even seem to be any qualitative relationship of this kind. Maximum activities for *N,N*-dimethyl-*p*-phenylenediamine and the corresponding radical cation Würster's Red have been shown to be of closely similar magnitude,[5] in spite of the fact that the ring π-electron density of Würster's Red is lower than that of the unsubstituted benzene ring, and thus lower than that of all of the non-substrates listed in Table 1.

The fact that Würster's Red, despite its low π-electron density, functions as a substrate for ceruloplasmin calls for an explanation. Examination of Table 1, which also gives energies of the highest occupied molecular orbital (EHOMO) of the respective compounds, shows that all of the substrates for ceruloplasmin, including Würster's Red, are characterized by exceptionally high EHOMO levels ($0.1-0.6\ \beta$), whereas non-substrates such as quinol, aniline, and 3,4-dihydroxyphenylalanine have more normal EHOMO values ($0.6-1.0\ \beta$). This observation suggests that a low ionization potential of the substrate is essential for the enzymatic reaction, and gives some support to the view that substrate binding takes place through the formation of a charge-transfer complex.

Further experiments are in progress in order to investigate whether the above characteristic property of the substrates reflects a quantitative relationship between EHOMO values and reaction rate parameters such as V and K_m. The experiments of Levine and Peisach were carried out before the influence of iron ions on the enzymatic reaction velocity was recognized,[6] and their kinetic data are not sufficiently reliable for such an analysis.

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Group-specific Adsorption of Glycoproteins

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Some of the plant proteins called phytohemagglutinins or lectins are known to form complexes with carbohydrates and glycoproteins.[1-4] Although such protein-carbohydrate interactions are usually revealed by studying mixtures in solution, these proteins can also interact with insoluble granular carbohydrates and as consequence be more or less strongly adsorbed.[5-7] The proteins can then be displaced from the adsorbent by an eluent containing competing soluble carbohydrate in appropriate concentration. Complementary adsorbents for carbohydrates can be formed by chemical fixation of such proteins to inert permeable supports and the adsorption and desorption of carbohydrates should be governed by the same principle.

Concanavalin A was chosen for the production of a specific carbohydrate adsorbent * since it is easily available in large quantities and shows affinity for certain carbohydrates.[8,9] Agarose was found to be a satisfactory matrix material for the fixation of concanavalin A.

Detailed studies of the adsorption properties of concanavalin A-agarose will be reported elsewhere but we present here the results of some orientation work done with human serum. Serum, containing a considerable number of glycoproteins of varying carbohydrate content was chosen as a model system to explore the feasibility of sorting out glycoproteins from a complex mixture. Some serum glycoproteins are reported to interact with concanavalin A.[10,33,11]

The adsorbent was prepared by coupling concanavalin A purified by the Sephadex adsorption method [5] to beads of cyanogen-bromide activated 2 % agarose gel.[12,13] The agarose beads were the commercial product Sepharose 2 B from Pharmacia Fine Chemicals, Uppsala, Sweden. Con-

* Patent pending.

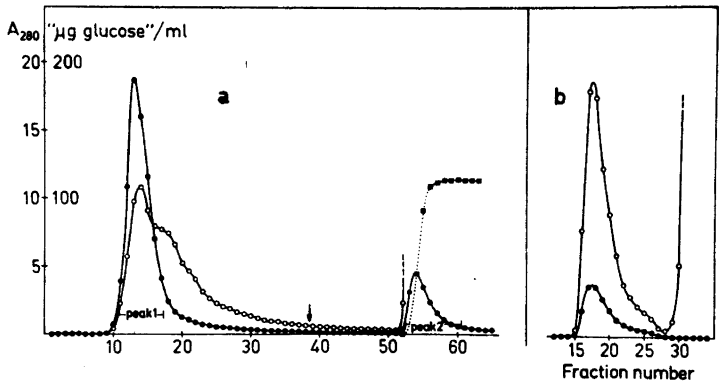

Fig. 1. a. Chromatography of human serum on concanavalin A-agarose. ● A_{280}, ○ neutral sugars as glucose equivalents, ■ neutral sugars, scale compressed 200 times to show the front of mannoside. *b.* Removal of the mannoside from the proteins of peak 2 of Fig. 1 a by gel filtration on a 3×16 cm column of Sephadex G-25 equilibrated with the phosphate-NaCl buffer used with the adsorbent column. Aliquots of 3 ml from fractions 53—63 were introduced into the bed in series, the last followed by buffer. Fraction volume: 3 ml. Scales and notations as in Fig. 1 a.

