acids with ratios between 0.1 and 40. The rate of increase in absorbance at 290 nm is directly proportional to phenylalanine concentrations at the molar ratios studied (Fig. 1A). Solutions with high ratios of phenylalanine to tyrosine (10 to 40), however, do not exhibit a linear relationship in the rate of increase in absorbance at 315 nm with tyrosine concentrations from 2 to 5 μM (Fig. 1B). Thus, plasma or serum tyrosine was determined at approximately 1 μM levels.

Table 1 demonstrates that analyses by our method and by the amino acid analyzer are comparable. Both phenylalanine and tyrosine values were slightly higher when measured by the phenylalanine ammonia-lyase assay, but they were not statistically significant within 20 donors (*11*). Phenylalanine ammonia-lyase has no catalytic activity on any of the common amino acids except phenylalanine and tyrosine, nor on the D-isomers or structurally related derivatives (*12*). Table 2 shows a comparative study of six patients with phenylketonuria. Plasma phenylalanine levels are comparable by all three methods, but values for tyrosine were considerably higher by the spectrofluorometric procedure for reasons unknown.

The results of this study demonstrate that phenylalanine ammonia-lyase can be used for the quantitative determination of plasma or serum phenylalanine and tyrosine. Although this method can be easily applied for screening of phenylketonuria, its use in the management of this disease is appropriate because it is rapid, sensitive, and requires only a small portion of blood. Patient No. 6, originally undetected by the Guthrie test, was diagnosed immediately by our enzyme method, and plasma concentrations of these two amino acids were monitored subsequently until the patient exhibited a stable pattern of plasma phenylalanine and tyrosine through dietary manipulation.

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7. The ratio of absorbance of *trans*-coumaric acid at 290 nm/315 nm is 1.48. Therefore, phenylalanine is determined by measuring the rate of increase of absorbance of *trans*-cinnamic acid at 290 nm according to the following formula: $\Delta A_{290} - (1.48)\Delta A_{315}$.
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Hepatic Binding Protein: The Protective Role of Its Sialic Acid Residues

Abstract. *Removal of sialic acid from a specific hepatic binding protein virtually abolishes its capacity to bind certain asialoglycoproteins. The loss of this capacity is the result of competition for the binding sites by galactosyl residues, of hepatic binding protein, that become terminal after desialylation.*

Removal of the terminal sialic acid residues of the carbohydrate chains of many mammalian serum glycoproteins exposes galactosyl residues as terminal. Intravenous injection of such a desialylated protein seems to be followed by a sequence of reactions that rapidly transfers the protein from plasma to hepatocytes. There appears to be a transient binding of the asialoglycoprotein to a component of the hepatic plasma membrane that is itself a glycoprotein with several terminal sialyl residues (*1*). A complex of a plasma asialoglycoprotein and the membrane glycoprotein can be formed in vitro, as well, but only if the terminal sialyl residues of the latter are not removed (*2*). Our study was directed toward understanding the functional significance of sialyl residues of the hepatic plasma membrane glycoprotein.

The relative affinities of the hepatic binding protein (HBP) for a number of sugars and glycoproteins were estimated from measurements of the capacity of each substance to inhibit the binding of [^{125}I]asialoorosomucoid (Table 1). The assumption is made that the lower the concentration required for 50 percent inhibition, the more tightly the inhibitor is bound to HBP.

