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# Effect of neutral resins on the production of dynemicins by *Micromonospora chersina*

KS Lam, JA Veitch, SE Lowe and S Forenza

*Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, Connecticut 06492, USA*

**Addition of Diaion HP-20 or Amberlite XAD-8 resin to the fermentation of *Micromonospora chersina* ATCC 53710 enhanced the production of dynemicin A by 4.7- and 6.9-fold, respectively. Addition of resin suppressed the production of other dynemicin analogs, which comprised 65% of the dynemicin complex in the fermentation.**

**Keywords:** dynemicins; antitumor antibiotic; Diaion HP-20; Amberlite XAD

## Introduction

A novel antitumor antibiotic, dynemicin A, was discovered in the fermentation broth of *Micromonospora chersina* ATCC 53710 [7]. Structural studies revealed that dynemicin A is a unique hybrid of an anthraquinone and an enediyne system [7,8] (Figure 1). The anthraquinone moiety of dynemicin A is identical to those of the anthracycline anticancer drugs daunorubicin and adriamycin [20]. The enediyne system of dynemicin A is similar to those of the esperamicin [2] and calicheamicin [11] class of potent antitumor antibiotics. The mechanism of action of dynemicin A may be similar to that of esperamicin-calicheamicin which involves a bioreductively-activated, highly efficient DNA strand scission [12,17–19,21,22].

As the production of dynemicin A in the original medium was very low, further development of this compound as an anticancer drug would have been difficult. Media development studies improved the production of dynemicin A by *M. chersina* from 0.1 $\mu$g ml$^{-1}$ in the original medium to 3.5 $\mu$g ml$^{-1}$ in a new medium, H881 [10]. However, based on the potency of dynemicin A, yields of 15–20 $\mu$g ml$^{-1}$ would be required for potential commercialization of this antitumor antibiotic. Further media formulation studies did not yield any meaningful improvement of dynemicin A production in the fermentation. This may be due to the fact that dynemicin A is an unstable metabolite containing the highly reactive enediyne chromophore. Further increase in the production of dynemicin A in the fermentation may lead to the formation of degradation products.

Dynemicin A possesses extremely potent activity against Gram-positive bacteria [7] and synthesis of dynemicin A by *M. chersina*, a Gram-positive filamentous bacterium, may be subject to end product inhibition. Since addition of neutral resins to the fermentations of several unstable and toxic antibiotics led to an increase in the production of these secondary metabolites [1,3–6,9,13,14], we examined the effect of neutral resins on the production of dynemicins. This communication describes the increase in the titer of dynemicin A produced by *M. chersina* as a result of resin addition, to levels significantly higher than those found in the improved medium reported previously.

Correspondence: KS Lam, Dept 114, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, Connecticut 06492, USA
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## Materials and methods

### *Microorganism*

The dynemicin-producing organism was *Micromonospora chersina* ATCC 53710. Frozen vegetative preparations were made by mixing a culture grown for 7 days in medium 53 with an equal volume of 20% glycerol/10% sucrose, frozen in dry ice/acetone bath and then stored at −80° C.

### *Media*

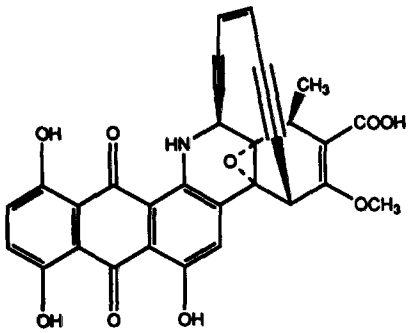
The seed medium was medium 53, which contained, in g L$^{-1}$ of deionized water: fish meal 10 g; dextrin 30 g; lactose 10 g; $CaSO_4$ 6 g; $CaCO_3$ 5 g. The production medium used was H881, which contained (g L$^{-1}$): soluble starch 10 g; Pharmamedia 5 g; $CaCO_3$ 1 g; $CuSO_4 \cdot 5H_2O$ 0.05 g; NaI 0.0005 g.

