Biosynthesis of "Drosopterins" by an Enzyme System from *Drosophila melanogaster*†

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ABSTRACT: The red eye pigment of *Drosophila melanogaster* consists of six complex pteridines known as neodrosopterin, drosopterin, isodrosopterin, fraction e, and aurodrosopterins (2); these pigments are greatly reduced in the purple mutant. Conditions for biosynthesis of these "drosopterins" are described and compared with those for the synthesis of sepiapterin. The enzymes are contained in a soluble, pteridine-free extract obtained between 40 and 60% saturated ammonium sulfate. The results indicate that sepiapterin synthase consists of two enzymes, the first of which provides a precursor for "drosopterin" biosynthesis. The evidence is (1) the purple mutant, low in accumulated sepiapterin and "drosopterins", is known to have ~10% of the sepiapterin synthase activity of wild type; (2) unlabeled sepiapterin does not cause isotope dilution of "drosopterin" synthesis; (3) the 600*g* pellet prepared from a wild-type head homogenate contains "drosopterin" synthesizing activity and no sepiapterin synthase, yet a heat-labile factor in this fraction stimulates sepiapterin synthesis in the 100000*g* supernatant of wild-type or *pr* flies; (4) sepiapterin and "drosopterin" syntheses require Mg^{2+}; (5) sepiapterin synthesis is stimulated by NADPH; "drosopterin" synthesis responds to either NADPH or NADH. Although "drosopterins" are complex pteridine-type pigments, we have demonstrated their biosynthesis by soluble enzymes. This allows us to consider investigation into the mechanism by which the amounts of these pigments are regulated.

Drosophila provides certain advantages for the study of the biosynthesis of the pteridine eye pigments: first, large amounts of pteridines are found in the eyes of *Drosophila*; second, the fly is a convenient source of enzymes for the study of the synthesis, interconversion, and control of synthesis of pteridines; and, third, a wealth of eye color mutants are associated with the pteridine eye pigments.

The eye pigments [with the possible exception of 7-hydroxypterin (isoxanthopterin[1])] are deposited in protein-containing granules present in the primary and secondary pigment cells of the ommatidia (Shoup, 1966). There are specific granules for the ommochrome pigments and for the pteridine pigments. The presence of the granules may depend upon the synthesis of the pigment (Shoup, 1966).

The major pteridines present in *Drosophila* are the "drosopterins", 7,8-dihydro-6-lactoylpterin (sepiapterin), 6-(L-*erythro*-1′,2′-dihydroxypropyl)pterin (biopterin), isoxanthopterin, and pterin. The structures of the "drosopterins" are not yet firmly established, but it appears that they are formed from two pteridine ring systems (Theobald & Pfleiderer, 1977). The structural interrelationships of "drosopterins" are of interest. Neodrosopterin is known to rearrange to a mixture of drosopterin and isodrosopterin in aqueous solutions (Rokos & Pfleiderer, 1975). Drosopterin and isodrosopterin appear to be closely related in that they have mirror-image circular dichroism spectra (indicating that they are stereoisomers (Rokos & Pfleiderer, 1975), but they are separable by cellulose thin-layer chromatography. Aurodrosopterin also consists of a pair of isomers analogous to drosopterin and isodrosopterin (Rokos & Pfleiderer, 1975). The "drosopterin" designated as fraction e by Schwinck & Mancini (1973) has not been characterized, but its color and R_f values resemble those of neodrosopterin.

It is now becoming accepted that dihydroneopterin triphosphate [H_2-neopterin-$(P)_3$] is the precursor of all pteridines. It is synthesized from GTP by the action of GTP cyclohydrolase 1 (Burg & Brown, 1968). This enzyme was first discovered in *Escherichia coli* and has been shown to be present in several organisms, including *Drosophila* (Fan et al., 1975). GTP cyclohydrolase occurs in two peaks of activity during the development of *Drosophila* (at pupariation and emergence), the latter being restricted to the head of the fly (Fan et al., 1976). Two eye-color mutants, raspberry (*ras*) and prune (*pn*), have been shown to have altered developmental profiles of GTP cyclohydrolase (Evans & Howells, 1978).

