BIOORGANIC CHEMISTRY 6, 465–471 (1977)

# Circular Dichroism Studies on the Structure of p-Nitrophenolate Cycloamylose Complexes

RAYMOND J. BERGERON

*Department of Chemistry, University of Maryland, College Park, Maryland 20742*

AND

PETER MCPHIE

*Laboratory of Biochemistry and Metabolism, NIAMDD, NIH, Bethesda, Maryland 20014*

*Received April 25, 1977*

Circular dichroism methods are used to determine the minimum contribution of induced optical activity in the substrate to the specific rotation of a number of cycloamylose–substrate complexes. The sign and magnitude of the Cotton effects induced in the guest molecules are explained in terms of a model in which the substrate enters the cycloamylose cavities nitro group first.

## INTRODUCTION

The cycloamyloses have proven to be excellent enzyme-active site models (*1*); recently a great deal of work has been focused on the nature of the forces responsible for binding of substrates in the cycloamylose cavity. The major thrust of these investigations has been to measure the stability of cycloamylose–substrate complexes in aqueous solution as a function of varying the host and guest structures. The procedure has been to monitor changes in either the cycloamylose or guest molecule or both as a function of changing cycloamylose–substrate ratios using either nmr, CD, ORD, or visible spectroscopy. While the interpretation of the changes observed in the nmr of the host and guest molecules on complexation is clear, i.e., that the changes actually indicate binding of the substrate in the cycloamylose cavity, interpretation of the corresponding changes observed in the visible spectra, CD, and ORD is, at best, tenuous unless used in conjunction with nmr data. However, even under these circumstances, the origins of any optical-rotation changes must be very carefully interpreted. A number of workers have attributed the optical-rotation changes observed at a particular wavelength to conformational changes produced in the cycloamylose by the substrate (*2–4*). Others have shown that optical activity is induced in the substrate on complexation (*5–7*).

In this paper we use circular dichroism data to determine the minimum contribution of induced optical activity in the substrate to the specific rotation of a number of cycloamylose–substrate complexes at 578 nm.

Complexes of *p*-nitrophenolates were chosen specifically because we had previously established their structure in aqueous solution using nmr (*8*). This made it possible to





ISSN 0045–2068

verify that our analysis of the circular dichroism spectra in terms of the cycloamylose–substrate structure in solution was correct. Takeo and Kuge have already given preliminary evidence of induced optical activity in *p*-nitrophenol–cycloamylose complexes (*6*).

## MATERIALS AND METHODS

The cyclohexa- and cycloheptaamyloses, *p*-nitrophenol, 3-methyl, and 2,6-dimethyl 4-nitrophenol were obtained from the Aldrich Chemical Co. Stock solutions were made up in sodium phosphate buffer, pH 11, $I = 0.5$ *M* and mixed in the required proportions several hours before measurements were made.

Specific rotations at 578 nm were measured in a Perkin–Elmer 241 polarimeter, with a 10-cm path length cell, at 25°C. Circular dichroism measurements were made in a Cary 60 recording spectropolarimeter with a 6001 CD attachment using 1-cm path length cells, again at 25°C. The usual precautions with regard to slit width and absorbance were observed. All other techniques were as before (*8–10*).

## RESULTS

*Binding of sodium p-nitrophenolate to cycloheptaamylose.* The addition to increasing concentrations of cycloheptaamylose of a $5.0 \times 10^{-5}$ *M* solution of sodium *p*-nitrophenolate was found to induce a positive Cotton effect in the spectrum of the dye, centered at 405 nm. The size of the Cotton effect increased with carbohydrate concentration (Fig. 1). The data could be approximated by a curve which assumed a

