. The BSA-BeP system at 77°K, when excited at 293 nm, fluoresced in the region of 310-370 nm, where there were two sharp peaks at about 328 and 342 nm on a broad protein fluorescence background. In addition, the normal fluorescence of BeP at 380-410 nm and the phosphorescence of

tryptophan residues of the protein at 405-460nm were observed at 77°K. The sharp fluorescence peaks in the UV region of 310-370 nm arise from the intrinsic energy levels of BaP. The two similar peaks at 338 and 353 nm (excited at 244 nm) were found in the fluorescence spectrum of BaP in EPA solvent (ether, isopentane, ethanol: 5: 5: 2). The excitation spectrum for the normal fluorescence at 380 nm corresponds to the absorption spectrum of BaP; whereas that for the 353 nm fluorescence had no resemblance with its absorption spectrum. These are probably from the two set of higher energy levels of BaP. Fluorescence from the second excited singlet state of BaP has been reported to appear at 389 and 394 nm (excited at 254 nm). As discussed in the following section, BaP interacting with BSA emitted the UV fluorescence in the region of 320-380 nm.

II. BaP - BSA Interactions

Our previous studies showed that the carcinogenic hydrocarbon BaP interacts with BSA to give rise to different products. Among them, species emitting in the UV region were further investigated.

a) Room temperature fluorescence

In addition to the three fluorescence peaks at 340, 357, and 378 nm, another peak was found at 330 nm when the BaP-BSA complex was excited at 280 nm. When excited at 305 nm, this fluorescence peak was previously observed only as a shoulder. These UV fluorescences were found to vary their intensities, depending on the energy of excitation. Higher energy excitation at 280 nm contributed more for the shorter wavelength fluorescence maxima at 330 and 340 nm; whereas the longer wavelength fluorescence maxima at 357 and 378 nm dominated when lower energy excitation at 305 and 313 nm was used. The benzene extract from the aqueous BaP-BSA complex showed the same UV fluorescences and their dependence upon excitation wavelengths. In contrast, the normal visible fluorescence (407, 430, 458 nm) of BaP in its complex with BSA maintained its vibronic intensities independent of the variation of excitation wavelengths (280, 305, 394 nm).

b) Low temperature fluorescence

The fluorescence and excitation spectra of the BaP-BSA complex were also studied at the liquid nitrogen temperature of 77°K. These spectra have been resolved more clearly at low temperature. When excited at 280 nm at 77°K, the major peak appeared very distinctly at 329 nm with a shoulder at 340 nm. The excitation spectra for the 330 and 340 nm fluorescences at 77°K exhibited a sharp maximum at 293 nm with an additional shoulder at 304 nm. (The peak and the shoulder are reversed at room temperature, where the major peak is at 307 nm with a shoulder at 296 nm). Furthermore, the excitation spectrum for the 358 nm fluorescence at 77°K revealed a new peak at 324 nm in addition to two known peaks at 294 and 308 nm. These results suggest that there should be more than one (possibly three) species responsible for these UV fluorescences. Longer wavelength (357 and 378 nm) emitting species may absorb around 325 nm, while shorter wavelength (330 and 340 nm) emitting species absorb in the region of 280-310 nm. It appears that the 330 nm fluorescent species which absorbs at about 295 nm, as well as the 325 nm absorbing species, increases their fluorescence yields

at 77°K. The visible fluorescences of the BaP-BSA complex also changed at 77°K: the second and third peaks at 430 and 456 nm were relatively higher compared to the first peak at 407 nm. This phenomenon may be due to the enhanced quantum yield at low temperature of hydroxy-BaP derivatives formed in this system.

c) Low temperature phosphorescence

The frozen aqueous BaP-BSA complex at 77°K did not show any phosphorescence spectrum due to BaP or any of its derivatives except for the phosphorescence of tryptophan residues in BSA. The dried residue of the benzene extract from the complex, dissolved in EPA, exhibited at 77°K phosphorescence with peaks at 407, 422 and 431 nm (excited at 310nm). The excitation spectrum consisted of a sharp peak at 310 nm. This phosphorescence differs completely from that of BaP or pyrene-type products which phosphoresce below 500 nm. Since the phosphorescence excitation maximum could be different from the fluorescence excitation maximum as in BaP, species responsible for the phosphorescence is not clearly defined.

