PHENYLALANINE HYDROXYLASELIKE ANTIGEN IN HUMAN CHORIONIC VILLI

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Phenylalanine metabolism in man is largely determined by functioning of the enzyme phenylalanine hydroxylase (PhH) (E.C. 1.14.16.1) of the liver. In addition, the presence of the corresponding protein product in minor quantities in a wide range of tissues was demonstrated by the writers previously [1, 2] and confirmed by detection of the mRNA of PhH by other workers [11]. The discovery of PhH in cells of chorionic villi is particularly interesting, not only for the development of a cheaper alternative to DNA diagnosis by prenatal diagnosis of phenylketonuria – a hereditary disease due to mutations in the structure of PhH, but also in order to determine the possible role of these cells in the organization of the placental barrier, which regulates the supply of phenylalanine to the fetus.

This paper gives the results of a search for an immunoreactive protein with antibodies against liver PhH in an extract of chorionic villi, and gives details of the physicochemical properties of this protein.

EXPERIMENTAL METHOD

Chorionic villi were obtained after abortions on medical grounds between 6 and 13 weeks of pregnancy. The material was quickly frozen to −70°C. Autopsy samples of human liver were obtained 2 h after death of the patients. Extracts of cytoplasmic and membrane proteins were prepared as described previously [3]. Monoclonal antibodies against hepatic PhH were obtained previously in R. G. H. Cotton's laboratory. The epitope of PH7 monoclonal antibodies is located in the PhH peptide incorporated into the composition of the N-terminal fragment consisting of 41 amino acid residues [13]. PH8 monoclonal antibodies react with the PhH peptide located between amino acid residues 139 and 155 [4] The epitope of PH9 monoclonal antibodies was located in the N-terminal fragment of PhH consisting of 15 amino acid residues [6]. Unidirectional electrophoresis of the proteins was carried out in 15% PAG by Laemmli's method [8]. Two-dimensional electrophoresis by O'Farrell's method was undertaken without any substantial modifications [9]. Immunoblotting of the proteins was done as described previously [3]. The sensitivity of the method was about 1 mg protein in a band. Peptide analysis of PhH was carried out after proteolysis of proteins in an extract of liver and chorionic villi by chymotrypsin, with the ratio protein:enzyme = 500:1 at 37°C. In the experiments with autolysis of the antigen, the liver extract was incubated without addition of chymotrypsin. After separation of the peptides thus formed by unidimensional electrophoresis in 15% PAG [8] the peptides of PhH were revealed by immunoblotting with PH8 monoclonal antibodies.

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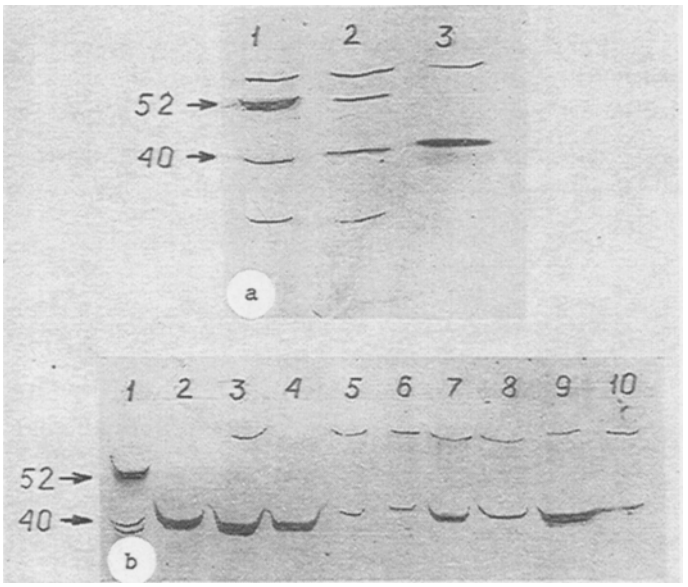

Fig. 1. Immunoblotting of extracts of chorionic villi with PhH monoclonal antibodies against liver phenylalanine hydroxylase a: 5 mg Protein of human liver extract after limited autolysis applied to lane 1; 30 mg protein of extracts of cytoplasmic and membrane fraction respectively applied to each lane 2 and 3; b: 5 mg protein of human liver extract applied to lane 1, 30 mg protein of extract of membrane fraction of chorionic villi at different times of pregnancy applied to lanes 2-10.

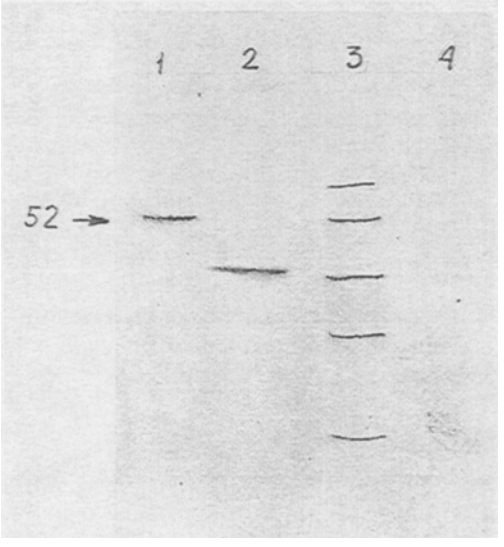

Fig. 2. Characteristics of peptides of PH-like antigen of chorionic villi and liver after proteolysis by chymotrypsin. 5 mg Protein of human liver extract applied to lane 1, 30 mg protein of extract of membrane fraction of chorionic villi applied to lane 2, the same specimens applied to lanes 3 and 4 as to lanes 1 and 2, but after limited proteolysis by chymotrypsin for 3 min.