Another known pteridine-synthesizing activity from *Drosophila* is sepiapterin synthase (Fan et al., 1975), which catalyzes the conversion of H_2-neopterin-$(P)_3$ to sepiapterin in the presence of Mg^{2+} and NADPH. The role of this enzyme in the synthesis of pteridines is implicated by the purple (*pr*) eye-color mutant. The *pr* mutant accumulates less sepiapterin and "drosopterins" than wild-type flies (Wilson & Jacobson, 1977b). Recently, our laboratory has reported that the *pr* mutant, and the allele pr^{bw}, have approximately 10–30% of the amount of sepiapterin synthase activity present in wild-type flies (Yim et al., 1977). Gene dosage data with pr^+ support the hypothesis that pr^+ is the structural locus for sepiapterin synthase. When *pr* or pr^{bw} is genetically combined with a suppressor mutation, suppressor of sable ($su(s)^2$), the levels of eye pigments accumulated are restored to wild-type levels,

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[1] Abbreviations used: H_2-neopterin-$(P)_3$, 3′-triphosphoester of 7,8-dihydro-6-(D-*erythro*-1′,2′,3′-trihydroxypropyl)pterin; sepiapterin, 7,8-dihydro-6-lactoylpterin; biopterin, 6-(L-*erythro*-1′,2′-dihydroxypropyl)pterin; isoxanthopterin, 7-hydroxypterin; Pipes, 1,4-piperazinediethanesulfonic acid. "Drosopterins" (with the quotation marks) refers to the class of six dipteridine-derived red eye pigments of *Drosophila*, while drosopterin (without the quotation marks) refers to the individual compound. Fraction e refers to the "drosopterin" compound as designated by Schwinck & Mancini (1973).

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as is the sepiapterin synthase activity. Different alleles of *su(s)*[2] have been found to have differing abilities to restore the sepiapterin synthase activity; when sepiapterin synthase activity is restored to approximately 30% of wild-type activity, the accumulation of drosopterins is the same as in wild-type flies (unpublished results).

We have studied "drosopterin" biosynthesis to clarify the role of sepiapterin synthase in the synthesis of these pigments. In this report we describe an in vitro assay for "drosopterin" biosynthesis which utilizes [U-^{14}C]H_2-neopterin-(P)$_3$ as the substrate and present evidence that sepiapterin is not a precursor of "drosopterins".

Materials and Methods

Drosophila melanogaster. Oregon-R flies were reared at 25 ± 1 °C on medium prepared as described by Lewis (1960). Flies were collected daily, so that all were 1 day old or less. After collection the flies were quickly frozen in liquid nitrogen; the heads were obtained as previously described (Wilson & Jacobson, 1977a) and stored in vials in liquid nitrogen for later use. The *pr*^{bw} flies used in these studies were reared and collected as above, but were stored at −80 °C for later use.

Enzyme Extracts; Centrifugal Fractionation. For fractionation by differential centrifugation, *Oregon-R* fly heads were homogenized in tube-and-pestle ground glass homogenizers (Kontes Co.) with 5 volumes of 50 mM Pipes (Calbiochem) buffer at pH 7.0. Grinding was limited to the minimum required to cause the disappearance of visible heads (approximately 10 strokes). The homogenate was centrifuged at 600*g* for 10 min, and the supernatant was saved for preparation of further fractions. The pellet consists of three layers, the uppermost having the uniform red color of the "drosopterins". This red layer was separated with a Pasteur pipet and buffer, resuspended, and centrifuged at 600*g* for 10 min. The pellet was washed again as above and was finally resuspended in an amount of buffer to equal the volume of the supernatant recovered after the 100000*g* centrifugation. This was done so the pellet preparations would contain roughly the same number of fly head equivalents as the 100000*g* supernatant. The first 600*g* supernatant was centrifuged at 15000*g* for 5 min; this pellet was washed twice and resuspended to the same volume as the 600*g* pellet. Then the first 15000*g* supernatant was centrifuged at 100000*g* for 60 min; this pellet was also washed twice and resuspended to the same volume as the 600*g* pellet. The 100000*g* supernatant was used directly. The above preparations were made fresh for each assay. All operations were done at 0–4 °C. The *pr*^{bw} whole-fly 100000*g* supernatant was obtained by homogenization of 1 g of flies in 4 mL of 50 mM Pipes at pH 7.0 with a tube-and-pestle ground-glass homogenizer and centrifugation at 15000*g* for 5 min and then at 100000*g* for 60 min.

