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Selection of Putative Immunogenic Peptides by Molecular Modelling of the Urease of *Helicobacter pylori*

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Key words: Molecular modelling, *Helicobacter pylori*, urease.

Abstract: Computational methods such as molecular modelling are becoming an increasingly useful means of rationalising experimental data and creating a hypothesis that can suggest new experiments. In this report we discuss the application of molecular modelling methods to aid the selection of feasible peptide epitopes of the urease enzyme from *Helicobacter pylori*, an important vaccine candidate. Surface exposure was chosen as a criterion for the selection of three peptides which each had different levels of accessibility according to the 3D model. Antibodies raised against these peptides were analysed for their immunoreactivity with the holo enzyme. Only one anti-peptide antibody showed good reactivity with the urease. Our findings emphasise that surface exposure of peptide is not the only important criterion for the selection of immunogenic peptides.

INTRODUCTION

Helicobacter pylori is a major cause of gastritis, peptic and duodenal ulcers and stomach cancer. Gastritis, peptic and duodenal ulcers can be treated effectively with a short course of antimicrobial agents and a proton pump inhibitor; however antibiotic resistant strains of *H. pylori* pose increasing problems for treatment efficacy. Vaccination may offer an alternative means of preventing and eradicating *H. pylori* infection. The urease enzyme (urease AB) of *H. pylori* is essential for the survival and colonisation of the stomach [1]. Of the several vaccine formulations currently being considered, the urease vaccine is the most extensively investigated. This vaccine consists of the holo-urease (urease AB) and a mucosal adjuvant, like cholera toxin. The urease B subunit was found to be the protective antigen in animal studies [reviewed in 2]. However urease vaccination of patients with duodenal ulcers or rhesus monkeys infected with *H. pylori* prior to immunisation did result in lower infection rates, but not in complete clearance of *H. pylori*. [3,4]. This failure

may be attributed to induction of local antibodies, which do not inhibit urease activity, or to the survival of *H. pylori* in the host despite an effective immune response against urease. We have investigated the structure of 3D models of the *H. pylori* urease to identify novel exposed regions on the ureB polypeptide chain and to determine the location of putative immunogenic peptides as well as the enzyme active site region.

MATERIALS AND METHODS

The amino acid (AA) sequence derived from ureAB of *H. pylori* 26695 (Acc.no. HPAE 000529) was used to construct a 3D model based on the known structure of the urease of *Klebsiella aerogenes* (pdb entry code 1KAU). Protein homology models have been constructed using the Modeler [5] module of the program insightII 98.0 (Molecular Simulations Inc.). The alignment step was trivial since the level of sequence identity between the urease sequences of the two species was of the order of 61%. The best model for each *H. pylori* sequence was then assembled into a trimer, using the *Klebsiella aerogenes* 3D structure as a guide; this trimer model was then used for calculations of the per residue solvent accessibility with the NACCESS program [6].

Three urease peptides were selected. peptide 1: ⁴²³GSVEVGKVA₄₃₁; peptide 2: ³⁸³KKEFGRLKEEK₃₉₄ and peptide 3: ¹³¹GGIDTHIHFI₁₄₅SPQQI₁₄₅. These peptides were conjugated to keyhole limpet haemocyanin (ISL, Paignton, UK). Rabbits were immunised with 200 µg conjugate suspended in complete Freund's adjuvant (SIGMA), followed by a booster injection of 200 µg conjugate in Incomplete Freund's adjuvant. Anti-urease IgG was produced by immunisation of rabbits with 250 µg recombinant urease (a gift from Oravax Inc., Massachusetts) and Titermax (CytRx Corporation, Georgia) as adjuvant. IgG antibodies to the three peptides and to recombinant urease were detected by ELISA.

RESULTS

Peptide 1 with the sequence ⁴²³GSVEVGKVA₄₃₁, which has previously been shown to have good reactivity with antibodies from patients sera [7], was located in the 3D model. This proved to be a region of moderate solvent accessibility. Peptide 2, ³⁸³KKEFGRLKEEK₃₉₄, proved to be an exposed loop in a helix-loop-helix motif in the 3D model. Peptide 3 contains the nickel binding site region of *H. pylori* urease, ¹³⁶HH₁₃₈, this tripeptide was found to be of low accessibility. Figure 1 shows

