CORTISOL METABOLITE BINDER I: IDENTITY WITH THE DIMETHYLAMINOAZOBENZENE BINDING PROTEIN OF LIVER CYTOSOL*

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Summary. Of the four macromolecules in liver cytosol that bind anionic metabolites of cortisol, the basic protein, designated "cortisol metabolite binder I", is identical to the protein that binds dimethylaminoazobenzene. This conclusion is based on double label experiments with the ^{14}C-labeled azo dye and tritiated cortisol. Correspondence of the two labels persists through fractionation steps leading to homogeneity: molecular sieve filtration of liver cytosol, column chromatography on DEAE Sephadex A-50, chromatography on cellulose-P columns and gel filtration on Sephadex G-75. In addition, there is convincing agreement in the reported values of molecular weight, sedimentation coefficient, isoelectric pH and amino acid composition. This demonstration provides, for the first time, evidence that carcinogens and corticosteroids have an identical site of action at the molecular level.

Various experiments have suggested an interrelationship in the liver between corticosteroids and certain carcinogenic substances. In particular, the presence of functioning adrenal glands appears to promote hepatic carcinogenesis by various carcinogens (1-5). In accordance with these observations, adrenalectomized rats fed 4-dimethylaminoazobenzene were relatively resistant to liver tumor induction (6,7). A logical explanation for this interdependence seemed to be that the corticosteroid was required to maintain the level of, or to induce, enzymes required to metabolize carcinogens to their active metabolite forms (for example, see review by Conney (8)). However, it has been observed

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that the corticosteroids are themselves fairly poor substrates for microsomal enzymes which metabolize carcinogens, sex hormones or insecticides (9). Within the microsomal compartment, however, a small percentage of intracellular cortisol binds preferentially to the smooth endoplasmic reticulum and is metabolized there (10). Consequently, there exists the possibility that carcinogens and corticosteroids interact elsewhere in the cell. In this communication we provide evidence that a common molecular site of interaction for the carcinogen, p-dimethylaminoazobenzene, and cortisol is the basic cortisol metabolite binding protein I (11) of liver cytosol. The possible relevance of this interaction to the mechanism of hepatic carcinogenesis is discussed.

Results and Discussion. Double label experiments with ^{14}C-p-dimethylaminoazobenzene and ^{3}H-cortisol: ^{14}C-Dimethylaminoazobenzene (specific radioactivity, 4.3 to 13.1 mg/100μC) and 1,2-^{3}H-cortisol (specific radioactivity, 44 C/mmole) were obtained chromatographically pure from the New England Nuclear Corporation. Male adrenalectomized rats (Charles River Breeding Laboratories) weighing 100 to 150 g were injected intraperitoneally 7 days after surgery with 2 x 10^{7} dpm of ^{14}C-dimethylaminoazobenzene in oil and allowed to metabolize with food and water ad libitum for 16 hours (12). At this time 6.6 x 10^{8} dpm ^{3}H-cortisol was injected intraperitoneally and the animals were decapitated 45 minutes later. The livers were perfused in situ and cytosol fractions were prepared by previously reported procedures (11,13-15). There was 1 to 4% of the dose of carcinogen radioactivity accumulated in the liver and about half of this was distributed in the cytosol. There was 15% uptake of cortisol radioactivity in liver with 40% of this in the cytosol. The chromatographic procedures for purifying the basic cortisol metabolite binding protein I to homogeneity (11) were followed and specific information concerning each step is given in the appropriate figure legend.

In Fig. 1 are depicted the results of gel filtration of the cytosol showing the separation of bound and unbound forms of radioactivity from cortisol and dimethylaminoazobenzene. Fifty-five percent of the carcinogen radio-

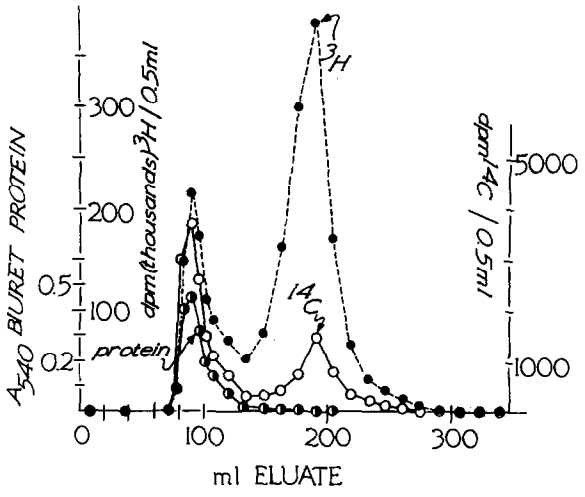

Fig. 1. Gel filtration of liver cytosol on a column (3 x 30 cm) of Sephadex G-25. Eluent was water. 9.5 ml cytosol containing 3.5 x 10^5 dpm ^{14}C and 4.2 x 10^7 dpm ^3H radioactivity was used in this particular experiment. Recovery of ^{14}C radioactivity in eluent was 70% and of ^3H was 100%.

