CARCINOGEN-PROTEIN COMPLEXES IN MAMMARY GLAND AFTER ADMINISTRATION OF 3-METHYLCHOLANTHRENE

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SUMMARY

Twenty-four hrs after i.p. administration of ^{3}H-3-methylcholanthrene (^{3}H-MCA) to 8-10 weeks old virgin or 14 days pregnant BALB/c mice, the macromolecules and ^{3}H-hydrocarbon-protein complexes in their mammary cytosols were extensively resolved according to molecular size. The profiles of the macromolecules at both stages of mammary development were generally similar, and contained at least five families of molecular size of modes: >300,000, 181,000, 88,000, 44,000 and 27,000 daltons. At least three species of ^{3}H-hydrocarbon-protein complex were present. The principal complex had a modal molecular weight of 83,000, and the two minor species >300,000 and 47,000. Only a small amount of complex similar to the principal species was produced in vitro by incubation of ^{3}H-MCA with cytosol at 1-4°, indicating that the carcinogen is probably metabolized prior to interaction in vivo with its principal target protein.

Chemical carcinogens interact with DNA, RNA and particular proteins during carcinogenesis. Which of these interactions may lead to oncogenesis is unknown (1,2). Certain polycyclic aromatic hydrocarbons, such as 3-methylcholanthrene (MCA), are carcinogenic in mammary glands in mice (3). In pursuit of the study of how chemical carcinogenesis in mammary gland may relate to abnormal organ development and differentiation, the mammary cytosolic macromolecules have been extensively resolved and the carcinogen-protein complexes therein examined. To our knowledge, we present here the first report of the multiplicity, amounts and description of the different complexes derived from mammary gland proteins and a carcinogen, and do so at two stages of mammary organ development.

MATERIALS AND METHODS

Mice of the BALB/c strain, bred in-house, were 8-10 weeks old virgins

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or 10-14 days into their first pregnancy. Each of 20 to 50 mice were injected intraperitoneally with 35 μCi of [G-^{3}H]-MCA, 7.9-12.5 Ci/mmole (Amersham/Searle Corp.), in 70 μl DMSO. After sacrifice and exanguination 24 hours later, the 2nd, 3rd, and 4th pairs of mammary glands were freed of lymphatic and connective tissues, rinsed in cold 0.25 M sucrose, and processed thereafter at 1-4°. The tissue was blotted on filter paper, finely minced with scapels, suspended 1:1 (w/v) in 0.25 M sucrose containing 2 mM dithiothreitol (DTT), and homogenized in a Potter-Elvehjem glass vessel by 10 up-down strokes of a motor-driven teflon pestle. The homogenate was centrifuged at 105,000 xg for 60-90 min in a 65 rotor of a Beckman Model L ultracentrifuge. Light yellow-pink cytosols, containing 7-10 mg protein/ml (Lowry), were concentrated in an Amicon ultrafiltration apparatus with UM-10 membrane, and centrifuged as above to yield clear amber cytosols. Experiments established that the ultrafiltration did not alter the qualitative nature of the profiles. The proteins and carcinogen-protein complexes of the mammary gland cytosols (1.5-2.5 ml; 15-40 mg protein/ml) were immediately resolved according to molecular size by filtration through columns (ca. 200 x 1.2 cm, i.d.) of fresh Sephadex G-200 gel equilibrated in 0.01 M Tris-Cl buffer, pH 7.4, at 1-4° (8). Fractions of 2.5 ml were eluted at 10-12 ml/hr, and radioactivity was counted by β-liquid scintillation in 15 ml of Aquasol 2 (New England Nuclear). Molecular weights at component modes in elution profiles were determined by filtration of standard macromolecules in the same packed column following each elution. The standard macromolecules were Blue Dextran, bovine plasma albumin, bovine γ-globulin, β-lactoglobulin, and cytochrome C (8). A plot of the log molecular weight (Y) against retardation ratio, V/Vo, (X) decreased linearly with a correlation coefficient of 0.995 according to the equation, $Y = -1.40\ X + 6.94$.

