POLYCYCLIC AROMATIC HYDROCARBON

-PROTEIN INTERACTIONS

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Abstract. As bovine serum albumin (BSA) undergoes acid- or base-induced conformational changes, the binding of benzo(a)pyrene (BaP) to BSA, as well as the type II UV fluorescence (380 nm) due to pyrene-like oxidation products, increases. The presence of fatty acids also enhances the binding of BaP to BSA at neutral pH, whereas the visible fluorescence of BaP is effectively quenched by fatty acids. L-Tryptophan, which is specifically bound to BSA, enhances the BaP binding and particularly the formation of pyrene-type products. Upon removal of oxygen, the production of the type I UV fluorescence (340, 357, 378 nm) probably due to BaP radicals is diminished and hydroxy-BaP derivatives are not formed. While BaP undergoes oxygen-dependent reactions with cysteine, non-carcinogenic benzo(e)pyrene does not react with cysteine. The BaP fluorescence of young collagen (from 4-6 weeks old rat tail tendon) is more intense than that of old collagen (from about 2 years old rat tail tendon). With increasing temperature, the former fluorescence decreases, whereas the latter increases. The denaturation temperature of both BaP-collagen and UV(365 nm)-irradiated BaP-collagen complexes does not differ from that of collagen itself.

I. BaP - BSA/SH-compound Interactions

pH. BSA undergoes pH-dependent conformational changes. Lowering the pH from 7 to 4 (N-F transition) and further down to 2, as well as raising from 7 to 9 (N-B transition), increased the binding of BaP to fatty acid-containing BSA (pH 4 > pH 9 > pH 2 > pH 7). The N-F transition involves a slight expansion of the protein resulting from a separation of intramolecular domains and the opening of a crevice. A further decrease of pH to 2 causes the protein to undergo a more complete expansion. The N-B transition involves a change in the tertiary structure of the molecule without any significant change in helical content.

The UV fluorescence (350 nm) of tryptophan residues in BSA at pH 7 shifted to shorter wavelengths and was quenched at increased acidity and basicity: pH 9 (343 nm) > pH 2 (337 nm) > pH 4 (347 nm) > pH 7 (350 nm). These changes indicate that the protein region around the tryptophan residues becomes more hydrophobic in the B (pH 9) and F (pH 4) states as well as in the acid expanded (pH 2) state. Both the visible fluorescence (408 nm) of BaP and the type II UV fluorescence (380 nm) of pyrene-like oxidation products were also affected by pH (pH 2 > pH 9 > pH 4 > pH 7). The effect of pH upon the type I UV fluorescence (340, 357, 378 nm) has not been studied, though strong acid (pH ~ 0) reduced this UV fluorescence.

Fatty acid and tryptophan. Serum albumin binds not only polycyclic aromatic hydrocarbons, but also a number of other ligands including fatty acids and L-tryptophan. Since both fatty acids and L-tryptophan (an essential amino acid) are bound to serum albumin under physiological conditions in the blood, the effect of these ligands upon BaP - BSA interactions have been investigated. The binding of fatty acids affects the conformational state of serum albumin and allosterically enhances the binding of certain aromatic ligands. In agreement with this concept, the presence of fatty acids in BSA was found to enhance the binding of BaP to BSA at pH 7. On the other hand, the visible fluorescence of bound BaP was effectively quenched by fatty acids. The formation of pyrene-like products from bound BaP was also decreased in the presence of fatty acids.

L-tryptophan is known to bind to BSA in competition with fatty acids. The effect of L-tryptophan upon BSA-BaP interactions was also found to be affected by fatty acids. Tryptophan slightly inhibited the binding of BaP to BSA in the presence of fatty acids, but enhanced it in the absence of fatty acids. The formation of pyrene-type products was increased by tryptophan particularly in the absence of fatty acids. On the other hand, tryptophan substantially reduced the visible fluorescence of BaP in both the presence and absence of fatty acids (probably because of the efficient conversion of BaP to pyrene-like products and other oxidation products). The importance of these findings in the quaternary system (BSA - fatty acid - tryptophan - BaP) is that the efficiency of the activation (oxidation) of BaP in interactions with BSA depends on the proportion of two important nutrients to be transported by serum albumin.

Oxygen. In order to determine the effect of oxygen upon BaP - BSA interactions, the degassed aqueous solution of BSA was mixed with BaP under vacuum. The rate and amount of BaP radical formation, as indicated by the appearance of the type I UV fluorescence, were much reduced compared to those in the presence of oxygen. In the absence of oxygen, the formation of hydroxy-BaP derivatives was not observed, as seen from the absence of enhanced fluorescences at 430 and 458 nm. It appeared that removal of oxygen also reduces the amount of soluble BaP in BSA. Upon exposure to air, the intensity of visible fluorescences increased much more rapidly than that of UV fluorescences. Oxygen appears to affect protein environments so that more hydrocarbon molecules can penetrate into the protein.