Preparation of Pteridine-Free Extract. Since a large amount of "drosopterin" biosynthesis occurs in the 600*g* and 100000*g* pellets, we devised a means of recovering some of this activity in a soluble form. It was found that activity could be extracted from the pellet fractions by use of 50 mM Pipes (pH 7.0) containing 0.5–1.5 M KCl. A pteridine-free preparation was obtained by the following procedure: 2.5 g of *Oregon-R* heads was homogenized in 12.5 mL of 50 mM Pipes (pH 7.0) containing 0.5 M KCl. The homogenate was centrifuged at 15000*g* for 10 min. The supernatant was saved, and the pellet was rehomogenized in and collected from 12.5 mL of the same buffer once, twice with buffer plus 1 M KCl, and twice with buffer plus 1.5 M KCl. The final pellet was discarded, and the proteins from the pooled supernatants were fractionated between 40% and 60% saturated $(NH_4)_2SO_4$. The pellet collected at 15000*g* for 30 min was drained, and dissolved with 1 mL of 50 mM Pipes (pH 7.0) containing 10% glycerol. The solution was applied to a Sephadex G-25 (Pharmacia) column (1.7 × 13 cm) which was eluted with the buffer in which the sample was dissolved at a flow rate of 4–6 mL/h, while 1-mL fractions were collected. The A_{280}-absorbing material eluted in the void volume was pooled and concentrated to 1.5 mL by vacuum dialysis (collodion bag, Schleicher and Schull, 25 000 mol wt cutoff). The resulting preparation had no visible pigment and was stored at −20 °C until use. All operations were carried out at 0–4 °C except the vacuum dialysis, which was at 23 °C.

Preparation of [U-^{14}C]H_2-Neopterin-(P)$_3$ and [3′-^{3}H]$_2$-Neopterin-(P)$_3$. [U-^{14}C]GTP (Amersham) was adjusted to a specific activity of 20 μCi/μmol with unlabeled GTP (sodium salt, Sigma). It was incubated at a concentration of 670 μM with 100 mM Tris-HCl (pH 8.5), 100 mM NaCl, 10 mM EDTA (pH 7.5) and pure GTP cyclohydrolase I prepared from *E. coli* (Yim & Brown, 1976) to produce [U-^{14}C]H_2-neopterin-(P)$_3$. The incubation was carried out in the dark at 42 °C for 90 min. The amount of H_2-neopterin-(P)$_3$ produced was determined from measurements of the release of [^{14}C]-formate by filtration of reaction aliquots through charcoal (Burg & Brown, 1968). Conversion was consistently greater than 95%. [8,5′-^{3}H]GTP (New England Nuclear), adjusted to 45 μCi/μmol, was the source of [3′-^{3}H]H_2-neopterin-(P)$_3$. An aliquot of the GTP cyclohydrolase reaction was used as the source of H_2-neopterin-(P)$_3$ for assays discussed below.

Phosphatase-treated [U-^{14}C]H_2-neopterin-(P)$_3$ was prepared by addition of 0.5 unit of bacterial alkaline phosphatase (Worthington, BAPC) to 30 μL of [U-^{14}C]H_2-neopterin-(P)$_3$ prepared as above and incubated 30 min at 42 °C.

