MONOSPECIFICITY OF ANTIBODIES AGAINST DIFFERENT FORMS OF CYTOCHROME B-450 STUDIED BY ROCKET IMMUNOELECTROPHORESIS

N. I. Gutkina and V. M. Mishin

UDC 612.351.11:577.152.112]-083.33

KEY WORDS: cytochrome P-450; multiple forms; antibodies; immunoelectrophoresis; monospecificity.

Research workers are currently involved in the study of the enzyme system responsible for oxidizing foreign chemical compounds (xenobiotics), among which are included toxic chemical factors polluting the environment, and drugs. The terminal oxidase of this system, located in the endoplasmic reticulum of the liver cells, is cytochrome P-450, which exists in many forms that differ in their catalytic, electrophoretic, and spectral properties. We know that xenobiotics can induce cytochrome P-450 selectively as regards the different forms of this enzyme [1, 10]. The most extensively studied inducers of cytochrome P-450, namely 3-methylcholanthrene (MCh) and phenobarbital (PbB), for example, induce the synthesis of two different forms: MCh- and PhB-cytochromes P-450 [3]. Other types of inducers also exist, and when they enter an animal's body, forms of cytochrome P-450 that differ from the MCh- and PhB-cytochromes P-450 are synthesized in the liver microsomes [6, 9].

One of the main approaches to the study of multiplicity of forms of cytochrome P-450 is the isolation of these forms in the pure state, the production of antibodies to them, and the study of induction of individual forms of cytochrome after introduction of different xenobiotics into the body, by immunochemical methods. By the use of antibodies, the quantity of particular forms of cytochrome can be determined actually in the microsomes. For quantitative immunochemical analysis monospecific antibodies are needed against the form of cytochrome which has to be assayed. However, difficulties do arise, connected with the obtaining of highly purified forms of cytochrome P-450 and of monospecific antibodies against these forms.

In this paper a technique of detecting monospecificity of antibodies cytochrome P-450 by rocket immunoelectrophoresis is suggested.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 150-200 g. MCh was injected intraperitoneally dissolved in sunflower oil for a period of 3 days (25 mg/kg) and PhB also was injected intraperitoneally, dissolved in physiological saline, for a period of 4 days (80 mg/kg). Liver microsomes were obtained by the usual method of differential centrifugation. PhB- and MCh-cytochromes P-450 were isolated by the method in [2], using the chromatographic adsorbents 1,8-diamino-octylsepharose-4B and DEAE-Sephacel. The cytochrome preparations contained 15-17 nanomoles cytochrome per milligram protein. Protein was determined by Lowry's method [5] and cytochromes spectrophotometrically [7]. Adult noninbred rabbits were immunized with the isolated cytochrome preparations by intradermal injection of the antigen [4]. Sera from 3-5 rabbits were taken 10 days after the intravenous reacting injection of the cytochrome preparations. Immunoglobulins were isolated from the sera by fractionation with ammonium sulfate. Rocket immunoelectrophoresis was performed as described in [8].

EXPERIMENTAL RESULTS

It is well known that by rocket immunoelectrophoresis it is possible to determine the quantity of antigen in a test sample, because the area of the shapes (rockets), formed by the immunoprecipitation boundary, is directly proportional to the quantity of antigens in the

Laboratory of Cellular Mechanisms of Adaptation, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 7, pp. 33-35, July, 1986. Original article submitted July 10, 1985.