EXPERIMENTAL RESULTS

The search for a PhH-like antigen in the chorionic villi was conducted by fractionation of the protein extracts by unidimensional electrophoresis followed by immunoblotting, using PH8 monoclonal antibodies, which react strongly with the denatured antigen. In some extracts of cytoplasmic proteins an antigen with electrophoretic mobility comparable with that of the liver antigen could be detected (Fig. 1a). However, its content in extracts of cytoplasmic proteins of the chorion was very low, comparable with the sensitivity of the method, and this evidently explains why this antigen could be found in only 6 of the 23 specimens of chorion studied.

An antigen with electrophoretic mobility corresponding to a molecular mass of 40-41 kilodaltons was detected in extract of membrane proteins of chorionic villi. This antigen was detected in all the specimens studied, although quantitatively they varied widely in its content (Fig. 1b). No relationship could be found between the antigen content and the time of pregnancy at which the chorionic villi were obtained. In control experiments with replacement of monoclonal antibodies by nonimmune mouse immunoglobulins no PH-like antigen was detected, evidence of the specificity of the reaction.

Since the molecular mass of the antigen in membrane protein extracts was 12 kilodaltons less than the molecular mass of the liver PG, it was interesting to determine the composition of the peptides formed after proteolysis of liver PH by chymotrypsin. It will be clear from Fig. 2 that one of the major peptides obtained after such a procedure has electrophoretic mobility identical with that of the antigen of the chorionic villi. These results are in agreement with data in the literature on removal of a peptide measuring 12 kilodaltons from N-end of the subunits of the liver PH under the influence of different proteases [5, 7]. The antigen of the membrane fraction of chorionic villi was highly sensitive to protease and was not detected in the extract even after incubation with chymotrypsin for a short time (Fig. 2).

Since the N-terminal fragment of liver PH contains antigenic determinants of two other monoclonal antibodies PH7 [13] and PH9 [6] with identified epitopes in the amino acid sequence of the subunits they were used to characterize the structure of the antigen discovered. None of these antibodies reacted with the antigen of the membrane fraction.

The homogeneity of the protein discovered was characterized by two-dimensional electrophoresis followed by immunoblotting; the results showed that the antigen of the membrane fraction was represented by a single spot. The presence of isozymes of liver PH on two-dimensional electrophoresis is known to be determined by phosphylation of the protein [12]. Consequently, the absence of isozymes of the antigen in chorionic villi during two-dimensional electrophoresis may also be associated with loss of the phosphylation site after removal of the N-terminal peptide.

The results evidently confirm expression of the PH gene in chorionic villi, which was found previously [11] by detection of illegal mRNA of PH. The translation product of this mRNA also was probably detected in the cytoplasmic fraction of the chorionic villi, but it was present in an extremely small amount, precluding a more detailed study of its properties.

For this reason the antigen found in the membrane fraction is particularly interesting. The showed previously that only the native enzyme is present in this fraction [3]. The methods of analysis used and also the method of collecting and keeping the material make the appearance of the antigen in the membrane fraction as a result of nonspecific proteolysis of the native PH unlikely. Later a more detailed study of its structure will have to be carried out, with particular reference to the use of fine methods of characterizing protein structure, including microsequencing. However, the results suggest that the antigen discovered is the product of expression of the phenylalanine hydroxylase gene.

The PH-like antigen of the membrane fraction of chorionic villi may possess important functions, for we know that the peptide of liver PH formed after removal of the N-terminal fragment is much more active than the native enzyme [5]. An argument in support of this view is given by the indications of the existence of specific

transport systems for phenylalanine in mammalian cell membranes [10] and detection of the PH-antigen in a tissue directly involved in regulation of the phenylalanine supply to the fetus.

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CHANGES IN NUCLEOID DNA STRUCTURE AND ADHESIVE PROPERTIES OF BLOOD LEUKOCYTES IN ANIMALS SOON AFTER IRRADIATION

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Much attention is currently being paid to the study of the molecular mechanisms of cellular adhesion. The structure of adhesion receptors and their connection with individual segments of chromosomes and concrete regions of DNA coding for receptor molecules or their fragments are being investigated [8, 10, 11]. Meanwhile it has been shown that during irradiation of cells significant disturbances of the supercoiled structure of DNA take place [9], which as we know are associated with expression, repair, and degradation of genetic material [1, 7]. These structural changes can be recorded by two-wave fluorescent analysis of the DNA of blood cell nucleoids [3, 4]. However, relations of the above-mentioned postradiation changes in DNA and functional properties of the leukocytes, especially their adhesive properties, have not been investigated.

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