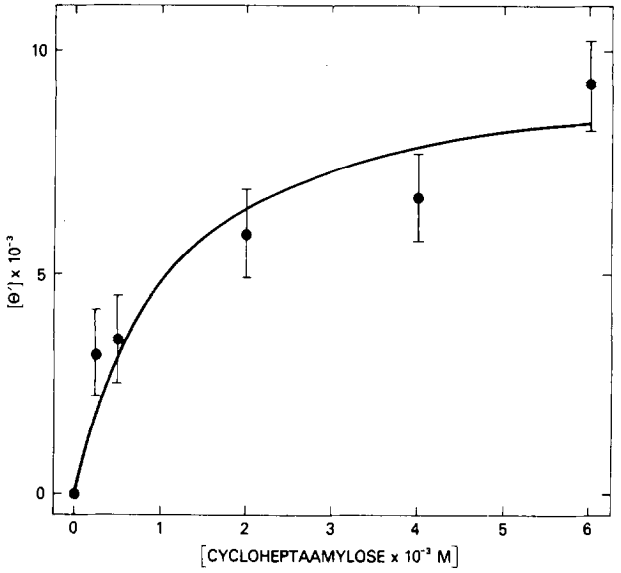


FIG. 1. The increase in reduced molar ellipticity at 405 nm with the addition of increasing concentrations of cycloheptaamylose to a $5.0 \times 10^{-5}$ *M* solution of sodium *p*-nitrophenolate, pH 11. The error bars show the noise in the instrument; repeated determinations fell within these limits. The curve assumes for the complex $[\theta'] = 9700°$, $K_{diss} = 1$ m*M*.

1:1 binding equilibrium between sodium p-nitrophenolate and cycloheptaamylose, $K_{diss}$ = 1 m*M*.

*Binding of substituted sodium p-nitrophenolates to cycloheptaamylose.* Similar experiments were performed to investigate the effect of substitution in the aromatic ring on the structure of the inclusion complexes. Both 3-methyl and 2,6-dimethyl sodium 4-nitrophenolates showed positive Cotton effects in the same region when complexed with cycloheptaamylose. On the other hand, sodium 3,5-dimethyl 4-nitrophenolate showed no optical activity in the presence of a large excess of host molecules. The results, corrected where necessary for the presence of unbound substrate using previously

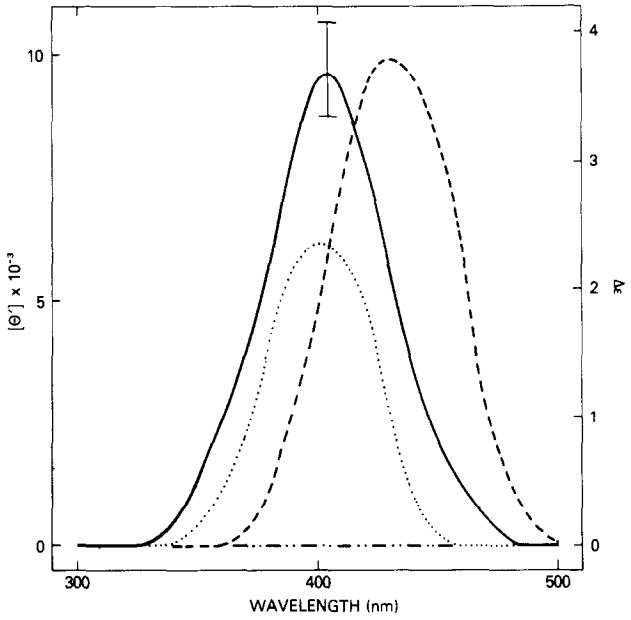


FIG. 2. The visible circular dichroism spectra of cycloheptaamylose complexes with sodium *p*-nitrophenolates, pH 11. —, sodium *p*-nitrophenolate; – –, sodium 2,6-dimethyl *p*-nitrophenolate; · · ·, sodium 3-methyl *p*-nitrophenolate; – · · –, sodium 3,5-dimethyl *p*-nitrophenolate.

determined equilibrium constants, are shown in Fig. 2. In control experiments, neither cycloheptaamylose nor the isolated sodium *p*-nitrophenolates showed any optical activity over the wavelength range shown.