Assay of Sepiapterin and "Drosopterin" Biosynthesis. Sepiapterin and "drosopterin" biosynthesis were determined in a reaction mixture which had a total volume of 70 μL with the following components: 50 mM Pipes (pH 7.5), 143 μM [U-^{14}C]H_2-neopterin-(P)$_3$ (or 131 μM [3′-^{3}H]H_2-neopterin-(P)$_3$), *Drosophila* extract, and cofactors or pteridines depending upon the experiment. Mg^{2+}, when present, was at a concentration of 14.3 mM; NAD^+ (Sigma), $NADP^+$ (Sigma), NADPH (chemically reduced, Sigma), and NADH (chemically reduced, Sigma) were used at concentrations of 2.5 mM. Isotope dilution experiments were done with sepiapterin at a concentration of 4 mM, neodrosopterin at a concentration of 1 A_{500} unit/mL (pH 5.5), and a mixture of drosopterin and isodrosopterin at 2 A_{500} units/mL (pH 5.5). The reactions were incubated in the dark at 42 °C for 60 min, and then 0.015 μmol of sepiapterin and 10 μL of a fresh fly-head extract (0.2 g of *Oregon-R* heads homogenized in 1 mL of 30% ethanol made pH 2 with concentrated HCl, then centrifuged) as a "drosopterin" carrier were added before the reaction tubes were placed in a boiling-water bath for 5 min. After the tubes were heated, 0.5 unit of bacterial alkaline phosphatase (Worthington, BAPC) was added to each, and they were then incubated at 42 °C for 10 min. After the tubes were centrifuged (Brinkmann table-top centrifuge), 40 μL of the resultant supernatant was subjected to two-dimensional cellulose thin-layer chromatography as previously described (Wilson & Jacobson, 1977a,b), except that the ammonium acetate concentration in the first solvent was increased to 2 g/100 mL of solvent. The sepiapterin and individual "drosopterin" spots were scraped into scintillation vials containing 1.5 mL of H_2O, and the amount of radioactivity was determined in a scintillation counter with 15 mL of 0.28% 2,5-bis[2-(5-*tert*-butylbenzoxazolyl)]thiophene (Packard) and

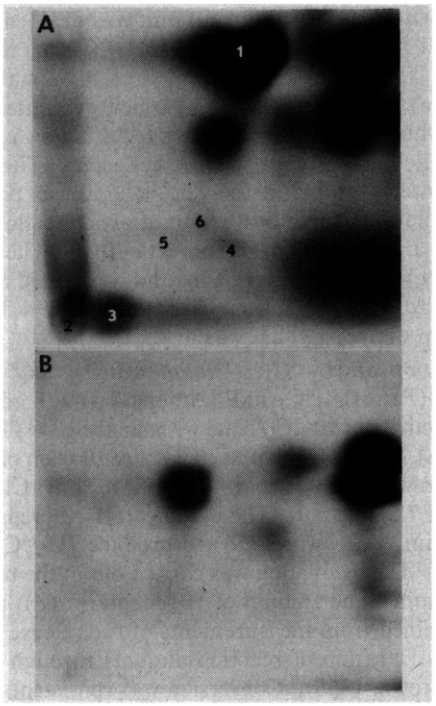

FIGURE 1: Autoradiogram of a thin-layer separation of 40 μL of a reaction which used pteridine-free extract, Mg^{2+}, and NADPH. (1) Sepiapterin; (2) neodrosopterin; (3) fraction e; (4) aurodrosopterins; (5) drosopterin; (6) isodrosopterin.

33% Triton X-100 in toluene. Kodak RP X-ray film was used for autoradiography.

Preparation of Neodrosopterin, Drosopterin, Isodrosopterin, and Sepiapterin. The "drosopterins" were prepared by the methods of Rokos & Pfleiderer (1975) except the initial extract was obtained by homogenizing 10 g of *Oregon-R* heads in 50 mL of 30% ethanol made pH 2 with concentrated HCl. The homogenate was centrifuged at 15000*g* for 10 min. The pellet was reextracted three times with 50 mL of the same solvent each time. The combined extracts were concentrated by rotary evaporation to form the initial extract.

