Recent advances in prokaryotic peptide transport

Peptide transport systems exist in most organisms, for example many prokaryotes, yeast, fungi, plants and animals including humans[1–3]. These transport systems serve an important function since peptides can be used as a source of nutrient amino acids. In addition, peptide transport systems have attracted interest as potential drug delivery systems[4–6]. Substances which normally do not permeate the cell can be attached to a peptide and transported into a cell via one of the peptide transport systems. Much of the recent work on peptide transport has been carried out on bacteria in which peptides may fulfil auxotrophic requirements for amino acids and may also be the only source of nitrogen or carbon[7,8]. In *Escherichia coli* and *Salmonella typhimurium*, three distinct peptide transport systems have been identified[9]. The Opp system transports oligopeptides of up to six amino acids, the Tpp system transports primarily tripeptides and the Dpp system transports mainly dipeptides. In general, after the peptide permeates the outer membrane of the bacterial cell, it is transported through the inner membrane into the cytoplasm by one of these systems. The genes for these three peptide transport systems have been mapped and, for two of the systems, the operons encoding the transport components have been cloned.

The oligopeptide transport system

The oligopeptide (Opp) transport system has been studied extensively. The energy source driving this system appears to be derived from high-energy phosphate bonds[10]. Genetic studies indicate that the Opp system comprises four genes. These genes, *oppA*, *oppB*, *oppC* and *oppD*, were localized to a single operon within a 5–6 kb segment of DNA at 35 min in *S. typhimurium* and at 27 min on the *E. coli* chromosome[11,12]. The *oppA* gene from both *S. typhimurium* and *E. coli* encodes a probable binding protein which has been localized to the periplasmic space[13]. These results are consistent with the earlier observation that oligopeptide ransport in *E. coli* is sensitive to osmotic shock, indicating a requirement for a periplasmic component[14]. The *oppA* gene product from both organisms is ≈52 kDa, which is rather large compared with periplasmic proteins from other transport systems[13]. This protein is expressed to a very high level in both organisms, relative to other periplasmic proteins[13]. Lastly, in *S. typhimurium*, the expression of *oppA* seems to be constitutive, i.e. not regulatable by any factor tested thus far[13].

The *oppB*, *oppC* and *oppD* gene products are probably associated with the inner membrane as determined by gene fusion experiments with the *lacZ* gene[9]. A single plasmid carrying the *oppB* and *oppC* genes was found to produce two plasmid-encoded inner membrane proteins of 53 and 56 kDa[9]. These proteins are presumed to be the gene products of *oppB* and *oppC*.

The *oppD* gene has been sequenced and its amino acid sequence subsequently deduced. It is highly homologous to the genes *hisP*, *malK* and *pstB* for membrane components from the histidine, maltose and phosphate transport systems, respectively[15]. In addition, it has been proposed that each of these proteins contains a nucleotide binding site. The evidence for this site is 3-fold[15]: (1) there is a consensus nucleotide-binding site on all four proteins; (2) the *oppD* protein is specifically eluted from an affinity column by ATP, not CTP or NADH; and (3) the nucleotide affinity analogue 5′-*p*-fluorosulphonylbenzoyladenosine specifically labeled the *oppD* protein. The presence of a probable nucleotide binding site on these proteins suggests that these transport systems derive their energy by hydrolysis of ATP (or a related nucleotide) and that these membrane components function in energy-coupling to their individual systems.

The tripeptide transport system

The tripeptide (Tpp) transport system uses di- and tri-peptides as substrates and displays a high affinity for hydrophobic peptides. Two genes, *tppA* and *tppB*, involved in tripeptide transport, have been identified in *S. typhimurium*[16]. The *tppB* locus is a structural gene for the transport system and maps to 27 min on the *S. typhimurium* chromosome[16]. The *tppA* gene regulates the expression of *tppB* and is located at 74 min on the chromosome. In addition, *tppA* is identical to the *ompB* gene which regulates the synthesis of proteins for the outer membrane[16]. In *E. coli*, a mutant in tripeptide transport has been isolated and located to 98.5 min[17].

In contrast to the Opp transport system, Tpp transport activity exhibits a complex regulatory pattern[18]. Generally, Tpp transport is much less active than Opp transport. However, under anaerobic conditions or in the presence of exogenous leucine, a 20–30-fold increase in Tpp transport activity was observed with *tppB–lacZ* operon fusions[18]. Using such fusions with the *tppA* gene, no induction was observed. In *tppA*− mutants, however, *tppB* expression was reduced approximately 20-fold. Hence, *tppA* is a non-inducible, positive regulator of tripeptide transport activity.

