glycoproteins, from an O non-secretor, probed with the antibody 1G7 (hybridoma culture supernatant diluted 1:50) in the presence of a range of inhibitors

Lane 1, control jejunal extract; lane 2, A; lane 3, precursor; lane 4, HLe(a-b-); lane 5, Le\(^e\); lane 6, lacto-N-tetraose; lane 7, lacto-N-fucopentaose I; lane 8, lacto-N-fucopentaose II; lane 9, lacto-N-difucohexaose I; lane 10, lacto-N-difucohexaose II; lane 11, lacto-difucooctaose; lane 12, lacto-N-neotetraose; lane 13, 2-fucosyl-lactose; lane 14, 3-fucosyl-lactose. Lanes 2-5 contain ovarian cyst blood group substances. Four other Le\(^e\) substances and one of two HLe\(^e\) substances also inhibited 1G7 binding. One other A substance, one other precursor and one B substance showed little or no inhibition. Titration experiments indicated that the Le\(^e\) substances were the most potent inhibitors.

FIONA GREEN,* PAMELA GREENWELL,* BEATRICE GRIFFITHS,* LAWRENCE DICKSON* and DALLAS SWALLOW*

*M.R.C. Human Biochemical Genetics Unit, The Galton Laboratory, University College London, Wolfson House, 4, Stephenson Way, London NW1 2HE, U.K., and *Division of Immunoochemical Genetics, M.R.C. Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ, U.K.

Various lines of evidence have suggested that ABH determinants are present on the glycoprotein hydrolases of the small intestinal brush border in man (Kelly & Alpers, 1973; Hermon-Taylor et al., 1977; Komoda & Sakagishi, 1978; Triadou et al., 1983). Triadou et al. (1983) used SDS/PAGE to separate human ileal brush-border membrane components before immunoblot analysis using ABO blood-group specific antisera and lectins. We have used detergent extracts of (post-mortem) jejunal tissue in a similar way, but we have extended these experiments by using monoclonal antibodies to isolate individual jejunal brush border hydrolases before further analysis.

The ABO blood groups of the individuals were determined by spleen red cell agglutination. Since there is good evidence that the amount of A, B and H antigens in stomach mucus is correlated with the ABH secretor status (Hartmann, 1941), this and the Lewis Le\(^e\) and Le\(^e\) groups were deduced by agglutination inhibition tests with extracts of stomach mucosa. The levels of the blood group glycosyltransferases, namely z-3-N-acetylgalactosaminyltransferase (A), z-3-galactosyltransferase (B), z-4-fucosyltransferase (Le), z-3-fucosyltransferase and z-2-fucosyltransferase (H), were also measured in the jejunal extracts (Watkins et al., 1981). Apart from two cases in which the quality of the post-mortem material tested was clearly poor, the A transferase was very active in A individuals, the B transferase in B individuals, and the level of the z-2-fucosyltransferase correlated well with the secretor status of the individual, though in a wider range of samples we have seen considerable overlap in the values. Acceptor specificity studies indicated greater preference for Gal\(^a\,1\)3GlcNAc than Gal\(^a\,1\)4GlcNAc in secretors but not in non-secretors.

The monoclonal antibodies used to isolate lactase, sucrase and aminopeptidase were isolated in the M.R.C. Human Biochemical Genetics Unit or were kindly supplied by Dr. H-P. Hauri, Basel, and their specificity checked using the procedures described by Swallow et al. (1985). Immuno-precipitation was performed as described by Swallow et al. (1985) except that rabbit anti-mouse immunoglobulins conjugated to CNBr-Sepharose (according to the manufacturer's instructions) were used in place of Staphylococcus protein A adsorbant. The precipitates were subjected to SDS/gel electrophoresis followed by direct staining of the gels with \(^{125}\)I-labelled lectins (Karlsson et al., 1983) or electrophoretic transfer on to nitrocellulose and staining with the mono-specific blood group antibodies or lectins as described by Swallow et al. (1986). The polyclonal blood group antisera were raised in rabbits at the M.R.C. Clinical Research

The ABO and Lewis antigens on the human intestinal hydrolases

suggests that this oligosaccharide contains the preferred structure bound by 1G7 and that this or a similar structure occurs on many jejunal glycoproteins of Lewis positive non-secretors. Since the specificity of this antibody appeared to be very similar to that reported for a monoclonal antibody 115C2 raised against human milk fat globule membranes (Hilkens et al., 1984; Gooi et al., 1985), we have made direct comparisons using 115C2 (kindly provided by John Hilkens) in parallel inhibition experiments. The pattern of inhibition was indistinguishable. A limited survey of tissue distribution using tissues from a non-secretor was also made using both antibodies and not more than trace amounts were seen in the membrane extracts of any other tissue tested (testis, lung, liver, skeletal muscle, heart muscle, kidney), but many glycoproteins present in human milk of both secretors and non-secretors carry this antigen.

We thank Dr. Winifred Watkins for her helpful advice.