### *Fermentation conditions*

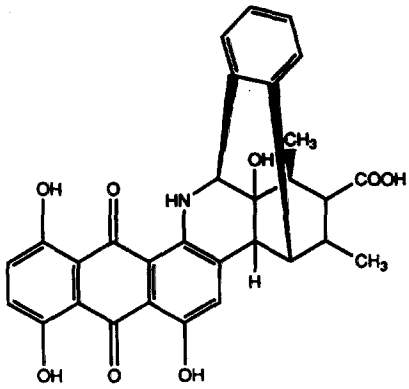
Four milliliters of the frozen stock of *M. chersina* was used to inoculate a 500-ml flask containing 100 ml of medium 53. The culture was incubated at 28° C on a rotary shaker at 250 rpm for 7 days. From this vegetative culture, 4% (v/v) was used to inoculate 500-ml flasks containing 100 ml of medium H881. These cultures were grown at 28° C on a rotary shaker at 250 rpm. Resin addition was made immediately following inoculation of the culture into production medium, unless stated otherwise. Diaion HP-20 resin was obtained from Mitsubishi Kasei America Inc (South Plainfield, NJ, USA). Amberlite resins XAD-2, XAD-7, XAD-8, IRC-50 and IRA-68 were purchased from Sigma Chemical Co (St Louis, MO, USA).

### *Extraction of fermentation products*

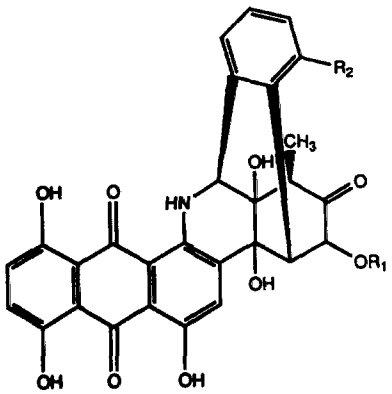
At various times during the fermentation cycle, a 3-ml aliquot of the whole broth resin mixture was removed from the flasks and mixed with an equal volume of ethyl acetate for 1 h. After centrifugation, the ethyl acetate fraction was concentrated 10-fold and analyzed by HPLC.

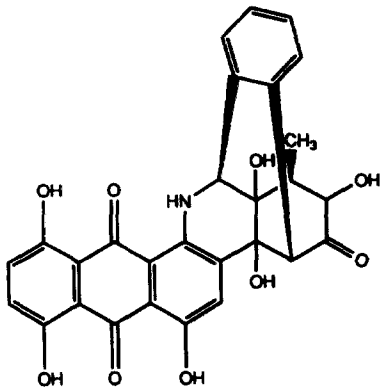



Dynemicin A




Dynemicin H




Dynemicin Q

| | $R_1$ | $R_2$ |
|---|---|---|
| Dynemicin M | $CH_3$ | H |
| Dynemicin O | $CH_3$ | OH |
| Dynemicin P | H | H |

**Figure 1** Structures of dynemicins

### *Analytical methods*

The production of dynemicins was monitored by HPLC using either a Novapak C-18 column (3.9 × 150 mm, Waters Associates, Milford, MA, USA) or a Microsorb 8-200 column (C-18, 4.6 × 100 mm, Rainin, Woburn, MA, USA). The eluant was monitored at 570 nm. The solvent system was 0.1% $H_3PO_4/CH_3CN$ (35 : 65) with a flow rate of 2.0 ml $min^{-1}$.

## Results and discussion

### *Production of dynemicins in medium H881*

HPLC analysis of the fermentation products of *M. chersina* grown in medium H881 is shown in Figure 2a. Dynemicin H (Figure 1), a reaction product of dynemicin A after visible light- or thiol-activation [16,17], was detected as the most abundant product of the fermentation. Dynemicin H is structurally similar to dynemicin A but the enediyne moiety was cyclized to a phenyl ring. Several other aromatized enediyne analogs, M, O, P, Q (Figure 1) [15], were also detected. Although dynemicin A possesses significant DNA-cleaving activity [16–18], these aromatized dynemicin analogs have no DNA-cleaving activity [16,17] further indicating that these analogs are degradation products of dynemicin A. The aromatized analogs comprised about 65% of the dynemicin complex. We have demonstrated that addition of a nonionic resin, Diaion HP-20, to the fermentation of *Actinomadura verrucosospora* increased the production of the highly reactive enediyne antibiotic esperamicin $A_1$ by preventing the degradation of the metabolite [9]. Therefore we decided to examine the effect of resins on the production of dynemicins.

