Labelling of antibiotic peptide - edeine B with ^{14}C

Janina Żupańska, Jadwiga Wąsowicz, Kazimierz Dzięgielewski[x]

Institute of Nuclear Research, Świerk - Otwock, Poland

Received April 12, 1977
Revised September 26, 1977

Summary

Edeine B with guanyl group labelled with ^{14}C was obtained by treating edeine A with a 6-fold excess of O-methylisourea-^{14}C. The radiochemical yield was 10,1%. The product was purified on a column, filled with Sephadex LH 20 and by preparative paper chromatography. The radiochemical purity of the obtained preparation was 97.7%.

Introduction

Edeine B is one of the components of the edeine complex, compounds of the antibiotic peptide group /1,2/. These compounds exhibit two-directional action: bacteriostatic on one hand, stimulating in RNA synthesis and inhibiting in DNA synthesis /3-5/, on the other hand.

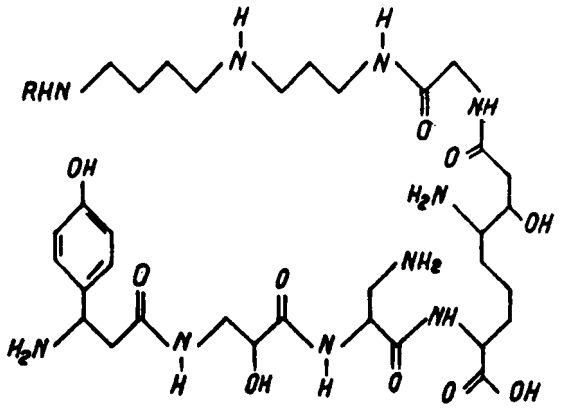
Owing to its biological activity edeine B is used in the studies of basic life processes. Labelling it with ^{14}C makes possible application of isotope techniques.

---

x/ Tarchomin Pharmaceutical Works "Polfa", Warsaw, Poland

0362-4803/78/0015-0079$01.00

Inactive edeine B can be obtained by biosynthesis i.e. isolation from the edeine complex produced by the culture of Bacillus brevis Vm4. It can also be obtained by synthesis i.e. quanylation of edeine A. The structure of edeine A and B proposed by Hettinger and co - workers /6,7/ is shown in fig 1.



R = H edeine A

R = -C/=NH/NH_2 edeine B

Fig 1.

According to these authors edeine B contains in the molecule equimolar amounts of the following aminoacids: glycine, isoserine, α,β-diaminopropionic acid, 2,6-diamino-7-hydroxyazelaic acid, β-tyrosine and spermidine substituted with a guanyl group. The presence of the guanyl group is specific for edeine B, which differs in this respect from edeine A obtained exclusively by biosynthesis. This also makes possible labelling of edeine B with ^{14}C by chemical synthesis. The guanylation reaction of spermidine in edeine A is carried out by means of O-methylisourea. According to Roncari and others /1/ the complete transformation of edeine A can be achieved by using a 25-30 fold excess of O-methylisourea /10/. However in the case of a radioactive synthesis the O-methylisourea is a labelled semiproduct and its use in such excess is uneconomical. Hence the purpose of the

present work was to find a more suitable ratio of O-methylisourea to edeine A, that would permit to obtain edeine B with good yield when extending the reaction time. The O-methylisourea used for this purpose was prepared according to Jezdić, Rajnovajn /8/ and Schuching and Barnes /9/.

