Peptides with different amino acid cores were synthesized and tested for interaction with common dyes and detection systems. Our goal was to discover the potential of 5-(and 6-)carboxybetamethylrhodamine (5[6]-TAMRA), fluoresceinithiocyanate (FITC), biotin and streptavidin to crossreact with individual amino acids.

To this end we designed 20 pep-tides of the sequence XXX[aa]XXX, where [aa] denotes five repeats of one of the 20 amino acids, and prepared them via SPOT synthesis [2]. Glycine was chosen as the flanking residue X to act as a spacer molecule. As analyses small peptides (gly-gly-gly) were solid phase synthesized and afterwards labelled with the detection compound of interest. The resulting amino acid library was then incubated with the glycine labelled detection system and evaluated via optical and fluorescent methods.

There are several methods commonly used to measure protein-protein interactions and binding affinities. Quite contrary to most of these methods, protein- and peptide arrays on cellulosic membranes or glass slides are suitable for high-throughput measurement, as they provide a higher density of probes and a multitude of peptide-protein interactions can be measured in parallel [1]. The most important application of the SPOT synthesis technique is to simultaneously detect a high number of peptides that have a strong binding affinity to defined targets. The validity of the results, however, depends on the ability of the detection system to indicate binding events whilst not interfering with the experiment itself through cross reaction. We tested three common peptide detection systems (TAMRA, FITC, Biotin/ Streptavidin) for their ability to interact with cellulosic bound peptide in the image. Our approach identified several amino acids interacting with different detection systems. These results will strengthen the reliability of the analysis of SPOT synthesis generated data in the future.

ABSTRACT

Solid phase peptide synthesis

Soluble peptides were synthesized (50 μmol scale) as amides on a multiple synthesizer AMS 422 according to the standard Fmoc protocol using TentaGel S RAM resin and PyBOP activation. All peptides were analysed by reversed phase HPLC and MALDI TOF.

Measurement of spot signal intensities

Analysis and quantification of spot signal intensities (SI) were conducted with the GeneSpotter software package. Genespotter has a fully automatic grid finding routine resulting in reproducible signal intensities. The spot signal is calculated from a circular region around the spot center detected in the image. The background signal for each spot is determined with a safety margin to this circular region.

OUTLINE

Biotin & Streptavidin

Streptavidin-POD, as a secondary detection system, was first tested on the membrane bound peptides to be able to differentiate between streptavidin interactions and biotin interactions. Then the biotin labelled peptide was incubated and detected via Streptavidin-POD. As the Figure shows, streptavidin is capable of crossreacting with positively charged amino acids like lysine and arginine. This interaction matrix set as a background reveals the weak crossreactivity capabilities of biotin. Mostly small hydrophobic (alanine, phenylalanine) and aliphatic (isoleucine, valine, leucine) amino acids as well as proline and the hydroxyl serine show a signal slightly above the background.

Cell-ligand peptide arrays were prepared according to standard SPOT synthesis protocols using a SPOT synthesizer as described in detail [2]. The peptides were synthesized on amino-

TAMRA

FITC and peptide autofluorescence

To get reliable results the signal intensities of the autofluorescence of peptides at 520nm (Fig) had to be set as background. The common background results from incomplete removal of the Fmoc protection group. Three amino acid cores (W, T and H) show signals above the average background. The aromatic tryptophan and the ring system of histidine have more than 6 valence electrons and are therefore capable of emitting light at 520nm. Both phenylalanine and tryptophan possess less valence electrons. Explaining the fluorescence of threonine still remains a challenge to theoretical chemistry.

References


RESULTS

METHODOLOGY