### *Effect of resins on the production of dynemicin A*

Initial studies were made comparing the addition of different concentrations (1% to 7%, w/v) of Diaion HP-20 immediately following inoculation of *M. chersina* into medium H881. From this study, a 1% concentration of resin was determined to be optimal, resulting in production of 16.2 $\mu$g $ml^{-1}$ of dynemicin A, 4.5-fold higher than that of

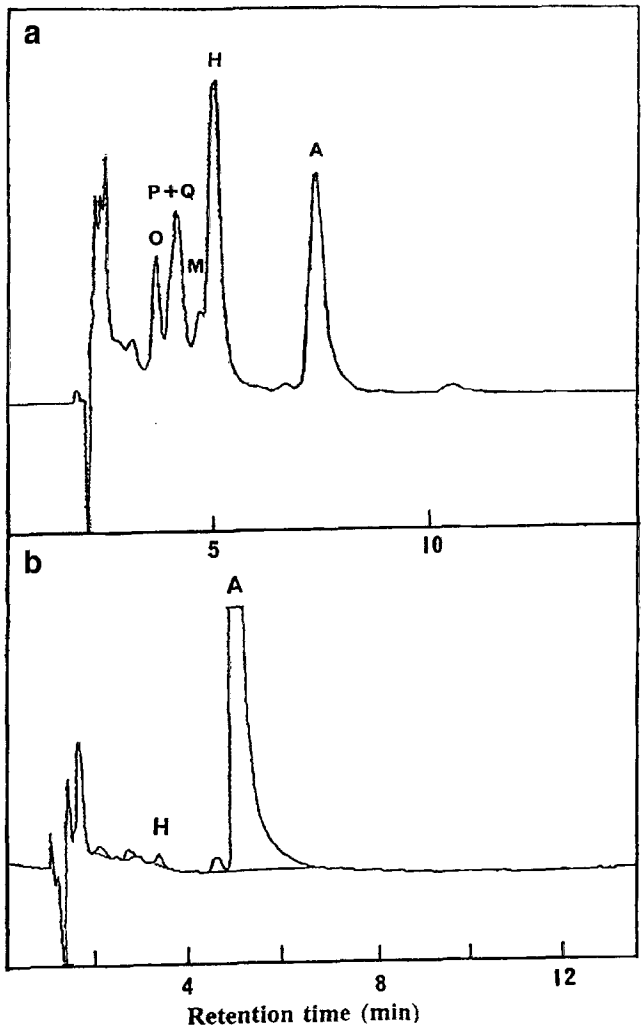



**Figure 2** Chromatograms of HPLC analysis of dynemicins produced by *M. chersina* ATCC 53710 in medium H881 (a) and medium H881 supplemented with 1% (w/v) Amberlite XAD-8 resin at 72 h (b). A Novapak C-18 column (3.9 × 150 mm) was used as stationary phase for the chromatogram shown in (a). A Microsorb 8-200 column (C-18, 4.6 × 100 mm) was used as stationary phase for the chromatogram shown in (b). A = dynemicin A; H = dynemicin H; M = dynemicin M; O = dynemicin O; P = dynemicin P; Q = dynemicin Q

the control with no resin addition (Table 1). Addition of resins at 4% or 7% to the fermentation completely inhibited the production of the antitumor antibiotic. Addition of high concentrations of resin to the fermentation may remove essential nutrients from the culture medium.

