Two Apparent Molecular Weight Forms of Human and Monkey Phenylalanine Hydroxylase Are Due to Phosphorylation

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Two-dimensional polyacrylamide gel analyses of purified human and monkey liver phenylalanine hydroxylase reveal that the enzyme consists of two different apparent molecular weight forms of polypeptide, designated H (Mr = 50,000) and L (Mr = 49,000), each containing three isoelectric forms. The two apparent molecular weight forms, H and L, represent the phosphorylated and dephosphorylated forms of phenylalanine hydroxylase, respectively. After incubation of purified human and monkey liver enzyme with purified cAMP-dependent protein kinase and [γ-32P]ATP, only the H forms contained 32P. Treatment with alkaline phosphatase converted the phenylalanine hydroxylase H forms to the L forms. The L forms but not the H forms could be phosphorylated on nitrocellulose paper after electrophoretic transfer from two-dimensional gels. Phosphorylation and dephosphorylation of human liver phenylalanine hydroxylase is not accompanied by significant changes in tetrahydrobiopterin-dependent enzyme activity. Peptide mapping and acid hydrolysis confirm that the apparent molecular weight heterogeneity (and charge shift to a more acidic pI) in human and monkey liver enzyme results from phosphorylation of a single serine residue. However, phosphorylation by the catalytic subunit of cAMP-dependent protein kinase does not account for the multiple charge heterogeneity of human and monkey liver phenylalanine hydroxylase.

Mammalian liver phenylalanine hydroxylase (phenylalanine 4-monooxygenase, EC 1.14.16.1) catalyzes the rate-limiting step in the hepatic conversion of phenylalanine to tyrosine (for reviews see Refs. 1–3). Purified phenylalanine hydroxylase from rat (4), human, and monkey (5) livers has two different molecular weight polypeptides, designated H (apparent Mr = 50,000) and L (apparent Mr = 49,000), as revealed by polyacrylamide gel electrophoresis in the presence of SDS. In addition to the molecular weight heterogeneity, two-dimensional polyacrylamide gel electrophoresis of human and monkey liver enzymes demonstrates that each apparent molecular weight class consists of three major isoelectric forms (6, 7). We have investigated the multiple forms of phenylalanine hydroxylase polypeptides to determine whether the polypeptides derive from post-translational modification of a single gene or from different genes. Previously, the L form was considered to be a degradative product of the H form, since preparations of the human enzyme which were low in activity contained more of the L apparent molecular weight class of polypeptides (6). However, recent evidence from this laboratory has demonstrated that the two apparent molecular weight forms of rat liver phenylalanine hydroxylase are encoded by two different mRNAs and hence may be products of different genes (8).

Phenylalanine hydroxylase can be phosphorylated (9) and it has been suggested that differences in the extent of phosphorylation may be responsible for the chromatographic heterogeneity of the enzyme on calcium phosphate (10, 11). In this paper we examine the influence of phosphorylation and dephosphorylation on the two-dimensional polyacrylamide gel electrophoretic patterns of human and monkey phenylalanine hydroxylase and demonstrate that the higher apparent molecular weight forms are phosphorylated and that the lower apparent molecular weight forms can be converted to the higher apparent molecular weight form by phosphorylation, but this change is not accompanied by significant alteration of enzyme activity.

EXPERIMENTAL PROCEDURES

Protein Purification—The catalytic subunit of cAMP-dependent protein kinase, referred to as protein kinase, was isolated from bovine heart muscle by the procedure of Sudgen et al. (12).

Human and monkey liver phenylalanine hydroxylase were purified from crude liver extracts by affinity chromatography using a monoclonal antibody (PH1-1) immunoaffinity column (13). The recovery of human and monkey enzyme in three recent preparations for each was 41, 59, and 52% and 50, 63, and 62%, respectively. The yield of human enzyme varied from 0.53 to 0.90 mg/20 g of liver in 3 recent experiments. The purified enzymes were approximately 95% homogeneous based on densitometric scans of stained patterns obtained from analytical SDS-polyacrylamide gel electrophoresis.

