An Investigation of some of the Properties of Biologically Significant Phosphorylated Compounds as Ligands of Copper

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Received January 5, 1985

Chromophores of many phosphorylated compounds of biological interest have extinction coefficients in the UV region that are either reported to be variable in a given report or have been reported in different reports to be different [1]. This variation in extinction coefficients leads to difficulty in the interpretation of the data presented, or in developing theoretical conclusions derived from this data. An example of this, is the successful use of a UV chromophore of ribulose-1,5-bisphosphate (RuBP) to determine the rate of catalysis of ribulose-1,5-bisphosphate carboxylase despite the observation that chromophore had variable extinction coefficient depending on the substrate's (RuBP) preparation bath [2]. The enzyme assay is useful; however, theoretical investigations on this chromophore's nature and possible biochemical and physiological investigations of the chromophore's reactions are difficult until more information can be obtained. Interaction between phosphoserine and transition metals that results in chromophore formation has been studied in much detail [3, 4]; however few other biologically and biochemically important phosphorylated compounds have been as well studied.

Our interest in the matter arose from concepts derived from the study of the controversy surrounding copper content and UV light activation of RuBPCase [5]. The concept investigated here is that the UV chromophores associated with RuBPCase could be the results of a transition metal(s)'s interaction with a phosphorylated ligand or ligands. To provide information on these matters an investigation on some of the properties of selected biologically important phosphorylated compounds when these compounds act as ligands of copper(II) was undertaken. Since, biological functions occur and most biochemical assays are performed within limited ranges of pH, substrate and metal cation concentrations, and since we were interested in specific biological and biochemical aspects of inorganic chemistry, complete equilibrium analysis of all equilibrium constants would not have served our immediate experimental objectives. However, we hope that the information presented here will serve to aid and promote further investigations of these interesting bioinorganic phenomena.

Results and Discussion

Metal ions can act as the Lewis acid function of a ligand–metal complex [6, 7]. Most ligands have basic properties, and for this reason, ligands are usually associated with hydrogen ions over a wide pH range. When ligands contain a large number of acidic groups, a large number of hydrogen ions may be displaced in the course of complex formation. Due to this, complex formation of a ligand and a metal ion usually results in decrease in pH of the solution containing such a complex. Alternatively, addition of a strong acid to such a solution results in a greater dissociation of a metal complex through reversal of the above reactions. For these reasons the decrease in pH that occurs on complex formation is frequently taken as a qualitative measure of the degree of interaction which takes place during complex formation.

In Fig. 1 the titration curves of some biologically important phosphorylated compounds in the absence (upper curve) and presence of 1:1 molar ratio copper chloride are shown. Comparisons between the upper and lower curves show that in the presence of copper(II) ions the titration curve is displaced to lower pH. This in turn demonstrates complex formation between these phosphorylated compounds and copper(II).

Since a change in optical absorption indicates complex formation (please see above), and complex formation is a function of pH there should be a change in optical absorption with increase in pH. This is illustrated in Fig. 2. The absorption was measured between 250 and 200 nm, but, with one exception, the figure illustrates absorption at 280 nm, since this is close to the absorption maxima in this region, and makes comparison with the ribulose-1,5-bisphosphate carboxylase assay method [2] more apparent. Only phosphothreonine data are presented at another wavelength (290 nm) since this complex has the lowest extinction coefficient in the pH range of interest, and 290 nm is the approximate absorption maxima. Copper chloride solution alone also forms a chromophore, but at higher pH (Fig. 2); this presumed to be due to interaction of copper(II) with hydroxide ions. The formation of these copper(II), pH dependent, chromophores offers a rational explanation of previously reported variable extinction coefficients of biologically important

