BINDING OF ^{125}I-LABELED β-LACTAM ANTIBIOTICS TO THE PENICILLIN BINDING PROTEINS OF *ESCHERICHIA COLI*

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^{125}I-Labeled derivatives of the β-lactam antibiotics cephalexin, cephradine, cefaclor and 6-α-aminopenicillanic acid have been obtained by reacting these compounds with (^{125}I)-Bolton-Hunter reagent.

The following target proteins were found in *Escherichia coli*: (1) The derivatives of cephalexin, cefaclor and cephradine preferentially interact with the high molecular weight penicillin binding proteins (PBP1a and PBP1b); (2) The ^{125}I-derivative of 6-α-aminopenicillanic acid is preferentially bound by the low molecular weight penicillin binding proteins 4 and 5/6. The iodinated derivatives showed a very high affinity of binding to their target proteins with apparent half-saturating concentrations in the nano-molar range.

The characteristics of the isotopically labeled β-lactams commercially available make them, in many cases, unsuitable to study the metabolism of penicillin binding proteins (PBPs). Their low specific activity and poor selectivity of binding make many experimental protocols too time-consuming to be feasible.

Following SCHWARZ *et al.*[1] and our own experience of using *N*-(3-(4-hydroxy-5-[^{125}I]iodophenyl)-propionyl)ampicillin, (abbreviated [^{125}I]ampicillin)[2,3,4], prompted us to synthesize other ^{125}I-labeled derivatives of commercial β-lactams, potentially useful for a more detailed study of the PBPs.

The results reported below indicate that the radioiodinated derivatives of cephalexin, cephradine, cefaclor and 6-α-aminopenicillanic acid, might be useful tools in the study of the physiology of at least some PBPs.

Materials and Methods

Bacterial Strains

The strains *Escherichia coli* MC6 (K-12, F$^-$, *thr*, *leu*, *proA*, *thyA*, *dra*, *drm*)[5] and W7 (F$^-$, *dapA*, *lysA*)[6] were used to obtain cell envelopes.

Binding of the ^{125}I-Labeled Derivatives to the Penicillin Binding Proteins

Purified cell envelopes were obtained and the binding experiments performed as described by SPRATT[7]. 25 μl aliquots of purified envelopes (15 mg/ml of protein) were reacted for 10 minutes at 37°C with different amounts of labeled antibiotic. After electrophoretic fractionation of the proteins, the labeled PBPs were detected by autoradiography on Kodak X-Omat X-ray film. The amount of antibiotic bound to each protein was quantified by counting the corresponding areas on the gels in a gamma-counter.

Measurement of the Binding of Unlabeled Antibiotics by Competition with [^{125}I]Ampicillin

The experimental protocol followed in competition experiments was essentially as above, except

that the envelopes were preincubated for 10 minutes at 37°C in the presence of the unlabeled β-lactam before the addition of [^{125}I]ampicillin (2,000 Ci/mM specific activity) to give a final activity of 50 μCi/ml.

