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Short Communication

A Simple and Rapid Chemical Method for the Determination of Cephalosporins

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ABSTRACT

A simple and rapid chemical assay for cephalosporins is described. It is a simple modification of the colorimetric determination of penicillins in which the narrow spectrum β-lactamase (penicillinase) is replaced by a broad spectrum β-lactamase (cephalosporinase) produced by *Enterobacter cloacae*. The method can be used for assay of fermentation broths as well as for pure cephalosporins.

Index Entries: Cephalosporins, determination of; β-lactamase; *Enterobacter cloacae*; broths, fermentation of; penicillin, colorimetric determination of.

A useful method for the colorimetric determination of penicillins was devised by Holm (*1*). It involves the enzymatic hydrolysis of penicillins into the corresponding penicilloic acids that, in the presence of mercuric chloride, reduce molybdoarsenic acid (*2,3*) to molybdenum blue; the latter is measured spectrophotometrically at 800 nm. The present work extends this method to the determination of cephalosporins. The situation with cephalosporins is somewhat more complex than with penicillins.

Cephalosporins are decomposed by β-lactamases not only to cephalosporoic acids (analogous to penicilloic acids), but, depending on the nature of the side-chain in the three-position, they break down to more stable products; these also reduce molybdoarsenic acid to molybdenum blue (4). By substituting the broad spectrum β-lactamase (which we call "cephalosporinase") from *Enterobacter cloacae* (Type IV, Sigma, St. Louis) for the penicillinase used in the Holm procedure, we have developed a useful assay for cephalosporins.

Using the conditions of the Holm assay, except for the use of cephalosporinase, we found most of the molybdenum blue that formed upon reaction with cephalosporin C was already present by 5 min, but that maximum absorption was reached only after 70 min (Fig. 1). The color remained constant for at least another 50 min. The time needed for incubation of cephalosporin C with the enzyme was found to be 10 min (Fig. 2) and the volume of cephalosporinase to be 50 μL (Fig. 3). Standard curves for cephalosporin C revealed a linear increase in molybdenum blue up to at least 250 μg/assay tube. It was found that the antibiotic produces some color even without enzyme, and, thus, a blank should be run with each sample and its value subtracted from the experimental value.

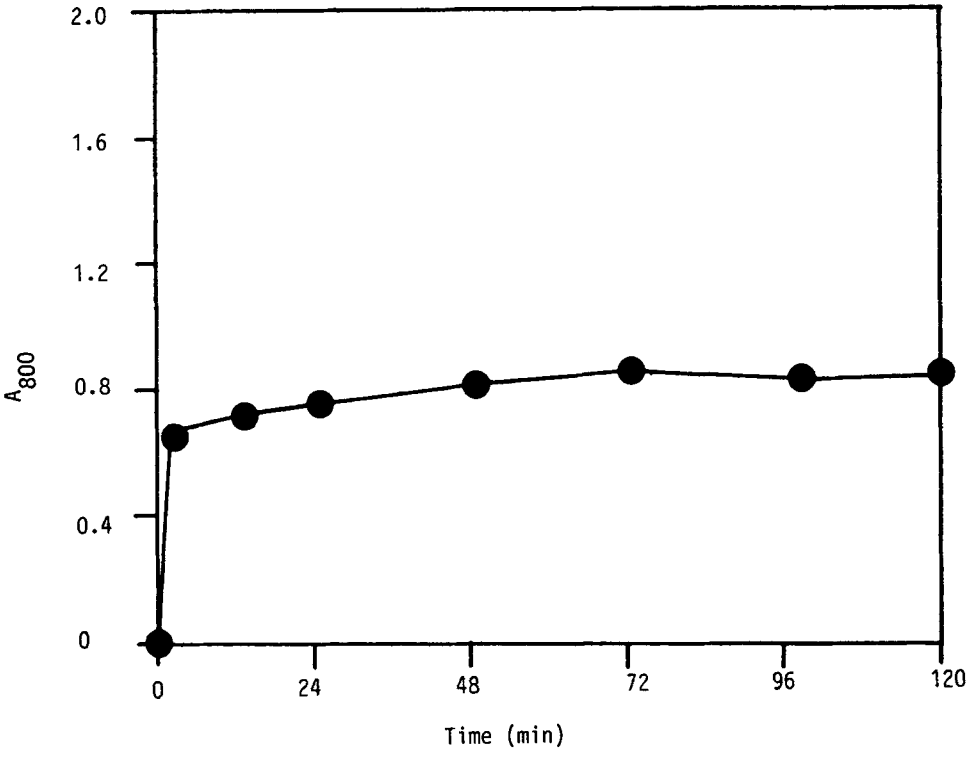

Fig. 1. Formation of molybdenum blue from cephalosporin C, measured spectrophotometrically at 800 nm.

