THE ORIGIN OF OXYGEN INCORPORATED DURING THE BIOSYNTHESIS OF PROSTAGLANDIN E_1 *

Ragnar Ryhage and Bengt Samuelsson

Department of Chemistry, Karolinska Institutet
Stockholm, Sweden

Received February 23, 1965

During the formation of prostaglandins from the C_{20}-unsaturated fatty acids (van Dorp et al. 1964; Bergström et al. 1964), three oxygen atoms are incorporated. In the PGE compounds, two of these atoms appear as hydroxyl groups and one as a keto group in the five membered ring. The incorporation of oxygen from ^{18}O-labeled oxygen gas during formation of PGE_1 from 8,11,14-eicosa-trienoic acid has now been determined using mass spectrometry in conjunction with gas chromatography.

Vesicular glands of sheep were used. The glands were minced in a Bucher medium, pH 7.4, in a tissue to buffer ratio of 1:3 and homogenized in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 700 x g for 10 minutes and the supernatant fluid was again centrifuged at 8.500 x g for 12 minutes. This 8.500 x g supernatant was used for the incubations. A benzene solution of 5 mg of 8,11,14-eicosa-trienoic acid-2-^{14}C (1.2 μC/mg) (Bergström et al. 1964) was evaporated to dryness in a 60 ml incuba-

* This work was supported by the Swedish Medical Research Council (Project Nos. F-0217-B-A and F-0220-B-A to Professor S. Bergström).

tion flask. The flask could be attached to a high vacuum system and had a side arm with a rubber stopper. About 30 ml of ^{18}O-oxygen gas (98.1% ^{18}O; Yeda Research and Development Co. Ltd., Rehovot, Israel) was introduced using a Toepler pump. The gland preparation (15 ml), repeatedly degassed by evacuation and introduction of argon, was introduced through the rubber stopper. After incubation for one hour at 37° with shaking, the flask was immersed in liquid nitrogen for removal of the oxygen. Five volumes of ethanol were added and after filtration, the solution was diluted with water, acidified with 2 N hydrochloric acid to pH 3 and then rapidly extracted with ether. The ether extract was washed with water until neutral and the ether was evaporated under reduced pressure.

The residue was separated by reversed phase partition chromatography as described earlier (Bergström et al. 1964). Radioactive material with an elution volume characteristic of PGE_1 was isolated in a yield of about 15%. The ethyl ester of the isolated PGE_1 was prepared by treatment with diazoethane and this product was converted into the dimethoxy derivative as previously described (Bergström et al. 1962). About 15 µg of this material after purification by silicic acid chromatography, was injected into the instrument for mass spectrometric analysis in conjunction with gas chromatography (Ryhage 1964). A column of 1% SE-30 on Chromosorb P (Horning et al. 1963) operated at 190° was used. The mass spectrum, shown in Fig. 1 A, was recorded on the main peak having a retention time of 14.8 minutes. This retention time corresponded to the single peak observed for the same derivative of the

reference PGE_1 (mass spectrum see Fig. 1 B).

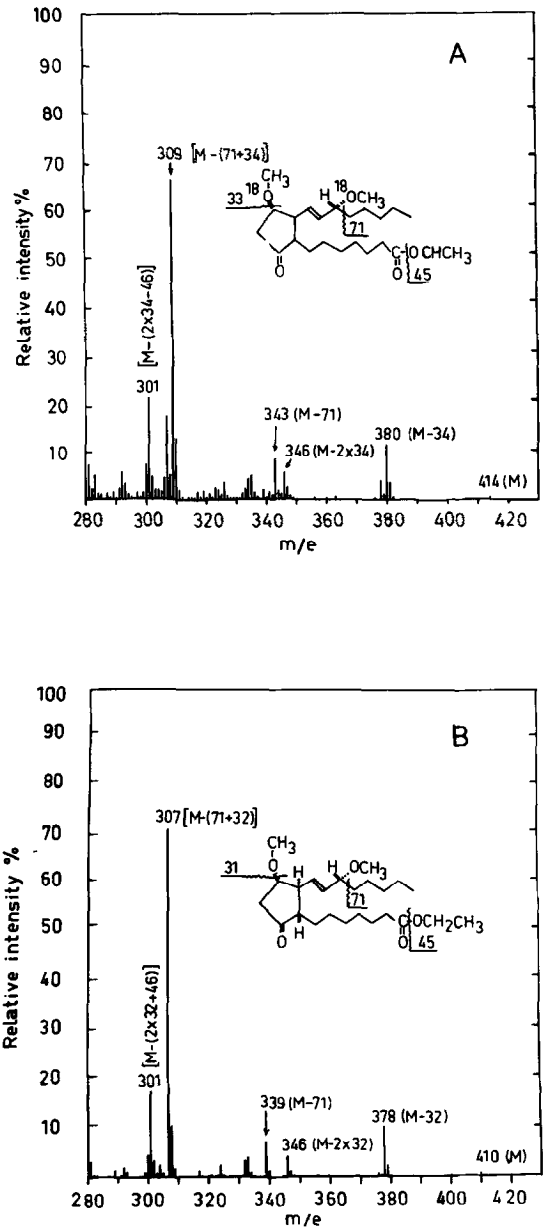

Fig. 1. Mass spectra of A) the dimethyl ether, ethyl ester of PGE_1 biosynthesized from 8,11,14-eicosa-trienoic acid in $^{18}O_2$ atmosphere and B) corresponding unlabeled derivative.

The ions retaining the two methoxy groups and the keto group (M, molecule ion and M-71) were four units higher in the compound biosynthesized in $^{18}O_2$ as compared with the reference. Furthermore, elimination of both methoxy groups (as CH_3OH) gave ions, which had the same m/e values for both compounds. These findings clearly demonstrated that the ^{18}O label was only present in the two methoxy groups and not in the keto group.

In summary the results show that in the biosynthesis of PGE_1 from 8,11,14-eicosa-trienoic acid the oxygens of the two hydroxyl groups are derived from oxygen gas.

The absence of ^{18}O in the keto group does not provide any conclusive evidence as to the origin of this oxygen, since exchange with the medium can be expected to take place (Hamilton and Westheimer 1959).

REFERENCES

Bergström, S., Danielsson, H., Klenberg, D. and Samuelsson, B., J. Biol. Chem. 239, PC 4006 (1964).
Bergström, S., Krabisch, L., Samuelsson, B. and Sjövall, J., Acta Chem. Scand. 16, 969 (1964).
van Dorp, D.A., Beerthuis, R.K., Nugteren, D.H. and Vonkeman, H., Nature 203, 839 (1964).
Hamilton, G.A. and Westheimer, F.H., J. Am. Chem. Soc. 81, 6332 (1959).
Horning, E.C., VandenHeuvel, W.J.A., Creech, B.G., in Glick, D. (Ed.), Methods of Biochemical Analysis, Interscience, New York, 11, 69 (1963).
Ryhage, R., Analyt. Chem. 36, 759 (1964).