Preparation of ^{125}I-Labeled Derivatives

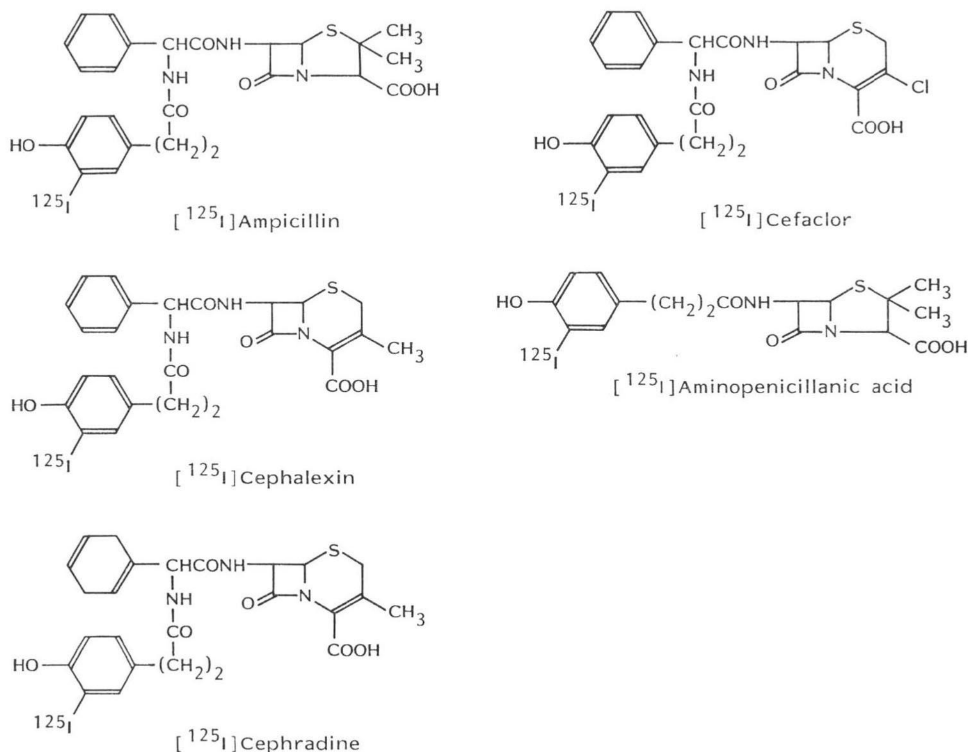
To obtain the radiolabeled derivatives, the selected antibiotics were reacted with (^{125}I)-Bolton-Hunter reagent (specific activity, 2,000 Ci/mM, The Radiochemical Center, Amersham) under conditions similar to those described by SCHWARZ *et al.*[1]. 0.5 ml of a 200 μg/ml solution of the selected β-lactam in 50 mM borate buffer pH 8.5, precooled at 2°C, were added to a vial containing 1 mCi of (^{125}I)-Bolton-Hunter reagent dried under a stream of nitrogen. The reaction mixture was incubated for 30 minutes at 2°C, plus 15 minutes at room temperature. We have developed a new method to recover the radioactive derivatives; the pH of the reacting solution was lowered to pH 2 with 1 N HCl, in the presence of 2 ml of ethyl acetate, and the mixture immediately vortexed for 30 seconds. The organic phase, containing the ^{125}I-derivative was separated, added to a test tube containing 2 ml of 50 mM phosphate buffer, pH 7.0 and mixed. Ethyl acetate remaining in the buffer was removed by a double extraction with petroleum ether. The aqueous phase, containing the radioactive derivative, was collected and aliquoted into samples of a convenient size, which were frozen and stored at −30°C until used. The structures of the ^{125}I-labeled derivatives obtained are shown in Fig. 1.

The antibiotics used were generous gifts from: Antibioticos S.A. (ampicillin and 6-α-aminopenicillanic acid), Lilly Indiana, España (cefaclor), and Squibb and Sons (cephradine).

Results

The patterns of binding of the ^{125}I-labeled derivatives of cefaclor, cephradine, cephalexin and 6-α-aminopenicillanic acid, to the PBPs of *E. coli*, were determined by standard binding assay. Fig. 2

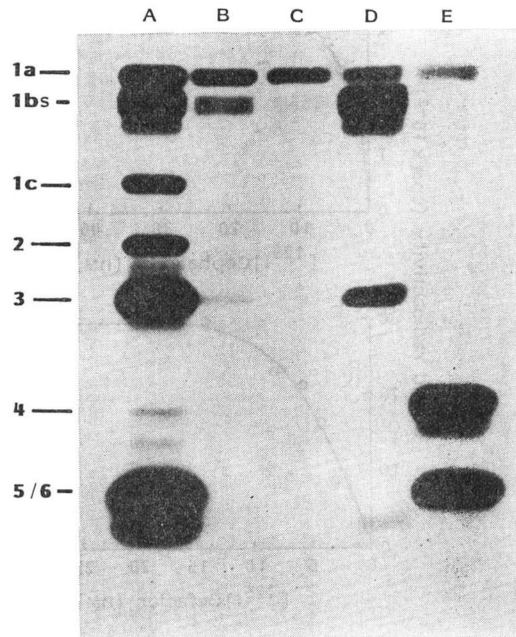
Fig. 1. Chemical structure of the ^{125}I-labeled derivatives of ampicillin, cephalexin, cephradine, cefaclor and 6-α-aminopenicillanic acid.



