PHOTOAFFINITY LABELLING OF A TYROSINE CHEMORECEPTOR IN *PSEUDOMONAS AERUGINOSA*

Roy BARBER, Sandy PRIMROSE and George DODD
Departments of Chemistry and Biological Sciences, University of Warwick, Coventry, CV4 7AL, England

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1. Introduction

The molecular mechanisms of several types of chemoreceptors have been recently investigated [1]. The receptor proteins involved in the sensing of the ligand can be identified in some cases by binding studies and another possible method appears to be chemical modification techniques. Photoaffinity labelling using aromatic azides is a particularly attractive chemical modification since molecules of this type are chemically unreactive in their ground state [2].

The aim of this work was to develop photoaffinity labelling methods suitable for identifying a variety of chemoreceptor proteins. Since the simplest chemoreceptors and also the best understood, are those in bacteria, we have chosen, initially, to study these. A variety of bacteria exhibit chemotaxis towards specific chemicals, quantitative assays are available and chemoreceptor proteins have been isolated [3,4]. Since aromatic amino azides are the best studied photoaffinity agents [2] we chose the aromatic amino acid tyrosine as a suitable stimulus for bacterial chemotaxis. The aim of this initial work was to see whether we could specifically photolabel the chemoreceptor protein for tyrosine using azido analogue. *Pseudomonas aeruginosa* was chosen as a bacterial species with a potential tyrosine receptor. It was felt that the ability of this species to metabolize a great variety of chemicals [5] might indicate that it had an unusual variety of chemoreceptors including one for tyrosine.

2. Materials and methods

All the compounds were pure on thin layer chromatography in several solvent systems and exhibited the expected NMR spectral characteristics and melting points. The following compounds were synthesized from L-phenylalanine by the method in [6]:

(1) *p*-nitrophenylalanine;
(2) *tert*-butoxycarbonyl-*p*-azidophenylalanine;
(3) *tert*-butoxycarbonylphenylalanine;
(4) *p*-azidophenylalanine.

All other materials used were of the highest purity available from the Sigma Chemical Co. and were used without further purification.

The organism used was *Pseudomonas aeruginosa* strain PA02, obtained from Dr M. Day, University of Warwick. A culture of the cells was kept in 1% tryptone broth (Oxoid) for $\leq$1 week at 4°C. The motile cells for the chemotaxis experiments were obtained by adding an aliquot of this culture to the tryptone broth and incubating overnight at 37°C with shaking. A sample of this culture was taken and the bacterial count was adjusted to 6×10^8 cell/ml. The bacteria were spun down in a bench centrifuge ($1450 \times g$, 10 min). The pellet was washed twice in chemotaxis medium (0.01 M potassium phosphate buffer, 0.01 mM potassium ethylenediamine tetraacetate (pH 7.0)).

The final pellet was resuspended in the chemotaxis medium to give a bacterial count of $\sim 6 \times 10^7$ cells/ml. Chemotaxis was measured using the conventional

capillary method [3]. The capillaries containing the attractant were incubated for 30 min in the bacterial suspension, the outside surfaces of the capillaries were washed carefully and the contents of the tube were blown into 10 ml tryptone broth. Aliquots of appropriate dilutions of the broth were plated out on tryptone agar, incubated overnight, and the colonies counted the next day. Only viable cells are counted in this assay. The photolabelling experiments were carried out by irradiating 1 ml of a bacterial suspension (5×10^6 cells/ml) in a 1 mM solution of *p*-azido-L-phenylalanine using the light from a 100 W tungsten lamp taken through a monochromator set at 364 nm. The suspension was irradiated at 15°C for 30 min. Under these conditions no obvious killing was observed.

3. Results and discussion

Pseudomonas aeruginosa exhibits positive chemotaxis towards several amino acids and sugars (tables 1, 2 and fig.1,2). In these experiments there was no attractant in the chemotactic medium and the mean number of cells which migrated into the capillary tube containing no attractant was 9980 (SD ± 8600) as measured from 20 determinations made on 12 cultures. The concentration of the attractant giving

Table 1
Chemotaxis in *P. aeruginosa*

Attractant		Concentrations giving maximum response
Sugars		
D-galactose	–	10^{-2} M
D-ribose	–	10^{-2} M
D-glucose	–	10^{-2} M
Maltose	–	10^{-3} M
L-Amino acids		
Aspartate	–	10^{-3} M
Glutamate	–	10^{-2} M
Cysteine	–	10^{-3} M
Alanine	–	10^{-2} M
Leucine	–	10^{-3} M
Histidine	–	10^{-3} M

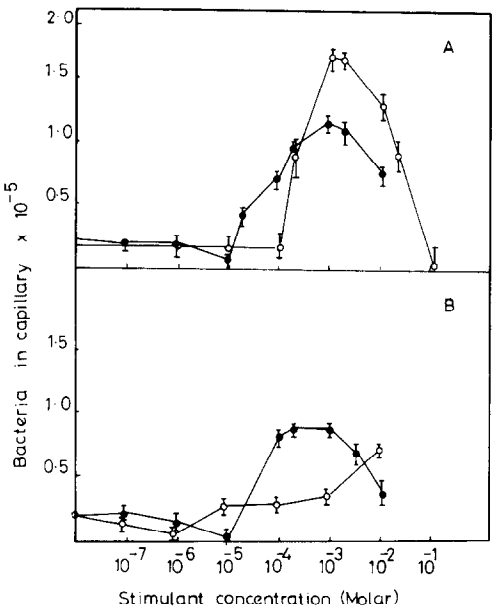

Fig.1. Chemotaxis of *P. aeruginosa* to amino acids. A: (○) L-phenylalanine; (●) *p*-azido-L-phenylalanine. B: (○) *p*-nitrophenylalanine; (●) BOC-*p*-azidophenylalanine. Error bars indicate the range of bacterial counts in three separate experiments.

