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W. Kabsch and C. Sander

Toward Peptide Vaccines: Prediction of Antigenic Peptides from Protein Sequences of Influenza Virus and Foot and Mouth Disease Virus

Peptide vaccines, synthesized or genetically engineered, may become a powerful tool against viral infection. They may be more efficient than conventional vaccines against viruses with rapid antigenic drift. Protein structure theory has aided the effort to develop such vaccines by selecting from the amino-acid sequence peptides likely to be antigenic.

We have combined algorithms for the prediction of protein secondary structure and analysis of antigenic determinants of proteins of known three-dimensional structure into a method for the prediction of antigenic peptides. The most likely candidates are peptides which as part of the protein are bent polar regions stabilized by hydrogen-bonded structure. Examples are surface loops between segments of helix/sheet^[1] and polar helix faces^[2].

Our predictions were tested experimentally in the case of influenza virus hemagglutinin^[1] and foot and mouth disease virus coat protein VP1^[2]. Two of the synthesized peptides (SKAFSNAYPYDVPDYASL^[1] and LRGLQVLAQKVARTL^[2], respectively) were successful in that they induced virus neutralizing antibodies and, in some animal tests, resulted in protection against challenge with the virus. The results on foot and mouth disease virus (FMDV) are particularly encouraging. Predictions on other viruses and bacteria are currently being tested.

In our method, secondary/tertiary structure prediction is a crucial part. Unfortunately, structure prediction methods used so far are relatively inaccurate^[3] (not better than 56% average accuracy for three states). We now have a new method for secondary structure prediction with a better than 60% rating. Its use will lead to a corresponding improvement in the prediction of antigenic sites.

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B. Kadenbach and A. Stroh

Influence of Variable Redox States of Glutathione on the Activity of Cytochrome c Oxidase

The activity of isolated pig heart cytochrome c oxidase (EC 1.9.3.1) is inhibited with partially oxidized glutathione. The rate of inhibition is proportional to the concentration of glutathione, whereas the extent of inhibition depends on the redox state of glutathione. Up to 10mM GSH or GSSG alone are without effect on the activity of cytochrome c oxidase. The steady state activity of cytochrome c oxidase is zero at a molar ratio GSH/GSSG of about 2:1. At physiologically occurring redox states of glutathione (95% reduced) the activity of cytochrome c oxidase is about 50% of the control. Similar inhibition of cytochrome c oxidase activity by glutathione was found with the reconstituted beef heart enzyme in proteoliposomes and with mitochondrial membranes from pig liver.

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V. Kaever and K. Resch

Characterization of Plasma Membrane-Bound Nucleotide Cyclases in Calf Thymocytes

The role of cyclic nucleotides in the regulation of lymphocyte growth and differentiation still remains controversial, despite an overwhelming literature dealing with intracellular levels of cAMP and cGMP under various conditions. One of the major reasons resides in the lack of adequate characterization of the key enzymes, adenylate cyclase (EC 4.6.1.1) and guanylate cyclase (EC 4.6.1.2) in the plasma membrane of lymphocytes.

Calf thymus lymphocytes were disrupted by nitrogen cavitation and plasma membranes isolated by differential centrifugation and subsequent sucrose density ultracentrifugation^[1].

As revealed by the chemical composition and the activities of some marker enzymes, the plasma membrane fraction proved to be highly purified. Nucleotide

cyclases were present in specific activities, basal being 13.7 pmol/(mg protein × min) for adenylate cyclase and 1.5 pmol/(mg protein × min) for guanylate cyclase. Adenylate cyclase effectors added directly (NaF, GTP, guanosine and molybdate. Basal as well as activities stimulated by imidodiphosphate exhibited kinetics. Activation by both affected K_m values, but activity of guanylate cyclase was not enhanced by the nonionic detergent Triton X-100. High doses of lysophosphatide indicated to be an inhibitor of adenylate cyclase indicated to be an activator by Hill equation with an n value of 2. In contrast Triton X-100 showed regular substrate inhibition but not affecting K_m . Preliminary experiments of adenylate cyclase nor guanylate cyclase were not changed in plasma membranes of lymphocytes, but rather in thymocytes.

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G. Kaiser and U. Heber

The Role of Vacuoles in the Regulation of Photosynthetic Products

In photosynthesis, the triose phosphate and phosphoglycerate are transported from the chloroplasts to the cytosol. Sucrose is synthesized in the cytosol. In kinetic work with mesophyll cells, vacuoles were rapidly isolated. It was found that the rate of transfer of sucrose from the cytosol across the vacuolar membrane may approach the rate of transport of a major nutrient among imported products of citrate, malate, glutamate, and other amino acids also significant. Phosphate transport from the vacuole.

Sucrose transport has been studied in isolated vacuoles from barley. It is carrier-mediated as shown