shows the patterns of binding of the new derivatives, compared to that obtained with [^{125}I]ampicillin. It is interesting to note that, whereas the derivatives of cefaclor, cephradine and cephalexin were preferentially bound to the high molecular weight proteins (PBPs 1a and 1b), that of 6-α-aminopenicillanic acid mainly interacted with the low molecular weight PBPs (4, 5/6). The binding of the ^{125}I-labeled derivatives to the PBPs of *E. coli* exhibited saturation kinetics as shown in Fig. 3. The concentration of the antibiotics required to reach half-saturation in the binding reaction was calculated from a number of experiments similar to that shown in Fig. 3. In these experiments, preparations of membranes from two different *E. coli* strains (MC6 and W7) were used. Results of this series of experiments are shown in Table 1.

Fig. 2. Binding of ^{125}I-labeled β-lactam antibiotics to the penicillin binding proteins of *Escherichia coli* MC6.

(A) [^{125}I]Ampicillin 25 nM; (B) [^{125}I]cephalexin 5 nM; (C) [^{125}I]cephradine 5 nM; (D) [^{125}I]cefaclor 5 nM; (E) [^{125}I]aminopenicillanic acid 5 nM.



The affinities of the original antibiotics (cefaclor, cephalexin, cephradine and 6-α-aminopenicillanic acid) to the PBPs of the same bacterial strains were determined by means of competition experiments for comparative purposes. Table 2 shows the concentrations of the antibiotics required to inhibit the binding of [^{125}I]ampicillin to the PBPs by 50% (ID_{50}), under conditions identical to those used in the studies of binding of the ^{125}I-derivatives. Similar ID_{50} values were found in analogous experiments using [^{14}C]benzylpenicillin (data not shown).

Table 1. Concentration of ^{125}I-labeled derivatives required to half-saturate the binding capacity of the PBPs in purified envelopes of *E. coli* W7 and MC6.

PBP	Half-saturating concentration (nM)									
	[^{125}I]Ampicillin		[^{125}I]Cephalexin		[^{125}I]Cephradine		[^{125}I]Cefaclor		[^{125}I]APA	
	W7	MC6	W7	MC6	W7	MC6	W7	MC6	W7	MC6
1a	21	19	3	3	15	6	ND**	ND**	ND**	ND**
1b	55	41	25	72	19	82	4	6	—	—
1c	9	6	—	—	—	—	—	—	—	—
2	14	11	—	—	—	—	—	—	—	—
3	8	4	ND**	ND**	—	—	ND**	ND**	—	—
4	ND**	ND**	—	—	—	—	—	—	2	7
5/6	ND*	ND*	—	—	—	—	—	—	ND*	ND*

ND: Not determined.
APA: 6-α-Aminopenicillanic acid.
* Half-saturating concentrations considerably higher than the maximum concentration tested.
** Weak final level of binding.

Fig. 3. Saturation kinetics of the binding of the ^{125}I-labeled derivatives of cephalexin, cephradine, cefaclor and 6-α-aminopenicillanic acid to their preferential target PBPs.

(A) Binding of [^{125}I]cephalexin to PBP 1a; (B) binding of [^{125}I]cephradine to PBP 1a; (C) binding of [^{125}I]cefaclor to PBP 1b; (D) binding of [^{125}I]aminopenicillanic acid to PBP 4.

