Vol. 136, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1986 Pages 438-444

Circular Dichroism Studies of Cobalt Substituted Lentil Lectin

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Received March 24, 1986

SUMMARY: A recent method has been developed to effect metal ion substitution at the Mn^{2+} site in the lentil lectin (Bhattacharyya et al. (1984) Biochem. Biophys. Res. Commun. 124, 857-862). We report here the preparation of cobalt substituted lentil lectin, containing Co^{2+} at the S1 site and Ca^{2+} at the S2 site. The cobalt derivative possesses full saccharide binding activity and can be used for spectroscopic studies. The near UV and visible CD spectra of the derivative are shown, and its spectral properties are compared with various cobalt complexes of concanavalin A.

1. INTRODUCTION

Lectins isolated from plants are cell agglutinating proteins, which have diverse and unusual biological properties that relate to their saccharide binding specificities (cf.1). Among the more widely used lectins are Con A[1] and CMLcH. Both proteins belong to the class of D-

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[1] Abbreviations used: Con A, Concanavalin A; CMPL, Con A in the native form in the locked conformation with Mn^{2+} and Ca^{2+} at the S1 and S2 sites, respectively; CCoPL, Con A in the locked conformation with Co^{2+} and Ca^{2+} at the S1 and S2 sites, respectively; CCoP, Con A in the unlocked conformation with Co^{2+} and Ca^{2+} at the S1 and S2 sites, respectively; CoP, Con A in the unlocked conformation with Co^{2+} at the S1 site; P and PL are the unlocked and locked conformation of Con A without metal ions; CMLcH, lentil lectin in the native form containing Mn^{2+} and Ca^{2+} at the S1 and S2 sites, respectively; CCoLcH, lentil lectin containing Co^{2+} and Ca^{2+} at the S1 and S2 sites, respectively; CD, circular dichroism; NMRD, nuclear magnetic resonance dispersion, the magnetic field dependence of nuclear magnetic relaxation rates, in the present case, the longitudinal relaxation rate, $1/T_1$, of solvent protons.

0006-291X/86 $1.50

glucose/D-mannose specific lectins, although they display different interactions with glycopeptides (2), and both require metal ions for their activity (3). Extensive NMRD (cf. 4) and CD studies (5-8) have been carried out to characterize the role of metal ions in the activation of Con A. CD studies of Ca^{2+}-Co^{2+}-Con A (CCoPL) and NMRD studies of Ca^{2+}-Mn^{2+}-Con A (CMPL) have led to the observation of two conformational states of the protein with different metal ion and saccharide binding properties.

We have recently developed a method of substituting different transition metal ions into the Mn^{2+} site of CMLcH, which results in fully active metalloprotein derivatives (9,10). In the present communication, we report the preparation of Ca^{2+}-Co^{2+}-LcH (CCoLcH) and its near UV and visible CD spectra. The results are compared with those for Con A.

2. MATERIALS AND METHODS

Seeds of lentil (Lens culinaris Med. sub. Macrosperma) were purchased from a local food store. Salts of diffent metals were the highest purity available from either Mallinckrodt or Fisher Scientific Co.

Preparation of CCoLcH. Native LcH was purified by affinity chromatography on Sephadex G-100 (11). CCoLcH was prepared by dissolving native CMLcH (containing Mn^{2+} and Ca^{2+}) at about 5 mg/ml in 10 mM sodium acetate buffer, pH 4.0, containing 100 mM $CaCl_2$ and 100 mM $CoCl_2$, and the solution allowed to dialyze at 37 ^{o}C against 10-fold excess of buffer for 16 days. Precipitates were removed by centrifugation, and the protein dialyzed against water and stored as a salt free lyophilizate. The yield of CCoLcH was 90%, the stoichiometric substitution of Co^{2+} was verified by atomic absorption spectroscopy (0.95 gm-atom/mole), based on a molecular weight of the monomer of 23.5 KD (12). Calcium was present in somewhat higher amount (1.2-1.4 gm-atom/mole). CCoLcH was found to be as active at precipitating

polysaccharides from P. pinus as the native protein, as well as possessing equal hemagglutinating activity (10). The generation of full activity (10), the unique spectral properties of the cobalt lentil lectin, the NMR characteristics (C.F.Brewer, unpublished data) as well as the metal stoichiometry (10) all indicate the positioning of Co^{2+} and Ca^{2+} to sites equivalent to the S1 and S2 positions of Con A, respectively.

Protein Concentrations. The concentration of native LcH was determined spectrophotometrically using the value $A^{1\%,1cm}=12.6$ at 280 nm (13). The same value was found for CCoLcH.

CD Measurements. CD spectra were obtained at ambient temperature (21 ^{o}C) with a Cary 61 spectropolarimeter using 1 cm path length cells. Standardization of the instrument was accomplished with a 1 mg/ml aqueous solution of d-10-camphor sulfonic acid, as specified by Varian Associates. Data are presented as molar ellipticities. Protein samples were prepared in a 0.1 M potassium acetate and 0.1 M KCl buffer, pH 6.4.

3. RESULTS AND DISCUSSION

We have recently found a means of effecting metal ion substitution at the transition metal ion site of CMLcH (9,10). In the present communication, Co^{2+} has been substituted for the Mn^{2+} ion in the native lectin, producing CCoLcH. The resulting derivative possesses the same molecular weight and hydrodynamic properties as the native lectin (dimer of molecular weight 47 KD), and equal saccharide binding activity (10). Furthermore, the near UV CD spectrum of CCoLcH is essentially the same as that of CMLcH, which demonstrates that the secondary and tertiary structures of the cobalt derivative of the protein are unchanged. Addition of methyl-α-D-mannopyranoside produces a decrease in the amplitude of the spectrum (Figure 1), which is also observed with the native lectin, and with the pea lectin (14).

