PROTEINS

The Mighty Photon

from our Molecular Biology Correspondent

THE use of the photon as a reagent, or as a trigger that will set off other reagents at a chosen point in space or time, is an approach that will undoubtedly find increasingly wide application in molecular biology. One—albeit qualified—advantage is that short-lived photochemically generated species are inclined to have very high reactivity, and when unleashed on an enzyme, for example, are apt to react even with aliphatic side chains. Too indiscriminate a reactivity of course restricts the value of a reagent, and Sperling and Elad (*J. Amer. Chem. Soc.*, **93**, 3839; 1971) have addressed themselves to ways of controlling the specificity of photochemical alkylation. They have found that on irradiation of a system containing a peptide, an unsaturated species, such as butene or toluene, and an initiator, substitution occurs at the α-carbon of glycine residues, butene giving rise to norleucine, toluene to phenylalanine. When proteins are allowed to react under these conditions, however, histidine, cystine, methionine, tyrosine and tryptophan residues also react. This is a consequence of the formation of various free radicals, and if radical scavengers are added to the reaction mixture the majority of these side reactions are suppressed. It proved impossible, however, to prevent instant death to the tryptophans. In ribonuclease, which has no tryptophan, glycine reacted and, though a certain amount of other damage occurred, 20 per cent of enzymatic activity was retained.

Of more immediate biological moment is the design of photochemically triggered affinity labels, which hold out the advantage that they will not react fruitlessly on their way to the specific binding site. Brunswick and Cooperman (*Proc. US Nat. Acad. Sci.*, **68**, 1801; 1971) have prepared analogues of the biochemical elixir, cyclic AMP, labelled with tritium and bearing diazomalomyl groups in the purine and in the sugar. On irradiation a ferociously reactive carbene is generated, which is expected to react with almost any group within reach. The analogues will bind to the enzyme phosphofructokinase, for which cyclic AMP is the natural activator. They both activate and compete with cyclic AMP, and after irradiation are covalently integrated into the enzyme.

This work now opens the way to the isolation of a labelled peptide and the characterization of the activator binding site. A more exciting prospect for the biochemist, however, is the use of the same reagents to label cyclic AMP receptors in cells, and indeed Brunswick and Cooperman have already succeeded in introducing a label into a protein component of rat testis. The reagent is thus permitted to traverse the membrane and diffuse through the cell to its target protein before it is irradiated and caused to react. The advantages of this strategy in labelling components in intact tissue need no labouring.

Erlanger and his colleagues have meanwhile continued their attempts to simulate the photochemical systems of nature in the laboratory. By the use of acetylcholine analogues capable of light-induced *cis-trans* isomerization they achieved partial control over the activity of an excitable tissue preparation, because the isomers will not in general be expected to possess precisely the same binding characteristics. Bartels, Wassermann and Erlanger (*ibid.*, 1820) have now brought this essay in biochemical engineering to a considerable state of refinement. They have, apparently by a process of serendipity, arrived at a species—an azobenzene derivative—of which the *trans* isomer is one of the most powerful known activators, and the *cis* isomer is largely, perhaps completely, inactive. They have moreover built into such a molecule the means for attaching it covalently to its receptor. This is based on the earlier observation by Karlin that affinity labelling of the acetylcholine receptor of the electroplax membrane could be achieved with a thiol-reactive function, if the tissue was first treated with a reducing agent to break a disulphide bond, evidently located hard against the binding site.

Substantial *cis-trans* interconversion of the azobenzene derivative can be achieved by ultraviolet irradiation, and is reversed by white light. Thus when the system containing electroplax tissue, stimulated by the *trans* isomer, is exposed to the near-ultraviolet light, precipitous depolarization ensues. Competition with carbamylcholine and curare occurs as expected. When the thiolreactive version of the analogue is covalently attached to the reduced membrane, the competitive effect of carbamylcholine is lost. This then is a completely self-sufficient light-regulated system. The reactive *trans* isomer is reported to inhibit acetylcholinesterase, but so equally does the *cis* form. The inferred message is that the acetylcholine receptor and acetylcholinesterase are distinct and unrelated forms.

Plasma Current Amplifier

IN next Monday's *Nature Physical Science*, P. C. Stangeby and J. E. Allen describe a new device which contains a narrow metal-walled electric discharge vessel and which is capable of high current amplification. The metal walls behave as a cathode and electrons are extracted from the metal by surface recombination with ions that arrive from the plasma.

According to the theoretical description of the device, the ion current travelling to the walls of the conducting cylinder is related to the discharge arc current in an expression involving the radius of the cylinder, the electron and ion masses and other constants which are of the order of unity. The cylinder is biased in such a way that the electrons produced by ionization of the neutral particles which carry them away from the wall are retained in the arc discharge; the ions can then return to the cylinder to pick up other electrons. The plasma current thus increases exponentially with distance down the cylinder and, for small cylinder radii and small ion masses, the multiplication factor is very large (for H_2 in a 5 mm diameter cylinder, for example, a current amplification of about 10^7 can be obtained).

The prototype apparatus used by Stangeby and Allen comprised a metal tube 6 mm in diameter and an anode which could be moved inside the tube. There were two alternative cathodes—a mercury pool type and a thermionic type. A 2 A current from the mercury pool cathode was made to flow across the mouth of the metal discharge tube and was collected by an auxiliary anode; the required current input to the metal tube itself was then tapped off.

The thermionic cathode gave similar experimental results, but one of its advantages was that the tube current could be measured directly with an ammeter. There was also a facility for introducing a quantity of argon gas to examine the effect of reducing the ion mass (which should increase the amplification). Measurements with the apparatus have shown that the current amplification at a mercury pressure of 1 mm Hg and an argon pressure a few hundred times this value is as expected theoretically. Although they did not measure the argon flow rate accurately, Stangeby and Allen found that an increased rate was associated with a distinct increase in the amplification.

There are probably several uses for current amplifying devices of this type, including applications to high power lasers; they may also be useful as high current controls.