A PAH metabolite-binding subdomain in human albumin.

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A major focus of our research is the characterization and quantitation of carcinogen-protein adducts. This was done in the past primarily by electron-capture NICI-GC-MS of derivatized adduct moieties following their hydrolytic release from the proteins. The actual analyses were thus carried out on small molecules identical to or structurally similar to the original carcinogen. Most experiments, in addition, were carried out with carcinogens of known human exposure that had been shown to form stable adducts with hemoglobin or albumin. More recently, the combination of electrospray ionization with tandem mass spectrometry has allowed the detection and characterization of adducted peptides. This technique can potentially reveal the presence of unknown carcinogen-protein adducts and, in some cases, allow characterization of the carcinogen-binding site on the protein.

We previously described preliminary results - for reaction of metabolites of cyclopenta[cd]pyrene, benzo[a]pyrene, and fluoranthene with human albumin - suggesting a common binding site, His(146) in albumin subdomain 1B, for these metabolites (Brunmark et al., ASMS, 1995). Reactions of at least 15 PAH metabolites with albumin have now been carried out by us or others. These include 7,8-dihydrobenzo[a]pyrene-7,8-diol 9,10 epoxide (1); 2,3-dihydrofluoranthene-2,3-diol 1,10b-epoxide (2); 1,2-dihydrochrysene-1,2-diol 3,4-epoxide (3); 6-methyl-1,2-dihydrochrysene-1,2-diol 3,4-epoxide (4); 5-methyl-1,2-dihydrochrysene-1,2-diol 3,4-epoxide (5); 3,4-dihydrobenzo[c]phenanthrene-3,4-diol 1,2-epoxide (6); 11,12-dihydrobenzo[g]chrysene-11,12-diol 13,14-epoxide (7); 11,12-dihydrodibenzo[a,l]pyrene-11,12-diol 13,14-epoxide (8); cyclopenta[c,d]pyrene 3,4-epoxide (9); benzo[a]pyrene 4,5-epoxide (10).

Proteinase K digest of CDE-HSA

![Proteinase K digest of CDE-HSA](image-url)
Human serum albumin was treated with solutions of the various epoxides or diol epoxides and the adducted proteins were then digested with Pronase, proteinase K or chymotrypsin to yield adducted peptides of various lengths. The digests were analyzed by tandem mass spectrometry using standard or capillary LC, or nanospray, for sample introduction.

Fragments characteristic of the adducting PAH metabolite were used in each case to identify which components of the digest represented adducted peptides. These peaks were then analyzed by MS/MS on the peptide ions generated by in-source CID. An example is shown above.

The smallest adducted peptide in each case identified the reacting amino acid, while the larger peptides provided sufficient sequence information to locate them within the protein. Digestion with Proteinase K typically yielded adducted peptides with the sequences RRHPY/RRHPYFYAPE or KYL/KYLY, uniquely located in subdomain Ib. Digestion with pronase yielded smaller peptides that generally established the binding sites as the histidine or lysine residues within the sequence. Compound 10 binds to a lysine, but its position could not be unambiguously assigned; one possibility is Lys(159), which is also in subdomain Ib. More than half the reactions were at His(146), but several compounds, including compounds 3, 7, 8, and 9, reacted at a second common binding site, Lys(137). Both of these sites are located in subdomain Ib, thus characterizing this as a principal binding location for metabolites of polycyclic aromatic hydrocarbons.

It is well established that specific amino acid residues in native proteins can be unusually reactive and that one possible explanation for this is the establishment of a noncovalent association, locating and maintaining the electrophile favorably near a nucleophilic site on an amino acid, prior to covalent bond formation. Albumin contains several bindings sites, including subdomain Ib. This subdomain is a primary binding site for long-chain fatty acids but has not been regarded as a drug binding site. It is now apparent from these and previous results, however, that subdomain Ib is the preferred binding site for epoxide and diol epoxide metabolites of polycyclic aromatic hydrocarbons. We can only speculate on the origins of this site-selectivity, but it now also appears to be strongly supported by the considerable range of compounds that have been investigated.

The fact that the number of peptides to which carcinogens might bind is limited now offers the possibility of carrying out this mass spectrometric screening process in reverse, i.e., using ions characteristic of the peptides rather than the hydrocarbons to flag adducts from the digests.

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