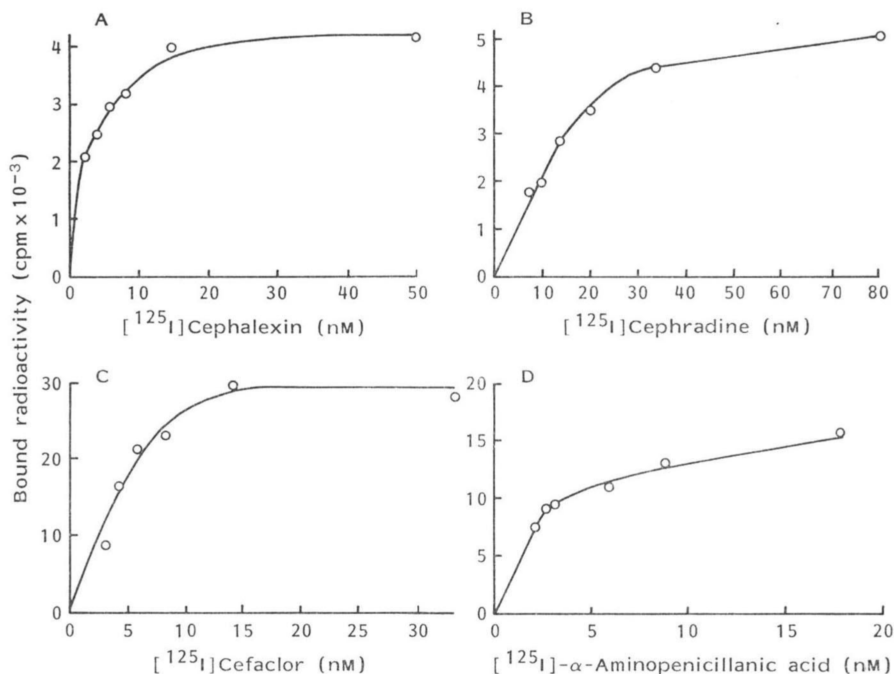


Table 2. Concentrations of β-lactam antibiotics required to inhibit by 50% the binding of [^{125}I]ampicillin to the PBPs in purified cell envelopes of *E. coli* W7 and MC6.

PBP	ID$_{50}$ (μM)									
	Ampicillin		Cephalexin		Cephradine		Cefaclor		APA	
	W7	MC6	W7	MC6	W7	MC6	W7	MC6	W7	MC6
1a	11	7	6	7	30	43	10	10	450	450
1b	56	30	>500	430	>500	500	400	300	>900	>1,000
1c	5	30	—	430	450	500	150	270	>900	>1,000
2	5	7	>500	580	>500	500	275	400	200	245
3	3	5	100	140	115	115	50	50	>900	>1,000
5	>500	590	>500	>580	>500	>580	500	>550	450	400
6	56	59	>500	>580	>500	>580	>500	>550	>900	>1,000

Discussion

Both, the ^{125}I-labeled derivatives of cefaclor, cephradine, cephalexin and 6-α-aminopenicillanic acid described in this paper, and the previously described derivative of ampicillin, have certain characteristics which make them useful tools to study the physiology of the PBPs. The main advantage of these derivatives is obviously their very high specific activity, which allows a considerable reduction in the time required to obtain autoradiographic data. Normally, exposure times of 1 ~ 2 days are enough. Furthermore, the use of these compounds also makes it easier to quantify the results. In most cases, the amount of bound antibiotic can be measured accurately on a gamma-counter.

A very interesting property, common to all the ^{125}I-labeled derivatives so far analyzed, is their ex-

tremely high affinity for their target PBPs. In fact, the apparent half-saturating concentrations calculated are usually between 2 and 3 orders of magnitude lower than the ID_{50}s reported for the unlabeled original antibiotics (compare Tables 1 and 2). There are some indications that it is the presence of the iodophenyl ring on the side chain of the iodinated derivatives which enhances the affinity of these compounds for certain PBPs.

Another striking property of all the iodinated derivatives described here is their high binding selectivity. The derivatives of cephradine, cephalexin and cefaclor were preferentially bound to the high molecular weight PBPs (1a and 1b) whereas the derivative of 6-α-aminopenicillanic acid interacted exclusively with the low molecular weight ones (PBPs 4 and 5/6). This characteristic might also be helpful to trace a single PBP during cell growth or during other kinds of experimental treatments.

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