The need for metabolic conversion of the carcinogen in the formation of specific complexes *in vivo* was evaluated. The mammary cytosols of normal mice (not administered carcinogen) were prepared in 1:0.75 (w/v) sucrose-DTT solution incubated with 5×10^{-9} M ^{3}H-MCA (0.063-0.125 μCi/ml) for 90 min at 1-4°. The presumed hydrophobic complexes thus generated were resolved by molecular sieving as described above.

RESULTS AND DISCUSSION

Molecular sieving of mouse mammary gland cytosol through tall, narrow columns of Sephadex G-200 gel constitutes a convenient, rapid, and efficient screen that provides information on the multiplicity, relative amounts, and molecular weights of the different species of carcinogen-protein complexes produced *in vivo* and *in vitro*. The method also provides an operational measure of the macromolecular bound carcinogen and its metabolites, separated from those that are free. Recoveries of macromolecules are good: 90% (86%-98%) of the absorbance at 280 nm and 85% (80-100%) of the radioactivity were recovered in the profiles in the *in vivo* experiments. However, the non-macromolecular

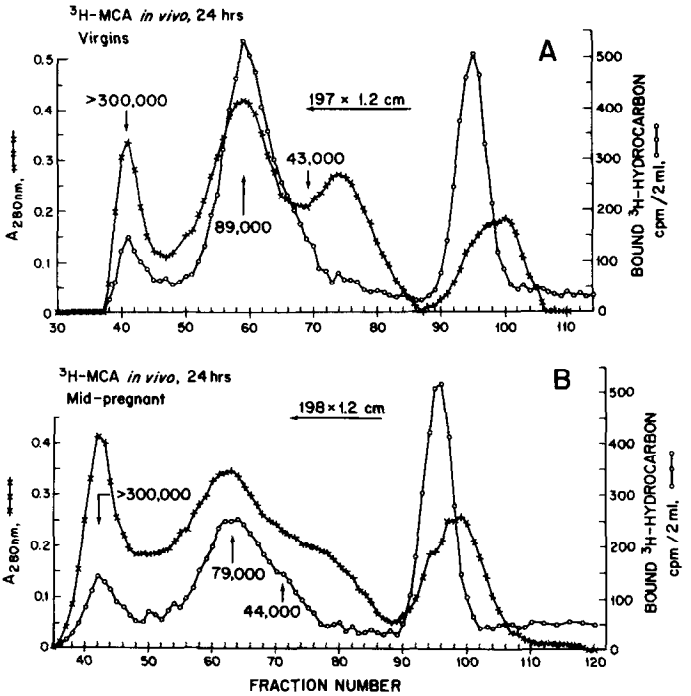

Fig. 1: Molecular size profiles of the cytosolic macromolecules and carcinogen-protein complexes of the mammary glands of mice administered ^{3}H-MCA in vivo. Upper (A): glands of virgin mice. Lower (B): glands of mid-pregnant mice. Column dimensions and direction of elution (arrow) are indicated.

^{3}H-polycyclic aromatic hydrocarbon, present in excess in the incubation experiments, was considerably adsorbed to the gel, lowering recoveries of radioactivity in the in vitro experiments to 35% (28-48%).

The cytosolic macromolecules of the mammary glands of the virgin mice and of the pre-lactating mice were resolved into at least five ranges of molecular size, as evidenced at 280 nm (Fig. 1). The modal molecular weights of the size families of macromolecules at the two stages of development were >300,000, 181,000, 88,000, 44,000 and 27,000. The profiles from the glands at the two stages of development were generally similar, except that the virgins contained significantly more of the 27,000 daltons size class. The last eluting component was non-macromolecular (6000 daltons).

Table 1. Carcinogen-protein complexes in mammary gland cytosols

Molecular weight	Amount, % Virgin	Mid-pregnant
In Vivo	(2)	(4)
>300,000	10	19
83,000 ± 4,000	64	46
47,000 ± 7,000	10	17
In Vitro	(3)	(4)
>300,000	40	53
90,000 ± 3,000	16	15
53,000 ± 3,000	25	19

In the molecular size profiles represented in Figs. 1 and 2, the molecular weights at the modes of the carcinogen-protein complexes were computed as described in Materials and Methods. The indicated standard deviations of the average molecular weights were determined from the aggregate of the number of experiments stated in the parentheses at the two developmental stages of mammary gland. The amounts of complexes are relative to the total radioactivity eluted with the macromolecules. The remaining minor complexes are not tabulated.

