through a mixed-bed ion exchange resin (AG 501-X8 (D), Bio-Rad), while the amino sugars were liberated from the glycoprotein in 4 N HCl at 100° for 6 hours. Glucose was found to be the major carbohydrate component, whereas xylose and hexosamine occurred to a lesser degree. The ratios of the three sugars varied from 3 to 5 moles of glucose and 1 to 2 moles of xylose and hexosamine per mole of the agglutinin. The mannose content decreased gradually with the degree of purity of the wheat germ agglutinin. The crystalline wheat germ agglutinin probably has no mannose or at best 1 mole per mole of the agglutinin.

More detailed analytical data on the amino acid and carbohydrate composition will be reported elsewhere.

Wheat germ agglutinin preparations obtained from a Sephadex G-75 (2) or a DEAE-cellulose chromatography gave two bands on polyacrylamide gel electrophoresis (Fig. 3C). The two bands were always of about the same relative intensity. However, a single band, corresponding to the slower moving band of the two (Fig. 3, A and C), was observed upon electrophoresis of the active material eluted from a carboxymethylcellulose column. The faster moving band (Fig. 3B), which was also obtained in pure form after chromatography on a carboxymethylcellulose column, was found to have no agglutinating activity for either L1210 leukemia cells or human red blood group Al or B cells. Amino acid analysis showed a clear difference between the two proteins, e.g., wheat germ agglutinin contained 40 residues of half-cystine and 3 residues of valine, whereas the faster moving band contained 14 residues of half-cystine and 22 residues of valine per 23,500 g. Therefore, the main impurity associated with wheat germ agglutinin up to the DEAE-cellulose step is probably not a subunit of wheat germ agglutinin or a similar kind of phytohemagglutinin.

A comparison with other agglutinins, concanavalin A and soybean agglutinin, both known to agglutinate also primarily but not exclusively transformed cells, indicates that these two agglutinins do not contain any cysteine, whereas carbohydrates have been observed in soybean agglutinin (mannose and glucosamine) but not in concanavalin A (11-13). On the other hand, only the nonagglutinating, inactive subunit of concanavalin A (active molecule, 55,000 and 100,000; inactive subunit, 27,000) (14, 15) has a molecular weight in the range of wheat germ agglutinin. The crystalline wheat germ agglutinin probably has no mannose or at best 1 mole per mole of the agglutinin.

An X-ray crystallographic study on wheat germ agglutinin is now in progress in Dr. R. Langridge’s laboratory at Princeton University, and preliminary results will be reported (16).

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maximum velocity of phenylalanine hydroxylase when BH₄ is used as cofactor.

For most of the assays the enzymatic rate was followed spectrophotometrically by measurement of the phenylalanine-dependent oxidation of TPNH (5). In these assays, the complete reaction mixture consisted of the following components in a final volume of 1 ml: potassium phosphate, pH 6.9, 0.1 M; TPNH, 0.2 mM; catalase (Boehringer-Mannheim Corp.), 100 μg; dihydropteridine reductase (purified through the calcium phosphate gel step, (1)), 200 μg; tetrahydropterin (concentration indicated in legends); phenylalanine (concentration indicated in legends) and phenylalanine hydroxylase (purified through Sephadex G-200 step and about 90% pure (6)), 18 μg. Although there was the same concentration of hydroxylase in each experiment, the basal and lysolecithin-stimulated rates varied, apparently due to a loss in activity from freezing and thawing the hydroxylase. The degree of lysolecithin stimulation, however, was nearly the same in all experiments. When turbid components such as lipid suspensions were present in the assay, the reaction rate was determined by a fluorometric modification of the nitrosonaphthol assay for tyrosine (7). In this assay, in addition to the components present for the spectrophotometric assay, glucose 6-phosphate, 2.0 mM; and glucose 6-phosphate dehydrogenase (Boehringer-Mannheim Corp.), 10 pg, were included to maintain the TPNH in its reduced state. All experiments were carried out at 25°C. Lysophosphatidylethanolamine is soluble in water in the concentrations used in the assay. The other lipids, however, are insoluble in water and were suspended by sonication (with a Sonifier Cell Disruptor, Branson Sonic Power Co., Conn.; 20 s at setting 3). Since the lysolecithin used was prepared commercially by treatment of egg white lecithin with phospholipase A, it was important to establish its purity. The lysolecithin migrated as a single spot in two thin layer chromatography systems. The adsorbant used was Silica Gel II. The solvent for one system was chloroform-methanol-acetic acid-water, 20.8:8:2.8:1.2 and the solvent for the second system was chloroform-methanol-40% aqueous methylamine-water, 18.2:9.8:1.4:1.4. The Rf values observed were similar to those reported for lysolecithin in the two systems (8). The concentration of the lipids was determined from the phosphorous content of ashed samples (9).

In the presence of BH₄, high concentrations of 1-propanol (1 M) stimulated phenylalanine hydroxylase several-fold. Since it was found that butanol was three times more effective than propanol, we studied compounds containing longer carbon chains. Fatty acids containing 14 carbons or less did not stimulate. Fatty acids containing 16 carbons or more were more effective than butanol in stimulating phenylalanine hydroxylase. The greatest stimulation was observed with phospholipids. Lysophosphatidylserine was the most active compounds tested.

