Purification of 20α-hydroxysteroid dehydrogenase from human erythrocytes

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Summary. 20α-hydroxysteroid dehydrogenase was found in the supernatant after hemolysis of human erythrocytes, and the enzyme was isolated from the membrane-free hemolysate on a DEAE-cellulose column. The NADPH-generating system was essential for the reaction.

It is known that red blood cells contain hydroxysteroid dehydrogenase which is active in several ways. 17β-hydroxysteroid dehydrogenase has been isolated from human red blood cells[3], and it has been shown that the enzyme is utilized to assess the transport of steroids into erythrocytes[4].

On the other hand, the presence of 20α-hydroxysteroid dehydrogenase activity has been described in the blood of several animals including humans[5]. Van der Molen and Groen observed the conversion of progesterone to 20α-hydroxypregn-4-en-3-one when progesterone was incubated in vitro with human whole blood or with an erythrocyte suspension[6]. However, no attempt to purify this enzyme from erythrocytes has succeeded.

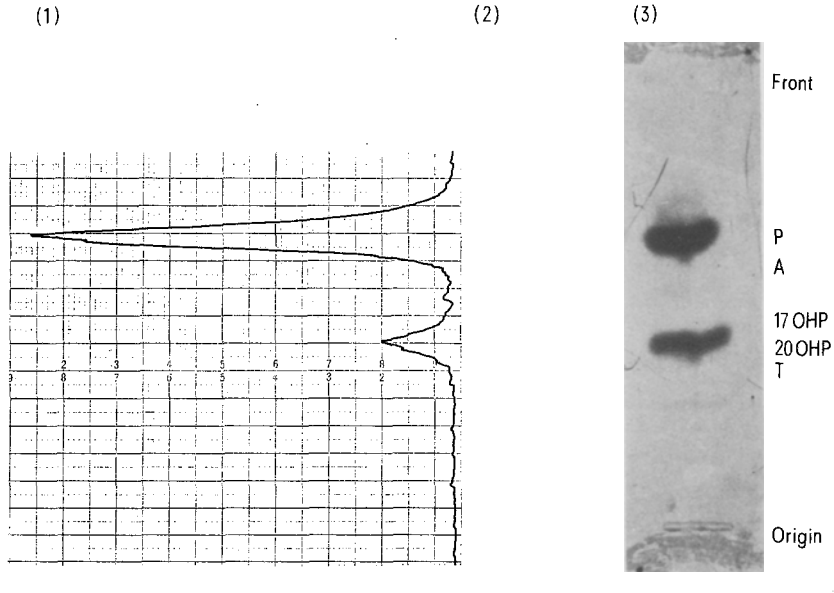
In this report, the conversion of progesterone to 20α-hydroxypregn-4-en-3-one is demonstrated in the hemolysate of human erythrocytes in the presence of an NADPH-generating system. The enzyme activity in the hemolysate was separated from hemoglobin by DEAE-cellulose chromatography.

Methods. Washed packed human red blood cells were hemolyzed by adding ten volumes of 2 mM phosphate buffer (pH 7.0) containing 1 mM EDTA. After dialysis of the hemolysates, they were centrifuged at 10,000 × g for 30 min. The supernatant was put on to a DEAE-cellulose column, which was equilibrated with 2 mM phosphate buffer (pH 7.0). The column was washed with 2 mM phosphate buffer (pH 7.0), and the enzyme was eluted with 100 mM phosphate buffer (pH 7.0). All of the procedures were carried out at 4–7 °C.

The enzyme preparation was incubated with 8 nmoles (0.025 μCi) of progesterone-4-^{14}C in a total volume of 5 ml of 0.1 M phosphate buffer containing 1 mM EDTA, 5 mM $MgCl_2$, NADPH-generating system (1 mM NADP, 10 mM glucose-6-phosphate and 2.5 units glucose-6-phosphate dehydrogenase). The enzymatic reaction was allowed to proceed for 2 h at 37 °C, and was terminated by extraction of the steroids with an excess of methylene chloride. Extraction, separation and elution of the steroids were performed essentially as described previously[7], and the radioactivity of the product, 20α-hydroxypregn-4-en-3-one, was determined. Protein was estimated by the method of Lowry et al.[8]. Hemoglobin concentration was measured by its absorbance at 576 nm[9].

Results and discussion. The 20 α-hydroxysteroid dehydrogenase was found in the stroma-free supernatant from human red cells. The rate of conversion of progesterone into its 20α-hydroxy compound in intact erythrocytes was essentially the same as that in the stroma-free hemolysate from the same amount of cells. The activity in the membrane preparation was very weak and this activity might come from the small portion of supernatant included. From the DEAE-cellulose eluted enzyme fraction, the hemoglobin was almost completely removed. The specific activity had increased to approximately 13-fold as compared with total hemolysate and the final yield of the activity was about 30% (table). This fraction can be stored at either 4 °C or −20 °C in 0.1 M phosphate buffer (pH 7.2) for at least 1 month with no appreciable loss of activity. The figure shows the separation of the reaction product. On the thin-layer chromatogram, the radioactive spots detected by radioautography were compatible with the UV-absorbing spots of 20-hydroxypregn-4-en-3-one and the substrate progesterone, and other metabolites were not detected. In the absence of an NADPH-generating system, no conversion of progesterone to the 20α-hydroxypregn-4-en-3-one by the enzyme fraction was observed. The NADPH-generating system alone did not catalyse conversion of progesterone.

