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

original nucleoside triphosphate. Identical results were obtained with synthetic UTP, obtained by courtesy of Professor A. R. Todd, Cambridge.

Inosine triphosphate (ITP) — previously found to be inactive [2] without the addition of a magnesium salt [3] — gave a volume change of actomyosin gel comparable in magnitude to that given by the other nucleoside triphosphates, but somewhat slower. No addition of magnesium salt was required, although the concentration of magnesium in the reaction mixture was below 4×10^{-7} *M*.

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Deamination of Adenosine Diphosphate by Adenylate Deaminase Preparations

Adam Deutsch and Roy Nilsson

Institute of Chemistry, University of Lund, Sweden

The deamination of adenine nucleotides by muscle preparations is generally assumed to proceed via adenosine 5′-monophosphate (AMP) through the action of a specific adenylate deaminase associated with myosin. In recent reports, evidence was presented for an additional route of deamination: direct deamination of adenosine diphosphate (ADP) by actomyosin gel [1] and washed myofibrils [2]. Deamination of ADP can likewise be effected by preparations of adenylate deaminase obtained from water extracts of skeletal muscle by the method of Kalckar [3]. Whereas only AMP is deaminated under the usual conditions [3] of the adenylate deaminase test (1—5 μg protein/ml and a reaction time of up to 60 min.), higher protein concentrations and longer reaction times lead to the deamination of ADP as well. 80—90 % deamination was observed in reaction mixtures containing 5 mg protein and 7 μmoles ADP per ml in 0.1 *M* succinate buffer, pH 6.0 at 20° C and using a reaction time of 4 hours. Ion exchange [4] and two-dimensional paper chromatography [5] were used for analysis of the deproteinized reaction mixtures. Inosine diphosphate (IDP) was found to be the main product of the deamination of ADP and was isolated as the barium salt from pooled fractions of the effluent from ion exchange columns and identified by chemical analysis (hypoxanthine : ribose : acid labile P : total P). Beside IDP, minor amounts of inosine 5′-monophosphate (IMP) were detected and a further inosine nucleotide fraction, which precedes IDP in the ion exchange effluent and contains hypoxanthine, pentose, acid labile and acid stable phosphate in the proportions 2 : 2 : 1 : 2, corresponding thus in its composition to an equimolar mixture of IMP and IDP, or to a diinosine triphosphate.

Experiments on the pH-dependence of ADP deamination by adenylate deaminase preparations point to a pH optimum of near pH 6, in harmony with previous findings on actomyosin gel [1].

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On the Deaminase Component of Actomyosin

Adam Deutsch and Roy Nilsson

Institute of Chemistry, University of Lund, Sweden

It has been shown previously [1] that dephosphorylation of adenosine triphosphate (ATP) by actomyosin is followed by deamination of the diphosphate initially formed, yielding inosine diphosphate (IDP) — or an IDP compound converted to IDP during isolation [2] — as the final reaction product. At 20° C and pH 6.8—7.1 (without addition of buffer), using 5—25 mg (dry weight) actomyosin gel and 5 μmoles ATP per ml, dephosphorylation is complete within 30 minutes, whereas deamina-