Experimental

Choice of guanylation conditions

The experiments were carried out using O-methylisourea labelled with ^{14}C. Samples were taken from the reaction mixture at defined time intervals and subjected to analysis.
The results are shown in the following table

The yield of edeine B in dependence on the amount of O-methylisourea-^{14}C and reaction time

Amount of O-methylisourea-^{14}C		Ratio of O-methylisourea-^{14}C to edeine A	Amount of edeine B-^{14}C in percent activity with respect to 1 mmole of O-methylisourea-^{14}C		
mmoles	mCi		24 h	48 h	72 h
0,15	0,22	3:1	29,1	68,7	77,1
0,2	0,28	4:1	59,5	60,8	78,6
0,25	0,36	5:1	84,0	92,5	-
0,3	1,3	6:1	81,6	94,2	100,2
1,2	24,0	6:1	-	96,0	98,0

At a ratio of O-methylisourea-^{14}C to edeine A of 3:1 and 4:1 even for a long reaction time /72 hours/ a mixture of both edeines was obtained, which contained considerable amounts of edeine A. Despite the use of a column filled with DEAE cellulose /11/ the separation of edeine A from edeine B was insufficient. At a ratio of 5:1 the reaction mixture purified on cellulose contained only trace amounts of edeine A. At a ratio of 6:1 the product did not contain edeine A and the separation on the cellu-

lose column was not necessary. On the other hand, independently of the ratio of O-methylisourea-^{14}C to edeine A, used in the reaction, the preparation contained also other unidentified radioactive substances of high molecular weight, which appeared on the chromatogram between the starting line and edeine B. These substances were separated from edeine B by preparative paper chromatography.

Guanylation of edeine A

Edeine A and edeine B sulphates of molecular weights 1260 and 1302, respectively, were supplied by Tarchomin Pharmaceutical Works "Polfa" /Poland/.

The synthesis of O-methylisourea-^{14}C was carried out using 2,3 mmoles of barium carbonate-^{14}C of a total activity of 105,3 mCi and a specific activity of 46 mCi/mmol. The radiochemical yield amounted to 58,0% and the specific activity of the product was 43,5 mCi/mmole.
0,8 mmole of O-methylisourea-^{14}C hydrochloride of a total activity of 24 mCi, diluted with 0,4 mmole of the inactive compound, was added to 0,2 mmole of edeine A sulphate.
The mixture was dissolved in a freshly prepared solution of a composition: 8,8 ml of water, 7 ml of ethanol, 3 ml of triethylamine, 1,6 ml of 1 N NaOH. The reaction was carried out at room temperature with continuas stirring up to the disappearance of edeine A /approx. 72 hours/. Every 24 hours the content of edeine A and B in the reaction solution his been determined by thin layer chromatography. After the reaction the mixture was acidified with 3 N hydrochloric acid to pH 3, concentrated to a volume of 10 ml and applied onto the column.

Separation on the column

Sephadex LH 20 and a chromatographic column K-26, supplied by Pharmacia, Sweden.
Automatic fraction collector type 301, Unipan, Poland.
The column was filled with the gel previously subjected to swelling in a water-methanol solution.
A disc of Whatman 3 paper was placed on the top of the column.
The reaction mixture was applied to this paper disc.
The elution was carried out with water under a hydrostatic pressure of 1 m H_2O at a flow rate of approx. 50 ml/h.
The volume of the bed was 120 ml. The first 100 ml of the solution formed one fraction, then 2 ml fractions were collected to the test tubes of the fraction collector.
Edeine B-^{14}C of a neutral reaction was eluted first, followed by an acid fraction containing the excess of O-methylisourea-^{14}C and inorganic salts as well as unidentified radioactive substances. The edeine B-^{14}C fraction of a volume of 80 ml was concentrated to 2,5 ml. The preparation obtained in the form of an aqueous solution showed a total activity of 3,25 mCi and a radiochemical purity of 86%.

Purification by means of paper chromatography

A chromatographic chamber Chropa Entwiklungskammer 0,02, supplied by Glasswerke Ilmenau, /GDR/ was used. 0,1 ml of the solution, of a concentration 80 mg/ml was applied to sheets of Whatman 3 paper of dimensions of 27x22,5 cm.
A mixture of isopropanol-25% ammonia-chloroform 12:8:1 was used as solvent. The chromatogram was developed for 17 hours and then subjected to autoradiography for 5 h.
The edeine B-^{14}C fraction was cut out and eluted for 48-72 hours. The eluate of a volume of 250 ml was concentrated to 6 ml.