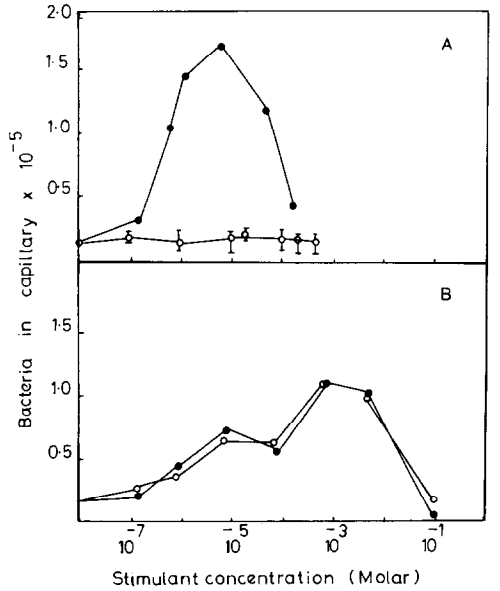

Fig.2. Effects of photolabelling on chemotaxis in *P. aeruginosa*. A: (●) Taxis to L-tyrosine before photolabelling; (○) taxis to L-tyrosine after photolabelling. B: (○) Taxis to L-aspartate before photolabelling; (●) taxis to L-aspartate after photolabelling. The errors in the bacterial count in these experiments was comparable to those shown in fig.1.

Table 2
Chemotaxis of *P. aeruginosa* towards phenylalanine and its derivatives

Attractants	Threshold concentrations	Concentrations giving maximum response
Phenylalanine	10^{-4} M	5.10^{-3} M
p-Nitrophenylalanine	10^{-5} M	10^{-2} M
BOC-*p*-nitrophenylalanine	10^{-7} M	10^{-3} M
Tyrosine	10^{-7} M	10^{-5} M
p-Azidophenylalanine	10^{-5} M	10^{-3} M
BOC-*p*-azidophenylalanine	10^{-5} M	10^{-3} M

Abbreviation: BOC, *tert*-butoxycarbonyl

maximum response, and the threshold concentration provide a guide to the sensitivity of the cell's chemoreceptors for the attractants.

Phenylalanine and its derivatives were good attractants. Of the phenylalanine derivatives tyrosine was clearly the best attractant having both a low threshold (10^{-7} M) and a low concentration (10^{-5} M) at the maximum response. Phenylalanine itself was a moderately good attractant (table 2) and substitution in the *para* position of the aromatic ring by either a nitro group or an azide group decreased the threshold concentration but resulted in a smaller number of cells attracted into the capillary at the maximum response. The concentration of attractant giving the maximum response for both *p*-azidophenylalanine and *p*-nitrophenylalanine was slightly lower than for the unsubstituted phenylalanine but was much higher than that of tyrosine. On the basis of its chemotactic properties *p*-azidophenylalanine seemed a possible lable for the tyrosine chemoreceptor.

We established suitable conditions for photolabelling of the tyrosine chemoreceptor in a preliminary investigation of the photochemical labelling of a protein. Esters of tyrosine are specific substrates of α-chymotrypsin with the aromatic ring fitting into a hydrophobic cavity in the active site. *p*-Azidophenylalanine, in the dark, was a competitive inhibitor of *N*-acetyltyrosine-*p*-nitrophenyl ester with $K_i \sim 10^{-5}$ M at pH 7.8 in 0.1 M Tris–acetate buffer. Photoinactivation experiments were performed by irradiating the enzyme–inhibitor mixture at 25°C under nitrogen, using a 100 W tungsten lamp with the light passing through a monochromator. At wavelength of 364 nm, well clear of the A_{max} of the protein, efficient photolysis was found. When the enzyme (2 mg/ml) and *p*-azidophenylalanine (4 mM) were irradiated under these conditions in Tris–acetate buffer at pH 7.8, the activity of the enzyme with *N*-acetyltyrosine *p*-nitrophenyl ester as substrate was reduced to 15% of the original value after 20 min. When tyrosine was used instead of *p*-azidophenylalanine no inactivation of the enzyme was found. This experiment established that the *p*-azidophenylalanine can act as a photoaffinity agent at a binding site for the tyrosine ring.

The photolysis conditions which were successful with the enzyme also worked with the cells of *Pseudomonas aeruginosa*. Irradiation with visible light rapidly killed the cells but monochromatic light of wavelength 364 nm had no effect. After photolysis with the affinity label, *p*-azidophenylalanine, the cells were washed in chemotactic medium to remove the label and its decomposition products. The washed cells were fully motile and although the chemotactic response to a non-aromatic amino acid, aspartate, remained unchanged, the response to tyrosine was completely abolished (fig.2).

These experiments show that *Pseudomonas aeruginosa* has a specific chemoreceptor for tyrosine and that this receptor can be specifically labelled using *p*-azidophenylalanine, a tyrosine analogue. A tritiated photoaffinity label will facilitate the isolation and characterisation of the chemoreceptor, and should also be of use with other species of bacteria and will aid in the design of photoaffinity labels for other chemoreceptors [8].

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