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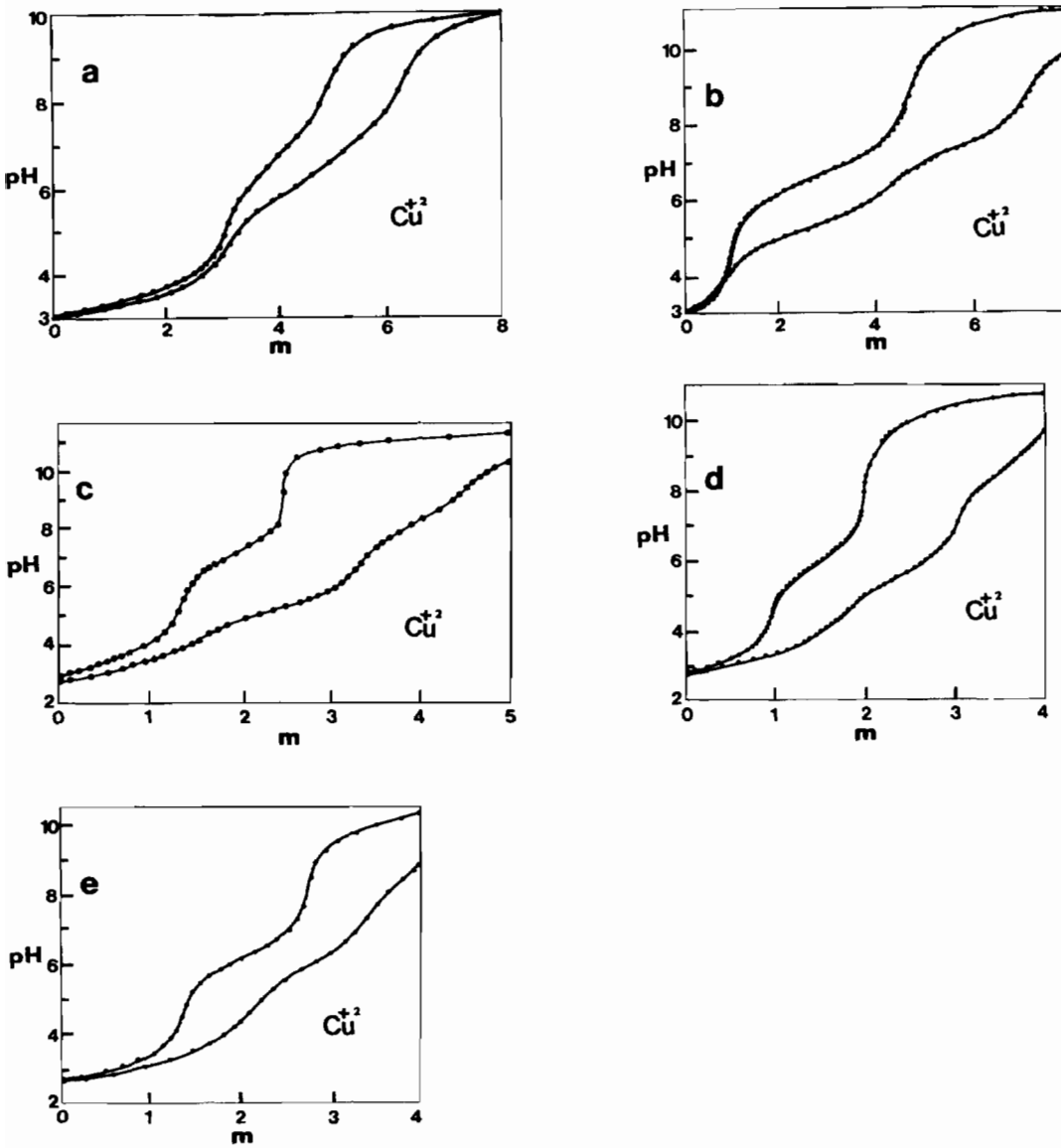

Fig. 1. Titration of some biologically important phosphorylated compounds in the presence and absence of copper chloride. In each figure the upper curve is without and the lower curve with copper chloride. Metal ligand ratios are 1:1, mol:mol; ligand: $CuCl_2$. Titrations were performed with an initial volume of 50 ml. Amounts of phosphorylated compounds were: (a) ribulose-1,5-bisphosphate (12.5 μmol), (b) fructose-1,6-bisphosphate (125 μmol), (c) 3-phosphoglyceric acid (150 μmol), (d) phosphothreonine (125 μmol), and (e) phosphoserine (125 μmol). The units for the abscissa are molar ratios of base to ligand and are indicated by m.

phosphorylated compounds [1, 2] and provides data on the ability of biologically important phosphorylated compounds to form copper(II) ligands. In addition these data strengthen the support of the concepts of metal cation chromophores as possible light receptors in biological systems [5].