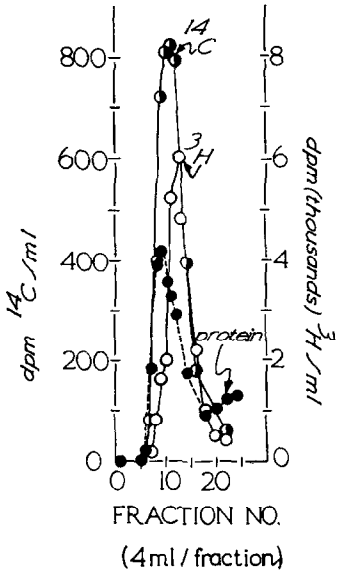

Fig. 2. Column (2 x 45 cm) chromatography of bound radioactivity on DEAE Sephadex A-50. Shown here is the region in which the basic cortisol metabolite binder I (11) elutes. The 6.5 ml sample, in this experiment, contained 1.2 x 10^5 dpm ^{14}C radioactivity and 8.6 x 10^6 dpm ^3H radioactivity. 31% of the ^{14}C eluted in the large binder region and 2.6% of ^3H radioactivity eluted in this region. Considerably more cortisol radioactivity is bound to the small binder III (11) not shown here. Elution was accomplished with 0.05 M Tris-HCl buffer, pH 7.5, with a linear gradient of KCl in 0.05 M Tris-HCl buffer.

activity and nearly 20 percent of cortisol radioactivity were in the bound form. This fraction was pooled, concentrated by ultrafiltration and chromatographed on a column of DEAE Sephadex A-50. Shown in Fig. 2 is the portion of the elution pattern in which the basic cortisol metabolite binding protein appears (11). Elution of protein-bound radioactivity from both labels occurs simultaneously although each form of radioactivity does not superimpose perfectly the other. However, when this fraction is concentrated and subjected to column chromatography on cellulose-P the two forms of radioactivity coincide (Fig. 3). Chromatography on Sephadex G-75, which leads to a homo-

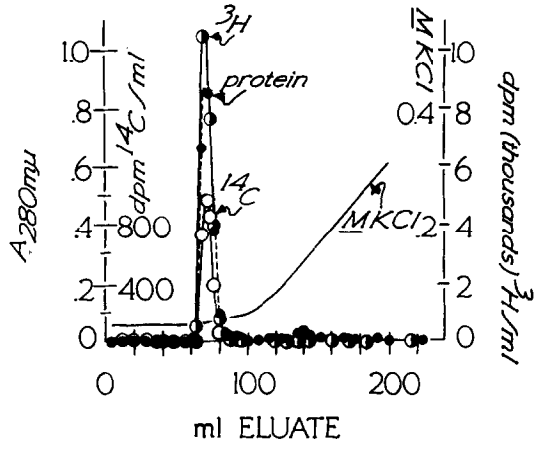

Fig. 3. Chromatography on a cellulose-P column (1.5 x 40 cm) of the protein peak from the cortisol metabolite binder I region of the DEAE Sephadex A-50 chromatogram. A linear gradient was established by mixing 150 ml each of Tris-HCl buffer and buffer with 1 M KCl (11). The sample applied to the column in 4 ml contained 1.5 x 10^4 dpm ^{14}C radioactivity and 1.1 x 10^5 dpm ^3H radioactivity. Percentage recovery was 28% in ^{14}C and 53% in ^3H.

geneous preparation (11), also results in alignment of the two forms of radioactivity (Fig. 4). This simultaneous purification of each form of bound radioactivity indicates that the cortisol metabolite binder I binds the steroid and the carcinogen.