 0007-4888/86/0007-0908$12.50

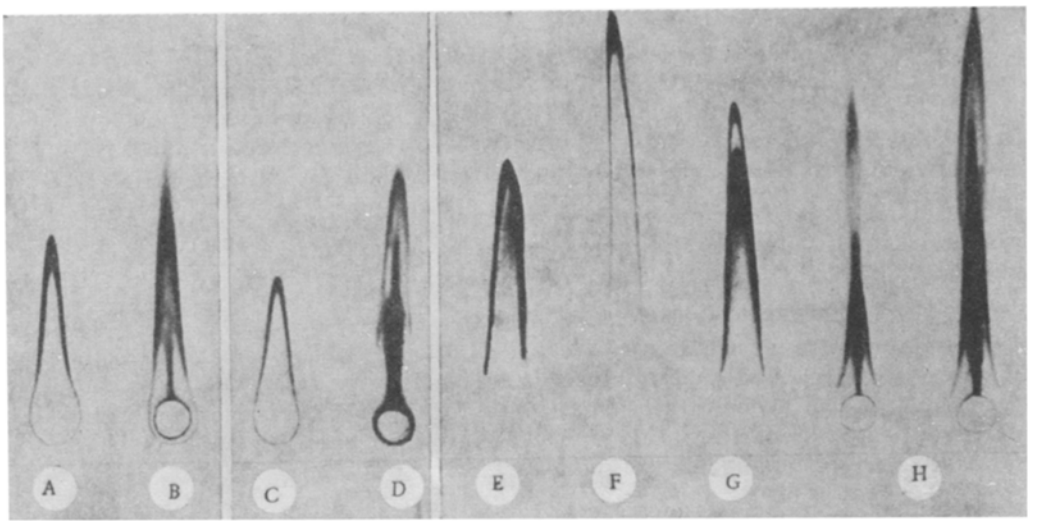

Fig. 1. Immunoelectrophoresis of isolated PhB- and MCh-cytochromes P-450 and rat liver microsomes induced by PhB, MCh, and arochlor-1254. A, B) agarose gel contained antibodies against PhB-cytochrome P-450 (0.2 mg/ml); well A contained PhB-cytochrome P-450 (0.01 mg/ml), well B contained PhB-microsomes (0.1 mg/ml); C, D) gel contained antibodies against MCh-cytochrome P-450 (0.2 mg/ml): well C contained MCh-cytochrome P-450 (0.01 mg/ml), well D contained MCh-microsomes (0.1 mg/ml); E-H) gel contained sum of antibodies against PhB-cytochrome P-450 (0.2 mg/ml) and MCh-cytochrome P-450 (0.1 mg/ml); well E contained PhB-cytochrome P-450 (0.02 mg/ml), well F contained MCh-cytochrome P-450 (0.02 mg/ml), well G contained sum of PhB-cytochrome P-450 (0.02 mg/ml) and MCh-cytochrome P-450 (0.01 mg/ml), and well H contained rat liver microsomes induced by arochlor-1254 (0.3 and 0.6 mg/ml).

starting well. A characteristic of the method is that it can be used with both monospecific and polyspecific antibodies. A rocket will be formed as a single precipitation band if monospecific antibodies are present in the gel and the starting well contains the total proteins among which the antigen is found, and also if the gel contains polyspecific antibodies and the starting well contains only one protein, reacting with particular antibodies. If the starting well contains all the proteins and they move into a gel containing different antibodies against them, several immunoprecipitation lines will be formed and the rocket will become double, treble, and so on. Thus if the total proteins are introduced into the starting well and a single rocket is obtained, it can be concluded that the antibodies used are monospecific. Conversely, the discovery of a single rocket during analysis of protein against antibodies known to be polyspecific is evidence of the presence of only one antigen protein in the starting well. The appearance of the rockets under the conditions described above is demonstrated in Fig. 1. Agarose gel A contains antibodies against an electrophoretically homogeneous form of cytochrome P-450 (PhB-cytochrome P-450), which is present in the starting well. The formation of a single immunoprecipitation line is evidence that, first, monospecific antibodies recognizing one of the several antigens present in the starting well are present in the gel, or second, that polyspecific antibodies are present in the gel but the starting well contains only one antigen, or third, that the gel contains monospecific antibodies reacting with one antigen, which is added to the starting well. The pattern of the rockets illustrated in Fig. 1B enables the choice to be made in favor of one of the alternatives mentioned above. Rat liver microsomes, induced by PhB, i.e., a preparation known to contain the total of all possible antigens, were introduced into the starting well. The same antibodies against PhB-cytochrome P-450 were added to the gel. The presence of a single precipitation line is evidence that antibodies recognizing one form of cytochrome (PhB- cytochrome P-450) were present in gel A and also in gel B. In Fig. 1, C and D, a similar experiment with antibodies against MCh-cytochrome P-450 also is illustrated. Well C contains MCh-cytochrome P-450, whereas well D contains rat liver microsomes induced by MCh. Here also the presence of a single rocket indicates the monospecific nature of the antibodies against MCh-cytochrome P-450, since microsomes, in the case of induction by MCh, contain several forms of cytochrome P-450, including that which we isolated in the pure form, and to which the antibodies were obtained. The pattern of immunoelectrophoresis with discovery of the double rocket is demonstrated in Fig. 1, E-H. In this case antibodies against botn PhB-cytodhrome P-450 and MCh-cytochrome P-450 were introudced into the gel.