Received 28 November 1986


Vol. 15

Fig. 1. Strips of nitrocellulose carrying bound separated jejunal glycoproteins from an O non-secretor, probed with the antibody 1G7 (hybridoma culture supernatant diluted 1:50) in the presence of a range of inhibitors.

\[
\begin{align*}
\text{Gal}^\beta 1 & \rightarrow 3\text{GlcNAc}^\beta 1 & \rightarrow 3\text{Gal}^\beta 1 & \rightarrow 4\text{Glc} \\
11.4 & & 11.3 & \\
\text{Fuc} & & \text{Fuc} & \\
\text{galactosyl} & & \text{fucosyl} & \\
\end{align*}
\]

Gal\(^a\,1\)3GlcNAc > Gal\(^a\,1\)4GlcNAc in secretors.
Centre and purified using the appropriate Synsors (Chembiomed Ltd., Alberta, Canada). Monoclonal anti-A was isolated in the M.R.C. Human Biochemical Genetics Unit. In one experiment lactase from two individuals of different blood group (A and O) was purified by immunoadfinity chromatography as described in Potter et al. (1985).

Using the A blood-group specific antibodies and the lectin from Helix pomatia we have shown that many different glycoproteins from five A secretors, one AB secretor and one (out of four) apparent A non-secretors carry abundant A antigen, and that the antigen is specifically carried on lactase, sucrase and aminopeptidase. Likewise the anti-B serum and the lectin from Bandeiraea simplicifolia (BS1) reveal that the B antigen is carried on the glycoproteins of all seven B secretors and the one AB secretor tested, and can be demonstrated specifically on the lactase, sucrase and aminopeptidase. No B non-secretors were available for study. Using anti-H serum and the lectin from Ulex europaeus, quantitative differences could be seen in the H antigen detected on these glycoproteins in secretors and non-secretors but this was less clear cut. Similar experiments in progress with anti-Lewis antibodies show that Le and/or Le are carried on this group of glycoproteins and can be specifically detected on sucrase, lactase and aminopeptidase. The presence of the Lewis antigens on the glycoproteins of the samples so far tested appears also to depend on the ABO, Lewis and secretor types of the individuals as expected.

Thus our results confirm those of Triadou et al. (1983) that the ABH antigens are structural determinants on the brush-border hydrolases of the human small intestine. This expression also appears to be dependent upon the secretor status, although there was one exception, an individual whose brush-border hydrolases carry the A determinant despite the lack of α-2-fucosyltransferase activity in the jejunum and their non-secretor status as judged by stomach mucus. This discrepancy is not easily explained by the quality of the post-mortem tissue, particularly since the levels of the α-3-N-acetylglactosaminyltransferase and α-3-fucosyltransferase were both high. It is known that the presence of the H antigen in certain human intestinal cells is not under the control of the Se gene (Mollicone et al., 1986) and it is possible that α-2-fucosyltransferase activities are present in the human gut which are not detectable by our standard assay procedures. It is intriguing but possibly coincidental that this exceptional individual was also Lewis negative.

Although jejunal aminopeptidase carries the ABH and Lewis determinants, aminopeptidase from kidney, which is a closely related enzyme, evidently carries only B product of the same gene locus, does not. It is noteworthy that the overall level of α-2-fucosyltransferase in kidney is low in comparison with jejunum, suggesting that this enzyme may not be expressed within the cells responsible for the synthesis of aminopeptidase.

We acknowledge the valuable assistance of Jean Nosade and Ira Islam. We are grateful for the ICRF fellowship awarded to P.G. We thank Dr. Winifred Watkins for her helpful advice.

Hartmann, G. (1941) Group Antigens in Human Organs, Munksgard, Copenhagen.


Received 28 November 1986

The preparation of biotin-labelled hyaluronidase

DIANE LACE, KENNETH S. DODGSON,* PETER GACESA and ANTHONY H. OLAVESEN†

Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, Wales, U.K.

Clinical trials in the U.K. using highly purified preparations of hyaluronidase have shown that the enzyme has some beneficial effect in the treatment of myocardial infarction (Flint et al., 1982; Henderson et al., 1982; Saltissi et al., 1982). However, there is considerable speculation as to the therapeutic mechanism of action of this enzyme (Maroko et al., 1977). Recent studies with 125I-labelled hyaluronidase have shown that enzyme, intravenously administered to the rat, is preferentially taken up by the liver (Eastman et al., 1985a); however, only 0.1% of the hyaluronidase is accumulated by the heart. Interestingly, studies using animals with induced myocardial infarction have shown that there is a preferential uptake of labelled hyaluronidase by the damaged myocardium as compared with normal heart tissue (Emslaw et al., 1985b).

The use of 125I-labelled hyaluronidase has precluded the use of autoradiographic methods for the specific localization of the enzyme within tissues. Also, the use of 125I-label may result in an anomalous distribution of enzyme within the whole animal and consequently a second method for the detection of enzyme would be desirable. In this paper we describe a method for the preparation of a biotinylated derivative of hyaluronidase. This preparation, in conjunction with appropriate streptavidin-conjugates (e.g. fluorescein-labelled streptavidin), will allow us to determine the sites of accumulation of the biotin-labelled hyaluronidase at the organ, cellular and subcellular levels.

Bovine testicular hyaluronidase (40 000 i.u./mg; Humphrey, 1957) was purified by the methods of Pope et al. (1976) and Gorham (1974). Enzyme activity was assayed by the method of Gacesa et al. (1981). Hyaluronidase was modified with biotinyl N-hydroxysuccinimide ester (BNHS) essentially by the method of Guesdon et al. (1979). Typically, hyaluronidase (1.2 mg) was dissolved in 0.48 ml of 0.1 M-NaHCO3, pH 8.3, to which 48 μl of N,N-dimethylformamide containing an appropriate quantity of BNHS had been added. The mixture was left for 4 h at 25°C before separation of the biotin-labelled enzyme from free biotin and BNHS by gel-permeation chromatography (Fig. 1). To estimate the stoichiometry of binding, preparations were car-