Phosphorylation of Phenylalanine Hydroxylase—The phosphorylation of phenylalanine hydroxylase by the protein kinase was carried out at 30°C in a reaction mixture (0.1 ml) containing 10 mM Tris-HCl, pH 7.6, 10% (v/v) glycerol, 0.1 mM EDTA, 10 mM magnesium acetate, 0.1% (v/v) 2-mercaptoethanol, 0.1 mM [γ-32P]ATP (4000 cpm/pmol), and 18.5 μg of protein kinase. From the reaction mixture, 5-μl aliquots were withdrawn, placed on phosphocellulose ion exchange filter paper squares, and washed as described previously (14). 32P incorporation into protein was determined by liquid scintillation counting. The stoichiometry of the acid-stable phosphorylation was calculated using a subunit molecular weight of 50,000 for phenylalanine hydroxylase. When activity measurements were to be made after phosphorylation, 2-mercaptoethanol was omitted from the reaction mixture, 0.1 mM ATP replaced radioactive ATP, and protein kinase was reduced to 7.5 μg.

The products of the phosphorylation reactions were isolated either by precipitation with 7% (w/v) trichloroacetic acid at 4°C or by immunoprecipitation, essentially as described previously (8).
Enzymatic Dephosphorylation of Phenylalanine Hydroxylase—Preparations of phenylalanine hydroxylase (10 μg) were incubated at 30°C with 0.4 unit of calf intestinal alkaline phosphatase (Boehringer Mannheim, Grade I suspension), 1 mM l-lysine, 0.3 mM MgCl₂, 0.1 mM phenylalanine in the presence of 0.03 mM phenylmethylsulfonyl fluoride and 0.03 mM ε-aminocaproic acid. The control samples were incubated without alkaline phosphatase. After 30 min, the reaction was stopped in the presence of 10 mM phenylalanine, and aliquots (5 μg) were removed for two-dimensional gel analysis (see text below), and the remainder was purified from alkaline phosphatase using the PH-1 immunoaffinity column (13). The phenylalanine hydroxylase was concentrated and phosphorylated to 30°C by the protein kinase as described above, and the stoichiometry of acid-stable radioactive incorporation of phosphate into purified phenylalanine hydroxylase was measured.

When activity measurements were to be made after dephosphorylation l-lysine and phenylalanine were omitted.

Enzyme Assays—Phenylalanine hydroxylase activity, after phosphorylation in the presence of protein kinase as above but replacing radioactive phosphate with 100 μM cold ATP or dephosphorylation in the presence of alkaline phosphatase as described above, was measured by following the conversion of [¹⁴C]phenylalanine to [¹⁴C]tyrosine at 25°C as described (6).

Gel Electrophoresis—One-dimensional electrophoresis was as described (8), but gels were run at constant current of 20 mA for 4 h.

Two-dimensional isoelectric focusing/SDS-polyacrylamide gel electrophoresis was performed as described (13). Isoelectric focusing was carried out at room temperature at a constant voltage (450 V) for 16 h followed by a further 800 V for 1 h. Second dimension SDS gels were 9% (w/v) polyacrylamide containing 0.1% (w/v) SDS (15). Electrophoresis was at a constant current of 25 mA for 5–6 h in 0.1% (w/v) SDS running buffer (16). All gel patterns are shown with the basic end of the isoelectric focusing dimension at the left and the lower molecular weight region of the second dimension at the bottom. For the location of ³²P-polypeptides, gels were autoradiographed after staining with 0.05% (w/v) Coomassie Brilliant Blue in methanol/water/acetic acid, 8:1:1 (v/v), and vacuum drying. The dried gels were then exposed to Kodak XAR-5 film using a DuPont Lightning Plus intensifying screen.