Information about the combining ratio of the metal ion and the ligands is required before quantitative measurements of stability of these metal chelate complexes can be made. When water-soluble chelate complexes are involved, it is sometimes not possible and frequently not convenient to prepare the pure substance, and to determine composition by analysis. When, as in this case, there is a change in the light absorption of a metal chelate relative to that of the free metal ion and ligand it is possible to determine metal chelate composition by relatively simple spectroscopic techniques. We have used the method of Job [8]. The Job procedure is based on the variation of the optical densities of solutions containing different ratios of metal ion to ligand. With this method (Fig. 3) we were able to determine

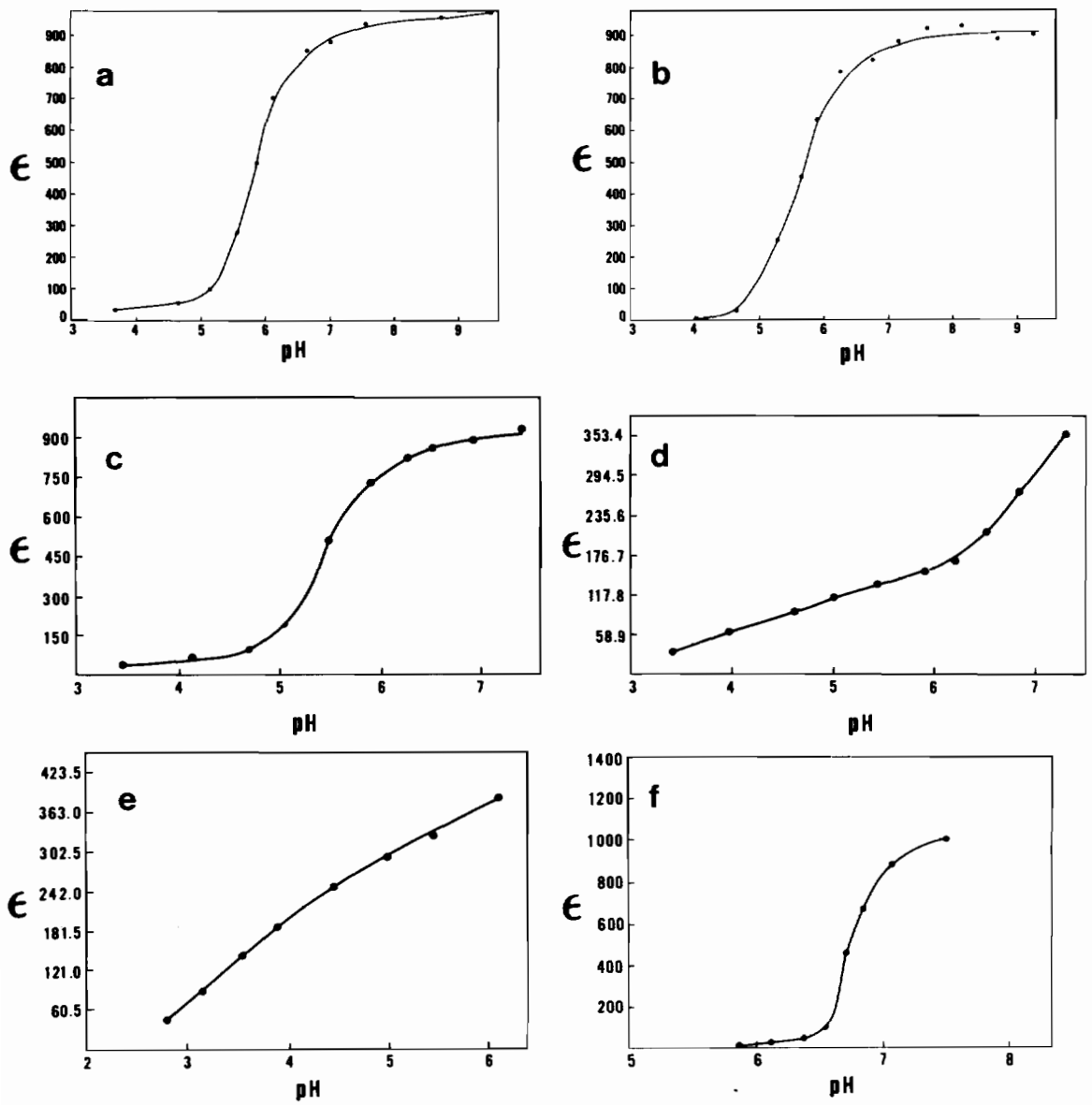

Fig. 2. Molecular absorptivity of biologically significant phosphorylated compound in the presence of copper chloride and at increasing pH. Copper chloride was saturating (see Fig. 3) in a 1:1; mol:mol; ligand to copper(II) ratio. The ligands and wavelengths measured are: (a) ribulose-1,5-bisphosphate (280 nm), (b) fructose-1,6-bisphosphate (280 nm), (c) 3-phosphoglyceric acid (280 nm), (d) phosphothreonine (290 nm), (e) phosphoserine (280 nm), and (f) copper chloride (for comparison) (280 nm).