SH-groups. The involvement of SH-groups in BSA - BaP interactions has not been substantiated, though the interaction of cysteine with BaP supports the reactivity of SH-groups with BaP. It was found by using Ellman's reagent for detecting the SH-group of BSA that about 20% of SH-groups disappeared when 10% BSA was incubated with BaP in the dark for several days. When 10% BSA was treated with the organic mercurial p-mercuriphenylsulfonate ($10^{-2}M$) to block the SH-group of BSA and subsequently treated with BaP, the formation of the type I UV fluorescence was inhibited, suggesting the inhibition of BaP radical formation. The structure of BSA can be changed by reducing disulfide bonds. Treatment of BSA with dithiothreitol for 1 hr at room temperature and pH 8 cleaves 14 of the 17 disulfide bonds of BSA. After this treatment, the reduced BSA was incubated with BaP. More BaP molecules were incorporated into the reduced protein, and the formation of pyrene-type products was enhanced.

SH-compounds. The non-carcinogenic or weakly carcinogenic hydrocarbon benzo-(e)pyrene, when mixed with cysteine in aqueous solution, did not show any detectable UV fluorescence, indicating little formation of its radical species. For the cysteine-BaP complex, the type I UV fluorescence appears as a structureless broad spectrum in the 310 - 370 nm range with a maximum at 330 nm. The intensity of this fluorescence is directly related to the concentration of cysteine (up to 4%). It has also been found that the formation of type I UV fluorescence is particularly sensitive to the presence of oxygen. This fluorescence was not clearly formed in a closed aqueous mixture (closed from atmosphere) of cysteine and BaP, kept at room temperature for one day. In an open system, however, the type I fluorescence was formed at 330 nm. This result indicates the involvement of oxygen in this reaction. After benzene extraction of free BaP from the aqueous mixture of cysteine and BaP (kept at room temperature for a few days), a species responsible for the type II fluorescence at 380 nm remained in the aqueous solution, indicating the formation of more polar pyrene-type products than BaP. The oxidation of BaP to these pyrene-like products occurred also with mercaptoethanol and more efficiently with dithiothreitol.

UV(365nm)-irradiation. Previous studies showed that UV(365 nm)-irradiation of the BaP - BSA complex induces binding of BaP photoproducts (hydroxy-BaP derivatives) to BSA as well as changes in the order of the type I UV fluorescence band intensities. Upon standing in the dark, hydroxy-BaP products underwent further changes, while the UV fluorescence returned to the original order. These changes were found to be reversed readily by short UV(365 nm)-irradiation. The changes in the type I UV fluorescence may suggest the presence of more than one UV-fluorescing components which are modified by UV irradiation or the modification of vibronic band intensities by environmental effects. When the BSA - BaP complex was irradiated with UV(365 nm) light, a new fluorescence at 455 nm (excited at 420 nm) appeared and increased at the expense of BaP fluorescence. Since the increase of this new fluorescence was in parallel with that of the ESR signal due to 6-oxo-BaP radical, this fluorescence originates most likely from that radical.

II. BaP-collagen Interactions

Age-related differences between young and old collagen were found in the fluorescence intensity of BaP-collagen complexes. Acid-soluble collagen (type I) was prepared from tail tendon of young rats (4-6 weeks) and old rats (about 2 years). The intensity of BaP fluorescence (407 nm) was about 3 times stronger in young collagen than in old collagen. Another difference was that the young collagen-BaP complex exhibited the type II UV fluorescence (380 nm).

The thermal denaturation-fluorescence profiles of the BaP-collagen complex also showed dramatic differences between young and old collagen. For old collagen, the BaP fluorescence intensity, when excited at 275 nm, increased with increasing temperature to 80°C, exhibiting a plateau in the temperature range of 35-45°C. When the solution was cooled to 25°C, the intensity decreased from 80 to 40°C and slightly increased from 40 to 25°C, where the intensity was higher by about 20% than its original value. For young collagen, the BaP fluorescence decreased showing a pronounced drop during the denaturation temperature, as the temperature increased from 25 to 60°C. Heating from 60 to 80°C slightly increased the intensity. Upon cooling from 80 to 60°C, the intensity decreased, while from 60 to 25°C, a slight increase occurred. The intensity was lower by about 25% than its original value at 25°C. In both collagens, no spectral shifts were observed during thermal denaturation. In contrast, the fluorescence (305 nm) of tyrosine residues simply decreased upon heating and increased upon cooling. The 380 nm UV fluorescence for the young collagen-BaP complex was temperature-independent. When BaP was directly excited at 387 nm, anomalous fluorescence changes seen by exciting at 275 nm (plateau for old collagen and sudden fall for young collagen) were not observed. Instead, gradual increase for old collagen and smooth decrease for young collagen took place with increasing temperature. Since tyrosine residues, present only at the non-helical terminal peptides of collagen, are excited by 275 nm UV light, these results suggest that during thermal denaturation the efficiency of energy transfer from excited tyrosine residues to bound BaP changes differently in young and old collagen.

Previously it was reported that the collagen-BaP complex, as well as its UV(365 nm)-irradiated complex, undergoes broader changes of CD ellipticity at 222 nm in the temperature range of thermal denaturation. This broadening appeared to lower the denaturation temperature, but was found to be caused by the exposure of collagen solutions to shorter UV radiation during heating experiments. When the sample cells were lifted up from the UV beam to avoid this exposure, the denaturation temperature did not change. This new result indicates that BaP bound to

the collagen molecule does not modify the conformation of the helical body of the protein, but rather affects the non-helical, terminal regions, inhibiting fibril formation.

Publications

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