## Complexing of ATP with molybdate

In experiments on the effect of KCl concentration on the ATPase activity of myosin we found, as had been reported by others, that there was an optimum ATP concentration above which the ATPase activity decreased. With increasing KCl concentrations inhibition began at progressively lower ATP concentrations. Further examination of the system showed that this apparent inhibition was due to the formation of a salt sensitive colorless complex between ATP and molybdate. This complex did not become colored upon the addition of reducing agent and catalyst.

Inorganic orthophosphate ($P_i$) was measured by the FISKE-SUBBAROW technique[1]. Five ml of the sample to be analyzed were added to two ml of 10% trichloracetic acid and five ml of the mixture were then added to one ml of 2.5% ammonium molybdate in 3 $N$ $H_2SO_4$. Color development was initiated by addition of 0.5 ml of 1-amino-2-naphthol-4-sulfonic acid (ANSA) in bisulfite solution[1]. The optical densities were measured at 750 mμ in a Beckman spectrophotometer ten minutes after the addition of ANSA. All concentrations of ATP, $P_i$, KCl, etc., refer to the original 5 ml test sample.

It was found that $P_i$ standards had optical densities which were independent of the concentration of KCl (or NaCl). When the orthophosphate blank in varying quantities of an ATP solution was measured it was found that the apparent blank decreased at high ATP concentrations. At 0.05 $M$ KCl there is an apparent loss of color at ATP concentrations greater than $7 \cdot 10^{-3}$ $M$. Table I shows the measured $P_i$ at two different concentrations of ATP as a function of KCl concentration. It is seen that in the presence of high ATP concentrations the estimation of $P_i$ can be in error even at low KCl concentrations. In the presence of $8 \cdot 10^{-3}$ $M$ ATP at 0.03 $M$ KCl, $P_i$ standards gave color values about 35% too low (contrasted to experiments without ATP), while in the presence of $4 \cdot 10^{-3}$ $M$ ATP at 0.6 $M$ KCl the color values were only about 5% too low. Increasing the amount of molybdate in the system increased the optical densities near to the expected values. We have obtained similar results with ADP and ITP. Although it had previously been suspected[2,3] that ATP and other organic phosphates did complex with molybdate, the present results show that at high KCl and at high ATP concentrations the complexing may be extensive enough to affect the sensitivity of the FISKE-SUBBAROW test unless proper precautions are observed. It seems possible that some of the reported salt dependent inhibitions of myosin ATPase by high substrate concentrations may be artifacts of the analytical method.

TABLE I

OPTICAL DENSITY (O.D.) OF THE $P_i$ IN TWO SAMPLES OF ATP

| *KCl moles/liter* | *O.D. for $10^{-3}$ M ATP* | *Corrected* O.D. for $5 \cdot 10^{-3}$ M ATP* |
|---|---|---|
| 2.05 | 0.488 | — |
| 1.05 | 0.658 | 1.31 |
| 0.55 | 0.768 | 1.31 |
| 0.25 | 0.850 | 1.30 |
| 0.05 | 0.938 | 1.30 |
| 0 (extrapolated) | 0.96 | 1.32 |

* Corrected O.D. = 2 × measured O.D.

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## Relations among cystine content, molecular weights and ultraviolet light sensitivity of proteins

A relationship connecting quantum yields, Ø, (for inactivation of specifically active proteins) with molecular weights, $M$, of proteins has been found, namely[1]

$$Ø = Q/M = 447/M. \quad (1)$$

Recent work with other enzymes[2] and antibodies[3] has supported the equation. SETLOW[4] has suggested another relationship*, namely that quantum yields are roughly proportional to the cystine content of enzymes. If both propositions are correct, one would expect to find that molecular weights of proteins and reciprocals of percent abundance of cystine tend to be directly related.

In Fig. 1 are plotted the reciprocal cystine contents of the ten proteins listed by SETLOW against corresponding molecular weights (the open circles). The expected trend is clear. In addition are plotted data for ten other globular proteins listed by TRISTRAM[5]. These proteins, except for the fact that both molecular weights and cystine contents are known, may be considered chosen at random; they are myoglobin, hemoglobin, serum albumin, globulin, $\beta$-lactoglobulin, ovalbumin, edestin, phosphorylase, conalbumin, ovomucoid and avidin. One of these, myoglobin ($M$ = 16,500), is without cystine. The others are represented as solid circles, Fig. 1. As the looked-for relationship is now vague overall, I refrain from speculating on what this may mean in terms of the stability of proteins except to say that the stability of some of the larger protein molecules may depend more on hydrogen bonding than on disulfide linkages.

At this point, and until many more data are available, it is not possible to decide which suggestion, equation (1) or that of SETLOW[1], is the more fundamental for the photochemistry of proteins.

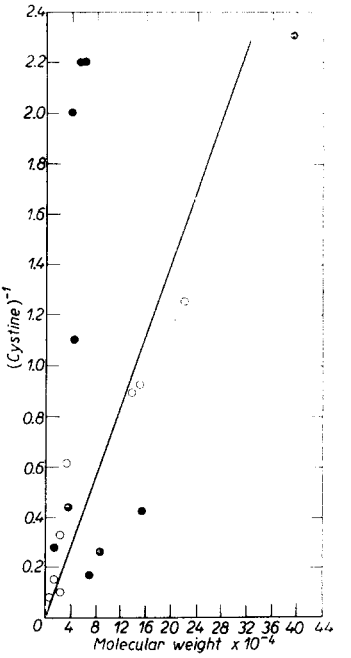


Fig. 1. Plot of reciprocals of cystine content of proteins [1/(g cystine in 100 g protein)] versus molecular weights. All data, except that for trypsin, is from a review by TRISTRAM[5]. Data for trypsin was kindly supplied by Dr. H. NEURATH prior to publication. The open circles are for the proteins listed by SETLOW and for which photochemical data are available.

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* This relationship only holds precisely at 2537 Å (R. SETLOW, private communication).
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