**Table 1** The effect of Diaion HP-20 addition on production of dynemicin A by *M. chersina* ATCC 53710 grown in medium H881

| Diaion HP-20 (%, w/v)[a] | Dynemicin A ($\mu$g ml$^{-1}$)[b] |
|---|---|
| 0 | 3.6 |
| 1 | 16.2 |
| 2 | 9.6 |
| 4 | 0 |
| 7 | 0 |

[a]Resin was added to the culture at the time of inoculation
[b]The titers of dynemicin A were determined at day 6 of the fermentation

As Diaion HP-20 is a fine mesh resin, the effect of larger mesh resins on dynemicin A was investigated (Table 2). The Amberlite resins XAD-2, XAD-7 and XAD-8 were all successful in enhancing production of dynemicin A, with the addition of XAD-8 resulting in the highest yield of 18.7 $\mu$g ml$^{-1}$, a 5.2-fold increase in production as compared with the control. Addition of IRC-50 and IRA-68 to the fermentation either decreased or completely inhibited the production of dynemicin A. Based on these data, resins Diaion HP-20 and Amberlite XAD-8 were selected for further study.

### *Effect of addition time of resins on the production of dynemicin A*

Addition of high concentrations of resin at the beginning of the fermentation inhibited the production of dynemicin A (Table 1). This suggests that resin addition at such an early time may bind essential metabolites. Amberlite XAD-8 and Diaion HP-20 resins were used in subsequent studies to determine the optimal time of addition. A concentration of 1% (w/v) resin was added to the fermentation at the time of inoculation or at 24, 48 or 72 h after inoculation. The optimal time for addition of Amberlite XAD-8 resin to the fermentation was at 72 h, before the onset of dynemicin A production, enhancing the titer of dynemicin A by 6.9-fold to 24.7 $\mu$g ml$^{-1}$. Addition of Diaion HP-20 was also optimal at 72 h, although the yield of dynemicin A (16.9 $\mu$g ml$^{-1}$) was lower.

### *Effect of resin on the production of aromatized dynemicins*

Addition of Diaion HP-20, Amberlite resins XAD-2, XAD-7 and XAD-8 to the fermentation successfully directed the fermentation towards the production of dynemicin A and led to a significant reduction in levels of the aromatized analogs. This effect was most marked with the addition of Amberlite XAD-8 (Figure 2b). Not only was dynemicin A production significantly enhanced, but formation of the aromatized analogs was suppressed, including production of dynemicin H with levels decreasing 30-fold. Dynemicin H, the major product in the control fermentation (no resin addition), is a degradation product of dynemicin A [16,17]. Therefore the increase in dynemicin A production in the resin-supplemented culture is in part due to the binding of dynemicin A to the resin, preventing its further metabolism and degradation to the aromatized analogs. The possible

**Table 2** The effect of various resins (1%, w/v) on the production of dynemicin A by *M. chersina* ATCC 53710 grown in medium H881

| Resins[a] | Dynemicin A ($\mu$g ml$^{-1}$)[b] |
|---|---|
| No resin | 3.6 |
| XAD-2 | 6.6 |
| XAD-7 | 6.7 |
| XAD-8 | 18.7 |
| IRC-50 | 0 |
| IRA-68 | 0.7 |
| HP-20 | 15.7 |

[a]Resins were added to the culture at the time of inoculation
[b]The titers of dynemicin were determined at day 6 of the fermentation

role of dynemicin A as an end product inhibitor was not investigated in this study. Since dynemicin A possesses extremely potent activity against Gram-positive bacteria [7], it may exert end product inhibition effect on *M. chersina*, a Gram-positive bacterium. HPLC analysis of the extract in the control fermentation with no resin addition (Figure 2a) indicated that the production of dynemicin A was 3.6 $\mu$g ml$^{-1}$ and comprised of 35% of the dynemicin complex. The total production of the dynemicin complex was therefore about 10.3 $\mu$g ml$^{-1}$. However, the titer of dynemicin A in the culture supplemented with XAD-8 resin at 72 h was 24.7 $\mu$g ml$^{-1}$, an additional 14.4 $\mu$g ml$^{-1}$ of dynemicin A was produced in the fermentation that may not be due to the stabilization effect of the resin on dynemicin A. The additional production of dynemicin A may be due to the relief of end product inhibition. Further work will be required to confirm this hypothesis. Nonetheless, the production of dynemicin A in the resin-supplemented cultures significantly increased the production of dynemicin A and met our targeted titer for a cost-effective large scale production process.

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