FORMATION OF A 5α HYDROXY STEROL BY SACCHAROMYCES CEREVISIAE

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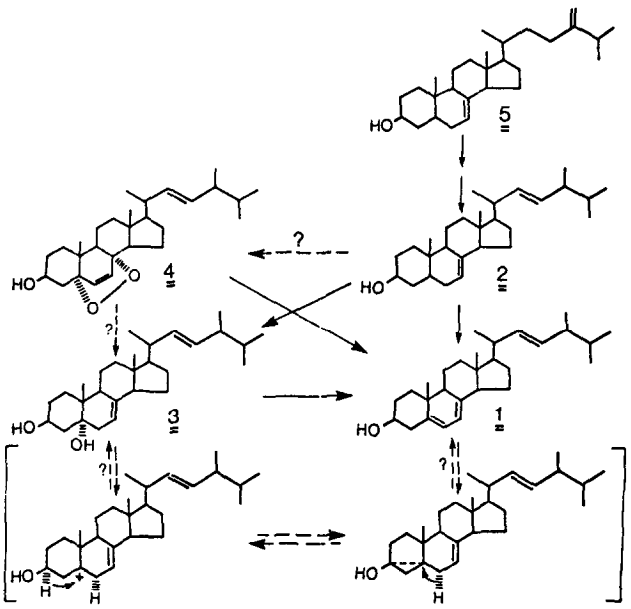
Summary

The incorporation of [28 ^{14}C] ergosta-7,24(28)-dien-3β-ol into ergosta-7,22-dien-3β,5α-diol by aerobically growing S. cerevisiae has established its presence in this organism. This, coupled with previous work, is considered to be substantive evidence for the operation of a hydroxylation-dehydration mechanism in the introduction of Δ^5 unsaturation in ergosterol biosynthesis in yeast.

Two alternative mechanisms have been proposed for the introduction of the Δ^5-double bond in ergosterol (1) during its biosynthesis in Saccharomyces cerevisiae. One[1] involves the direct elimination of two hydrogens from C_5 and C_6 (i.e., 2→1) while the second[2,3] involves a hydroxylation-dehydration mechanism (i.e., 2→3→1).

Akhtar et al.[1] reported the incorporation of [5α,6α-$^3H_2$] ergosta-7,22-dien-3β-ol (2) into ergosterol with the complete loss of both the 5α and 6α tritiums. They also observed a significantly lower incorporation of [3α-^{3}H] ergosta-7,22-dien-3β,5α-diol (3) in whole yeast cells under anaerobic than under aerobic conditions. These investigators concluded that the introduction of the Δ^5 unsaturation in ergosterol proceeded via a cis removal of 5α and 6α-hydrogens rather than via a hydroxylation-dehydration mechanism.



Topham and Gaylor reported that a soluble enzymatic system[2], obtained from yeast, anaerobically converted [3α-^{3}H] - 3 to ergosterol with loss[3] of the 3α-^{3}H. Since this label was lost in the cell free system, it was suggested[3] that the apparent low incorporation obtained[1] in the anaerobic whole cell conversion of [3α-^{3}H] - 3 was due to loss of label and not low conversion. Based on the foregoing, Gaylor[3] postulated a hydroxylation-dehydration mechanism (3→[ ]→1) for the introduction of the Δ^5-unsaturation. Critical to the evaluation of this proposal is the demonstration of the production of the requisite 5α-hydroxysterol intermediate in yeast. This is particularly pertinent since enzymes responsible for steroidal modifications involved in ergosterol production in S. cerevisiae have considerable latitude with respect to substrate structure. We have recently shown[4] that the enzymatic system(s) that introduce the Δ^5-unsaturation fall into this category. Ergosterol substrates with $\Delta^{7,22}$, $\Delta^{7,22,24(28)}$ and $\Delta^{7,24(28)}$ unsaturation are readily converted to the corres-

ponding $\Delta^{5,7}$ compounds in whole yeast cells. Indeed, Gaylor's cell free system[3] accepted, with equal facility, the $\Delta^{7,22}$-3β,5α-diol, 2, and 5α,8α-dioxy-ergosta-6,22-dien-3β-ol (4).

We have established the elaboration of 3 from ergosta-7,24(28)-dien-3β-ol (5) in aerobically growing yeast. Labelled ergosta-7,24(28)-dien-3β-yl acetate[5] was synthesized from 3β-acetoxy-cholest-7-en-24-one[6] by a Wittig reaction with [methylene-^{14}C]-triphenylphosphorane[7]. Subsequent hydrolysis (2% K_2CO_3 in 10% water-ethanol) gave [28-^{14}C]-5 (4.5×10^5 cpm/mg). This compound, [28-^{14}C]-5, (1.5×10^5 cpm) was incubated for 10 hrs. with aerobically growing yeast that had been depleted of sterols by anaerobic pretreatment.

The cells were harvested by centrifugation, washed with phosphate buffer to remove exogenous sterol, and finally mixed with unlabelled 3, synthesized by the method of Gaylor[2,8]. Base hydrolysis of the mixture (2 g wet cells/100 ml 15% KOH in water-ethanol, 80:20) was followed by hexane extraction and acetylation of the crude sterol fraction. The acetylated mixture was separated on an alumina column to give sterol acetates (hexane-benzene, 1:1) and 3β-acetoxy-ergosta-7,22-dien-5α-ol[8] (benzene: ether, 3:1). After crystallization (8X from ethyl acetate-methanol) to constant activity (1.7×10^2 cpm/mg), an incorporation of 0.2% into 3 based on added carrier and activity recovered in the acetate fraction, was calculated.

We have previously reported the conversion of [28-^{14}C]-5 to the $\Delta^{7,22}$-dienol-2 and this sterol, in turn, to ergosterol in this culture[4]. Since Akhtar[1] has reported the efficient conversion of 3 to ergosterol in *S. cerevisiae* under aerobic conditions, the present experiment establishes the participation of the $\Delta^{7,22}$-dien-3β,5α-diol-3 in ergosterol production.

The efficient conversion of 2 and 3 to ergosterol by aerobically growing cells[1] with retention of the [3α-^{3}H] label may indicate that two mechanistically different systems are available in yeast for the introduction of Δ^5 unsaturation.

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References

1. M. Akhtar and M.A. Parvez, Biochem. J., 108, 527 (1968).
2. R.W. Topham and J.L. Gaylor, Biochem. Biophys. Res. Comm., 27, 644 (1967).
3. R.W. Topham and J.L. Gaylor, ibid, 47, 180 (1972).
4. M. Fryberg, A.C. Oehlschlager and A.M. Unrau, ibid., 48, 593 (1972).
5. Compounds were fully characterized by mp, i.r., u.v., nmr and mass spectroscopy.
6. W. Sucrow and B. Raduchel, Chem. Ber., 102, 2629 (1969).
7. M. Fryberg, A.C. Oehlschlager and A.M. Unrau, Tetrahedron, 27, 1261 (1971).
8. See also R.B. Clayton, H.B. Henbest and E.R.H. Jones, J. Chem. Soc., 2015 (1953). K.M. Sivanandaiah and W.R. Nes, Steroids 5, 539 (1965).