The inhibition of binding by HBP of

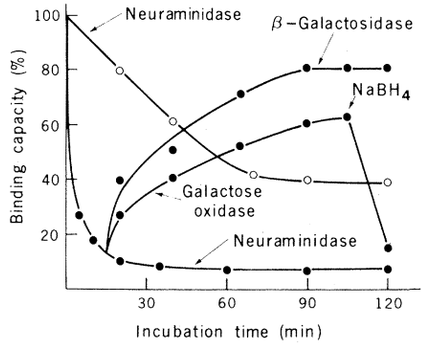

Fig. 1. Effect of enzymatic treatment on the binding capacity of HBP. Hepatic binding protein (2 mg/ml) and neuraminidase (*Clostridium perfringens*; Sigma) (100 milliunit/ml) were incubated at 22°C in 0.05*M* Na acetate buffer (*p*H 6.2) and 0.15*M* NaCl. After 15 minutes of incubation, two portions of the mixture were removed and either β-galactosidase (*Diplococcus pneumoniae*; provided by G. Ashwell) or galactose oxidase (*Polyporus circinatus*; Sigma) was added to a final concentration, respectively, of 100 milliunit/ml or 40 unit/ml. After 90 minutes of incubation with galactose oxidase, 5 μg of $NaBH_4$ were added. The samples of 5 μl that were taken for the determination of the binding capacity of HBP for [^{125}I]asialoorosomucoid (•) and [^{125}I]DOSM (○) were each diluted 40-fold with buffer, as a consequence of which there was no necessity for removal of enzymes present. Binding of [^{125}I]DOSM to HBP, after incubation for 10 minutes as described for asialoorosomucoid (Table 1), was estimated by precipitation of the complex with an equal volume of a saturated solution of ammonium sulfate, adjusted to *p*H 7.8, and filtration on a Whatman GF/A glass disk to remove unbound [^{125}I]DOSM. Desialylated ovine submaxillary mucin was labeled by conjugating it with ^{125}I containing *N*-hydroxysuccinimide ester of 3-(4-hydroxphenyl)propionic acid, by the method of Bolton and Hunter (*8*). The unreacted ester was removed by exhaustive dialysis against water. The labeled protein had specific activities ranging from 0.1 to 0.2 μc per microgram.

Table 1. Inhibitory effect of various carbohydrates and glycoproteins on binding of [^{125}I]asialoorosomucoid to the hepatic binding protein. The inhibitor was added to 0.6 μg of [^{125}I]asialoorosomucoid (0.075×10^{-3} mM) in an assay buffer (tris-Cl, 0.05M, pH 7.8; $CaCl_2$, 0.01 M; NaCl, 0.1M; Triton X-100, 0.5 percent; and bovine serum albumin, 0.1 percent), prior to the addition of 5 to 8 μg of HBP, prepared as previously described (*3*), making the total volume 0.2 ml. After incubation for 10 minutes at 25°C, 0.3 ml of a 0.6 percent solution of γ-globulin, and 0.5 ml of 20 percent (weight to volume) polyethylene glycol (PEG-6000; Fisher) in tris-Cl (0.1M), $CaCl_2$ (0.01M), and NaCl (0.1M), at pH 7.8, were added. The suspension was filtered on a Whatman GF/A glass disk under reduced pressure and washed with an 8.0 percent solution of PEG in the assay buffer without Triton X-100. Radioactivity of the disks was measured; appropriate blanks deposited about 0.5 percent of the total radioactivity on the disks. Desialylated orosomucoid, fetuin, and ceruloplasmin were prepared as in (*4*); and DOSM as in (*5*). Asialoorosomucoid, labeled with 2 mc of carrier-free Na^{125}I (Amersham/Searle) by a modification of the method of Greenwood *et al.* (*6*), possessed specific activities of 0.5 to 0.8 μc/μg in different preparations. Total protein was determined by the method of Lowry *et al.* (*7*). At a concentration of 250 mM none of the following monosaccharides showed any inhibition of [^{125}I]asialoorosomucoid binding to HBP: D-glucose, D-mannose, L-fucose, galactosamine, or N-acetylglucosamine.