Recent work has identified a gene, *oxrC*, which is required for anaerobic induction of *tppB* and maps at 98 min on the *S. typhimurium* chromosome (C. F. Higgins, pers. commun.). The gene responsible for the induction of most anaerobic respiratory enzymes is *fnr*. Since the anaerobic induction of *tppB* is not *fnr*-dependent[18], there seem to be two independent anaerobic regulatory pathways.

The dipeptide transport system

The dipeptide (Dpp) transport system is the least understood of the three peptide transport systems discussed here. Although initially reported as specific for dipeptides[19], recent evidence shows that it can also transport certain tripeptides[9]. Mutants in Dpp transport have been isolated and located to 13 min on the *E. coli* chromosome[19]. However, another report states that Dpp mutations map to 82 min (C. F. Higgins, pers. commun.). In triple mutants (Opp⁻, Tpp⁻, Dpp⁻), no peptide transport is detectable, suggesting that the three systems are responsible for essentially all the peptide transport observed in bacteria.

Concluding remarks

The prokaryotic peptide transport systems are proving to be complex both in their organization and regulation. A more thorough knowledge of them should be a significant step towards understanding the mechanism(s) by which solutes are transported.

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Emerging Techniques

Photoelectron microscopy

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Imaging photoemitted electrons from cell components and markers is the electron optics equivalent of fluorescence microscopy. It can be used to extend immunofluorescence to problems in cell biology requiring higher resolution.

In 1941 Coons *et al.* introduced the technique of fluorescence emission combined with labelled antibodies and an optical microscope to visualize and identify antigenic sites in biological specimens[1,2]. The power of immunofluorescence microscopy is that it combines the natural selectivity of the immune system with the contrast provided by fluorescence markers and the morphological information that only a microscope can provide. A dramatic increase in the use of this approach has occurred, due in part to refinements in the techniques and advances in immunology such as the use of monoclinal antibodies[3]. However, as important as this technique is, there is a firm limit on the resolution that can be obtained (about 200 nm). This is imposed by the wavelength of light and is much greater than the molecular dimensions of cellular components. From the efforts of many laboratories to increase resolution have emerged labelling techniques adapted for the established transmission- and scanning-electron microscopy methods (TEM and SEM) and also one technique new to biology, photoelectron microscopy (PEM), which is the subject of this article.

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Basic principles

Photoelectron microscopy is the electron optics analog of fluorescence microscopy. The idea is to retain the contrast inherent in an emission experiment, i.e. imaging bright objects against a dark background, while increasing the resolution by reducing the wavelength of the emitted radiation. The essentials are compared in Fig. 1. Fluorescence involves the absorption of light ($h\nu'$) by the specimen followed by emission of light at longer wavelengths ($h\nu'$). A portion of this fluorescent light is transmitted by a glass objective lens focused on the specimen, and the image is enlarged by an ocular (projector lens). The result is an image of the biological specimen in which bright areas correspond to regions rich in fluorescence emission. Photoelectron microscopy is similar except that UV radiation is used to eject electrons, and the electrons are accelerated and focused by an electron lens system.

As with fluorescence microscopy, the basic principles and instrumentation involved in photoelectron microscopy can be traced back to early work in the non-biological sciences. In the classical physics literature, the emission of electrons by light is simply referred to as the photoelectric effect. This is the same phenomenon that Einstein explained as a quantum effect in 1905. Electron lenses were added in the 1930s and several emission microscopes have been built for use in metallurgy and material science[4–6]. The first photoelectron images of organic and biological specimens were reported in 1972 (Ref. 7) and more recently a high-resolution instrument has been built for biological studies[8]. UV radiation from 100 w short arc lamps is focused on to the specimen which is maintained at cathode potential (−30 to −40 kV). The emitted electrons are accelerated and then passed through a conventional electron optics system. The enlarged image is recorded on film or displayed on a TV monitor for real-time viewing. The microscope is an oil-free ultra-high vacuum design, to avoid contamination of the biological surface structures. The lateral resolution of current photoelectron microscopes approaches 10 nm. It is hoped that this resolution of PEM can be improved to 4–5 nm, i.e. sufficient to map cell surfaces to single-protein resolution. The ultimate resolution is set by the diffraction limit of the emitted electrons, about 1 nm for a 1 eV electron. However, an advanced corrected electron optics system would be required to reduce the aberrations before this diffraction limit could be reached.

It could be said that PEM is just about at the point that fluorescence microscopy reached in 1950 (Ref. 9). The instrumentation and applied physics aspects are successful, if not complete, and biophysical experiments have been performed to determine the photoelectron quantum yields of a wide variety of biological surface components such as amino acids, saccharides, lipids and chromophores. Preliminary photoelectron micrographs have been obtained of a number of biological specimens, including cultured cells, sperm cells, red cell ghosts, thin sections, cytoskeletal preparations, bacteria,