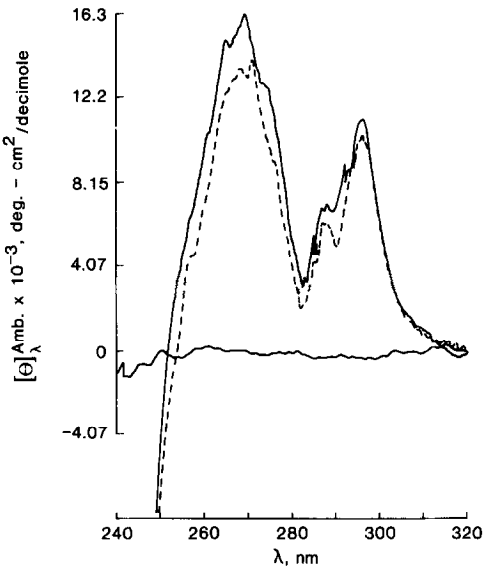

Figure 1. The near UV CD spectrum of CCoLcH (——), and with 6 mM methyl-α-D-mannopyranoside (– – –) at 21 °C in .1 M potassium acetate, .1 M KCl buffer, pH 6.4. Concentration of CCoLcH was 1.44 mg/ml.

The preparation of cobalt substituted lectins offers the opportunity of studying the structural properties of the proteins by observing the extrinsic Cotton effects in the visible region of their spectra. Cobalt substituted Con A complexes have been used in extensive studies of the effect of metal ion and saccharide binding on the structural properties of the lectin. In conjunction with NMRD studies (4,15), CD studies of CCoPL (5,7) have identified two conformational states of the protein, called unlocked (P) and locked (PL), which differ both in their metal ion and saccharide binding properties. The ground state free energies of the two conformations differ by only a few kcal M^{-1}, with the sign of this difference determined by metal ion binding (15). Apo-Con A at equilibrium is primarily in the unlocked conformation, which weakly binds metal ions and saccharide. The fully metallized protein is in the locked conformation, which tightly binds metal ions and possesses full saccharide binding activity. Sequential binding of metal ions to the S1 (transition metal ion) and S2 (calcium ion) sites of P induces a conformational transition in the protein leading to the locked ternary complex.

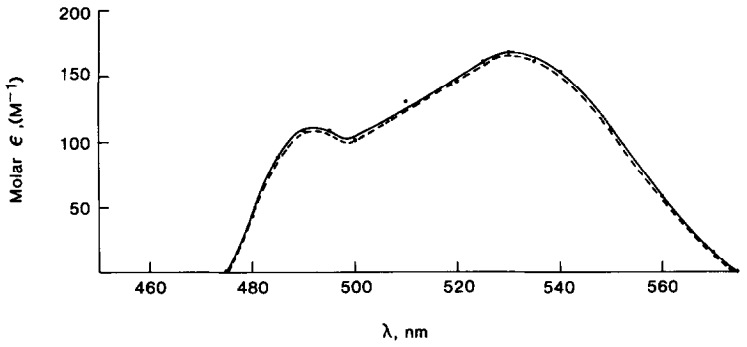

Figure 2. The visible CD spectrum of CCoLcH (——), and with 0.9 mM 3-O-methyl-D-glucose (– – –) at 21 °C in .1 M potassium acetate, .1 M KCl buffer, pH 6.4. Concentration of CCoLcH was 32.1 mg/ml.

Three cobalt complexes of Con A have been identified and characterized by their visible cobalt CD spectra: CoP, CCoP, and CCoPL (7). CoP, the binary unlocked complex, exhibits bands centered at 470, 505, and 530 nm. Binding of Ca^{2+} to CoP to form CCoP, the ternary unlocked complex, results in a loss of the 535 nm band, with two bands remaining at 485 and 505 nm. CCoPL, the locked ternary complex which forms from CCoP, has two bands at 483 and 505 nm, with reduced molar ellipticities relative to CCoP.

The visible CD spectrum of CCoLcH is shown in Figure 2. Saccharide binding to the protein has little effect on the spectrum, which is similar to the behavior of CCoPL (5). A strong band is observed near 535 nm with a shoulder near 500 nm. It has not been possible thus far to prepare apoLcH, since the demetallized protein is unstable and aggregates (10). However, the visible CD spectrum of CCoLcH possesses interesting similarities and differences with those of the cobalt substituted Con A complexes. The major band near 535 nm of CCoLcH is similar to the band at 530 nm for CoP, as is its molar ellipticity. However, the bands near 475 nm present in all three cobalt complexes of Con A is missing in CCoLcH. The fact that a band at 535 nm is present in the lentil complex, together with the observation that the protein binds monosaccharides such as methyl-α-D-mannopyranoside about 10 times more weakly than CMPL (Bhattacharyya, unpublished results), suggest that

the conformation of the lentil lectin resembles in some way the conformation of the unlocked binary complex of Con A, CoP, particulary around the metal ions. Since all of the metal ion binding residues are conserved in the two lectins except for substitution of Tyr 12 at the Ca^{2+} site in Con A for a Phe in LcH (16), differences in the visible CD spectra of the cobalt complexes of the two proteins may result from a combination of substitution at this position, and either different protein conformational states, or local configurational differences in the metal ion binding residues.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Bert Olson and B.L. Gilbert of IBM T.J. Watson Reserach Center for the metal ion analysis. C.F.B. acknowledges helpful conversations with Drs. Seymour Koenig and Rodney Brown, III, of the IBM T.J. Watson Research Center. The present work was supported in part by Grant CA 16054, awarded to C.F.B. by the National Cancer Institute, Department of Health, Education and Welfare, and NIH/NCI Core Grant P30 CA 13330.

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