The phosphorylation of phenylalanine hydroxylase polypeptides on nitrocellulose paper by the protein kinase was carried out under the same conditions as described above. After 30 min of incubation, the ³²P-labeled nitrocellulose papers were washed 3 times and stored overnight in 75 mM phosphoric acid. The washed nitrocellulose papers were then air-dried and autoradiographed with Kodak XAR-5 film using a DuPont Lightning Plus intensifying screen. The upper and lower apparent molecular weight forms of the phenylalanine hydroxylase were located using rabbit anti-rat liver phenylalanine hydroxylase serum and ¹²⁵I-protein A after decay of ³²P as described below. The phosphorylated nitrocellulose papers were incubated overnight at room temperature in 3 ml of 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 3% (w/v) serum albumin containing 25 μl of rabbit anti-rat liver phenylalanine hydroxylase serum. The papers were then washed at room temperature once with 50 ml of 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, twice with 50 ml of 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.2% (v/v) Nonidet P-40 on a gyratory shaker, and once with 50 ml of 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl. The washed nitrocellulose papers were then incubated for 60 min at room temperature with 3 ml of 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 3% (w/v) serum albumin containing 0.5 μCi/ml (3.7 × 10⁶ cpm/ng) of ¹²⁵I-protein A (Amersham International).

The phosphorylated nitrocellulose papers were washed as described above, air dried, and autoradiographed.

Phosphoamino Acid Analysis—[³²P]Phenylalanine hydroxylase was phosphorylated in the presence of protein kinase as above but replacing radioactive phosphate with 100 μM cold ATP or dephosphorylation in the presence of alkaline phosphatase as described above. After dephosphorylation, aliquots (5 μg) were removed for two-dimensional gel analysis (see text below), and the remainder was purified from alkaline phosphatase using the PH-1 immunoaffinity column (13). The phenylalanine hydroxylase was concentrated and phosphorylated to 30°C by the protein kinase as described above, and the stoichiometry of acid-stable radioactive incorporation of phosphate into purified phenylalanine hydroxylase was measured.

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RESULTS

Two-dimensional Gel Pattern of Human and Monkey Phenylalanine Hydroxylase—The two-dimensional gel pattern of both purified human and monkey liver phenylalanine hydroxylase exhibited two apparent molecular weight forms, each of which contained three isoelectric forms (Fig. 1, thin layer plates (Merck) using electrophoresis and chromatography at pH 1.9 (pyridine:acetic acid:water, 2:20:978 (v/v)) in the first dimension and chromatography (1-butanol:pyridine:acetic acid:water, 15:10:3:12 (v/v)) in the second dimension.

Phosphopeptide Analysis—[32P]Phenylalanine hydroxylase (10 µg) was dissolved in 0.1 ml of 0.1 M NH₄HCO₃ and incubated with 0.5 µg of trypsin (toxophenylalanyl chloromethyl ketone treated; Boehringer Australia) for 16 h at 30°C. Peptide were separated on cellulose thin layer plates (Merck) using electrophoresis (1000 V for 25 min) at pH 1.9 (pyridine:acetic acid:water, 2:20:978 (v/v)) in the first dimension and chromatography (1-butanol:pyridine:acetic acid:water, 15:10:3:12 (v/v)) in the second dimension.

Phosphorylation of Phenylalanine Hydroxylase by the Protein Kinase—When the purified human and monkey liver enzyme were incubated with [γ-32P]ATP and Mg²⁺ in the presence of the protein kinase the 32P-polypeptides co-migrated with the H form polypeptides (apparent Mᵣ = 50,000). Each of the charge species of the H form was labeled with 32P (Fig. 1, C and D). There was no 32P associated with the L form polypeptides (apparent Mᵣ = 49,000). No detectable alteration in electrophoretic mobility and no incorporation of 32P occurred when the protein kinase, ATP or Mg²⁺, was omitted as a control.