Sepiapterin was prepared by homogenization of 5 g of sepia mutant heads in 25 mL of 30% ethanol made pH 2 with concentrated HCl. The sample was centrifuged at 15000*g* for 10 min, concentrated by rotary evaporation, and applied to a Sephadex G-25 column (1.7 × 40 cm) which was eluted with water. The peak sepiapterin fractions were rotary evaporated to dryness and redissolved in a minimal amount of water. The sample contained a few minor blue fluorescent contaminates as resolved by two-dimensional cellulose thin-layer chromatography.

Results

Figure 1 shows the pattern of radioactive spots obtained when [U-^{14}C]H_2-neopterin-$(P)_3$ was incubated with the pteridine-free extract. The radioactive spots (1–6) correspond in position and shape to sepiapterin and the five visible "drosopterin" spots. If the extract was heated at 100 °C for 5 min before incubation, no radioactive spots were found in these positions. The amount of radioactivity incorporated into the "drosopterin" and sepiapterin spots increased with time and increasing enzyme concentration. Radioactivity from [3′-^{3}H]H_2-neopterin-$(P)_3$ was incorporated into sepiapterin but not the "drosopterins". When [U-^{14}C]H_2-neopterin-$(P)_3$ was incubated with bacterial alkaline phosphatase to produce [U-^{14}C]H_2-neopterin and the latter used as substrate, no radioactivity was incorporated into the six pteridines.

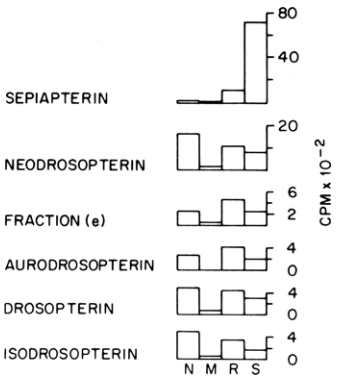

FIGURE 2: Ability of various subcellular fractions prepared as described under Materials and Methods to support sepiapterin and "drosopterin" biosynthesis. Fifteen microliters of each fraction was used in the standard assay. Mg^{2+}, NADPH, $NADP^+$, and NAD^+ were the cofactors used. (N) The 600*g* pellet; (M) 15000*g* pellet; (R) 100000*g* pellet; (S) 100000*g* supernatant.

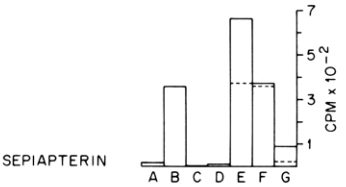

FIGURE 3: Stimulation of sepiapterin synthase in *Oregon-R* head 100000*g* supernatant and pr^{bw} whole-body 100000*g* supernatant by an *Oregon-R* head 600*g* pellet, and inactivation of stimulation by heat treatment of the 600*g* pellet. The assay employed 15 μL of each extract; Mg^{2+}, NADPH, $NADP^+$, and NAD^+ were the cofactors used. (A) *Oregon-R* head 600*g* pellet; (B) *Oregon-R* head 100000*g* supernatant; (C) heat-treated A; (D) pr^{bw} whole-fly 100000*g* supernatant; (E) A + B; (F) C + B; (G) A + D. (—) Obtained; (- - -) additive.

When planning these investigations we were aware of the role of sedimentable granules in *Drosophila* eye pigmentation (Shoup, 1966) and consequently decided to determine the activity for "drosopterin" synthesis in fractions prepared by differential centrifugation of a head homogenate. Sepiapterin and "drosopterin" syntheses appear to be distributed differently in the various centrifugal fractions (Figure 2). The 600*g* pellet contains a red layer that is enriched in the "drosopterins" and shows considerable "drosopterin" synthesizing activity and yet essentially no sepiapterin synthesis. Since Yim et al. (1977) had shown that the *pr* mutant is low in sepiapterin synthase and the "drosopterins", it was assumed that sepiapterin is a precursor of the "drosopterins". To test if sepiapterin synthase was limiting in the 600*g* pellet, a mixture of the pellet and the 100000*g* supernatant was assayed. The effect on "drosopterin" synthesis was additive but, surprisingly, sepiapterin synthase activity was nearly twice the sum of these individual values (Figure 3). Since the stimulatory effect could be eliminated by heating the 600*g* pellet in a boiling water bath for 5 min, we believe the stimulatory effect is due to an enzyme. Krivi and Brown have fractionated sepiapterin synthase into two proteins, one that is Mg^{2+} dependent and one that is NADPH dependent (Brown et al., 1979). If these two proteins represent two steps of sepiapterin synthesis, we propose that only the first is necessary for "drosopterin" synthesis. The 600*g* pellet would contain the enzyme that catalyzes the first step, but not that for the second, while the 100000*g* supernatant would contain both. If the enzyme for the first step is limiting for sepiapterin synthesis in the 100000*g* supernatant, then mixing the 600*g* pellet with the 100000*g* supernatant would result in synergistic synthesis of sepiapterin. Since the pr^{bw} mutant accumulates less "drosopterins" than the wild type and shows lowered sepiapterin synthase levels, the above hypothesis would