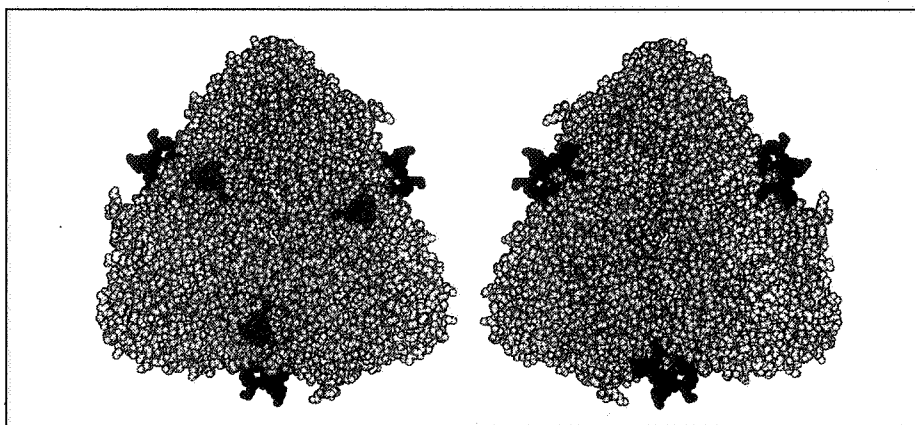


Fig. 1: Space-filling representation of the 3D structural model of a UreAB trimer, with the UreB sequence taken from accession number HPAE 000529. Both faces of the triangular disc-like structure are displayed here. Peptide regions 1 and 2 are shown as light and dark grey respectively.

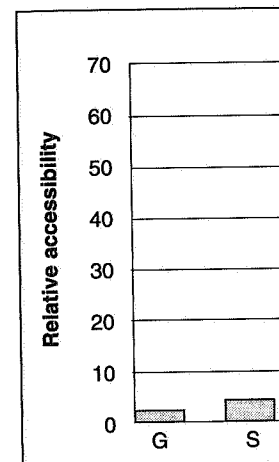


Fig. 2: Per residue plot of the equivalent Gly-X-Gly

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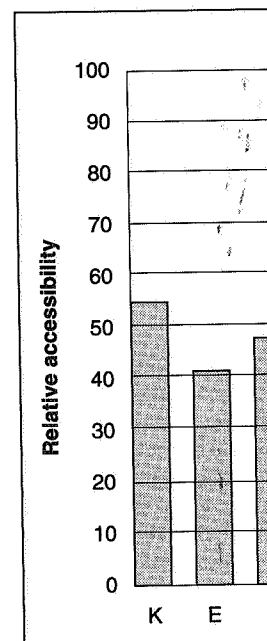
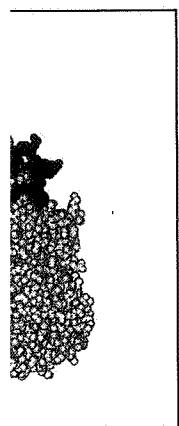


Fig. 3: Per residue plot of the equivalent Gly-X-Gly

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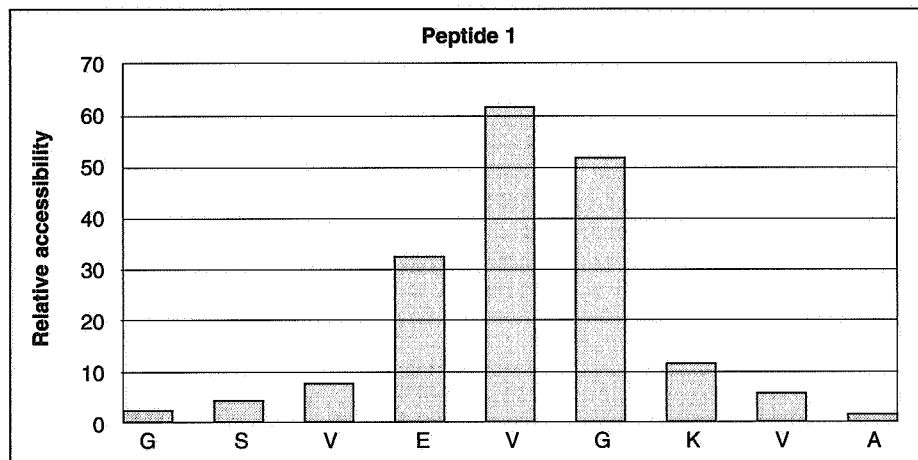


Fig. 2: Per residue plot of the solvent accessible surface area for the peptide 1 region, relative to that in an equivalent Gly-X-Gly tripeptide. This region is considered to be of moderate accessibility.

a space-filling representation of a model trimer for UreAB, in which the UreB sequence is that with accession number HPAE 000529. This figure shows two opposite sides of the trimer, which is triangular in profile with a thickness of approximately half the trimer width. The tripeptide nickel-binding site is not visible