Since it was shown that hemoglobin has oxidereductase-like activity in human erythrocytes[10], it was uncertain



Separation of 20α-hydroxypregn-4-en-3-ones from progesterone by chromatography on a silica gel thin layer plate. *1* Scanning of radioactivity; *2* autoradiogram; *3* the spots under UV-light of the same TLC plate. The marker steroids indicated: P, progesterone; A, androstendione; 17OHP, 17α-hydroxyprogesterone; 20 OHP, 20α-hydroxypregn-4-en-3-one; T, testosterone.

whether the conversion of progesterone was catalyzed by hemoglobin or by a specific enzyme. It was reported that the 20α-hydroxysteroid dehydrogenase activity disappeared from hemolysates during purification of 17β-hydroxysteroid dehydrogenase by $(NH_4)_2SO_4$ precipitation[3]. However, the presence of a specific enzyme for this converting activity is apparent, because no hemoglobin was detected in our enzyme preparation.

Concerning human blood, it has been thought that the steroid metabolic system could have a rather large capacity[6]. Although it is not possible to predict this enzyme activity in blood in vivo quantitatively from the present results, the total activity in this system is considered to be 10–25% of that of human testes[11], and 1–4 times as much as in human placenta[12].

The physiological significance of the presence of 20α-hydroxysteroid dehydrogenase activity in erythrocytes is still not known. In testes, a role for 20α-hydroxysteroid dehydrogenase has been described in the regulation of steroid biosynthesis by the feed-back of products of this enzyme towards progesterone[11]. This steroid dehydrogenase in erythrocytes may have a role in supporting the metabolic regulation of the steroids.

Partial purification of 20α-hydroxysteroid dehydrogenase activity from a human hemolysate fraction

Fraction	Total protein (g)	Total activity*	Specific activity**	Total hemoglobin (μmoles)
Total hemolysate	15.3	115.5	7.55	1.26
Centrifuged supernatant	11.7	137.9	11.8	1.00
membranes	3.74	13.2	3.53	0.29
DEAE-cellulose eluated	0.39	38.2	97.9	<0.000.2

*pmoles 20α-hydroxypregen-4-en-3-ones/min; **pmoles 20α-hydroxypregen-4-en-3-ones/min/g.

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Mating patterns of virgin and inseminated *Drosophila melanogaster* of different alcohol dehydrogenase *(Adh)* genotypes

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Summary. Female choice mating experiments showed virgin female *D. melanogaster* of the 3 *Adh* genotypes chose heterozygous (Adh^F/Adh^S) males most commonly. Inseminated females chose mates randomly, but the likelihood of a female remating was genotypically dependent.

The description of mating patterns in natural populations of *Drosophila* is complicated by a common incidence of multiple inseminations[2–8]. Multiple insemination may be of considerable evolutionary significance as it has the potential to influence the level of genetic variability in a population[3] and may influence components of fitness such as fertility and fecundity[5,9] although this has not been demonstrated in all studies[10,11]. Furthermore, the choice of a 2nd mate may be influenced by previous mating experience[12,13]. These, and other, mating characteristics may contribute to the maintenance of enzyme polymorphisms in *Drosophila*[8,14–16]. This paper reports the results of female choice experiments designed to test the effect of the initial mating choice on subsequent mating behaviour and to compare the mating patterns of Adh phenotypes in the initial and subsequent matings.

Materials and methods. The Adh genotypes of females collected individually from the 'Chateau Tahbilk' vineyard population[17] were determined by cellogel electrophoresis[18]. Homozygous (Adh^F/Adh^F or Adh^S/Adh^S) females were progeny tested and iso-female strains started from females inseminated by males of the same *Adh* genotype as their own. 10 iso-female strains of each homozygote were pooled to generate the 2 pure breeding strains used in this study. Heterozygotes were produced by crossing these strains.

Individual 2–3-day-old virgin females of each genotype were placed with 3 2–3-day-old males, 1 of each genotype, in a 10×2.5 cm cylindrical glass vial. Prior to each trial males of each genotype were marked with 1 of 3 coloured UV-fluorescent dusts, thereby making them visibly distinguishable. The colour used for each genotype was randomized between experiments. A 30-min mating period was allowed. When mating occurred the genotype of the successful male was ascertained and at the cessation of copulation the female was placed in a vial containing standard medium. Inseminated females were held until 7–10 days old and then the mating choice procedure was repeated. 200 virgin females of each genotype were initially tested over a 9-generation period. At 3-generation-intervals 7–10-day-old virgin females were tested as in the original female choice experiment, a total of 45 being tested for each genotype.