NADPH- AND NADH-DEPENDENT SYSTEM OF BENZ(a)PYRENE HYDROXYLATION.

II. RELATIONSHIP TO LIPID PEROXIDATION

N. P. Belevich, L. F. Dmitriev,
and I. I. Ivanov

UDC 612.351.11:577.152.
1'133].014.46

KEY WORDS: hydroxylation of benz(a)pyrene; peroxidation of lipids; microsomal oxidation.

The xenobiotics are metabolized in the liver by an enzyme system in which the key components in the chain of electron carriers is cytochrome P-450, and in which predominantly NADPH is used as the source of reducing equivalents.

The purpose of this communication is to examine the distribution of reducing equivalents in microsomes metabolizing benz(a)pyrene (BP) and, in particular, the utilization of NADH in reactions of BP hydroxylation and lipid peroxidation (LPO). This is an important problem in connection with the study of the contribution of NADH to BP hydroxylation, depending on various factors influencing electron transport in the NADPH oxidation chain.

EXPERIMENTAL METHOD

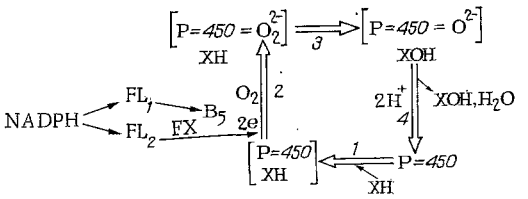
Male Wistar rats weighing 150-180 g were used. The animals' liver was perfused with cold 0.15 M NaCl and homogenized in 0.25 M sucrose and 1 mM EDTA in a Potter's homogenizer. The resulting homogenate was divided into two parts, into one of which a solution of α-tocopherol in ethanol (2 mg/ml) in the ratio of 1:50 by volume was injected. By using homogenate without and with α-tocopherol, microsomes I and II were isolated by the method of low-speed centrifugation in the presence of Ca^{2+} [8]. The intensity of LPO was estimated from the accumulation of malonic dialdehyde [11]. The intensity of BP hydroxylation was determined fluorometrically [2].

EXPERIMENTAL RESULTS

Kinetic curves of BP hydroxylation in microcomes obtained under different conditions of isolation are shown in Fig. 1. It will be clear from Fig. 1 that the rate of NADPH-dependent BP hydroxylation was lower in I than in II microsomes. However, the fastest possible rate of BP hydroxylation was observed in I microsomes when NADH was present in the incubation medium.

The most acceptable explanation of these two facts, in the writers' opinion, is that which assumes the presence of two active centers FL_1 and FL_2 on NADPH-dependent reductase. This hypothesis is supported by data according to which one of the flavins (FAD or FMN) of NADPH-dependent reductase functions as a carrier of two electrons, whereas the other can function only as a carrier of a single electron [6].

The results can be interpreted on the basis of this subdivision, which determines the role of flavins in the hydroxylation of a foreign hydrocarbon as the following scheme 1 illustrates:



M. V. Lomonosov Moscow State University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 2, pp. 158-160, February, 1981. Original article submitted April 18, 1980.

 0007-4888/81/9102-0136$07.50

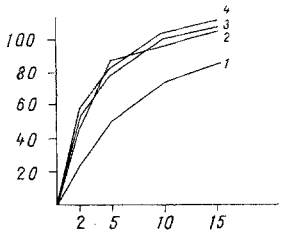


Fig. 1

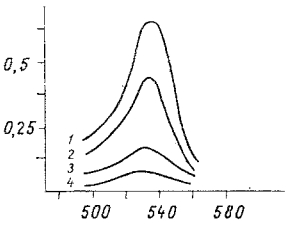


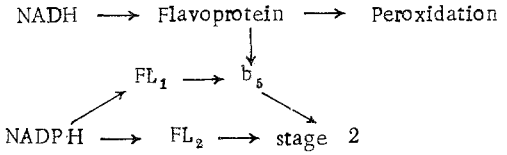
Fig. 2

Fig. 1. BP hydroxylation. Abscissa, time of incubation (in min); ordinate, utilization of BP (in relative units). 1) I microsomes without NADH, 2) with NADH, 3) II microsomes without NADH, 4) with NADH. Incubation medium: 0.15 M KCl, 10 mM Tris HCl, pH 7.4, 5 μm $MgSO_4$, 0.6-1 mg protein/ml, and 0.6 μM BP. Concentration of NADPH and NADH was 50 μM. Unmetabolized part of BP extracted with acetone and decrease in BP concentration determined from decrease in intensity of fluorescence. Error of quantitative determination of BP did not exceed 7%.

