Poster Session: Complexes of Aminoacids, Peptides and Proteins

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Comparative Studies on the Adsorption of Urease to Porous and Nonporous Aluminium Oxides

PETER GRUNWALD

Institut für Physikalische Chemie, Universität Hamburg, Laufgraben 24, D-2000 Hamburg 13, F.R.G.

The adsorption of enzymes and other proteins has for a long while played an important role in the field of chromatography [1]. Beyond that, the adsorption of proteins, as well as their covalent attachment to carriers is known as a method for preparing immobilized enzymes. The application of carrier bound urease in the fields of analytical chemistry and medicine [2, 3] is well known.

Most of the adsorption equilibria examined can be described quantitatively in terms of the Freundlich or Langmuir adsorption isotherm. We studied the adsorption equilibria between the enzyme urease and some aluminium oxide modifications and found that all followed the Langmuir theory with one exception: in the case of α-Al_2O_3 (corundum) the quantity of enzyme adsorbed to it as a function of enzyme supply steeply increases in the range of small enzyme concentrations and, going through a maximum again decreases to a saturation value. A plot of the activity of the adsorpt shows the same course. At unfavourable α-Al_2O_3 to enzyme ratios the activity of the adsorbed urease is low, an effect resulting from the spreading of the protein molecule on the non-porous plain surface. However, at the adsorption maximum the residual activity is nearly 100%. Accordingly, the loss of activity of urease adsorbed to porous aluminium oxide modifications (γ-Al_2O_3) is considerable. Even though these surfaces are much larger and contain a large number of hydrophilic groups the adsorbed quantity of enzyme per square metre is 5 to 6 fold smaller than when α-Al_2O_3 is applied as adsorbent.

For an explanation of this anomalous adsorption behaviour, at present two working models are discussed. If it is assumed that the structure of the urease molecule deviates from the spherical shape, the adsorption maximum could be a result of a particular orientation of the urease molecules, which is especially favourable for the conservation of catalytic activity and is dependent on enzyme concentration in solution [4]. A second proposal for interpretation is based on investigations concerning the association of urease molecules in solutions of different concentrations [5] as well as the dissociation of an urease monomer in several active subunits [6], and on calculations about the thickness of the adsorbed protein layer. Assuming that in the range of the adsorption maximum coverage of the surface is 100%, the thickness of the layer is identical with that of a 4 n subunit of urease. Therefore, a kind of 'dissociative adsorption' of the enzyme to plain α-Al_2O_3 surfaces cannot be excluded.

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U2

Coordination Catalysis: the Oxidation of Amino Acids and Peptides by Dioxygen in the Presence of Transition Metal Ions

A. DEMPSEY and D. A. PHIPPS*

Department of Chemistry and Biochemistry, Liverpool Polytechnic, Byrom Street, Liverpool, L3 3AF, U.K.

The modification of ligand reactivity which occurs when amino acids and related ligands are coordinated to certain transition metal ions is now well known [1]. Increased reactivity is demonstrated both by the base catalysed racemisation of amino acids and by the oxidation of the amino acid to a keto acid with dioxygen as oxidant [2]. In the case of labile complexes, *e.g.* with Cu^{2+} or Zn^{2+}, oxidation serves to increase racemisation *via* the formation of a coordinated Schiff base which can undergo subsequent reactions [2, 3].

Although such oxidation has been reported [2] for the Cu^{2+}–L-alanine system certain mechanistic details remained unclear. This system has been re-investigated and the role of oxygen (and the intermediary of superoxide and peroxide intermediates) re-examined. The role of peroxide derived from oxygen is related to the effect of hydrogen peroxide added separately to the system and the general nature of the oxidation for aliphatic amino acids has been demonstrated.

The role of the keto acid and metal ion in this pathway, are significant and it has been shown that oxygen uptake is reduced by excess keto acid and varies, as expected, with the choice of metal ion. With copper further oxidation occurs yielding other carbon fragments including carbon dioxide and acetic acid.

The reaction is also shown to occur with dipeptides, but to be relatively inefficient for tripeptides.

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U3

The Correlation between the Activity of Urease Immobilized to Anodized Sheet Aluminium and the Anodizing Conditions

P. GRUNWALD

Institut für Physikalische Chemie, Universität Hamburg, Laufgraben 24, D-2000 Hamburg 13, F.R.G.

The fixation of proteins to water insoluble materials has become an important field in chemistry and related disciplines [1]. Preference is often given to inorganic carriers because of their resistance to bacterial degradation. Moreover, the investigation of the interaction between proteins and nonbiological surfaces is of great practical interest with respect to the increasing use of prosthetic materials in the body [2].

Recently, we could show with the enzyme urease as an example that anodized sheet aluminium is a suitable carrier material for enzyme immobilisation [3, 4]. Further, the anodizing process leads to an adsorbens with a homogeneous surface, which is required for the interpretation of adsorption experiments. In this paper the influence of the different anodizing parameters on the activity of urease adsorbed to anodized sheet aluminium is described. The results are discussed with regard to the surface structure that has been studied by scanning electron micrographs.

The anodizing of aluminium was carried out in sulfuric acid as electrolyte. The rolled Al-sheets were placed between two cathodes of lustrous carbon. A dc power supply allowed anodizing at constant voltage and constant current. The investigated parameters were: the concentration of sulfuric acid (c_a), the applied voltage U and current density (i), the anodizing temperature (T_a), the anodizing time (t_a), and the contact time (t_{ad}) between anodized sheet aluminium and the enzyme solution. We found that optimum activities are obtained if the anodizing conditions are: $c_a = 26$ wt%, $t_a = 50$ min, $T_a = 308$ K, and $i = 85$ mA/cm^2. The connection between the single anodizing parameters and the activity of the adsorbed enzyme is as follows: in the starting experiment with $U = 18$ Volt, $t_a = 30$ min, $t_{ad} = 40$ min, $T_a = 298$ K and c_a between 2 and 40 wt% the best results were achieved with $c_a = 26$ wt%; the corresponding i-value was 50 mA/cm^2. Higher acid concentrations lower the current density at which optimum activities are obtained. The activity itself differs only negligibly. At t_a-values less than 20 min the activity of the immobilized enzyme is low. For $30 \text{ min} < t_a < 50$ min the activity is raised by a factor of 1.5 to 2, dependent on c_a. If T_a is 308 K instead of 298 K the activity is three times as high as in the starting experiment. A nearly tenfold activity results if i is raised up to 80 mA/cm^2, and the activity increases again by a factor of 2 if t_{ad} is extended by 100 min. The importance of the contact time t_{ad} for the acitivity of the adsorbed enzyme distinctly increases for $i > 50$ mA/cm^2 as a consequence of the increasing porosity of the anodized sheet aluminium. The complex influence of the anodizing parameters on the surface structure and its correlation to the activity of the adsorbed enzyme is conclusively interpretable by means of scanning electron micrographs.

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U4

Stereochemistry of Pyridoxal–Amino Acid Model Systems

LUIGI CASELLA and MICHELE GULLOTTI

Dipartimento di Chimica Inorganica e Metallorganica, Centro CNR, Via Venezian 21, 20133 Milan, Italy

Pyridoxal phosphate is the essential cofactor for most of the enzymic reactions undergone by amino acids during metabolism [1]. All these reactions proceed through the formation of a Schiff base, such as I, followed by cleavage of one of the bonds to the amino acid α-carbon atom. The nature of the substrate and the conformation of the apoenzyme dictate which of the bonds to C_α is broken and control the further course of the reaction. The stereospecificity of the reaction depends on the ability of the dissymmetric apoenzyme to bind the