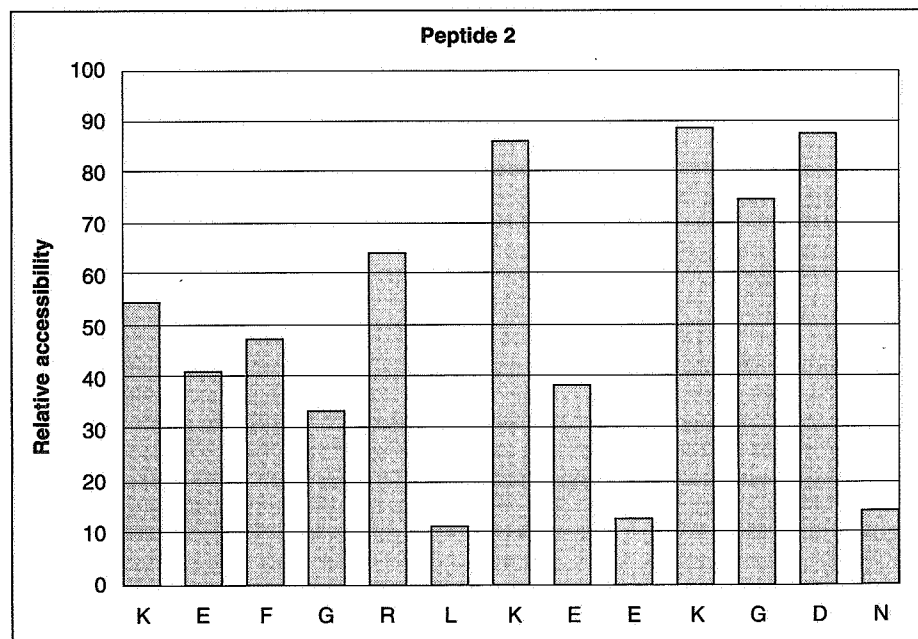


Fig. 3: Per residue plot of the solvent accessible surface area for the peptide 2 region, relative to that in an equivalent Gly-X-Gly tripeptide. This region is considered to be highly solvent accessible.

Table 1: Serum IgG titres^a of rabbits immunised with urease peptides tested in ELISA using recombinant urease and peptides as antigens.

IgG specificity	Recombinant urease	Homologous peptide
Anti-peptide 1	1:800	1:14000
Anti-peptide 2	1:6400	1:51200
Anti-peptide 3	1:25600	1:51200

^a cutoff OD = 0.25

in a space-filling representation because it is buried within other sections of the protein structure. In Figure 1, peptides 1 and 2 are shown as light and dark grey respectively. The latter is clearly in an exposed region at the periphery of the structure. Figures 2 and 3 show plots of the per-residue solvent accessible surface of these two peptides, relative to that in a Gly-X-Gly tripeptide. Antibody reactivity between individual sera varied considerably. In this report we only include sera which showed the highest reactivity to urease. The titres of anti-peptide antibodies with the homologous peptides and with recombinant urease are shown in Table 1. Anti-urease IgG did not react with peptides 1 or 2, and reacted only weakly with peptide 3 (results not shown).

DISCUSSION

The poor reactivity of peptide antibodies with the urease indicates that the conformation of amino acids in peptides 1 and 2 is likely to differ from their conformation in the holo urease. This hypothesis is strengthened by the lack of reactivity of anti-urease IgG with these peptides. An unexpected finding is the high reactivity of anti-peptide 3 antibodies with holo urease. Our model predicted that peptide 3 would not be surface exposed, and therefore antibodies to this peptide were not expected to react with the holo-urease. We explain this finding with two assumptions: (i) the peptide covers the active centre of the urease. Since this centre and its immediate environment are well conserved among ureases of other bacterial species [8], we assume that the amino acids are essential for the conformation, and therefore the conformation of this peptide is compatible with the conformation of the active centre in the enzyme; (ii) coating antigens to solid surfaces i.e. ELISA plates may result in some degree of protein denaturation [9]. This may then allow peptide 3, which is buried in the holo enzyme, to be much more exposed as a result of adsorption to plastic surface of the ELISA plates, and therefore accessible to antibodies. Furthermore, the methodology presented here does not explicitly consider the effects of major histocompatibility complex (MHC) class II binding of peptide antigens, and the subsequent production of cytokines by helper T-cells on the immunogenicity of the peptide. One way in which computational methods may be of assistance here is in the prediction of peptide binding specificity to MHC class II using matrices of amino acid propensity for many MHC allotypes [10].

CONCLUSIONS

It is hoped that the results of these modelling methods can be used in future experiments. In the applications, these methods have been used to identify exposed regions of the enzyme. The anti-peptide antibodies are important for recognition of the native enzyme. Further experiments are planned to test the antibodies with the native enzyme.

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CONCLUSIONS

It is hoped that the results obtained here have demonstrated that computational modelling methods can be valuable for rationalising experimental data, and indeed that such methods can be used for constructing a hypothesis that can suggest new experiments. In the applications described here 3D protein homology models have been used to identify exposed regions of the amino acid sequence in a bacterial enzyme. The anti-peptide antibodies demonstrate that conformation appears more important for recognition of epitopes in the holo-urease than surface exposure. Further experiments are planned to investigate the interaction of anti-peptide 3 antibodies with the native enzyme.

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