Fig. 2. Lipid peroxidation by microsomes. Abscissa, wavelength (in nm); ordinate, optical density. 1) NADPH + NADH, 2) NADPH, 3) NADPH + BP, 4) NADPH + NADPH + BP.

The process of hydroxylation in scheme 1 is conventionally subdivided into four stages, one of which (stage 2) is catalyzed by flavin FL_2. This flavin which can transport two electrons in a short time interval, evidently catalyzes both reduction of the heme of cytochrome P-450 and the activation of oxygen, thereby resulting in hydroxylation of the hydrocarbon. The other flavin FL_1, functioning with the formation of a stable semiquinone, gives up electrons to cytochrome b_5.

In this scheme the NADPH → FL_1 chain does not participate in the hydroxylation reaction (XH → XOH) and electron transport in this chain must introduce a definite change into the stoichiometric ratio $\Delta NADPH:\Delta O_2:\Delta XOH$. In other words, the transfer of electrons by FL_1 reduces the intensity of NADPH-dependent hydroxylation, but this decrease can be compensated if NADH takes part in the reaction. An example of this is hydroxylation of BP in I microsomes. Participation of NADH in the hydroxylation of the hydrocarbon (synergic action of NADH) must depend on deviation of this stoichiometry from the theoretical value of 1:1:1, and this in fact takes place [9]. In the presence of NADH every electron carried by the NADPH → FL_1 chain can form a pair with the electron transported in the NADH oxidation chain, and so ensure the maximal level of hydroxylation of the foreign hydrocarbon. This situation is reflected in scheme 2:



It follows from the results that the stage catalyzed by FL_2 is the limiting stage in I microsomes obtained by the low-speed method under ordinary conditions, i.e., in this case hydroxylation of BP is evidently determined by electron transport in the NADPH → FL_2 chain. In I microsomes NADH exerts a synergic action, and stage 2 ceases to be the limiting stage only in the presence of NADH. The use of α-tocopherol in the separation process allows II microsomes to be obtained, in which stage 2 is not the limiting stage even in the absence of NADH. In such microsomes BP is hydroxylated by reducing equivalents on account of oxidation of NADPH in the principal chain, which incorporated FL_2.

The observed effect is evidently associated with the fact that during isolation definite injury to the microsomal membranes occurs, and this effects interaction between the carriers in the NADPH oxidation chain. The character of this injury is not yet clear but the possibility cannot be ruled out that endogenous phospholipases present in the membranes are activated by Ca^{2+} ions [10] and so causes appreciable injury to the microsomal membranes. Meanwhile, during separation of II microsomes the probability of such injury is much less, because phospholipase A_2 is effectively inhibited by α-tocopherol [7].

The results indicate that participation of the necessary quantity of NADH in BP hydroxylation in I microsomes is guaranteed despite the fact that NADH can be utilized in other ways and, in particular, in reactions involving endogenous substrates. For example, NADH can stimulate LPO, as is confirmed by data showing the effect of NADH on LPO under conditions when the NADPH concentration is below the saturating level for this reaction (Fig. 2). These data reflect the quantity of LPO products formed during incubation with NADPH in a concentration of 50 μM. The use of NADPH in higher concentration (100 μM) led to intensification of LPO. The same effect (in the presence of cyanide) was observed in medium containing 50 μM NADPH and 50 μM NADH. Cyanide evidently inhibits the competitive pathway of NADH oxidation associated with contamination of the microsomal fraction.