the composition of copper(II) complexes with: fructose-1,6-bisphosphate, ribulose-1,5-bisphosphate, phosphoserine, phosphothreonine and 3-phosphoglyceric acid. These ratios were 1:3, 1:3, 1:1, 1:1 and 1:2, respectively. We conclude that the simplest explanation of these ratios is that copper(II): forms a complex with ribulose-1,5-bisphosphate, and fructose-1,6-bisphosphate at a ratio of 1:3 because the phosphate groups are distant enough from each other that both of phosphates cannot act as ligand in one complex yet may interact to inhibit formation of 1:4 complexes; forms a complex with 3-phosphoglyceric acid at a ratio of 1:2 because the phosphate and acid groups are close enough to form a chelate; phosphoserine and phosphothreonine forms the complex in a ratio of 1:1 since NH– acts as a coordinating group. In addition, because of the analogy between titration curves of Fig. 1 for phosphoserine and phosphothreonine to other reported data [9] 1:2 metal ligand complexes, as well as the demonstrated 1:1 complexes, are inferred for these phosphorylated amino acids, as has been shown previously [9].

The displacement of the titration curve of free phosphorylated ligand on addition of copper(II) shows that there is a strong interaction between

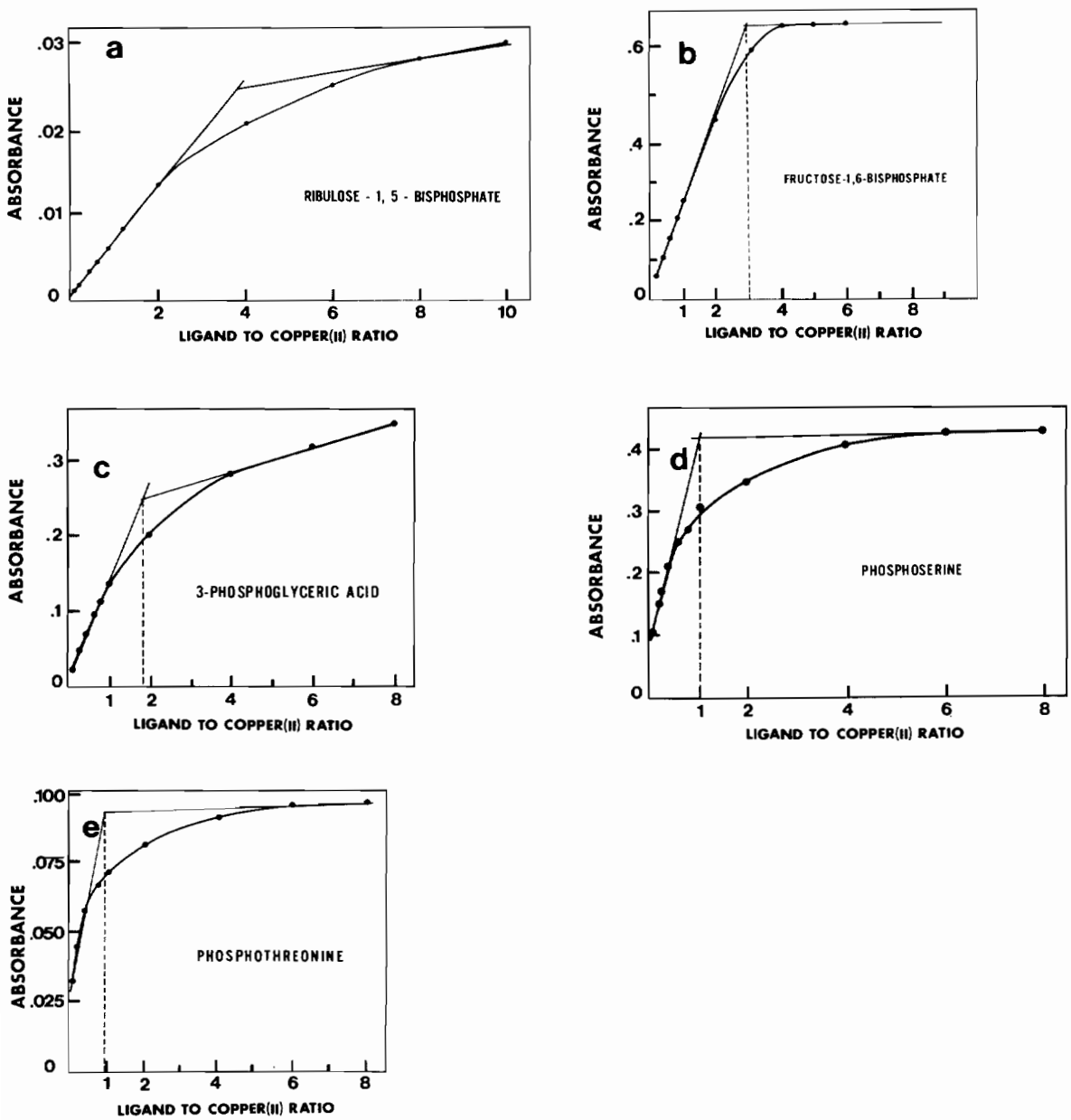

Fig. 3. Determination of chelate metal–ligand ratio by the method of Job [8]. In this method optical absorption changes are measured in the copper chloride solution as additions of the ligand are added. The intercept of the asymptotes indicates the ratio of ligand to copper(II) in the chelate metal–ligand complex. The abscissa indicates the proportions of ligand to copper chloride (molar basis). The initial copper chloride concentrations and wavelength observed were (a) ribulose-1,5-bisphosphate, 0.1 mM $CuCl_2$, 280 nm; (b) fructose-1,6-bisphosphate, 1 mM $CuCl_2$, 280 nm; (c) 3-phosphoglyceric acid, 1 mM $CuCl_2$, 280 nm; (d) phosphothreonine, 1 mM $CuCl_2$, 257 nm; (e) phosphoserine, 0.1 mM $CuCl_2$, (280 nm). Certain accomodations had to be made with the pH used and in the absorbance of threonine. The pH was between 5.5 and 6.5 and was not buffered; since, addition of buffers would have added other ligands and addition of base to $Cu(OH)_2$ formation. Threonine has lowest extinction coefficient and was observed at 257 nm.

