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Configurational determination of neopterin from sepia mutant of D. melanogaster.

Quite a large amount of unconjugated pteridines are found in D. melanogaster, and some of these pteridines were observed to be transformed from purine. But it is not fully clarified yet by what pathways these pteridines are synthesized in insects. Recently an enzyme, cyclohydrolase, was reported in D. melanogaster (Fan and Brown 1976) as in E. coli (Burg and Brown 1968) and golden hamster (Fukushima et al. 1975), which catalyzes the formation of dihydro neopterin (2-amino-4-hydroxy-6-trihydroxypropyl-pteridine) triphosphate from GTP. And in insects, neopterin-compound might also be a key intermediate in the formation of pteridine from purine, though neopterin was not found in insects except in honey bees (Rembold and Bushmann 1963).

We have found and isolated neopterin from the sepia mutant of D. melanogaster as reported in DIS 51 (Katoh and Arai 1974). There are four stereoisomers of neopterin: D- and L- erythro-neopterin and D- and L- threo-neopterin (Fig. 1). Neopterin obtained from sepia flies was shown to be erythro- form by paper chromatography (Katoh and Arai 1974). We tried, in this study, to determine the configuration (D- or L-) of neopterin isolated from se flies by using circular dichroism (CD).

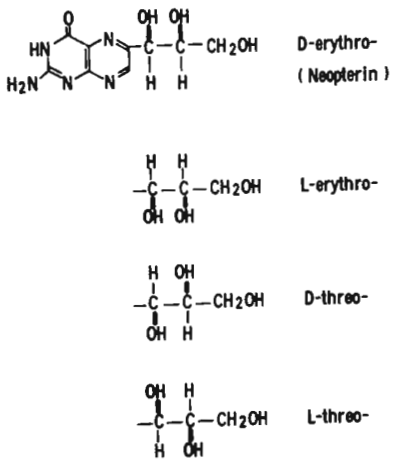

Fig. 1. Four isomers of neopterin.

Neopterin was isolated from se flies in higher purity than in a previous report (Katoh and Arai 1974) by the modified method. 100 g of se flies were extracted with 5 vol of 50% ethanol at 90°C. After the column chromatographies on Ecterola-cellulose (7x30 cm, pH 7.5) and phospho-cellulose (5x48.5 cm), the blue fluorescent fraction mainly containing biopterin was collected and was applied to paper chromatography using 36 sheets of Toyo filter paper No. 50 (40x40 cm) in the ascending method with n-propanol/ethylacetate/water (7:1:2 v/v). The neopterin area was cut off and suspended in distilled water. The extract was then applied to ethanolyzed-cellulose column (3x24 cm) as reported (Katoh and Arai 1974). The neopterin fraction was obtained from the last column chromatography on Sephadex G-25 fine (2x50 cm). About 40 μg of neopterin was finally yielded.

CD measurements were obtained with Automatic Recording Spectropolalymter J-20 (Japan Spectroscopic Co. Ltd.). The measurements were made at 20°C in cells with 5 mm lightpaths on Scale 1. Authentic neopterins (1.4×10^{-4} M) and neopterin from se flies were assayed in final 0.1 N HCl solution. Fig. 2 shows their CD spectra. D-erythro-neopterin has positive bands at 247 nm and 310 nm, and negative bands at 223 nm and 269 nm. On the other hand, L-erythro-neopterin shows the mirror image of the CD pattern of D-erythro- form (Fig. 2). Therefore, neopterin in se flies is determined to be D-erythro-neopterin from this measurement.

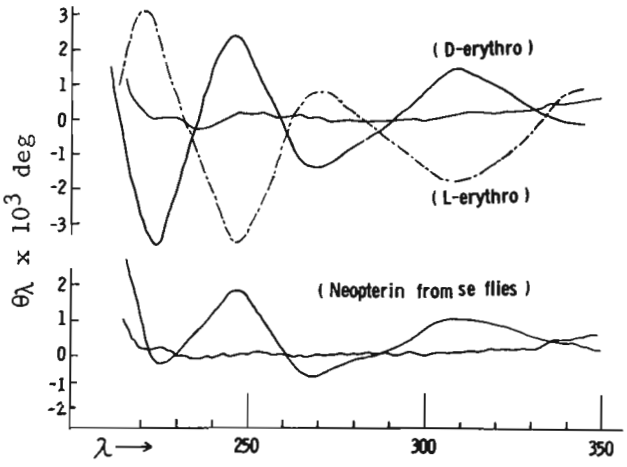

Fig. 2. CD-spectra of authentic D- and L-erythro-neopterin (1.4×10^{-4}M) and of neopterin from se flies. (in 0.1 N HCl, 20°C, 5 mm cell lightpath, scale 1)

