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

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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

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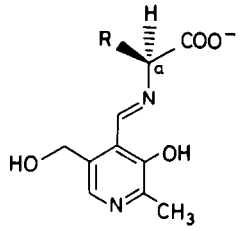
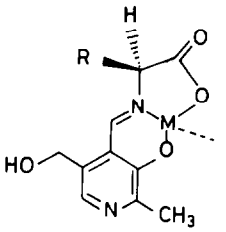
Pyridoxal phosphate is the essential cofactor for most of the enzymic reactions undergone by amino acids during metabolism [1]. All these reactions proceed through the formation of a Schiff base, such as I, followed by cleavage of one of the bonds to the amino acid α-carbon atom. The nature of the substrate 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

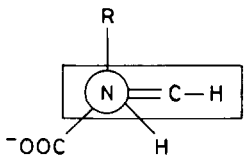


I

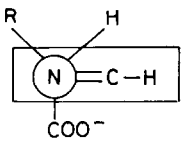


II

complex I in such a way as to allow a stereochemical differentiation between the groups at the amino acid C_α. The understanding of the stereochemical course of pyridoxal-dependent reactions usually relies upon the determination of the stereochemistry of the products in the hypothesis, put forward by Dunathan [2], that in the substrate–cofactor complex the bond to be broken is oriented perpendicular to the plane of the extended conjugated π system [3]. Direct evidence of the structure of the substrate–cofactor complex might however be obtained if we could relate the features of *e.g.* the CD spectra of I to the conformation of the C_α–N bond. We have shown that this is easily achieved in model metal complexes such as II, since their CD spectra correlate with the mode of binding of the amino acid residues [4, 5]. In particular, it is invariably found that the predominant conformation of the amino acid chelate ring of II contains the side chain R in the axial disposition. This conformation involves a ring chirality of sign λ for L-amino acids and is identified by Cotton effects of negative sign within the azomethine CD band. In free pyridoxal–amino acid Schiff bases like I a much wider range of conformations about the C_α–N bond is theoretically possible and correlations between CD spectra and conformations are more difficult to assess. The CD spectra of the Schiff bases I actually vary with the nature (polar, nonpolar, aromatic) of the L-amino acid side chain. However, a careful analysis of the chirality of the dominant interacting chromophores shows that the predominant conformers of I are restricted within the narrow range depicted by III and IV [6]. These CD results agree with previous NMR conformational studies [7].



III



IV

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U5

The Properties and Structures of Glutathione–Cu(II) Complexes and SOD Activity

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The properties of Cu(II) complexes of reduced and oxidized glutathione ligands, respectively, were examined by potentiometric titration, electron spin resonance and visible absorption spectroscopy. Three Cu(II) complexes for reduced glutathione, [blue (I), green (II), and violet (III)], and three Cu(II) complexes for oxidized glutathione were obtained, respectively. The physiological concentration of reduced glutathione in human erythrocytes is 2 m*M* and that of the oxidized form is 4 μM. Thus, the complexation of reduced glutathione and Cu(II) is of biological interest.

Reduced glutathione and the Cu(II) 1:1 system below pH 6 forms a polymerized Cu(I) complex which does not dissolve even at high pH. Above pH 6, however, soluble Cu(II) complexes are obtained. The pH-dependent frozen solution ESR spectra reveal the presence of four Cu(II) species, namely, I, II, III and $[Cu(OH)_4]^{2-}$ (IV). The complexes I, II and III are interconvertible with protonation and deprotonation of peptide in the following manner.

$$2Cu(II) + 2GH_4SH \underset{pH \sim 6}{\rightleftharpoons} I \underset{pH \sim 9}{\rightleftharpoons} II \underset{pH \sim 10.5}{\rightleftharpoons} III \underset{pH \sim 12}{\rightleftharpoons} IV$$

The structure of III has been reported before [1]. The results show that the complexes, I, II, and III are binuclear, and the ligand coordinates with Cu(II) as its oxidized form. The coordination of Cu(II) to I at physiological pH involves the glutamic amine