*Binding of sodium p-nitrophenolates to cyclohexaamylose.* Addition of cyclohexaamylose to sodium *p*-nitrophenolate solutions was found to induce positive Cotton effects in the visible spectra of the unsubstituted and the 3-methyl and the 2,6-dimethyl compounds. As in the cycloheptaamylose case, no optical activity could be detected with sodium 3,5-dimethyl 4-nitrophenolate cyclohexaamylose mixtures or with any of the isolated compounds. The results, again corrected for unbound substrate, are shown in Fig. 3. It was found that the intensity of the Cotton effects with the different guests varied much more than in the cycloheptaamylose complexes. For the sodium 2,6-dimethyl and the unsubstituted sodium *p*-nitrophenolate complexes, the signal-to-noise ratio was sufficiently good that a second smaller Cotton effect, negative in each case, was detected in the ultraviolet region.

*Optical-rotation changes accompanying binding.* In several cases, addition of sodium *p*-nitrophenolates to cycloamylose solutions, in the same concentration ranges as shown in Figs. 2 and 3, was found to cause an apparent increase in the specific rotation of the cycloamylose, measured at 578 nm. The significance of these measurements will be dealt with below.

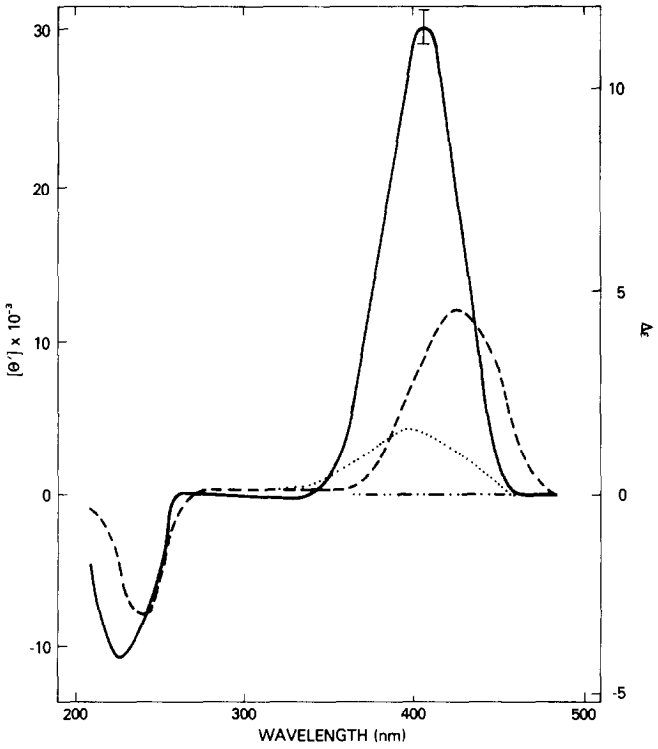


FIG. 3. The circular dichroism spectra of cyclohexaamylose complexes with sodium *p*-nitrophenolates. Symbols as in Fig. 2.

## DISCUSSION

A number of workers have measured cycloamylose–substrate binding constants by observing changes in optical rotation as a function of varying the cycloamylose–substrate ratio while holding the cycloamylose concentration constant. The method has an advantage over the visible-spectra techniques in that binding constants can be determined for nonabsorbing substrates and in absorbing solvents. This is particularly important in view of Siegel and Breslow's exciting new discovery that cycloamyloses bind substrates in solvents like dimethyl sulfoxide (*11*). The danger in the method lies in a misinterpretation of the origins of the optical-rotation changes. Some workers have taken optical-rotation changes generated on complex formation to indicate conformational changes in the cycloamylose. The remainder of this section will deal with an analysis of the circular dichroism spectra in terms of the complexes structure and with

an evaluation of the contribution of the induced optical activity in the substrates to the specific rotation of the complexes at 578 nm.