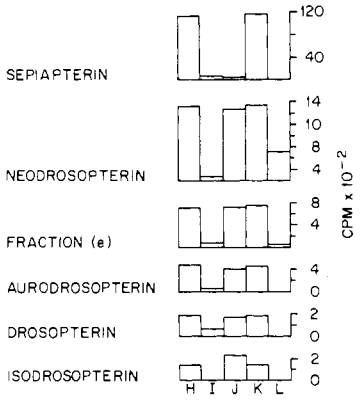

FIGURE 4: Effects of cofactors on sepiapterin and "drosopterin" biosynthesis. Thirty microliters of the pigment-free extract was used in the standard assay. The cofactors present in each reaction are shown. (H) Mg^{2+} + NADPH + NADH; (I) NADPH + NADH; (J) Mg^{2+} + NADH; (K) Mg^{2+} + NADPH; (L) Mg^{2+}.

predict that pr^{bw} is lacking the enzyme for the first step of sepiapterin synthase. When the *Oregon-R* 600*g* pellet was mixed with 100000*g* supernatant from a whole-fly pr^{bw} extract, a synergistic incorporation into sepiapterin was seen, supporting our hypothesis. Further evidence for the common precursor hypothesis came from studies with the pteridine-free extract as a source of enzyme.

When these studies were initiated, the 600*g* pellet and 100000*g* supernatant of crude head extracts were employed. Although "drosopterin" synthesis could be detected, the crude extracts presented several technical problems: storage at −20 °C resulted in loss of activity, large amounts of pteridines present in the crude extract precluded the possibility of performing meaningful isotope dilution experiments, and the presence of various cofactors interfered with determination of cofactor requirements. These difficulties led us to develop the procedure for the preparation of the pteridine-free extract described under Materials and Methods. The pteridine-free extract may be stored for at least 3 weeks at −20 °C without significant loss of activity.

By use of this soluble system, the various cofactor requirements were determined (Figure 4). Sepiapterin synthesis requires both Mg^{2+} and NADPH. When Mg^{2+} and NADH are present in a reaction, only slight conversion of H_2-neopterin-$(P)_3$ to sepiapterin is seen; yet, synthesis of the "drosopterins" occurs at the same level as if NADPH were present. This is evidence that sepiapterin is not a "drosopterin" precursor. When Mg^{2+} is left out of the reaction, however, both sepiapterin and "drosopterin" synthesis are decreased, suggesting that a common enzymatic step exists for the production of both classes of pteridines.

When NADPH and NADH are both left out of a reaction, sepiapterin synthesis is negligible and "drosopterin" synthesis is greatly reduced. Since neodrosopterin synthesis, in contrast to the other "drosopterins", is only reduced by little more than 50% in the absence of NADPH and NADH, we are still investigating the cofactor requirements. A possible reaction scheme in agreement with the above results is shown in Figure 5.

Isotope dilution experiments provide additional support for the common precursor hypothesis. When unlabeled sepiapterin is present during "drosopterin" biosynthesis, no dilution of the isotope entering the "drosopterins" is seen. While it is possible that there is no equilibration between the unlabeled sepiapterin and the enzymatically produced sepiapterin or that sepiapterin is converted by a first-order reaction under the conditions used, the most likely explanation is that sepiapterin is not a precursor of the "drosopterins".

