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USE OF ORGANIC DEVELOPERS ON DEAE-CELLULOSE FOR THE PURIFICATION OF THE PEPTIDE ANTIBIOTIC MYCOBACILLIN FROM ITS ASSOCIATED PEPTIDE COMYCOBACILLIN

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BUFFERS but not organic solvents are usually employed in biochemical separations by ion-exchange chromatography. However, aqueous organic solvents have sometimes been used as developers in the process[1]. Use of non-aqueous organic solvent mixture has been reported for ion-exchange chromatography of polar lipids only which are insoluble in water[2,3]. Mycobacillin, an antifungal cyclic tridecapeptide, was isolated as a homogeneous compound in this laboratory[4]. It has recently been observed that mycobacillin prepared now from the culture filtrate of *Bacillus subtilis* B_3 according to the earlier method is not a pure compound but it contains another peptide subsequently named comycobacillin (unpublished). Thus, antibiotic has recently been purified from the mixture[5] by chromatography on DEAE-cellulose using Tris-HCl buffer (10 mM) at pH 7.5. As the method is a little cumbersome, involving further isolation of the antibiotic from the aqueous buffer, a rapid method of purification has been developed and is reported here.

The antibiotic preparation as used for further purification was prepared according to the earlier method[4] from the fermented broth of *B. subtilis* B_3. DEAE-cellulose (medium) was obtained from Sigma Chemical Company, USA and was used as such without any pretreatment. Silica gel G-60 for TLC was obtained from E. Merck, West Germany. Benzene, methanol and glacial acetic acid used were of E. Merck, India and were used as such.

The purity of the antibiotic preparation was tested by TLC on silica gel using *n*-propanol—25% aqueous ammonia (2:1 v/v). The spots were located by exposing the plates to iodine vapour[5].

For DEAE-cellulose chromatography, about 200 mg of the cellulose exchanger (hydroxylated form) was suspended in benzene-methanol (90:10 v/v), shaken well and then poured into a column having internal diameter 1 cm. The suspension was then allowed to be packed under gravity. In charging the column 10 mg of the antibiotic was dissolved in 0.1 ml methanol and to the solution was added 0.6–0.7 ml benzene (added until the solution became slightly turbid) which was