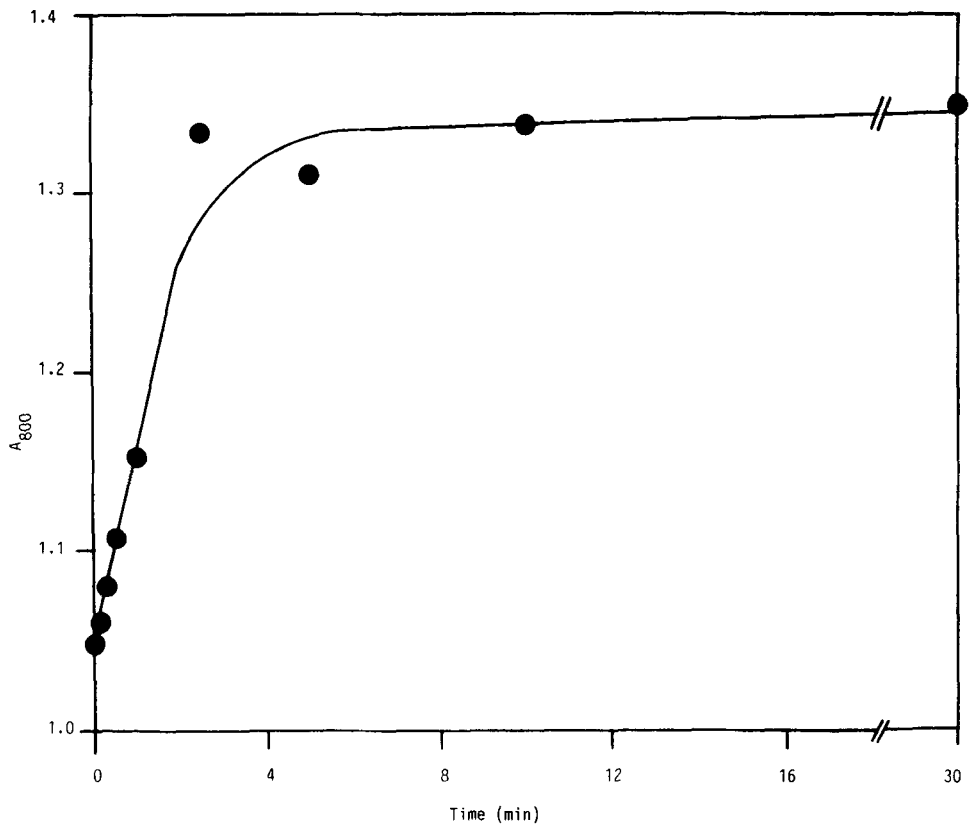

Fig. 2. Influence of duration of cephalosporinase treatment on molybdenum blue formation. The cephalosporinase was used at 50 μL/assay tube. The cephalosporin C was used at 26 μg/assay tube.

The standard deviation of the assay was found to be 4.2%. Other cephalosporins (cephamycin C, cephalexin, cephalothin, and cephaloridine) were tested and found to yield amounts of molybdenum blue similar to that from an equimolar concentration of cephalosporin C (data not shown).

We next followed a cephalosporin fermentation, using *Cephalosporium acremonium* C-10 (*Acremonium chrysogenum* ATCC 48272), by both the disk-agar diffusion bioassay and the chemical assay. The bioassay employed the β-lactam super-sensitive mutant, *Escherichia coli* ESS. As can be seen in Table 1, the values with the chemical assay were very similar to those of the bioassay. Thus, the present method can be used to assay fermentation broths. The chief advantage of the chemical assay is that it is much faster.