Phosphatase Treatment of Phenylalanine Hydroxylase—Since only the H forms incorporated 32P it was of interest to determine if the H forms could be converted to the L forms by dephosphorylation. Incubation of human liver enzyme with calf-intestinal alkaline phosphatase for 30 min at 30°C caused a marked reduction in the amount of H form (apparent Mᵣ = 50,000) polypeptides and a corresponding increase in the L form (apparent Mᵣ = 49,000) polypeptides (Fig. 2A). No new isoelectric forms were generated by the phosphatase. Incubation in the absence of phosphatase did not alter the polypeptide pattern (Fig. 2B). Addition of 32P human phenylalanine hydroxylase in the presence of phosphatase (data not shown). These results indicate that the H form (apparent Mᵣ = 50,000) represents the phosphorylated form of phenylalanine hydroxylase.

Phosphorylation of Phenylalanine Hydroxylase Transferred to Nitrocellulose—The treatment of phenylalanine hydroxylase with alkaline phosphatase converted it from the H form (apparent Mᵣ = 50,000) to the L form (Mᵣ = 49,000). In order to investigate this further we examined directly the capacity of the L form of phenylalanine hydroxylase to act as a substrate for the protein kinase. Both human and monkey liver phenylalanine hydroxylase were separated into their different apparent molecular forms by two-dimensional gel electrophoresis and transferred onto nitrocellulose. After incubation of the nitrocellulose bound protein with the protein kinase and [γ-32P]ATP, only the L form of the phenylalanine hydroxylase was phosphorylated (Fig. 3A). No 32P was associated with the H form polypeptides (apparent Mᵣ = 50,000). The location of the L and H forms of the enzyme was determined by probing the nitrocellulose filters with rabbit anti-phenylalanine hydroxylase serum (6) and IBI-protein A (Fig. 3, C and D) after decay of the 32P. The protein kinase phosphorylated each of the different charged forms of the L

### Table I

| Apparent molecular weight class | Human isoelectric point | Monkey isoelectric point |
|--------------------------------|-------------------------|--------------------------|
|                                | %                       |                          |
| 49,000                         | 6.5 (50)                | 6.35 (45)                |
|                                | 6.4 (34)                | 6.25 (31)                |
|                                | 6.2 (16)                | 6.15 (22)                |
| 50,000                         | 6.3 (45)                | 6.2 (43)                 |
|                                | 6.2 (33)                | 6.1 (33)                 |
|                                | 6.1 (22)                | 6.05 (22)                |

*p values were determined as described under “Experimental Procedures.”

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2 Preliminary pI values were assigned to the different isoelectric forms by determining the pH gradient established under the isoelectric focusing conditions used (see “Experimental Procedures”). These values indicate the isoelectric point at which the proteins would focus at equilibrium.
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FIG. 3. Phosphorylation of phenylalanine hydroxylase on nitrocellulose. Human and monkey liver phenylalanine hydroxylase was subjected to two-dimensional gel analysis and electrophoretically transferred to nitrocellulose. Human liver (A) and monkey liver (B) enzymes were phosphorylated by the catalytic subunit, and the 32P-polypeptide species were detected by autoradiography. After decay of 32P, the nitrocellulose filters were probed with rabbit anti-phenylalanine hydroxylase sera and 125I-protein A. C, autoradiogram of human liver phenylalanine hydroxylase; D, autoradiogram of monkey liver phenylalanine hydroxylase. IEF, isoelectric focusing.

FIG. 4. Stoichiometry of phosphorylation. Preparations of human and monkey liver phenylalanine hydroxylase incubated in the absence of alkaline phosphatase (■—■, human; ■—■, monkey) or in the presence of phosphatase (□—□, human; ○—○, monkey) were phosphorylated by the cAMP-dependent protein kinase as described under "Experimental Procedures." The progress of the reaction was monitored by the withdrawal of aliquots (5 μl of reaction mixture containing 100 μg/ml of protein) at intervals, and the 32P incorporated was determined as described under "Experimental Procedures."
tein kinase phosphorylates a single serine residue in both human and monkey phenylalanine hydroxylase.