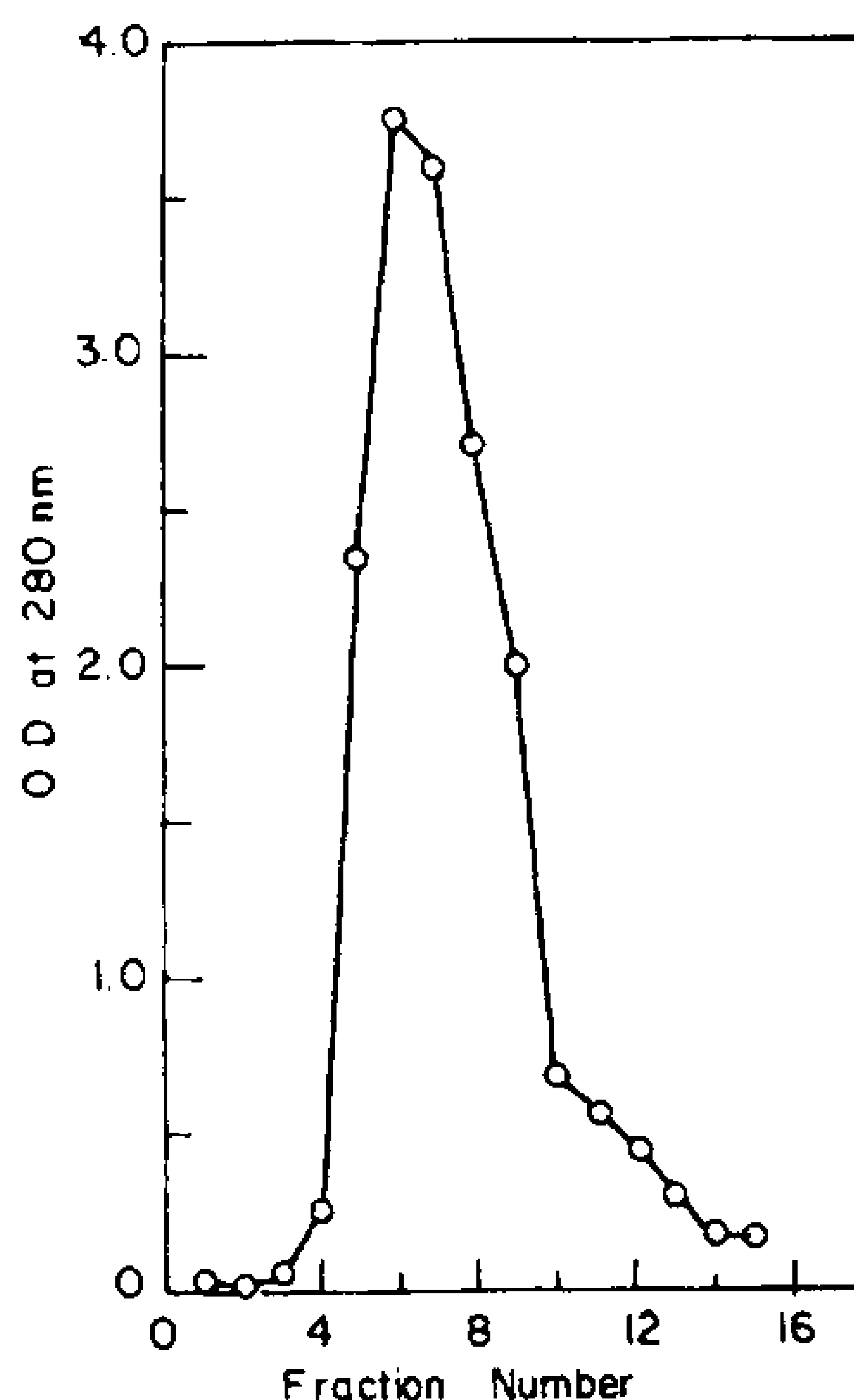

Figure 1. Purification of mycobacillin on DEAE-cellulose column.

Table 1 *Separation of mycobacillin from co-purified mixture of mycobacillin and comycobacillin*

	Co-purified mixture[1]	DEAE-cellulose
R_f values of the constituent peptides[2]		
1. Mycobacillin	0.70	0.70
2. Comycobacillin	0.60	ND
Content of the constituent peptide (mg/mg peptide)[3]		
1. Mycobacillin	0.65	1.00
2. Comycobacillin	0.35	ND
Yield of purified mycobacillin	—	92%

1. Prepared according to Majumder and Bose[4].
2. On the thin layer chromatogram developed with the solvent system *n*-propanol—25% ammonia (2:1 v/v).
3. Comycobacillin has no antifungal activity but it stimulates the antifungal action of mycobacillin (unpublished) so the antibiotic content is measured in terms of serine (present only in mycobacillin) in the acid hydrolysate of the mixture.
ND = Not detectable.

then centrifuged and the supernatant was charged to the top of the column (4 × 1 cm). The column was then eluted with benzene-methanol-acetic acid (glacial) (85.5:9.5:5.0 v/v). The eluent was collected in 0.5 ml fractions which were monitored by measuring absorbance at 280 nm (figure 1). The positive fractions were pooled and evaporated under reduced pressure. Sometimes it was necessary to dissolve it in the minimum volume of ethanol and then to reprecipitate it, by adding ten volumes of cold (0°C) ether.

The purified antibiotic was free from comycobacillin. About 6 mg of the pure antibiotic was thus obtained (table 1). The method was convenient both in respect of time and labour. Regarding the mechanism of purification it is felt that the antibiotic, being a polar peptide containing a number of aspartic acid and glutamic acid residues[6], benzene and methanol have created an optimum condition just enough to retain it, in the column along with comycobacillin (pure cellulose does not retain the peptide mixture under identical conditions). The subsequent use of acetic acid could ensure selective elution of the antibiotic from its association with comycobacillin. Thus this method would be useful for the purification of a polar peptide on DEAE-cellulose column.

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ENZYMES OF NITROGEN ASSIMILATION IN DEVELOPING ENDOSPERM OF NORMAL AND OPAQUE-2 MAIZE (*ZEA MAYS* L)

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THE average absolute activities (per endosperm) of nitrogen assimilatory enzymes, glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrgenase (GDH), and glutamate oxaloacetate transaminase (GOT) were substantially higher in developing opaque-2 maize endosperm as compared to normal maize endosperm. The activity of nitrate reductase (NR) was, however, very low both in normal and in opaque-2 developing endosperm. The present study suggests that low protein content in opaque-2 maize endosperm at maturity is not due to limitation of the key enzymes of nitrogen assimilation.

It is generally recognized that the improvement of nutritional quality in opaque-2 maize is due to the suppressed synthesis of major storage protein zein[1-3] which is extremely deficient in lysine. It is not known as to why the net reduction in protein content in endosperm occurs. It has been speculated that the increase in RNase activity results in degradation of mRNA towards the later stages of endosperm development and consequently protein synthesis is reduced[4-6]. In addition to the regulation of mRNA synthesis, the levels of different amino acids could also play an