The details of the assay are as follows. The reagents used for the colorimetric assay of cephalosporins are the same as those used for penicillins (*1*), except for the replacement of penicillinase by cephalosporinase from *E. cloacae*. The assay employs 100 μL of sample, containing 2.5–250

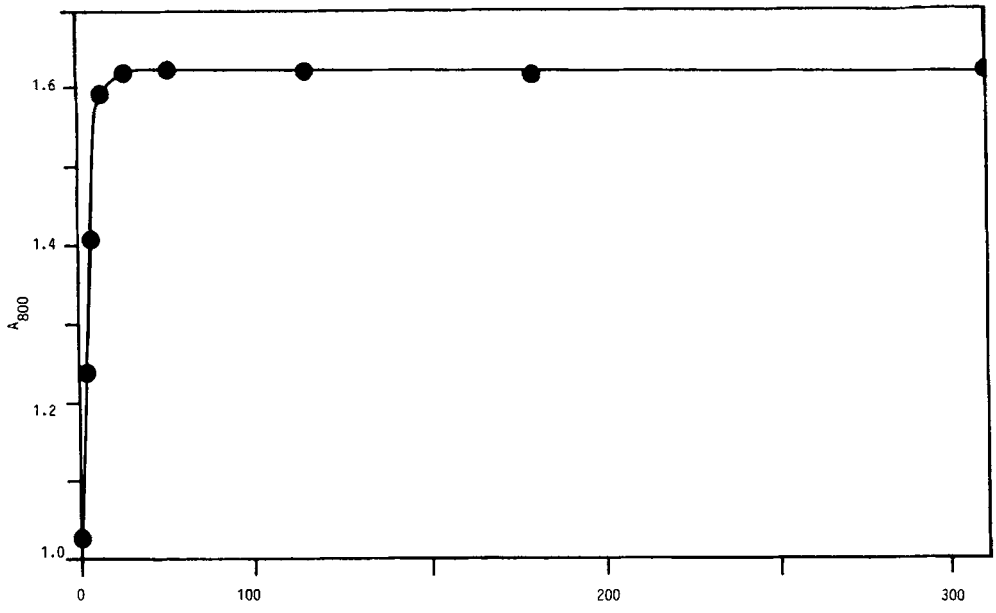

Fig. 3. Influence of the amount of cephalosporinase on molybdenum blue formation. Incubation with cephalosporinase was for 10 min. Cephalosporin C was used at 26 μg/assay tube.

μg of a cephalosporin, 1 mL of phosphate buffer (0.3*M*, pH 7.5), and 50 μL of the cephalosporinase solution (containing 50 μg cephalosporinase in phosphate buffer). A control reaction is carried out without cephalosporinase, and its value is subtracted from those containing the enzyme. After a 10-min incubation at room temperature, 1 mL of molybdoarsenic acid–mercuric chloride reagent is added. The reagent

TABLE 1
Comparison of Chemical and Microbial Assay of Cephalosporins[a] Produced During a Fermentation

Fermentation time, h	Cephalosporins[a] μg/mL	
	Microbial assay	Chemical assay
24	33	50
48	760	600
72	3.250	3.500
96	6.200	6.600
120	10.500	11.000

[a]The organism used here, *Cephalosporium acremonium* strain C-10 (*Acremonium chrysogenum* ATCC 48272), makes a mixture of predominantly cephalosporin C and desacetylcephalosporin C, with only traces of penicillin N.

is prepared by dissolving 50 g of ammonium molybdate $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$ in 700 mL of deionized water and then adding 42 mL of concentrated sulfuric acid (with cooling), followed by addition of a solution containing 6 g of sodium arsenate ($Na_2HAsO_4\cdot 7H_2O$) in 100 mL deionized water; the final vol is adjusted to 1 L. The solution is stored at 37°C for at least 24 h. Just before using, the stock solution of molybdoarsenic acid is mixed with an equal vol of a stock solution of mercuric chloride, containing 0.7 g $HgCl_2$/L. The resulting blue color is measured after 70 min of incubation at 800 nm in the Gilford Model 2600 spectrophotometer. In the case of mixtures of penicillins and cephalosporins, we recommend determining the total β-lactam titer by using cephalosporinase (which can hydrolyze both cephalosporins and penicillins) and subtracting from this value the penicillin titer that can be determined by the same assay with penicillinase replacing cephalosporinase, i.e., the assay of Holm (*1*).

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