copper(II) and the corresponding ligand. All complexes of biologically active phosphorylated compounds studied absorb UV light at wavelenghts between 250 and 300 nm. The absorption intensity changes with pH. Using a spectrophotometric method we found that copper(II) forms a complex with fructose-1-bisphosphate, ribulose-1,5-bisphosphate, phosphoserine, phosphothreonine, and 3-phosphoglyceric acid in ratios of 1:3, 1:3, 1:1, 1:1, and 1:2 respectively. This type of complex formation is capable of explaining previously reported variability in UV absorption of these

phosphorylated compounds [1, 2] and may have some significance in explaining related biochemical, biophysical and physiological information [5].

Experimental

Fructose-1,6-bisphosphate, ribulose-1,5-bisphosphate, phosphoserine, phosphoserine and 3-phosphoglyceric acid were obtained as their corresponding sodium salts from Sigma Chemical Company (Saint Louis, MO). All chemicals used were reagent grade or better. All solutions were aqueous.

Titration. The titration was done using a Corning digital pH meter model 101 fitted with a combination glass electrode. Titrations were performed manually using NaOH. The UV spectra were obtained with a Cary 14 recording spectrophotometer. The spectra were obtained using one cm path length quartz cells and the wavelengths examined were the region from 200 to 350 nm. Determination of metal chelate composition was done spectroscopically using the method of continuous variations introduced by Job [8].

Acknowledgements

This work was supported by the Daley Research Institute, Sigma the Chemical Company and the Welch Foundation. We thank N. Daley for editorial work, M. Nash for figure design and preparation, E. Abbott for helpful suggestions and K. Miller for manuscript preparation. The support of National Clonal Germplasm Repository is also acknowledged. Contribution of the Oregon Agricultural Experimental Station in cooperation with the USDA-ARS. Technical paper no. 7441.

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