ISOLATION OF AN ADRENAL Δ5→4 3-KETO-CHOLESTENE ISOMERASE

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Talalay et al (1) have isolated a Δ5→4 3-keto-steroid isomerase from Pseudomonas Testosteroni grown on testosterone. The isomerase is active on C19 (Δ^5-androstene 3,17-dione) and C21 (Δ^5-pregnene 3,20-dione) steroid substrates. The potential importance of such an enzyme in steroid hormone biosynthesis led Ewald (2), Kruskemper (3) and their colleagues to partially separate fractions having the C19 and C21 isomerase activity from beef adrenal. Indeed, not only in hormone synthesis, but also in cholesterol general metabolism, a Δ5→4 3-ketoisomerase active on C27 sterol (Δ^5-cholesten-3-one) could be of importance. This paper will describe the isolation of such an enzyme from beef adrenal, in which its presence has already been detected by Ewald (4). Actually, the present work is preliminary to studies on the conformation-activity relationship of enzymes, acting upon steroid substrates and/or on which steroids could act as effectors. For such studies, the Δ5→4-ketosteroid isomerase was chosen, since its activity, at least in bacterias, does not require any other molecule than steroid and protein (1).

Experimental

250 g of beef adrenal cortex are hand homogenized with mortar and pestel in 500 ml of potassium phosphate buffer, 0.3 M, pH 7, at 3°C. The pH and t° are kept constant throughout the isolation procedure. After a 10 minutes centrifugation at 1,450 g, the supernatant is centrifuged 60 minutes at 75,000 g, giving a new supernatant (A 1). The extract of the sediment with 100 ml of the phosphate buffer is centrifuged 10 minutes at 250 g, and the supernatant centrifuged 60 minutes at 75,000 g, giving a new supernatant A 2; the new sediment is treated identically giving finally a supernatant A 3. The A fractions are pooled, dialyzed for 20 hours against a solution of 20% $(NH_4)^2SO_4$, brought to pH 7, and then centrifuged 20 minutes at 30,000 g. The sediment (P) is extracted with 100 ml of potassium phosphate buffer, 0.1 M, and the extract is dialyzed at 3°C during 20 hours against a 0.005 M potassium phosphate buffer. The same dialysis is performed with the supernatant S. P and S are centrifuged separately at 30,000 g, for 20 minutes and the extracts of the sediments by 100 ml of 0.1 M potassium phosphate buffer are centrifuged 15 minutes at 20,000 g. The sediments are extracted for 1 hour with 100 ml of potassium phosphate buffer 0.1 M, sodium deoxycholate 2.55×10^{-3} M, and the extracts are centrifuged 15 minutes at 20,000 g. All supernatants are pooled and dialyzed against 0.1 M potassium phosphate buffer (2).

Thereafter, the protein is adsorbed on calcium phosphate gel (1), recovered with 0.3 M potassium phosphate buffer, pH 7, and dialyzed against potassium phosphate buffer 0.03 M. The protein is then dialyzed against 65% $(NH_4)^2SO_4$, pH 7, during 24

hours; after centrifugation for 20 minutes at 25,000 g, the sediment is taken up in phosphate buffer 0.03 M, dialyzed against phosphate buffer 0.001 M, and chromatographed on a DEAE cellulose (Serva) column, 20 cm high and 1.9 cm diameter. After washing with 0.02 M phosphate potassium buffer, the protein is eluted with phosphate potassium buffer 0.1 M.

The content of the tubes of the proteic peak is dialyzed against 60% $(NH_4)^2SO_4$, pH 7, during 24 hours. After centrifugation for 20 minutes at 25,000 g, the sediment is dissolved in a minimum volume of 0.03 M potassium phosphate buffer.

Results

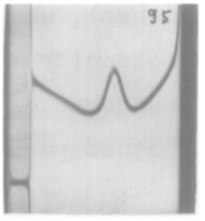

Figure 1
Ultracentrifugation in potassium phosphate buffer, 0.3 M, pH 7.2; concentration : 7.9 mg/ml; velocity 59,780 cpm. Photograph at 95 mn. Angle 50°

The isolated protein presents one peak after ultracentrifugation for 100 minutes; $S_{20W} = 5.1 \pm 0.1$ (fig. 1). Electrophoresis on polyacrylamid gel gives one band, pH_i 6.8. The immunoelectrophoresis (fig. 2) confirms the homogeneicity. The aminoacid analysis (to be published) reveals a percent composition very different from the bacterial enzyme (1). The extinction coefficient, $E_{1\%} = 9.80$ at 278 mμ.

Activity assays show no C19 and C21 isomerase activity. With the C27 substrate, the activity is 18 unit/mg, the unit being defined as the amount of enzyme isomerizing 1 μmole of substrate per minute at 37°C.

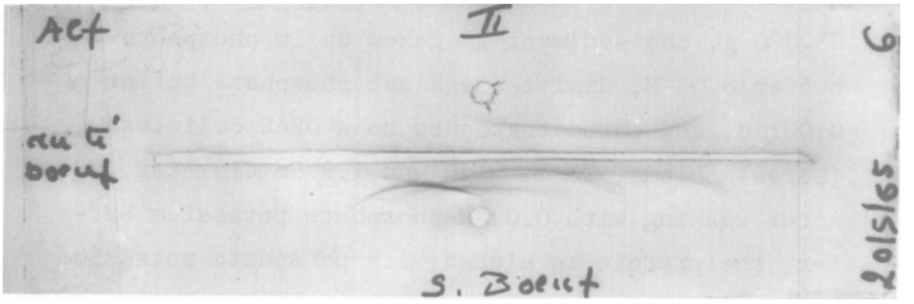

Figure 2
Immunoelectrophoresis with
rabbit serum anti beef serum.

Discussion

The purified protein fraction appears to be a single protein. From molecular sieving on Sephadex 200 and Biogel 300 columns (to be published), and from the Svedberg constant value, the molecular weight appears several times greater than that of the Talalay microbial enzyme, the only other purified preparation so far described.

It should be mentioned that during the first steps of the procedure (2), C19 isomerase activity was found; at the same time, some C27 isomerase activity was detected. After the phosphate gel adsorption, the C19 activity was still present, but it was lost after DEAE chromatography, whereas the C27 activity was enhanced. It is not known if there is a distinct C19 isomerase which has been inactivated during the chromatographic step, or if there is a single protein whose activity was modified during the chromatography.

References

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Acknowledgments

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