OPTICAL ROTATIONS OF THE DIASTEREOISOMERS OF dl,L-METHYLENETETRAHYDROFOLATE

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The chromatographic resolution of dl,L-methylene-tetrahydrofolate into the two diastereoisomers has been recently described by Kaufman, Donaldson and Keresztesy (1963). Although these authors referred to the diastereoisomer which they found fully active for the growth of Pediococcus cerevisiae as l,L-methylenetetrahydrofolate, they did not report measurements of optical rotations. Blakley (1963) isolated the methylenetetra-hydrofolate formed by reaction of formaldehyde with enzymically synthesised tetrahydrofolate and found that this product was a substantially pure diastereoisomer since it reacted with an approximately equimolar amount of NADP in the presence of methylenetetrahydrofolate dehydrogenase. This diastereoisomer was found to be dextrorotatory ($[\alpha]_D^{20} = +145°$), i.e., the d,L-isomer. In this communication we present some results which indicate that the enzymically active methylenetetra-hydrofolate obtained by chromatographic resolution of dl,L-methylenetetrahydrofolate is also dextrorotatory.

The resolution of dl,L-methylenetetrahydrofolate was carried out on TEAE-cellulose on lines similar to those described by Kaufman et al. (1963) with a few modifications. TEAE-

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cellulose prepared from DEAE-cellulose by the method of Porath (1957), was equilibrated with 0.4 M ammonium bicarbonate buffer (pH 8.0) and then washed with water to remove excess bicarbonate. The TEAE-cellulose was then equilibrated with 4 mM ammonium bicarbonate buffer (pH 8.0) and the column packed under gravity in a cold room at 2° to 5°. A 0.1 M solution of dl,L-tetrahydrofolate was prepared as described earlier (Ramasastri and Blakley, 1962) and dl,L-methylenetetrahydrofolate was formed in 20 ml. of a reaction mixture containing 200 μmoles of dl,L-tetrahydrofolate, 10 mM tris-HCl buffer (pH 7.4), 50mM formaldehyde and 50 mM mercaptoethanol. After incubating the solution for 15 to 20 minutes at room temperature in darkness, it was cooled to 0° and was immediately applied to the TEAE-cellulose column (3 x 60 cm.). All subsequent operations were carried out at 2° to 5°. Gradient elution was performed by the use of 450 ml. of 4 mM ammonium bicarbonate buffer (pH 8.0) in a closed mixing chamber, and 0.4 M ammonium bicarbonate buffer (pH 8.0) in the reservoir. Both the buffers contained 50 mM mercaptoethanol and 25 mM formaldehyde. Fractions of 10 ml. were collected and the concentration of methylenetetrahydrofolate in them was estimated by measurement of absorbancy at 295 mμ after suitable dilution (for the molar extinction coefficient, see Blakley, 1960). Samples were diluted with 4 mM ammonium bicarbonate buffer containing 50 mM mercaptoethanol and 25 mM formaldehyde.

Significant amounts of methylenetetrahydrofolate appeared in fractions 135 to 195 under the experimental conditions; the pattern of elution is shown in Figure 1. Spectra recorded in a Cary No. 14 spectrophotometer of samples from every fifth tube were typical of 5,10-methylenetetrahydrofolate with

λ_{max} at 295 mμ.

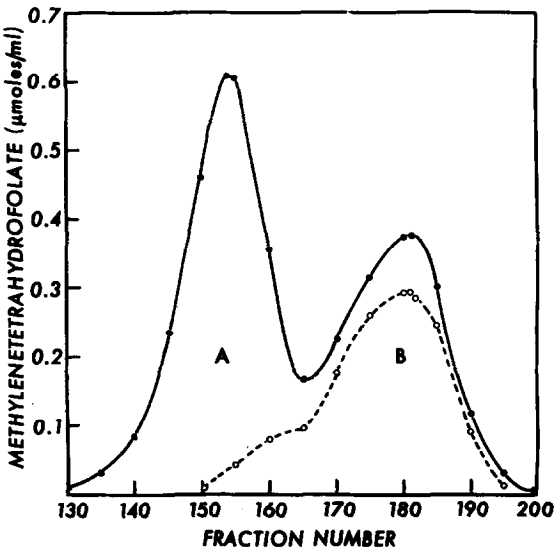

Figure 1. Chromatographic resolution of dl,L-methylenetetrahydrofolate. The experimental details are given in the text. The solid line represents concentration of methylenetetrahydrofolate as calculated from the absorbancy at 295 mμ, and the broken line represents the concentration as measured enzymically (see text).

The concentration of the active isomer of methylenetetrahydrofolate in fraction 150 and later fractions was measured enzymically with methylenetetrahydrofolate dehydrogenase purified from bakers' yeast (Ramasastri and Blakley, 1962) by measurement of methenyltetrahydrofolate formed by acidification of the reaction products and calculation from the molar extinction coefficient at 350 mμ (Ramasastri and Blakley, 1963).

It can be seen (Fig. 1) that the pattern of resolution of dl,L-methylenetetrahydrofolate under the present experimental conditions followed that reported by Kaufman et al. (1963). The compound that is first eluted (peak A) was essentially inactive as substrate for methylenetetrahydrofolate dehydrogenase, and the active diastereoisomer was associated with peak B. The results of enzymic assays showed that the concentration of the active

diastereoisomer of methylenetetrahydrofolate in fraction 170 and later fractions was 80 to 85% of that calculated from the absorbancy.

The optical rotation of material in fractions 153, 181 and 182 was measured at room temperature (23.5°) in a Perkin Elmer photoelectric polarimeter Model 141, which had an accuracy of ± 0.002°. The specific rotations, calculated on the basis of the concentrations derived from the absorbancy measurements and of a molecular weight of 453, were −82° ± 8° for the inactive diastereoisomer of methylenetetrahydrofolate (fraction 153) and +165° ± 11° (fraction 181), and +163° ± 11° (fraction 182) for the active diastereoisomer.

The inactive diastereoisomer appears to be relatively uncontaminated by the active diastereoisomer (Fig. 1). The profile of the elution pattern suggests that it is unlikely that the inactive diastereoisomer is the major contaminant of the active diastereoisomer, particularly since the purity of the latter was constant from fraction 170 onward. The apparent low recovery of the active diastereoisomer, as judged by the enzymic assay, may be due to low assay values due to unfavourable assay conditions (cf. Ramasastri and Blakley, 1963). Such errors would not affect the optical rotation values. Alternatively, some decomposition of the compound not reflected in significant spectrum changes may have occurred. A considerable rate of decomposition of both diastereoisomers, with resultant spectrum changes, was in fact observed when elution was performed with ammonium bicarbonate buffer, pH 9.6, in absence of formaldehyde and mercaptoethanol, but this decomposition progressively decreased the positive rotation of the enzymically active diastereoisomer and the negative rotation of the inactive

diastereoisomer. This implies that if the apparent 80 to 85% purity of the active diastereoisomer is due to decomposition, the true optical rotation of this compound is positive and greater than the mean value of 164° obtained in the present experiment. The present report, therefore, confirms the earlier observation by Blakley (1963) that the active diastereoisomer of methylenetetrahydrofolate is the d,L-isomer.

The two enzymically active tetrahydrofolic acid derivatives for which the optical rotations have been measured so far are 5-formyltetrahydrofolate (Cosulich, Smith and Broquist, 1952) and tetrahydrofolate (Mathews and Huennekens, 1960), the enzymically active diastereoisomers of both of which are reported to be laevorotatory. The reason for the reversal of the optical rotation of tetrahydrofolate on combination with formaldehyde is an interesting problem of stereochemistry.

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