Table I (Experiment 2) shows that low levels of lysolecithin and lysophosphatidylethanolamine stimulated phenylalanine hydroxylase about 20-fold when the enzyme was saturated with its substrates (phenylalanine, BH₄, and oxygen). Lysolecithin stimulation was studied in greater detail because this compound is more readily available than lysophosphatidylethanolamine. Lysolecithin produced detectable activation at 0.01 mM and full activation at 0.15 mM (Fig. 1). Lysolecithin obtained from two different commercial sources gave nearly the same degree of stimulation for a given concentration of the lipid. The data in Table I (Experiment 1) also demonstrate that at high concentrations, phosphatidylethanolamine and sphingomyelin also stimulated the hydroxylase. Phosphatidylethanolamine and lysophosphatidylethanolamine had no effect. Egg white lecithin was highly inhibitory and this inhibition was overcome by lysolecithin. In the presence of lecithin, however, a higher concentration of lysolecithin was required to give full activation. It should be mentioned that lysolecithin cannot substitute for the phenylalanine hydroxylase-stimulating protein that was recently described (10). Indeed, a synergistic effect is observed when phenylalanine hydroxylase-stimulating protein and lysolecithin are both added to the hydroxylase.²

**Table I**

_Effect of phospholipids on activity of phenylalanine hydroxylase_

The cofactor was BH₄ at a concentration of 0.03 mM and the phenylalanine concentration was 1.0 mM. In Experiment 1 the rates were determined from the production of tyrosine in a 4 min incubation. In Experiment 2 the initial rates were measured by the continuous spectrophotometric assay for TPNH oxidation.

| Phospholipid          | Concentration (mM) | Enzymatic rate (μmol/min) |
|-----------------------|--------------------|---------------------------|
| **Experiment 1**      |                    |                           |
| None                  | 0.16               | 0.4                       |
| Lysolecithin²         | 0.03               | 1.2                       |
| Lecithin              | 2.0                | 90                        |
| Phosphatidylethanolamine| 3.2               | 1.1                       |
| **Experiment 2**      |                    |                           |
| None                  | 0.05               | 4.0                       |
| Lysolecithin²         | 0.016              | 3.5                       |

² From Sigma Chemical Company, St. Louis, Mo.
³ From General Biochemicals, Chagrin Falls, Ohio.
⁴ From Supelco Inc., Bellefonte, Pa.

² D. B. Fisher and S. Kaufman, unpublished observations.
Fig. 2 (left and center). a, phenylalanine hydroxylase activity as a function of phenylalanine concentration with and without 1 mM lysolecithin. The cofactor was BH₄ at 0.06 mM. b, the reciprocal of the velocities obtained with lysolecithin depicted in a were plotted versus the reciprocal of the phenylalanine concentrations.

Fig. 2a shows that the saturation curve for phenylalanine was sigmoidal when BH₄ was used as cofactor (as previously reported (2)). Lysolecithin converted the kinetics for phenylalanine saturation to the hyperbolic type with substrate inhibition above 0.1 mM phenylalanine (Fig. 2b). Lysolecithin stimulated the maximum velocity 50-fold and reduced the Kₘ for phenylalanine from 0.3 to 0.2 mM. At rat serum levels of phenylalanine, 0.075 ± 0.0075 mM (n = 30), lysolecithin stimulated the rate of phenylalanine hydroxylation over 100-fold when the natural cofactor was employed.

When the synthetic pterin, DMPH₄, was used as the cofactor, lysolecithin increased the maximum velocity only 1.15-fold (Fig. 3). Under these conditions, lysolecithin decreased the Kₘ for phenylalanine from 1.3 to 0.8 mM. A comparison of the results in Fig. 2 with those of Fig. 3 shows that without lysolecithin the rate of hydroxylation in the presence of DMPH₄ was 12 times the rate obtained with BH₄, but with lysolecithin the rate of hydroxylation was four times faster with BH₄ than with DMPH₄. We also studied the effect of lecithin in the presence of DMPH₄. With this synthetic cofactor even 5 mM lecithin did not inhibit the hydroxylase. Therefore, both the activation by lysolecithin and inhibition by lecithin occur only with the natural cofactor BH₄.

It is known that a large number of enzymes can be activated by phospholipids (11, 12). The mechanism of activation, however, is obscure. Since we have recently found that surfactants, such as sodium dodecyl sulfate, can also activate phenylalanine hydroxylase, the activation by phospholipids of this enzyme is probably related to their detergent properties. It should be noted that, in contrast to the effect of phospholipids, the activation by SDS is seen only within a narrow concentration range; the maximum stimulation requires three times more SDS than phospholipid, and concentrations of SDS greater than 0.5 mM inactivate the enzyme.

Our results demonstrate that two naturally occurring phospholipids, lysolecithin and lecithin, are remarkably effective activators and inhibitors, respectively, of rat liver phenylalanine hydroxylase and suggest the possibility that these lipids might regulate the in vivo activity of the hydroxylase. Since 2% of liver phospholipids is lysolecithin (13), its concentration in this tissue (assuming uniform distribution within the liver cell) would be 3 mM. Even if only a few per cent of the total lysolecithin were available to the hydroxylase, therefore, it could lead to marked activation (see Fig. 1). On the other hand, the inhibitor, lecithin, is present in much higher concentrations (about 50% of the total liver phospholipids (13)). The critical factor that might determine the physiological outcome of these opposing effects (i.e., activation by lysolecithin and inhibition by lecithin) is phospholipase A and an enzyme present in liver (14) which can catalyze the conversion of the inhibitor, lecithin, to the activator, lysolecithin.

The large activation of phenylalanine hydroxylase by lysolecithin may find important applications. The activation could facilitate the detection of the hydroxylase in tissues where it has not been found previously. It should also permit a more accurate assessment of the extent of the hydroxylase deficiency in phenylketonuria. If more sensitive assays in the presence of lysolecithin can detect hydroxylase activity in livers from phenylketonuric patients, the possibility would then be opened for in vivo enhancement of this residual activity with lysolecithin administration. Studies are underway to explore some of these possibilities and also to determine whether lysolecithin, or a related compound, plays a similar role in activating the other two pterin-dependent hydroxylases, tyrosine and tryptophan hydroxylases.

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