Activity of Human Liver Phenylalanine Hydroxylase—It was of interest to test the influence of phosphorylation and human phenylalanine hydroxylase activity since studies have shown that the rat counterpart is activated by phosphorylation (9–11). We have found no reproducible stimulation of human phenylalanine hydroxylase activity following phosphorylation when tested over a range of tetrahydrobiopterin cofactor concentrations (0.1–50 μM). There was a small decrease in phenylalanine hydroxylase activity (26%) with removal of phosphate by alkaline phosphatase treatment.

DISCUSSION

The results presented here clearly demonstrate that the L form (apparent \( M_\text{r} = 49,000 \)) and H form (apparent \( M_\text{r} = 50,000 \)) of human and monkey phenylalanine hydroxylase represent the dephosphorylated and phosphorylated forms of the enzyme, respectively. The H and L forms can be interconverted with cAMP-dependent protein kinase and alkaline phosphatase. The difference in SDS gel mobility leading to the apparent shift in molecular weight results from the incorporation of a single phosphate. The molecular basis of this effect is not known although it has been observed in several systems by others (18–20). The effect of phosphorylation on mobility in SDS is opposite to that expected for the simple addition of two negative charges. One interpretation of the results is that phosphorylation leads to a reduction in the amount of SDS bound by the enzyme and thereby reduces its electrophoretic mobility. In addition to the apparent molecular weight change, the phosphorylation of the phenylalanine hydroxylase was also associated with a charge shift to a more acidic pl on two-dimensional gels. Similar changes in the pl following phosphorylation have been observed by others (16, 18, 21, 22). Phosphorylation of human and monkey phenylalanine hydroxylase on a single serine residue by the cAMP-dependent protein kinase is analogous to the findings for rat liver phenylalanine hydroxylase (9, 23).

The mechanism generating the two apparent molecular weight forms in primate phenylalanine hydroxylase should be contrasted with that found with the rat enzyme. All three enzyme preparations show two apparent molecular weight forms of which the lower molecular weight forms were previously assumed to be due to proteolysis (6). In this paper we demonstrate that the two molecular weight forms of human and monkey phenylalanine hydroxylase result from protein phosphorylation. In the rat, however, we have previously demonstrated that the two molecular weight forms are iso-

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FIG. 5. Phosphopeptide map of \([^{32}P]\)phenylalanine hydroxylase. Human (A) and monkey (B) liver enzymes were phosphorylated by the protein kinase, and samples were treated for two-dimensional peptide mapping as described under "Experimental Procedures." The origins are represented by O.

enzymes encoded by different mRNAs (8). More recent analysis has revealed that the two proteins of different apparent molecular weight are coded by allelic genes (24). Preliminary results indicate that both of these forms of the rat phenylalanine hydroxylase can be phosphorylated.3

Our finding that phosphorylation of human phenylalanine hydroxylase activity was not altered by protein phosphorylation was surprising in the light of the results obtained with the rat enzyme (8–11). This negative result, however, does not exclude the possibility that under different assay conditions or in vivo, phosphorylation may have an influence.

In contrast to the results obtained here, Abita et al. (25) reported that purified human phenylalanine hydroxylase was not phosphorylated by the cAMP-dependent protein kinase. There are major differences in the purification procedures used by Abita et al. (25) and those used here. However, it is not clear why the form isolated by these workers could not be phosphorylated. One possibility is that there are allelic forms (24) that differ in their capacity to act as substrates. Preliminary amino acid analysis of the preparations used in the study indicate that the level of methionine is lower than the 10.8 residues reported by Abita et al. (25). Furthermore, treatment of the human phenylalanine hydroxylase with cyanogen bromide resulted in only 4 peptides as indicated by SDS-gel electrophoresis.4

The approach used in this study of testing the capacity of phenylalanine hydroxylase to act as a substrate for the protein kinase following transfer from a denaturing slab gel to nitrocellulose paper was unexpectedly successful. Although immunological identification of transferred proteins is regularly used, the use of a protein kinase as a probe to screen nitrocellulose bound protein has not been reported previously. We expect this approach will be applicable to other proteins but should be cautiously applied since the method may mask phosphorylation sites or alternatively expose sites that are not normally phosphorylated. In the experiments reported here no new sites were exposed on the enzyme bound to the nitrocellulose; otherwise both molecular weight forms of the enzyme would have been phosphorylated.