The mammary gland cytosols of the mice intraperitoneally injected with ^{3}H-MCA contained at least three species of carcinogen-protein complexes (Fig. 1). The same three kinds of complexes, as judged by their molecular weights, were apparently present in the virgin and mid-pregnant glands, though in somewhat different amounts. The principal complex had a modal molecular weight of 83,000, while those of the two minor species were of >300,000 and 47,000 daltons. Table 1 presents the relative amounts of the complexes, and their molecular weights and standard deviations in six experiments carried out in vivo. Inasmuch as the mammary glands were not perfused, the question arose as to

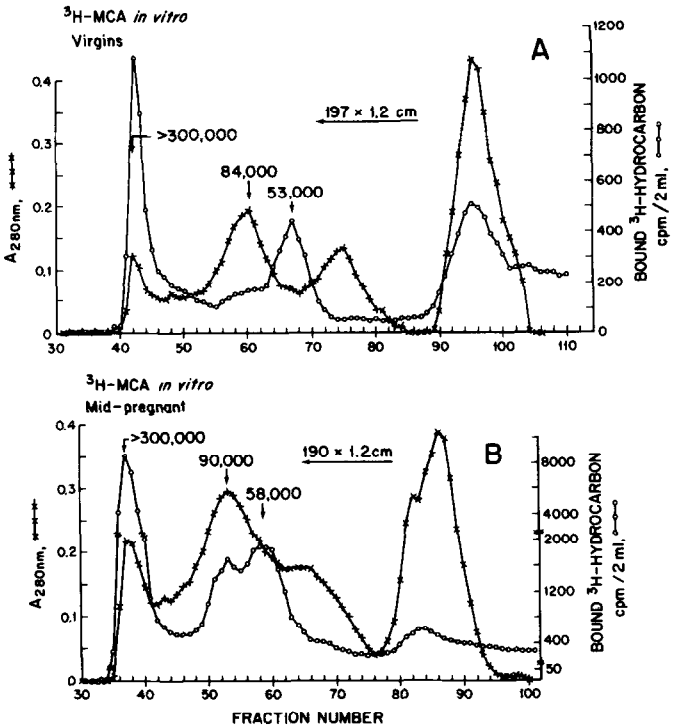

Fig. 2: Molecular size profiles of the carcinogen-protein complexes produced by in vitro incubation of ^{3}H-MCA with cytosols of mouse mammary glands at 1-4°. Upper (A): glands of virgin mice. Lower (B): glands of mid-pregnant mice. Column dimensions and direction of elution (arrow) are indicated.

whether the complexes may actually originate from blood rather than mammary tissue. We will report elsewhere on the complexes in blood cytosol, and the basis of the conclusion that they differ from those reported here.

The carcinogenic polycyclic aromatic hydrocarbon, MCA, is probably metabolized prior to its interaction in vivo with its principal target protein in the cytosol of virgin and pre-lactating mammary glands. This judgement derives from the finding that the principal carcinogen protein complex formed in vivo is of the molecular size (83,000 daltons that is produced in least relative amount in vitro by incubation of

^{3}H-MCA with mammary gland cytosol at 1-4° (Fig. 2 and Table 1). Quantitatively, the amounts of the individual complexes produced *in vitro* were rather different from those arising *in vivo*. Qualitatively, however, the complexes produced *in vitro* and *in vivo* were of analogous molecular sizes. Accordingly, hydrophobic interactions may provide a basis in part for the specificity of the different carcinogen-protein interactions in mammary gland cytosol *in vivo*.

During chemical carcinogenesis in other organs, carcinogens complex with proteins both covalently and non-covalently. Among these are the complexes of ligandin, which belongs to the gluthathione S-transferase (4), and other proteins (5-7). We recently reported evidence in support of a model in which the principal target protein of a liver carcinogen acts as a cytoplasmic receptor protein containing activated carcinogen. The complex of protein and carcinogen electrophile is presumed to protect the reactive carcinogen in an intramolecular hydrophobic environment isolated from cellular nucleophiles, and to deliver the activated carcinogen to cell nuclei for covalent interaction with principal macromolecule(s) (7). The nature and roles of the present reported carcinogen-protein complexes of mammary gland are now to be determined.

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