Summary

Cytochrome P₄₅₀ in the mitochondria of the adrenal cortex functions in the monooxygenation reactions for the biosynthesis of various steroid hormones, such as cholesterol side chain cleavage, hydroxylation at 11β-position and that at 18-position of the steroid structure. The cytochrome is firmly associated with the mitochondrial membrane and therefore can be isolated only by the aid of ionic or non-ionic detergent. Recently, two cytochromes P₄₅₀ each catalyzing a specified reaction have been purified to a homogeneous state, that is, P₄₅₀ₛₐ₃₅ having cholesterol side chain cleavage activity and P₄₅₀₁₈₉ having 11β-hydroxylation activity. The properties of these purified P₄₅₀’s as well as the other components of the monooxygenase system, adrenodoxin and adrenodoxin reductase, are, therefore, summarized and compared to those of P₄₅₀ in the mitochondrial preparation in situ.

Among many findings, both purified cytochromes P₄₅₀ were revealed to be a low-spin type hemoprotein and their spin states were changed to a high-spin state by being complexed with the corresponding substrate. The binding of a substrate also facilitated the reduction of the cytochrome and appeared to increase the stability of the oxygenated form of cytochrome P₄₅₀. These effects are important from the point of view that the primary role of the heme of cytochrome P₄₅₀ is the activation of molecular oxygen. In addition, the results of our detailed kinetic studies on the transfer of electrons from adrenodoxin to cytochrome P₄₅₀ in the reconstituted system have also been described. Finally, the topology of adrenodoxin and the reductase were shown to be on the inner mitochondrial membrane by a peroxidase-labeled antibody method.

Physiological Role of Cytochrome P₄₅₀ in Adrenal Cortex

The adrenal glands consist of two functionally and structurally different tissues, the cortex and the medulla. Between two, the cortex has been known to occupy a key position in the biosynthesis of steroid hormones. Corticosteroids, which are so designated because of the biosynthesis in the adrenal cortex, are of three kinds: mineral corticoids being secreted from zona glomeruloza, glucocorticoids from zona fasciculata and androgenic corticoids from zona reticularis. They are known to be responsible for the regulation of K⁺, Na⁺ and water concentration, for the regulation of carbohydrate metabolism and for the secondary sex characteristics, respectively.

Figure 1 shows a simplified scheme for the biosynthetic pathway of steroid hormones with cholesterol as the starting material, where various monooxygenase reactions were indicated by bold arrows. Cholesterol is largely stored in the adrenal gland and is ready for the biosynthesis of cortical hormones, while cortical hormones themselves are not stored in the gland in any significant amount. The terminal oxygenases in the monooxygenase reactions have been identified to be a hemoprotein which shows a particular absorption maximum at 450 nm when
CO is ligated to its reduced form. It is therefore called cytochrome P₄₅₀ and is handled in this article.

The stoichiometry of these monooxygenase reactions is generally expressed by the following equation:

$$\text{NADPH} + \text{H}^+ + \text{S} + \text{O}_2 \rightarrow \text{NADP}^+ + \text{SO} + \text{H}_2\text{O}$$

where S denotes a substrate to be monooxygenated. As can be seen, the reaction requires the reducing equivalent in the form of NADPH as well as molecular oxygen and a substrate to be monooxygenated. During the reaction, one atom of molecular oxygen is incorporated to a substrate while the other atom is reduced to water and therefore the reaction is also referred to as a mixed function oxidase reaction. Besides cytochrome P₄₅₀, a specific electron-transfer system composed of adrenodoxin, an iron-sulfur protein, and adrenodoxin reductase, a flavoprotein, is necessary to convey electrons from NADPH to cytochrome P₄₅₀.

Of the steroids in Figure 1, those synthesized in adrenal gland are restricted to so-called corticosteroids, while androgens and estrogens are exclusively produced in other organs, such as corpus luteum, ovary, placenta, and testis. Among many cytochromes P₄₅₀ functioning in the adrenal cortex, those responsible for cholesterol side chain cleavage reaction (P₄₅₀ccc), 11β-hydroxylation,