Isotope dilution experiments were also done in an attempt to answer questions about the interconversion of the various "drosopterin" species. No decreases in the amount of radioisotope-labeled "drosopterins" synthesized are seen when either unlabeled neodrosopterin or unlabeled drosopterin and isodrosopterin are added to reactions. Since drosopterin and isodrosopterin can arise nonenzymatically from neodrosopterin in aqueous solutions (Rokos & Pfleiderer, 1975), kinetic considerations could explain the failure of neodrosopterin to dilute the isotope entering drosopterin and isodrosopterin. Because the structural relationship between the two forms of aurodrosopterin is analogous to that between drosopterin and isodrosopterin (Rokos & Pfleiderer, 1975), and because fraction e is similar to neodrosopterin in color and in chromatographic behavior, we are also considering the hypothesis that fraction e nonenzymatically rearranges to the aurodrosopterins. Schwinck & Mancini (1973) have reported that

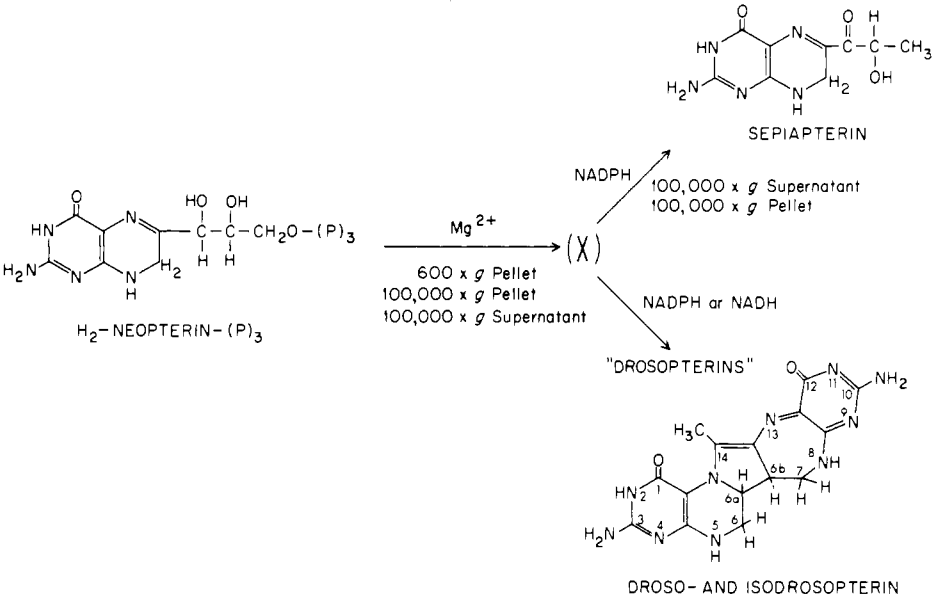

FIGURE 5: Postulated reaction sequence for sepiapterin and "drosopterin" biosynthesis and the differential centrifugation fractions in which the steps are recovered. The structures of drosopterin and isodrosopterin are after Theobald & Pfleiderer (1977).

fraction e and aurodrosopterin act genetically as a group. The pteridine-free extract synthesizes relatively more fraction e and aurodrosopterin than the crude extracts, with neodrosopterin and fraction e being the major products. While this does not reflect the relative amounts of "drosopterins" found in vivo, the possibilities may be raised for the nonenzymatic conversion of neodrosopterin and fraction e to other "drosopterins" in the fly. If such nonenzymatic conversion occurs during extraction of the "drosopterins", the distribution measured after chromatography may not represent the distribution in vivo. We have observed that when there is a delay between stopping the enzymatic reaction and application of the reaction mixture to thin-layer plates, there occurs a lesser amount of neodrosopterin and fraction e and a greater amount of drosopterin and isodrosopterin. The problem of interconversion among the "drosopterins" is currently under investigation.