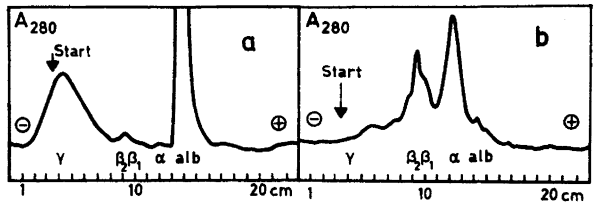

Fig. 2. Zone electrophoretic analysis of the material from peaks 1 and 2 of Fig. 1 a. To the left: Peak 1. To the right: Peak 2 after concentration to about 1 % total protein. The electrophoresis was performed in 0.1 M Tris-HCl buffer of pH 8.6. (Tube length: 36 cm, diameter: 0.3 cm, current: 4 mA, voltage: 1160 V, temp: 20°C, sample volume: 4 µl, time: 40 min). Positions of the various main components in the serum run under identical conditions are indicated in the figures.

canavalin A seems to be rather unstable in solution at pH 8, where the coupling yield is satisfactory. Therefore, the concanavalin A solution (1.4 g in about 100 ml) was adjusted up to this pH immediately before mixing with the activated agarose (100 ml when sedimented, activated with 2 g BrCN). The pH of the reaction mixture was kept at 8.0 for 2 h by addition of acid. The gel was then drained on a filter and unbound protein was washed out with 1 M $NaHCO_3$ of pH 8.0. Residual reactive groups on the agarose were hydrolyzed by washing with an acetate buffer of pH 5.

Ten milliliters of dialyzed serum was introduced at a speed of 20 ml/h into a bed (26×2 cm) of concanavalin A-agarose equilibrated with 0.02 M phosphate buffer pH 7.0, containing 1.0 M sodium chloride. The effluent was collected in 5 ml fractions. After washing with 180 ml of the buffer, displacement of glycoprotein was effected with the same buffer containing 0.1 M methyl α-D-mannopyranoside. The absorbance at 280 nm was measured and the distribution of neutral sugars was determined by the phenol-sulfuric acid method.[15] Two well-separated peaks were obtained (Fig. 1a).

The column could be regenerated simply by washing with the starting buffer. We have observed that the displacement could

not be effected by galactose, a sugar known not to inhibit the concanavalin A-carbohydrate interaction.[9]

Electrophoretic analysis in free solution by the revolving tube method of Hjertén [15] revealed that the non-adsorbed Peak 1 contained essentially albumin and γ-globulin and only very small amounts of components of intermediate mobilities (Fig. 2a). The adsorbed material of Peak 2 contained α- and β-globulins together with prealbumin and some fast γ-globulin which according to the behaviour in the ultracentrifuge appears to be IgM (Fig. 2 b). A β-component ($S_{20}=4.4$), probably hemopexin, was found at the rear of Peak 1 which showed a higher carbohydrate content than the leading protein peak. No lipoproteins were found in Peaks 1 or 2.

Relative to protein, Peak 2 contained about 8 times as much carbohydrate as Peak 1. Apparently, concanavalin A-agarose acts as a group specific adsorbent for the serum components of high carbohydrate content. Concanavalin A-agarose and similar adsorbents might therefore be useful for the fractionation of serum proteins.

The fractionation can be improved in several ways. For example: (1) by using concentration gradients of a specific desorbent or even an unspecific desorbent such as hydrogen ions, (2) by the use of selective desorbents specific for different carbohydrate groups, and (3) by using in a sequence adsorbents prepared from different phytohemagglutinins having specific affinities for different carbohydrates.

Biospecific adsorption might thus be generally applicable in the chemistry of carbohydrates and glycoproteins.

After the preparation of this manuscript a paper has appeared [16] in which the synthesis of concanavalin A-agarose is described. The utility of the adsorbent for the fractionation of carbohydrate polymers and hog gastric blood group substance is also illustrated.

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Synthesis of 2,6-Dimethylphenylcarbamoylmethyl Carbamate and a *C*-Methyl Derivative

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Because of their potential pharmacological interest 2,6-dimethylphenylcarbamoylmethyl carbamate (1a) and α-(2,6-dimethylphenylcarbamoyl)ethyl carbamate (1b) have been prepared. The synthesis followed conventional routes. α-Chloro-2,6-dimethylacetanilide (2a) or the corresponding bromopropionanilide derivative (2b) were transformed into the α-hydroxy compounds (4a and b) *via* the acetates (3a and

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