PhB- and MCh-cytochromes P-450 were introduced in the wells E and F, respectively, both these cytochromes into well G, and rat liver microsomes induced by arochlor-1254, which induces the synthesis of both PhB- and MCh-forms of cytochrome P-450, into well H. Figure 1 shows clearly that polyspecific antibodies prepared artificially recognize homologous antigens, and give a picture of a double rocket in the case of a mixture of isolated preparations of cytochrome P-450 and rat liver microsomes induced by arochlor-1254.

Thus rocket immunoelectrophoresis, as applied to isolated forms of cytochrome P-450 and microsomal preparations, can be used to assess the monospecificity of antibodies on the basis of pattern of immunoprecipitation lines.

Incidentally, Ouchterlony's double immunodiffusion method is widely used for the qualitative study of induction of microsomal enzymes. However, this method is not sensitive enough when determining monospecificity of antibodies if the preparation of immunoglobulins obtained against a certain form of cytochrome P-450 contains antibodies against other antigens, and in the sample for testing, these antigens are present in a small quantity. The rocket immunoelectrophoresis technique possesses higher resolving power.

We consider that in most cases antibodies to a certain form of cytochrome P-450 can be regarded as monospecific if they give rise to a single rocket during analysis of a microsomal preparation known to contain the total number of forms of cytochrome P-450.

LITERATURE CITED

1. F. P. Guengerich, G. A. Dannan, S. T. Wright, et al., Xenobiotica, 12, 701 (1982).
2. F. P. Guengerich and M. V. Martin, Arch. Biochem., 205, 365 (1980).
3. N. Harada and T. Omura, J. Biochem. (Tokyo), 89, 237 (1981).
4. T. Kamataki, D. H. Belcher, and R. A. Neal, Molec. Pharmacol., 12, 921 (1976).
5. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 19, 265 (1951).
6. S. L. Newmann and P. S. Guzelian, Biochem. Pharmacol., 32, 1329 (1983).
7. T. Omura and R. Sato, J. Biol. Chem., 239, 2379 (1964).
8. C. B. Pickett, R. L. Jeter, J. Morin, and A. Y. H. Lu, J. Biol. Chem., 256, 8815 (1981).
9. D. E. Ryan, S. Iida, A. W. Wood, et al., J. Biol. Chem., 259, 1239 (1984).
10. D. E. Ryan, P. E. Thomas, L. M. Reik, and W. Levin, Xenobiotica, 12, 727 (1982).

EFFECT OF RUTHENIUM RED ON Ca^{++}-INDUCED β AND γ STATES OF COMUTON REGULATION OF RESPIRATION AND OXIDATIVE PHOSPHORYLATION IN RAT MITOCHONDRIA

G. M. Élbakidze, I. M. Élbakidze, and A. G. Gachechiladze

UDC 612.262:261.398.145.1].014.46

KEY WORDS: mitochondria, comuton, Ca^{++} ions, ruthenium red.

The writers showed previously that the tissues of rats [3, 5] and other vertebrates [1] contain a mechanism regulating energy metabolism in the tissue [4]. Its effectors are tissue-specific peptides known as comutons [6]. It has been shown that comuton regulation may exist in three states: in the α-state, expressed as tissue-specific stimulation of the respiration rate of mitochondria (MCh) in medium with succinate before addition of ADP (v_4), the β-state, when rotenone-insensitive uncoupling of oxidative phosphorylation of MCh is observed, and the γ-state, when this uncoupling becomes rotenone-stimulated [7]. In experiments *in vitro* the β- and γ-states of comuton regulation can be induced by brief preincubation of MCh from rat liver and kidney with Ca^{++} ions in the presence of 3 mM inorganic phosphate (P_i) [7]. To study the mechanism of the regulating action of Ca^{++} on the character of comuton control of oxidative

Sector of Tissue Control of Metabolism, Research Institute for Biological Testing of Chemical Compounds, Kupavna, Moscow Region.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 7, pp. 36-38, July, 1986. Original article submitted June 19, 1985.

0007-4888/86/0007-0910$12.50