Our results demonstrate that the multiple charged forms of both apparent molecular weight forms (H and L) do not result from charge heterogeneity following phosphorylation by the cAMP-dependent protein kinase. The molecular basis of the different charged forms of human and primate phenylalanine hydroxylase remains to be determined.

3 S. C. Smith, unpublished results.
4 S. C. Smith, and W. J. McAdam, unpublished results.
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REFERENCES
1. Goodwin, B. L. (1979) in Aromatic Amino Acid Hydroxylases and Mental Disease (Youdim, M. B. H., ed) pp. 5–76, John Wiley and Sons, New York.
2. Kaufman, S. (1977) Adv. Neurochem. 12, 1–132.
3. Cotton, R. G. H. (1977) Int. J. Biochem. 8, 333–341.
4. Kaufman, S., and Fisher, D. B. (1970) J. Biol. Chem. 245, 4745–4750.
5. Cotton, R. G. H., and Danks, D. M. (1976) Nature (Lond.) 260, 63–64.
6. Choo, K. H., Cotton, R. G. H., Danks, D. M., and Jennings, I. G. (1979) Biochem. J. 181, 285–294.
7. Cotton, R. G. H., Jennings, I. G., Choo, K. H., and Fowler, K. (1980) Biochem. J. 191, 777–783.
8. Mercer, J. F. B., Hunt, S. M., and Cotton, R. G. H. (1983) J. Biol. Chem. 258, 5854–5857.
9. Abita, J.-P., Milstien, S., Chang, N., and Kaufman, S. (1976) J. Biol. Chem. 251, 5310–5314.
10. Donlon, J., and Kaufman, S. (1977) Biochem. Biophys. Res. Commun. 78, 1011–1017.
11. Donlon, J., and Kaufman, S. (1980) J. Biol. Chem. 255, 2146–2152.
12. Sudgen, P. H., Holladay, L. A., Reimann, E. M., and Corbin, J. D. (1976) Biochem. J. 159, 409–422.
13. Choo, K. H., Jennings, I. G., and Cotton, R. G. H. (1981) Biochem. J. 199, 527–535.
14. Glass, D. B., Masaracchia, R. A., Feramisco, J. R., and Kemp, B. E. (1978) Anal. Biochem. 87, 566–575.
15. Laemmli, U. K. (1970) Nature (Lond.) 227, 680–685.
16. O’Farrell, P. H. (1975) J. Biol. Chem. 250, 4007–4021.
17. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354.
18. Steinberg, R. A., O’Farrell, P. H., Friedrich, U., and Coffino, P. (1977) Cell 10, 381–391.
19. Gershon, R. L., and Krebs, E. G. (1980) J. Biol. Chem. 255, 9375–9379.
20. Lasky, S. R., Jacobs, B. L., and Samuel, C. E. (1982) J. Biol. Chem. 257, 11087–11093.
21. Garrison, J. C., and Wagner, J. D. (1982) J. Biol. Chem. 257, 13135–13143.
22. Drickamer, K., and Mamon, J. F. (1982) J. Biol. Chem. 257, 15156–15161.
23. Wretborn, M., Humble, E., Ragnarsson, U., and Engstrom, L. (1980) Biochem. Biophys. Res. Commun. 93, 403–408.
24. Mercer, J. F. B., Grimes, A., Jennings, I., and Cotton, R. G. H. (1984) Biochem. J. 218, 891–898.
25. Abita, J.-P., Blandin-Savoja, F., and Rey, F. (1983) Biochem. Int. 7, 727–737.