

Table 1. Inhibitory effect of various carbohydrates and glycoproteins on binding of [¹²⁵I]asialoorosomucoid to the hepatic binding protein. The inhibitor was added to 0.6 μg of [¹²⁵I]asialoorosomucoid (0.075 × 10⁻³ mM) in an assay buffer (tris-Cl, 0.05M, pH 7.8; CaCl₂, 0.01M; 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), CaCl₂ (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 Na¹²⁵I (Amersham/Searle) by a modification of the method of Greenwood *et al.* (6), possessed specific activities of 0.5 to 0.8 μCi/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 [¹²⁵I]asialoorosomucoid binding to HBP: D-glucose, D-mannose, L-fucose, galactosamine, or N-acetylglucosamine.

Inhibitors	Concentration required for 50 percent inhibition (mM)
<i>Carbohydrates</i>	
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
<i>Desialylated glycoproteins</i>	
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

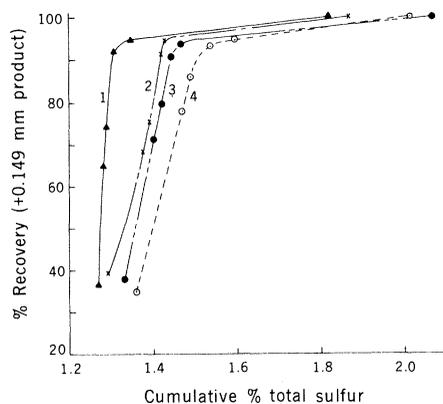


Fig. 1. Washability curve comparing sulfur value and recovery of various methods of size reduction. Curves are described in Table 1.

consist decreases, the amount of liberation of mineral matter and the cost of separation increase.

Chemical comminution (fracture) provides a unique way of crushing coal for significant liberation of mineral matter without excessive size reduction. Coupled with conventional physical separation techniques, which rely on a difference in density or surface properties between the coal and the mineral matter, it has considerable potential as a pre-combustion coal desulfurizing method. Chemical comminution consists of the treatment of coal with a chemical (usually ammonia gas or a concentrated aqueous ammonia solution). Selective breakage of the coal ensues which appears to take place along the maceral [coal lithotype (6)] boundaries and other deposits within the material such as pyrite bands. Although the mechanism of the phenomenon is not fully understood, the rate of breakage is increased by evacuation of the reaction vessel before chemical treatment and by treatment at elevated pressure. Also, as the coal rank increases the chemical fragmentation decreases, perhaps due to differences in micro- or macroporosity, maceral content, cleat system, swelling ability, mineral matter distribution, or other factors. Chemical reactions between the ammonia and the coal do not appear to be significant. Nitrogen analysis before and after chemical treatment indicates an increase of approximately 0.2 percent in overall nitrogen content in one lower-rank seam coal, with many coals having no detectable increase. These results suggest that ammonia used to comminute coal can be easily recovered after treatment. The mechanism should be better understood after further studies of the fracturing process under different reaction conditions and with different types of coal.

Comparisons of mechanically crushed and chemically comminuted coal sam-

ples indicate that, at a particular size distribution, more liberation of pyrite sulfur and comparable liberation of ash is possible with chemical comminution. Table 1 and Fig. 1 provide a typical comparison of mechanical and chemical breakage and sulfur liberation for an Illinois No. 6 seam coal. Similar results have been found with Redstone, Pittsburgh, and Upper Freeport seam coals (7, 8) and some Iowa coals (9).

To obtain representative samples, 45.3-kg lots of coal were used for the comparisons. Each curve in Fig. 1 was confirmed by analyzing a duplicate 45.3-kg sample. After the indicated size reduction, a representative sample (45.3 kg for curves 1 and 4, 13.6 kg for curve 3, and 3.2 kg for curve 2) was analyzed by float-sink analysis at various specific gravities (3). Each point in Fig. 1 represents the sulfur analysis and recovery of the product in the size class > 0.149 mm ($+ 0.149$ mm) at a particular density (specific gravity). To consider recovery based on feed rather than product, the amounts of product in the size class < 0.149 mm ($- 0.149$ mm) noted in Table 1 must be considered. For example, curve 1 represents 96 percent of the feed, while curve 2 represents only 78 percent.

Figure 1 shows that the chemically comminuted sample (curve 1), although it has a larger size consist than the two mechanically crushed samples (curves 2 and 3), has less sulfur at a particular recovery or more recovery at a particular sulfur value than the mechanically crushed samples. Ash curves (not illustrated) have also been developed for the Illinois No. 6 seam coal samples, and these curves indicate that, unlike sulfur liberation, ash liberation is correlated directly with the size consist (order of increasing ash liberation = run of mine chemical comminution $<$ mechanically crushed to 9.51-mm top size $<$ mechanically crushed to 1.41-mm top size).

The results cited above show that selective breakage by chemical comminution can be used to desulfurize coal without excessive size reduction and without creating large amounts of fines. A preliminary assessment of the economics indicates that the cost of a flow sheet including chemical comminution would be less than that of a conventional deep coal cleaning system, the savings resulting from higher yields of clean coal and a simpler cleaning system.

With many eastern coals, chemical comminution can provide a product that will be environmentally acceptable for direct combustion. For any particular

Table 1. Comparison of the size distribution of Illinois No. 6 seam coal crushed by mechanical and chemical methods. The curves are shown in Fig. 1: curve 1, chemically comminuted; curve 2, mechanically ground to top size of 1.41 mm; curve 3, mechanically ground to top size of 9.51 mm; and curve 4, run of mine. The run of mine sample had a top size of approximately 38.1 mm. Chemically comminuted coal was treated with gaseous ammonia at 25°C and 9 atm for 3 hours.

Size (mm)	Coal (cumulative percent by weight)			
	Curve 1	Curve 2	Curve 3	Curve 4
9.51	96		100	37
6.35	91		90	26
4.00	78		82	19
2.38	59		72	14
2.00	53		67	13
1.41	39	100	55	10
0.841	25	83	38	7
0.595	19	70	30	6
0.297	9	40	16	4
0.149	4	22	9	2

coal this will depend on the caloric value and the proportions of pyritic and organic sulfur. Other applications of chemical comminution include coal preparation for gasification, liquefaction, coking, and blending schemes; coal size reductions that require low amounts of fines; and use in mine processes such as degassing or in situ gasification (10).

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