reaction between R-1-P and GDP has been determined by following the formation of G-6-P. The optimum conditions for the preparation of RDP have been worked out. With the same enzyme system evidence has also been obtained for a reaction between GDP and deoxyribose-1-phosphate or galactose-1-phosphate. Preliminary experiments with pigeon liver acetone powder confirm the indications obtained by Saffran and Scarano [2] for RDP to be an intermediary in 5-adenylic acid formation from ^{14}C-adenine.

1. Klenow, H. *Arch. Biochem. Biophys.* **46** (1953) 186.
2. Saffran, M. and Scarano, E. *Nature* **172** (1953) 1949.

Studies on the Inhibition of D-Amino Acid Oxidase

Otto Walaas and Eva Walaas

Medicinska Nobelinstitutet, Biokemiska avdelningen, Stockholm, Sweden

Studies on D-amino acid oxidase from pig kidney purified according to Negelein and Brömel [1] have been made. The flavineadenine dinucleotide (FAD) used was a pure sample obtained from baker's yeast by inophoresis on paper as the final step in the purification. The coupling between the apoenzyme and FAD caused no shift in the absorption band and no quenching of the fluorescense. Thus, D-amino acid oxidase is fluorescent and is similar to Straub's diaphorase in this respect.

Inhibition of enzyme activity by different anions and by partial structural analogs of the FAD molecule has been demonstrated. Anions were inhibitory in the following order:

$$J^- > Br^- > NO_3^- > Cl^- > PO_4^{--} > SO_4^{--} > CH_3COO^- \gg F^-$$

Thus, the anions of strong acids were most effective as inhibitors. At a concentration of 1.4×10^{-7} *M* FAD 50% inhibition was exerted by 8.5×10^{-2} *M* NaCl. The inhibition was reversible in the presence of high FAD concentration, suggesting competition of the inhibitor with the prosthetic group.

In similar experiments strong inhibition by adenylic acid, ATP and FMN was observed. 50 % inhibition was given by concentration of approximately 5×10^{-4} *M*. The inhibition by DPN was somewhat weaker, while adenosine, adenine and hypoxanthine exerted inhibition of the same order of magnitude at concentrations of 6×10^{-3} *M*. Cytosin and uracil were slightly inhibitory while riboflavin was without effect.

The experiments indicate that the phosphoric ester groups and the adenine part of the FAD molecule are of importance in the coupling to the apoenzyme. A possible physiological significance by some of these inhibitors in regulation of enzyme activity is suggested.

1. Negelein, E. and Brömel, H. *Biochem. Z.* **300** (1939) 225.

Distribution in Rabbit Livers of Intravenously Injected Iron

K. Agner, R. Bonnichsen and G. Hevesy

Chemical Department, Serafimerlasarettet, Biochemical Department, Medical Nobel Institute, and Institute for Biochemistry, Stockholms Högskola, Stockholm, Sweden

Rabbits weighing from 1.5 to 1.7 kg were injected with various amounts of a high molecular weight iron-carbohydrate complex (Astra) and sacrifized after different time intervals. The livers were homogenized and fractionated according to Hogeboom, Schneider and Pallade [1] and the iron determined in the different fractions.

The results show a considerable increase in ferritin in all the fractions. In the nuclei and the mitochondria there was also a large increase of a water-insoluble hydrolyzeable fraction (hemosiderin). Notable also was a very marked increase of bound hemin (cytochrome b) in the microsome fraction.

1. Hogeboom, G. H., Schneider, W. C. and Pallade, G. E. *J. Biol. Chem.* **172** (1948) 619.

The Combination of Flavin Mononucleotide and Riboflavin with the Protein of "The Old Yellow Enzyme"

Hugo Theorell and Agnar P. Nygaard

Medicinska Nobelinstitutet, Biokemiska avdelningen, Stockholm, Sweden

In a forthcoming paper *(Acta Chem. Scand.)* we have presented some kinetic data on the reversible dissociation of the old yellow enzyme (OYE) into FMN and apoenzyme obtained by the aid of fluorescence measurements. In water, the dissociation constant *K* of this

system was too low to be measured ($> 10^{-12} M$). However, in the presence of certain salts we have found the enzyme to dissociate readily. The effect is due to the anions, and seems to be correlated to the acidic strengths of the corresponding acids. Thus, they may be arranged in the following order:

Bromide > Nitrate > Chloride > Sulfate > Phosphate > Acetate

Chloride has more than 1 000 times as strong an effect on the dissociation velocity constant (k_2) as acetate.

