and electrospray ionization mass spectrometry (2). Similar abnormal species were observed for not only transferrin but also other serum glycoproteins including α_1-antitrypsin, orosomucoid, α_2-HS-glycoprotein, antithrombin III, plasminogen, thyroxine-binding globulin, fibrinogen, and lutropin (3). These results indicate that CDG syndrome is a defect in glycoprotein biosynthesis caused by impairment of *N*-linked olicosaccharide transfer. We are now investigating the primary deficient enzyme causing CDG syndrome, and the relationship between pathological characteristics and biological function of these carbohydrate deficient glycoproteins.

(1) J. Jaeken *et al.* (1984) *Clin. Chim. Acta,* **144**, 245–247.
(2) K. Yamashita *et al.* (1993) *J. Biol. Chem.* in press.
(3) I. Yuasa *et al.* (1993) *Clin. Chim. Acta* in press.

S7.7

The Role of Carbohydrate on the Biological Activity of Erythropoietin

J. C. Egrie, J. R. Grant, D. K. Gillies, K. H. Aoki and T. W. Strickland
Amgen, Inc., Amgen Center, Thousand Oaks, CA. 91320-1789, USA.

Erythropoietin is a sialylglycoprotein which is responsible for stimulating red blood cell synthesis in mammals. The protein contains three *N*-linked and one *O*-linked oligosaccharide chains. When the recombinant protein is expressed in Chinese Hamster Ovary (CHO) cells, molecules containing from 2–14 sialic acid residues are produced. We have fractionated the CHO-produced recombinant erythropoietin based on charge, which has resulted in the isolation of forms containing between 4–14 sialic acid residues (Isoforms 4–14). When assayed in the exhypoxic polycythemic mouse *in vivo* bioassay, the isolated isoforms were found to vary in their activity over an approximately 20-fold range, with isoforms containing lower numbers of sialic acid residues exhibiting the lower activity. These results were confirmed in longer-term assays designed to measure the effect of the isolated isoforms on the hematocrit of mice. In order to determine if these differences in bioactivity are due to differences in receptor affinity or serum clearance, the isoforms were assayed in a radio-receptor assay using OCIM-1 cells and their pharmacokinetic parameters were determined. The isoforms having fewer sialic acids have greater affinity for the receptor, but lower serum half-life. In contrast, the isoforms having higher sialic acid content have a lower affinity for the receptor and a longer serum half-life. These results indicate that the carbohydrate moieties of erythropoietin have significant effects on the bioactivity of erythropoietin and that the serum clearance is the primary determinant of *in vivo* bioactivity.

S7.8

On the Oligosaccharide Moiety of Angiotensin I-Converting Enzyme

B. Baudin, C. Coudert, A. Tahraoui, B. Bénéteau-Burnat and J. Giboudeau
Biochimie A, Hôpital Saint-Antoine, 75571 Paris Cedex 12 and Chimie Biologique, UER Pharmacie — Paris V, 75006 Paris, France.

Angiotensin I-converting enzyme (ACE, EC 3.4.15.1) is a glycoprotein located on vascular endothelial cells as an ectoenzyme controlling both systemic and tissular renin-angiotensin systems. We studied ACE oligosaccharide moiety searching its involvement in physico-chemical and catalytic properties of the enzyme. ACE was purified to electrophoretic homogeneity from pig and rat lungs and from porcine serum as previously described (1). These enzymes contained 8.6, 8.8 and 11.9% of sugars, respectively, with molar ratios indicating a mixture of oligomannosidyl and *N*-acetyl-lactosaminyl chains for all the forms which are only *N*-glycosylated, but sialylated and fucosylated. The plasma isoform was essentially more sialylated and had a lower pI (4.3–4.6) than tissular ACE (2). Further, pig lung ACE was enzymatically deglycosylated; endoglycodisase F2 was more effective than *N*-glycanase, the former modifying ACE Mr from 175 to 154 KDa on SDS-PAGE, also increasing pI from 4.5–4.7 to 5.2–5.4 but still with 3-4 bands on IEF; deglycosylation did not modify Stokes radius, as measured on size-exclusion HPLC, nor enzymic activity. Neuraminidase treatment did not diminish electrophoretic mobility nor enzyme activity but significantly increased pI as did complete deglycosylation, which shows that sialic acid is partially responsible for acidic ACE pI and that glycans are not the support of IEF microheterogeneities. Lung ACE glycosylation was further studied on agarose-bound lectins. Native enzyme completely bound to Concanavalin A and elution was partial in 0.2 M α-methylmannoside, whereas binding on Wheat Germ Agglutinin was incomplete and elution effective in *N*-acetylglucosamine; deglycosylated ACE did not bind onto the lectins.

These data indicate that oligosaccharide moiety of pulmonary ACE is not involved in its activity or overall shape but explains acidic properties; oligomannosidyl glycans seem to be preponderant, but the membrane-bound form isolated from lung could be contaminated by intraendothelial forms maybe richer in sialylated *N*-acetyl-lacticosaminyl glycans for active secretion in the blood stream and protection against hepatic lectins.

(1) B. Baudin, A. Tahraoui, F. C. Baumann, D. Robic, L. Drouet and Y. Legrand. *Protein Expr. Purif.*, **2**, 412–419 (1991).
(2) A. Tahraoui, B. Baudin, D. Robic, B. Fournet and J. Giboudeau (in preparation).

S7.9

Glycosylated Analogues of Vasopressin-Synthesis, Conformational Studies and Biological Investigations

*J. Kihlberg**[a], J. Åhman[a], B. Walse[b], A. Nilsson[c], H. Olsson[c] and C. Söderberg-Ahlm[c]
Organic Chemistry 2[a] and Physical Chemistry 2[b], Chemical Center, The Lund Institute of Technology, P. O. Box 124, S-221 00 Lund, Sweden; Research Department[c], FERRING Pharmaceuticals, Box 30561, S-200 62 Malmö, Sweden.