L-threo-neopterin besides D-erythro-neopterin is reported in naturally occurring materials. It is the D-erythro- form of dihydroneopterin triphosphate that is synthesized from GTP by cyclohydrolase in D. melanogaster, and this product is further converted to sepiapterin by other enzyme systems (Fan et al. 1975). The finding of D-erythro-neopterin in D. melanogaster in this study really supports that neopterin (D-erythro-dihydroneopterin triphosphate) is an important intermediate when unconjugated pteridines are formed from GTP.

References: Burg, A.W. and B.M. Brown 1968, J. Biol. Chem. 243:2439; Fan, C.L., G.C. Krivi and G.M. Brown 1975, Biochem. Biophys. Res. Commun. 67:1047; Fan, C.L. and G.M. Brown 1976, Biochem. Genet. 14:259; Fukushima, K., I. Eto, D. Saliba and T. Shiota 1975, Biochem. Biophys. Res. Commun. 65:644; Katoh, S. and Y. Arai 1974, DIS 51:70; Rembold, H. and L. Bushmann 1963, Justus Liebigs Annln. Chem. 662:1406.

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The effect of trypsin on "survivability" of imaginal disks of D. melanogaster.

In connection with the works concerning dissociation of imaginal disks subjected to the action of trypsin solution, it was of interest to discover "survivability" of these disks under conditions of different concentrations of trypsin solution. For this purpose leg imaginal disks were chosen from the larvae of D. melanogaster of the "Berlin wild" line at the age of 72 hours and were put into trypsin solutions of different dilutions. 0.25% trypsin solution was used as an initial material to prepare the working solutions of 1:1, 1:2, and 1:8 in physiologic Ringer's solution (NaCl 7.500 g, KCl 0.287 g, $CaCl_2 \cdot H_2O$ 0.287 g, distilled water to 1.0 liter). After 5 minutes' treatment of imaginal disks by trypsin solution at room temperature they were washed for one minute with 20% solution of bull serum prepared on the basis of Ringer's solution. Using the standard techniques of transplantation of imaginal disks (1), the latter were transplanted into larvae of the same age and line. In the control, selected disks of the same age were exposed for 5-10 minutes in Ringer's solution and then transplanted into the larval hosts.

For further analysis only those larvae were used that survived for 4 hours after transplantation, since the mortality in the first hours is presumed to be conditional on the imperfection of surgical techniques (2). The larval hosts with implanted disks were placed in tubes with the standard forage for drosophila (agar, raising, treacle) and kept at room temperature. Part of these larvae were allowed to pupate and imagos emerged. The adult flies were dissected and the presence of disk-implants was established. These disks were examined under a microscope to determine the elements of leg tissue. Results are given in Table 1.

Table 1. "Survivability" of leg imaginal disks after treatment with trypsin solution of different dilution.

Dilution of 0.025% trypsin solution	Number of surviving larvae	Number of adult flies (%)	Number of "survived" disks with regard to imago (%)	Number of "survived" disks with regard to larvae (%)
1:1	48	28.6	0.0	0.0
1:2	114	10.5	8.3	0.9
1:8	337	23.7	56.2	16.0
Control	791	40.0	55.0	22.3

This table shows that the increase of dilution of trypsin solution led to the increase of "survivability" of disks, both with regard to adult flies and with regard to surviving larvae; but at the same time this increase did not yield a concrete result in respect to the number of flies concerning surviving larvae. We are inclined to explain all this by unregistered technical conditions of operation rather than by peculiarities of the given trypsin dilution. In the selected disks we did not discover any significant qualitative difference (for example, appearance of allotypical elements) between the experimental and control groups.

References: (1) Ephrussi, B. and G. Beadle 1936, Amer. Nat. 70:218-225; (2) Shivertaker, L. 1970, DIS 45:188-189.

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Manifestation of mutation singed on the homoeotic limbs, caused by the action of homoeotic mutations Nasobemia and aristapedia at different temperatures.

The mutation singed (ns,1-21.0) (twisted bristles) is manifested unequally on bristles of different sizes. Specifically, its expressivity is more marked on large bristles in comparison with small ones. As a result of the effects of some mutations on the homoeotic structures (1,2,