Discussion

In the past, hypotheses about "drosopterin" biosynthesis have relied upon genetic results and quantitation of the "drosopterins" accumulated in various mutants. Schwinck & Mancini (1973) divided several eye-color mutants into three classes based on the relative amounts of different "drosopterins" resolved by cellulose thin-layer chromatography. They proposed that the biosynthesis and accumulation of different "drosopterins" are under genetic control.

The in vitro assay of "drosopterin" biosynthesis has become possible because of three major developments: (1) H_2-neopterin-$(P)_3$ is recognized as the precursor of pteridines and this precursor is readily produced by use of purified GTP cyclohydrolase from *E. coli* (Yim & Brown, 1976); (2) high resolution, fairly rapid, reproducible separation of pteridines is possible using cellulose thin-layer chromatography (Wilson & Jacobson, 1977a); (3) sepiapterin synthase was discovered (Fan et al., 1975) and its role in "drosopterin" biosynthesis was recognized (Wilson & Jacobson, 1976; Yim et al., 1977).

Several lines of evidence support the idea that "drosopterin" biosynthesis is enzyme mediated: (1) the substrate required is H_2-neopterin-$(P)_3$ and not H_2-neopterin; (2) Mg^{2+} is required; (3) NADPH or NADH greatly stimulates synthesis of "drosopterins"; and (4) the activity is heat labile, precipitable in ammonium sulfate, nondialyzable, and elutes in the void volume of a Sephadex G-25 column.

Both genetic and biochemical evidence support the idea that sepiapterin synthesis and "drosopterin" synthesis are related. In the *pr* mutant "drosopterin" and sepiapterin are greatly reduced in *Drosophila* heads (Wilson & Jacobson, 1977b). This mutant also has low amounts of sepiapterin synthase (Yim et al., 1977). In this report it is shown that, when the 100000*g* supernatant of the *pr* mutant was supplemented with the 600*g* pellet enzymes of wild type, a superadditive rate of sepiapterin synthesis occurred. Since the 600*g* pellet enzymes of wild type make little or no sepiapterin but do make "drosopterins", it seems likely that a common enzyme, found in the 600*g* pellet, is involved in the synthesis of sepiapterin and the "drosopterins". This, along with the finding that adding nonradioactive sepiapterin to the reaction mixture caused no reduction in the rate or amount of "drosopterin" synthesis, indicates that sepiapterin is not a precursor to "drosopterins". Brown et al. (1979) reported that sepiapterin synthase can be separated into two protein fractions both of which are required for sepiapterin production. It would appear that their Mg^{2+}-requiring fraction is the same as that which we found in the 600*g* pellet.

Since it has been proposed previously that pr^+ is the structural locus for sepiapterin synthase (Yim et al., 1977), it is logical to modify this and propose that pr^+ is the structural locus for the enzyme that catalyzes (the Mg^{2+}-requiring step). The effect of gene dosage for pr^+ was examined by comparing sepiapterin synthase activities. A ratio of 2 was found when flies with two doses of pr^+ were compared with flies with one dose, but the ratio was only 1.2, instead of 1.5, when three doses were compared with two doses. This might be expected if pr^+ is the structural locus for the Mg^{2+}-requiring enzyme that synthesizes the precursor, since the possibility exists that with three doses of this enzyme, the second step (NADPH-requiring) becomes limiting.

In a possible scheme for the biosynthesis of "drosopterins", it is necessary to recognize that (1) the 3′ hydrogens of H_2-neopterin-$(P)_3$ do not appear in the final products and (2) there are three carbons less in drosopterin than there are in two molecules of H_2-neopterin-$(P)_3$. The removal of the 3′ hydrogens could be a consequence of (1) the loss of the 3′ carbon or larger portions of the side chain of H_2-neopterin-$(P)_3$, or (2) the oxidation of the 3′ carbon. Since the 3′ hydrogens do appear in sepiapterin, it is necessary to consider such alterations as occurring at some stage subsequent to the production of the common precursor. The mechanism by which the number of carbon atoms is reduced by three in the formation of "drosopterins" must also be sought. Identification and structure determination of the "drosopterin–sepiapterin precursor will shed light on these questions.

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