In addition, Ketterer et al. have isolated the protein of liver cytoplasm which binds dimethylaminoazobenzene (12). The physical-chemical properties of this protein (12) are compared with those of the basic cortisol metabolite

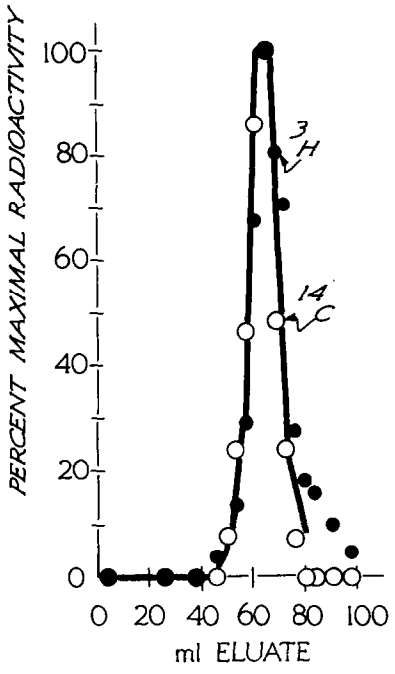

Fig. 4. Further purification of the cortisol metabolite binder I on Sephadex G-75 (11) after chromatography on cellulose-P. The column was 1.5 x 68 cm and elution was by 0.05 M Tris-HCl, pH 7.5.

binding protein in Table 1. It is clear that isoelectric point by two different techniques, sedimentation coefficient and molecular weight from gel filtration agree very closely. Furthermore, the amino acid composition for each protein (Table 2) agrees closely and a statistical comparison gives a rank concordance (16) of +0.9 in a statistical range wherein -1.0 indicates complete rank discordance and +1.0 indicates complete rank concordance. In contrast, is the comparison of the amino acid analysis of the cortisol binder with the "peak A" of liver cytosol which binds radioactivity from N-2-fluorenylacetamide-9-^{14}C (17). When the amino acid analysis for this fraction and the cortisol metabolite binder are equalized on the basis of total basic amino acid residues, the rank concordance statistic is -0.05 suggesting discordance. That these proteins are not identical is further supported by a non-alignment in molecular weight. On the other hand, the cortisol metabolite

Table 1. PHYSICAL-CHEMICAL CHARACTERISTICS OF CORTISOL METABOLITE BINDER I (CMBP I) AND DIMETHYLAMINOAZOBENZENE BINDING PROTEIN (DMABP) OF LIVER CYTOSOL.

PROPERTY	CMBP I*	DMABP+
pI	8.9 (electrofocusing)	8.4 (free-boundary electrophoresis in barbital-NaCl buffer)
$S_{20,w}$	3.47	3.5
Molecular weight by gel filtration	50,000 ± 6,000	45,000

*Data from Morey and Litwack (11).

+Data from Ketterer et al. (12).

binder and the fraction binding 3-methylcholanthrene (18) may be identical (19). All of these comparisons show clearly the identity of the basic cortisol metabolite binding protein I and the dimethylaminoazobenzene binding protein of liver cytosol.

Similarities of the cortisol metabolite binder I with other binding fractions may exist, although the organic anion-binding protein of rat liver (20) and a cortisol binding fraction of liver cytosol having an S value of 4 (21) have not been purified sufficiently to enable comparison of physical-chemical characteristics or amino acid analyses. If the latter fraction is identical to the basic cortisol metabolite binding protein I, then a role for this binder as an intracellular transporting protein shuttling between the cytosol and the nucleus can be hypothesized. This idea will be tested with the purified binder and isolated, surviving liver nuclei. Further speculation would require the bound steroid for this transport, thus the presence of the bound steroid would be needed to transport the carcinogen

Table 2. AMINO ACID COMPOSITION OF CMBP I AND DMAB PROTEINS

AMINO ACID	CMBP I*	DMABP+
Asp	28	34
Thr	12	11
Ser	12	13
Pro	16	16
Glu	36	39
Gly	20	18
Ala	24	26
Val	20	17
Meth	12	14
Ileu	20	18
Leu	44	46
Tyr	12	12
Phe	16	17
Lys	28	33
His	4	5
Arg	20	21
Try	2	ND
Cys	Trace	2

*Data from Morey and Litwack (11).

+Data from Ketterer et al (12). Rank correlation (16) : $r_s = 1 - \left[\frac{6\ Sd}{n(n^2-1)}\right] = +0.9$ The numbers for each amino acid represent the number of residues from each protein per 43,000 molecular weight. ND= not determined.

to the nucleus. In the absence of steroid (adrenalectomy) the carcinogen could still bind to the binder but might not be transported to the nucleus.

This postulate would explain the observed interdependence of carcinogenesis in liver and the presence of functional adrenal glands and will be tested soon.

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