Anions also in general decrease the association velocity constant (k_1) of FMN and apoenzyme. The effect appears to depend mainly on the charge of the anions. Thus, versene, citrate, sulfate and secondary phosphate are strong inhibitors, whereas primary phosphate, chloride and acetate have very small effects on k_1. The inhibiting effect may be due to the combination of the anion with positive groups of the protein which are essential for the attachment of FMN. Monovalent anions like chloride and acetate counteract the inhibitory effects of the polyvalent anions, probably by displacing them from their binding sites on the protein. These salt effects on the FMN-protein system resemble the salt effects on anionic exchangers of the weakly basic type, and it is likely that FMN and protein are attached through electrostatic forces, the protein furnishing the positive, FMN the negative charges. FMN has been suggested to be joined to the protein by two linkages, the phosphate group and the NH (3) group of the *iso*alloxazine ring being involved. Since the fluorescence of riboflavin like that of FMN is quenched by coupling to the apoenzyme, we have been able to study the attachment of the *iso*alloxazine group separately. Strong effects of anions were observed also in the riboflavin-protein system.

The affinity of riboflavin for the protein is very weak as compared to the tight binding in the FMN-protein system. Thus, the dissociation constant in water is of the order of magnitude $5 \times 10^{-6} M$ as compared to $10^{-12} M$ or less for FMN-protein. This correlates with the low enzymatic activity of riboflavin as compared to FMN.

Changes in pH give interesting effects in the system FMN-protein. k_1 decreases with increasing acidity along a curve which essentially coincides with the second dissociation step of the phosphate group in FMN ($pK' \sim 6.0$). One may conclude that both the acidic groups of the phosphoric acid residue must be dissociated in order to enable FMN to combine to the protein. In the alkaline range, k_1 for FMN + protein increases to a maximum around pH 9.0 (23.5°) or 9.3 (13.5°). The steep drop in k_1 on the alkaline side of the k_1-maximum is probably due to the loss of positive charges on the protein which are essential for the attachment of the FMN to the protein.

We have reason to believe that similar pH- and salt effects are of general importance in all biological systems involving proteins + phosphorylated compounds (coferments, nucleic acids, phosphatides).

Reaction of Guanosine Triphosphate and Uridine Triphosphate with Actomyosin Gel

Rolf Bergkvist and Adam Deutsch

Institute of Chemistry, University of Lund, Sweden

Interaction of adenosine triphosphate (ATP) with actomyosin gel has been shown to result in a volume constriction of the gel with dephosphorylation of ATP-predominantly to the diphosphate stage — followed by deamination of the adenine nucleotide.

Following the recent isolation of guanosine triphosphate (GTP) and uridine triphosphate (UTP) from rabbit muscle [1], a study of the reaction of the new triphosphates with actomyosin was undertaken. On actomyosin gel, both GTP and UTP produce a volume constriction; equimolar amounts of ATP, GTP and UTP give volume effects of the same order of magnitude and of the same time course. The reaction was generally performed at 20° C and a pH of approx. 7.0 (without addition of buffer), the reaction mixture containing 10 mg thrice reprecipitated actomyosin (dry weight) and 1—2 μmoles sodium nucleoside triphosphate per ml. Analysis of the deproteinized reaction mixture — after a reaction time of 30—60 minutes — showed the presence of orthophosphate in quantities corresponding to approx. one-third of the total phosphorus originally present in the form of triphosphate. Ion exchange chromatography gave guanosine diphosphate and uridine diphosphate respectively, corresponding to 90 % conversion of the original triphosphates; no deamination of guanosine nucleotide could be detected.

Summarizing, our results show that the reaction between actomyosin gel and ATP can be reproduced with GTP or UTP, both with respect to the resulting volume change in the protein and dephosphorylation of the