In the presence of BP, LPO in the microsomes is inhibited, possibly as a result of the antioxidant action of 3-OH-BP formed during the hydroxylation reaction. As will be clear from Fig. 2, the decrease in the intensity of LPO was particularly marked in medium containing NADPH and NADH. The effect of BP in this case was evidently connected with intensification of 3-OH-BP formation. This intensification in turn was the result of utilization of the NADH oxidation chain and the NADPH → FL_1 chain in the reaction of BP hydroxylation.

The main component of these chains responsible for switching the reducing equivalents to cytochrome P-450 is cytochrome b_5. There is evidence [5] of the existence of two cytochrome b_5 pools, differing in the degree of their inhibition by antibody and, perhaps, in the localization of the protein in their membrane. The functions performed by the two cytochromes are evidently similar and the existence of two cytochromes is linked with the need for two different carriers: one in the oxidation chain of NADPH, the other in that of NADH [1].

During interaction between BP and the system responsible for its metabolism, a situation may thus arise when the participation of both NADPH and NADH in the reaction is necessary to guarantee high intensity of BP hydroxylation. Such a situation may arise, in particular, when microsomes obtained by the low-speed method are used, for the intensity of NADPH-dependent hydroxylation of BP in such microsomes can vary appreciably depending on the isolation conditions, which determine the degree of modification of the membranes. In all probability the difference between the kinetic curves of hydroxylation is the result of a change in the NADPH-dependent flavoprotein:phospholipid:cytochrome P-450 ratio, which determines the efficiency of electron transport through FL_2 to cytochrome P-450. A change in this ratio may take place *in vivo* also, when carcinogenic hydrocarbons are administered to animals, as a result of which synthesis of a cytochrome of P-450 type (P-448) is induced, but there is no appreciable increase in the activity of NADPH-dependent reductase [4].

When BP is administered to animals the demand of the hydroxylase system for reducing equivalents rises sharply [2]. This demand is satisfied, in all probability, both by NADPH formed in the pentose phosphate cycle and by participation of coenzymes in the NADPH form in the reaction. Since the reducing power of the NADH/NAD system in the cytoplasm is comparatively low, the question of the participation of NADH in BP hydroxylation must be examined in close connection with the functioning of the oxidase system and, in particular, with its oxidation of NADH formed in the Krebs cycle [3]. Spatial separation of the oxygenase and oxidase systems cannot be an obstacle to interaction between these systems, for NADH can be carried through the mitochondrial membrane into the cytoplasm by means of shuttle mechanisms. However, such interaction, if it takes place, can lead to a disturbance of the existing electron acceptor–donor equilibrium. This is of great importance for the maintenance of cell structure and, consequently, the regulation of cell differentiation and metabolism.

LITERATURE CITED

1. A. I. Archakov, Microsomal Oxidation [in Russian], Moscow (1975).
2. L. F. Dmitriev and I. I. Ivanov, Dokl. Akad. Nauk SSSR, 236, 471 (1977).
3. L. F. Dmitriev and I. I. Ivanov, Vopr. Med. Khimii, No. 4, 451 (1979).
4. T. E. Gram, B. I. Sikic, C. L. Litterst, et al., in: Microsomes and Drug Oxidations, Oxford (1977), p. 527.
5. I. Jansson and J. B. Schenkman, Arch. Biochem., 178, 81 (1977).
6. T. Iyanagi, F. K. Anan, Y. Imai, et al., Biochemistry (Washington), 17, 2224 (1978).
7. A. S. Pappu, P. Fatterpaker, and A. Sreenivasan, Biochem. J., 172, 349 (1978).
8. J. B. Schenkman and D. L. Cinti, Life Sci., 11, 247 (1972).
9. H. Staudt, F. Lichtenberger, and V. Ullrich, Eur. J. Biochem., 46, 99 (1974).
10. M. Waite and L. M. van Deenen, Biochim. Biophys. Acta, 137, 498 (1967).
11. E. D. Wills, Biochem. J., 113, 333 (1969).