Inhibitors	Concentration required for 50 percent inhibition (mM)
Carbohydrates	
D-Galactose	27.5
Stachyose	17.5
p-Nitrophenyl-α-galactopyranoside	9.0
p-Nitrophenyl-β-galactopyranoside	9.0
Thiodigalactoside	9.0
N-Acetylgalactosamine	2.0
Desialylated glycoproteins	
Fetuin (bovine)	1.30×10^{-3}
Ceruloplasmin (human)	0.95×10^{-3}
Orosomucoid (human)	0.075×10^{-3}
Submaxillary mucin (ovine)	0.003×10^{-3}

asialoglycoproteins varies with the specific carbohydrate and, for any one carbohydrate, is greatly enhanced when the sugar is part of a glycoprotein. Thus, N-acetylgalactosamine has the greatest affinity for HBP of any carbohydrate tested, while desialylated ovine submaxillary mucin (DOSM), a glycoprotein whose carbohydrate moiety consists almost solely of terminal N-acetylgalactosaminyl residues, is bound to HBP orders of magnitude more tightly than is N-acetylgalactosamine.

The progressive removal of sialyl residues from HBP initially has a strikingly different effect on the capacity of this protein to bind asialoorosomucoid and DOSM. After 20 minutes of incubation with neuraminidase the binding capacity of HBP for asialoorosomucoid is almost completely abolished, while that for DOSM is reduced by only 20 percent (Fig. 1). This suggests that the binding sites, which are the same for DOSM and asialoorosomucoid, are not the sialyl residues of HBP. Confirmation of this conclusion is obtained if the terminal galactosyl residues of desialylated HBP are either oxidized to aldehydes or removed, after which the affinity of the protein for asialoorosomucoid is restored significantly. In the former instance, furthermore, this restored binding capacity can again be abolished if the terminal galactosyl aldehyde groups are reduced by borohydride. The mechanism by which the binding capacity of HBP, following desialylation, is thus diminished appears to be analogous to that which operates when any of the inhibitors with terminal galactosyl residues is present in the reaction mixture (Table 1).

The inhibitory effect of desialylated HBP on its own binding capacity is observed whether the protein is in solution or is a part of the plasma membrane (*3*). This implies either a sufficiently close juxtaposition of galactosyl residues and binding sites on HBP to permit intramolecular competition or a sufficient mobility of HBP molecules within the membrane to permit the binding of the galactosyl residues of one desialylated HBP molecule to the binding sites of another.

Thus it seems reasonable to speculate that terminal sialic acid residues may play two different protective roles in glycoprotein metabolism. (i) In circulating glycoproteins they mask the galactosyl residues that are the groups by which the protein is bound to HBP and thereby permit continued intravascular survival of these proteins (*1*). (ii) In the membrane glycoprotein HBP, sialic acid also masks galactosyl residues, thereby preventing them from competing for the binding sites of this receptor protein.

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Desulfurization of Coal by Use of Chemical Comminution

Abstract. *Chemical fracturing (comminution) of coal provides selective breakage, which may be used to liberate inorganic sulfur from it without resorting to excessive mechanical size reduction. The technique can be used for economic precombustion desulfurization and may have many other applications in coal utilization.*

One of the major impediments to increased use of the plentiful supply of eastern coal available in the United States is its high sulfur content. Sulfur occurs in coal in two significant forms—inorganic (pyrite and sulfates) and organic. The organic sulfur is chemically bound to the coal, and the majority of it can be removed only during or after combustion, during chemical conversion processes such as liquefaction or gasification, or by some chemical extraction processes with drastic reaction conditions (*1*). Sulfates are usually of only minor significance except in lignites, while pyrite comprises, on the average, half or more of the total sulfur and can account for most of the total sulfur in some major eastern U.S. coal reserves (*2*). Pyrite occurs in many forms and sizes in coal, and much of it can be removed by physical cleaning methods (*3*) and chemical leaching processes (*4*). Of the processes mentioned above, physical coal preparation (although it can remove only pyrite) is the least expensive and most highly developed commercially (*5*).

Conventional physical coal preparation consists of mechanical size reduction, which results in the liberation of the pyrite sulfur and other mineral matter, followed by a separation step, the cost of which is dependent on the size consist of the crushed coal. In general, as the size