Publications

- Srinivasan, B.N. and Fujimori, E. (1980). Benzo(a)pyrene radicals and other derivatives formed in interactions with cysteine/serum albumin, in Polynuclear Aromatic Hydrocarbons, Battelle Press, Columbus, Ohio. pp. 319-331.
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Polycyclic Aromatic Hydrocarbon - Protein Interactions
(Contract No. DE - AC02 - 77 EV04374)

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Comprehensive Report

1. Main research accomplishments

Oxidative modification of the carcinogenic hydrocarbon benzo(a)-pyrene (BaP) has been found to occur in association with the SH-protein bovine serum albumin (BSA). In this interaction, a BaP radical, a pyrene-like product and UV-fluorescent species, as well as 6-oxo-BaP radical and hydroxy-BaP, were produced. The new BaP radical exhibited a widely separated six-line electron spin resonance spectrum, different from that of the known 6-oxo-BaP radical. The pyrene-type product showed spectral characteristics typical of pyrene, namely a first fluorescence peak at 380 nm with two distinct excitation maxima at 330 and 345 nm. From low temperature emission studies, UV-fluorescent products were found to fluoresce separately at 330 nm (preferentially excited at 295 nm) 340 nm (excited at 310 nm), 357 and 378 (excited at 325 nm) and to phosphoresce at 400-440 nm (excited at 310 nm). With these reactions, the SH-group of the protein was consumed. The interaction of BaP with the SH-compound cysteine also led to the oxygen-dependent formation of BaP radical and pyrene-like products. In contrast to BaP, the very weak carcinogen benzo(e)pyrene (BeP) did not react with cysteine. With BSA, no BeP radical was also formed, but the formation of a pyrene-type product was found, differing from the cellular metabolism of BeP, in which the reactive pyrene-type diol-epoxide is not formed. Furthermore, comparative studies with the non-SH-protein, type I collagen, revealed the absence of BaP modification in this protein, though the properties of collagen molecules such as fibril formation in vitro were affected by bound BaP.

2. Plans for continuation and possible new objectives

Studies on the modification and activation of carcinogenic hydrocarbons in interactions with SH-proteins will be continued. The formation of various products (UV-fluorescent products, pyrene-type compound and radical species) has been found in BaP-BSA interactions, but the separation of each product has been only partial and incomplete. Both radical and UV-fluorescent products can be extracted by benzene. Their other common features led to the assumption that both could be the same molecule. However, fluorescence and excitation spectral studies particularly at low temperature suggest that UV-fluorescent species consist of more than one

compound. Further efforts for the separation of these products from excess amounts of BaP will be continued along with studies on substituted BaP derivatives. Substitution of methyl or fluoro groups into the BaP ring may affect the ability of radical formation. In contrast to radical and UV-fluorescent species, the pyrene-type product, which is also formed from BeP-BSA interactions, can not be extracted with benzene. Its possible binding to the protein will be explored for both BaP and BeP. In consideration of the involvement of SH-groups in interactions with hydrocarbon metabolites and other carcinogenic hydrocarbons, the reactions of BaP-7, 8-diol-9, 10-epoxide, benz(a) anthracene and 7, 12-dimethyl-benz(a)-anthracene with BSA/glutathione will be investigated.

During the course of our investigation on the interactions between BaP and BSA/cysteine, we have been contemplating possible extension of our studies to other SH-proteins. Considering the functional importance of reactive SH-groups, we have now a new plan to extend our investigation to erythrocyte membrane proteins. Being highly lipophilic, polycyclic aromatic hydrocarbons can readily partition into membranes. Membrane proteins contain a large number of SH-groups for various functions. SH-groups may also play a structural role in hydrophobic bonding of proteins to lipids. Thus, interactions of hydrocarbons with SH-proteins could be enhanced in biomembrane environments. This study also serves as a model for membrane-bound protein-hydrocarbon interactions. Furthermore, the red cells are available in large quantities, while SH-groups are also ubiquitous in these cells. The erythrocyte study also provides the opportunity to extend our future research to erythrocyte nuclei and nuclear proteins.

3. Graduate and post-doctoral

This institute is a private research organization and is not associated with universities. So far one research associate (Ph.D.) and one research assistant (M.S.) have participated in this project.

4. Bibliography

Srinivasan, B.N. and Fujimori, E. (1979). Benzo(a)pyrene-serum albumin/cysteine interactions: fluorescence and electron spin resonance; Chem.-Biol. Interact. 28, 1-15.

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5. Present state of knowledge, significance and needed future investigations

There has been a particular interest in the studies of metabolic activation of polycyclic aromatic hydrocarbons and the binding and modification of DNA by activated hydrocarbons. Proteins are also important targets in chemical carcinogenesis. Although binding of hydrocarbons to proteins such as serum, microsomal, nuclear and cytoplasmic proteins has been demonstrated, the mode of interaction or activation of hydrocarbons in association with proteins, as well as modification of proteins by activated hydrocarbons, has not fully been investigated. In particular, the role of reactive SH-groups, known to form both superoxide radical and thiyl radical, in oxidative modification needs to be elucidated. (Interestingly, many types of cancer cells lack the essential SH-amino acid cysteine either by losing their ability to produce or by abnormally using up their own cysteine.) It is therefore of significance to further investigate these problems important in carcinogenesis.

6. Federal support

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Dept. of Energy (DE-AC02-77 EV0 4374) "Polycyclic aromatic hydrocarbon-protein interactions;" \$74,345 (includes indirect costs) (March 1980 - Feb. 1981).