None of the sodium *p*-nitrophenolates, nor the cycloamyloses considered here, show any circular dichroism above 200 nm. However, in a number of cases, mixing the host and guest molecules was found to result in sizable circular dichroism signals in the visible and ultraviolet regions. The variation of the intensity of this signal with concentration showed its appearance to be a direct consequence of complex formation. The value of $K_{diss} = 1$ m$M$ determined for the binding of sodium *p*-nitrophenolate to cycloheptaamylose by this method is in good agreement with the value $1.4 \pm 0.3$ m$M$ found using both visible spectroscopy and nmr (*7–9*). Furthermore, that sodium 3,5-dimethyl *p*-nitrophenolate, which is known by other techniques (*8*) not to form a complex with cycloamyloses, showed no optical activity with a large molar excess of either host or guest molecules, confirms that circular dichroism spectra only result from complex formation.

Since the cycloamyloses show no absorbance bands above 200 nm, the observed circular dichroism of the complexes must arise from the spectrum of the sodium *p*-nitrophenolates. The wavelength maxima of the *p*-nitrophenolate bands are such that they can confidently be assigned to the first primary ($^1L_a$) and second primary ($^1B_b$) transitions of the spectrum (*12*).

Horata and Uedaira (*7*) tried to explain the circular dichroism spectra of cycloheptaamylose complexes of naphthalene derivatives using the Kirkwood–Tinoco coupled oscillator model (*13*). Their calculations were only partly successful. Nevertheless, a number of generalizations can be made from their results, which are in seeming agreement with the present data. In this theory, other things being equal, the rotational strength of an induced Cotton effect depends on the Coulombic interaction energy between a point dipole representing the absorbing transition and point dipoles representing transitions in the perturbing groups. From this, three main points follow:

(i) The sign of the induced Cotton effect depends on the direction of the electric dipole moment of the observed transition. An electric dipole moment on the axis of the cycloamylose ring gives a positive Cotton effect, while one perpendicular to the axis, a negative one. In every case, we observe a positive effect associated with the $^1L_a$ transition. Since this transition is polarized along the sodium *p*-nitrophenolate molecule (*12*), this is more consistent with the guest molecule entering the host nitro or hydroxyl group first, rather than sideways. In agreement with this model, the perpendicular polarized $^1B_b$ transition gave a negative Cotton effect.

(ii) The rotational strength of an induced Cotton effect will decrease with the third power of the distance between the observed and perturbing dipoles. The much higher intensity seen with the sodium *p*-nitrophenolate cyclohexaamylose complexes compared to the cycloheptaamylose complexes indicates that the aromatic molecule is much more strongly perturbed by the former (cavity diameter, 6 Å) than the latter (cavity diameter, 7.5 Å).

(iii) The induced rotational strength depends on the degree of penetration of the cycloamylose cavity, by the guest, being a maximum when its point dipole is close to the center and decreasing markedly with displacement to either side.

The results shown in Figs. 2 and 3 can be rationalized on this basis, if it is assumed that the guest enters the host nitro group first. Inspection of molecular models shows

that penetration of the sodium phenolate into the tight cyclohexaamylose cavity will be slightly reduced by substitution in the *ortho* position and greatly reduced by substitution *meta* to the hydroxyl group. In the case of the looser cycloheptaamylose cavity, *ortho* substitution is found to make little difference, whereas *meta* derivatives are again hindered. These restrictions are reflected in the large decrease in the size of the Cotton effects seen with cyclohexaamylose on progressing from sodium *p*-nitrophenolate to the 2,6-dimethyl and the 3-methyl derivatives (Fig. 3). The trend is less marked in the case of the *β*-cycloamylose complexes (Fig. 2). Similar conclusions concerning the relative orientations of the host and guest molecules were derived from changes in their nmr spectra (*8*).