The edeine B was then precipitated by acidifying the eluate with 1 N H_2SO_4 to pH - 5,5 and adding it dropwise to 190 ml of ethanol. The precipitate was filtered and washed thrice with 5 ml of absolute methanol when leaving a layer of alcohol on the top of the precipitate.

The precipitate under the methanol layer was transferred to a dessicator and dried under vacuum over P_2O_5. 153 mg /0,12 mmole/ of edeine B-^{14}C was obtained.

Its total activity amounted to 2,43 mCi and its specific activity to 20,6 mCi/mmole. The radiochemical yield with respect to the O-methylisourea-^{14}C, was 10,1%. The radiochemical purity was 97,7% as determined in the solvents described below.

Regeneration of barium carbonate-^{14}C.

The fraction containing O-methylisourea-^{14}C, inorganic salts und the unidentified substances of low molecular weight, of a total activity of 76 mCi was subjected to regeneration by combustion in a closed vacuum system. The gas evolved, which consisted largely of CO_2-^{14}C and hydrogen chloride was absorbed in 20% sodium hydroxide solution. The barium carbonate-^{14}C precipitated from this solutions had a total activity of 35 mCi. The radiochemical yield was 46%.

Chromatographic analysis

The analysis of samples taken during guanylation of edeine A and those obtained by elution from the Sephadex column as well as the analysis of the final preparation was carried out by means of ascending paper chromatography. Samples and standards /5-10 ug/ were applied to strips of Whatman 3 paper 2 cm in diameter. The chromatograms were developed using the following solwent: isopropanol-25% ammonia - chloroform /12:8:1/ at room temperature for 17 hours. The chromatograms were dried by means of stream of warm air /approx. 50° C/.

The inactive substances were located by spraying the spots with 0,3% ninhydrin solution in ethanol.

The position and the relative activity of the substances labelled with ^{14}C were determined with the aid of a Berthold model LR 2732 chromatogram scanner. Rf value of 0,17 and 0,31 for edeine B and A, respectively, were found.

The results obtained by paper chromatography were confirmed by thin layer chromatography under the following conditions;

mobile phase: isopropanol-25% ammonia-water /6:4:3/;

thin layer: DC-Alufolie Kieselgel GF_{254} /Merck/;

The samples and standards /5-10 ug/ were applied to a plate 0,25 mm thick and developed at room temperature for 3 hours. The thin layer chromatograms were dried and developed in the same as the paper chromatograms.

The obtained Rf values were 0,28 and 0,36 respectively for edeine B and edeine A.

The radioactivity was measured by means a liquid scintillation counter /12/.

References

1. Biochemistry 5:2153 /1966/
   Roncari G.; Kurylo-Borowska Z.; Craig L.C.
2. Biochemistry 7:4147 /1968/
   Hettinger T.P.; Craig L.C.
3. Acta Microb. Pol. 17:59 /1968/
   Chmara H.; Borowski E.
4. Acta Microb. Pol. 15:223 /1966/
   Chmara H.; Borowski E.
5. Acta Microb. Pol. 17:241 /1968/
   Borowski E.; Chmara H.
6. Biochemistry 9:1224 /1970/
   Hettinger T.P.; Craig L.C.

7. Biochemistry 7:4153 /1968/
Hettinger T.P.; Kurylo-Borowska Z.; Craig L.C.

8. J. Lab. Comp. 12:127 /1961/
Jezdić V.; Rajnovajn J.

9. J.Am.Chem. Soc. 72:3817 /1950/
Schuching S.V.; Barnes F.W.

10. Pol. Pat. 178.161
Borowski E. and others

11. Pol. Pat. 165.549
Borowski E. and others

12. Nukleonika 20:5 /1975/
Chyliński A.; Radoszewski T.