Because of its dispersive nature, optical rotation measured at any wavelength contains contributions from all Cotton effects arising from substrate, cycloamylose, and complex, even those arising from spectral transitions at distant wavelengths. Consequently, a change in optical rotation at one wavelength cannot be assigned to any particular chromophore. Circular dichroism is an absorptive phenomenon, occurring only at those wavelengths at which the optically active species have spectral transitions. Thus, the origin of the activity can be much more readily identified.

In our earlier binding studies we measured the optical rotation changes generated at 578 nm as a function of changing cycloamylose–substrate ratios while holding the cycloamylose concentration constant. We chose this wavelength because it represented the best trade off between the power output, i.e., signal-to-noise ratio and change in optical rotation. To determine the origin of these changes, the optical rotatory dispersion in the guest molecules were calculated using the Kronig–Kramers transform (*14*). It was found that it makes a sizable contribution at the wavelength of interest. The apparent changes in specific rotation of the cycloamylose arising from superposition of those rotations onto the unperturbed rotation of the carbohydrate are also shown in

TABLE 1

THE OBSERVED AND CALCULATED CHANGES IN SPECIFIC ROTATION, $[\alpha]_{578}$, ON COMPLEX FORMATION BETWEEN CYCLOHEXAAMYLOSE AND SODIUM *p*-NITROPHENOLATE

| | $\Delta[\alpha]_{578}$ | |
|---|---|---|
| Guest molecule | Observed | Calculated from Fig. 3 |
| Sodium *p*-nitrophenolate | +131° | +250 ± 30° |
| Sodium 2,6-dimethyl *p*-nitrophenolate | +112° | +115 ± 30° |
| Sodium 3-methyl *p*-nitrophenolate | 0 | +32 ± 30° |

Table 1. These studies confirm that most of the change, in specific rotation, on cycloamylose–substrate complexation is due to induced optical activity in the substrate. This means that any conclusion drawn from changes in optical rotation on cycloamylose complexation regarding changes in the cavities conformation must be made very cautiously, in the absence of supporting evidence.

## ACKNOWLEDGMENT

Acknowledgment is made to the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support (PRF 3127 G12) of this research.

## REFERENCES

1. D. W. GRIFFITHS AND M. L. BENDER, *Advan. Catal.* **23**, 209 (1973).
2. H. SCHLENK AND D. M. SAND, *J. Amer. Chem. Soc.* **83**, 2312 (1961).
3. D. A. REES, *J. Chem. Soc. B*, 877 (1970).
4. W. SAENGER, M. NOLTEMEYER, P. C. MANOR, B. HINGERTY, AND B. KLAR, *Bioorg. Chem.* **5**, 187 (1976).
5. K. SENSSE AND F. CRAMER, *Chem. Ber.* **102**, 509 (1969).
6. K. TAKEO AND T. KUGE, *Die Stgrke* **9**, 281 (1972).
7. K. HARATA AND H. UEDAIRA, *B. Chem. Soc. Jpn.* **48**, 375 (1975).
8. R. BERGERON AND R. ROWAN, *Bioorg. Chem.* **5**, 290 (1976).
9. R. BERGERON AND M. CHANNING, *Bioorg. Chem.* **5**, 289 (1976).
10. R. BERGERON, M. A. CHANNING, G. J. GIBEILY, AND D. M. PILLOR, *J. Amer. Chem. Soc.* **99**, 5146 (1977).
11. B. SIEGEL AND R. BRESLOW, *J. Amer. Chem. Soc.* **97**, 6871 (1975).
12. H. H. JAFFE AND M. ORCHIN, "*Theory and Applications of U.V. Spectroscopy*" Wiley, New York, 1962.
13. I. TINOCO, JR., *Adv. Chem. Phys.* **4**, 113 (1962).
14. A. MOSCOWITZ, *in* "Optical Rotary Dispersion" (C. Djerrasi, Ed.), McGraw–Hill, New York, 1960.