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The effect of ATP on the CD spectrum of membrane fraction from oxyntic cells

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SUMMARY

A vesicular preparation derived from acid-secreting cells of dog gastric mucosa was studied in a Cary 60 modified for simultaneous CD and absorbance measurements. Significant changes in the spectrum were obtained on addition of MgATP, but not MgAMP, which were only partly due to changes in scattering, *etc.* Thus ATP may induce conformational changes in the protein constituents of the vesicle membrane.

Gastric mucosa of various species contains a membrane-bound ATPase insensitive to Na^+ or K^+, but stimulated by HCO_3^- and inhibited by SCN^-, an inhibitor of acid secretion[1-3]. This enzyme is localized in the microsomal fraction[4], derived from the acid-secreting cells[3] and indeed in the smooth-surfaced vesicles of that fraction. Various lines of evidence point to this enzyme playing a critical role in acid secretion[5,6], hence it was of interest to determine whether any conformational changes could be detected in the vesicular fraction obtained from dog gastric mucosa as a result of ATP addition. Since some evidence has been presented for such changes in the circular dichroism (CD) spectrum of $(Na^+ + K^+)$-ATPase particles[7], a similar approach was used to study the gastric ATPase preparation. When changes are observed in the CD spectra of membrane suspensions, the fundamental question is whether the effects result from a change in the state of aggregation or whether they result from change in the conformation of molecules comprising the membrane. This requires proper analysis of the CD data and corresponding absorption data in order to obtain results relevant to molecularly disperse systems.

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The vesicular fraction banding at a sucrose density of 1.12 was isolated as previously described from dog gastric mucosa[5]. Enzyme activities were measured using the phosphate analysis procedure of Yoda and Hokin[8] and protein was measured by the method of Lowry *et al.*[9]. Electron microscopy of the fraction was performed using OsO_4 fixation in order to check the purity of the preparation, and with negative staining in order to assess whether any effects of MgATP could be detected morphologically. CD measurements were carried out on a Cary 60 spectropolarimeter with Model 6001 attachment for CD and an accessory for simultaneous measurement of absorption[10]. The chosen pathlength was $1.02 \cdot 10^{-2}$ cm and the protein concentration of the sample was adjusted to about 1 mg/ml. When treated with sodium dodecyl sulfate, at a final concentration of 0.4%, 0.2 ml of the original suspension was used, diluted to 1 ml with Tris acetate buffer. Accordingly, a $5.2 \cdot 10^{-2}$ cm cell was used for CD measurements for the sodium dodecyl sulfate preparation.

In order to check the effects of ATP on the preparation, 10 μl of a solution of 100 mM ATP containing 100 mM $MgCl_2$ were used and the final volume of 1 ml was achieved using as buffer 10 mM Tris acetate, giving a final concentration of 1 mM ATP. Again a cell of $1.03 \cdot 10^{-2}$ cm pathlength was used for the measurements on the suspension and the longer pathlength cell for the sodium dodecyl sulfate-treated system.

Electron microscopic preparations showed that the preparation consisted almost entirely of smooth-surfaced vesicles of about 0.2 μm in diameter[5]. The HCO_3^--ATPase activity is also localized in this fraction[5]. Negative staining showed changes occurring with the addition of ATP, resulting in an apparent swelling and loss of definition of the membrane forms.

The CD spectrum of the original preparation of the oxyntic cell membranes shows two troughs at 223 nm and 208 nm, respectively, a crossover at 202 nm and a peak near 192 nm. Upon treatment with sodium dodecyl sulfate, no improvement of the

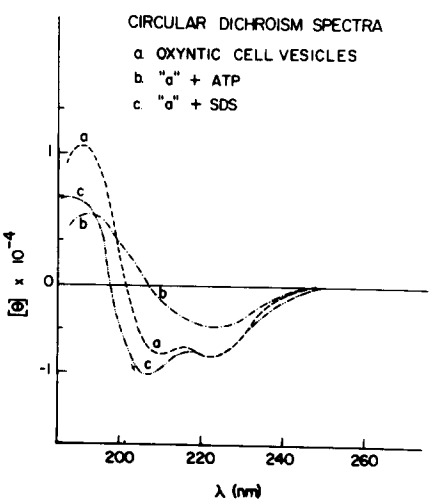

Fig. 1. The CD spectrum of vesicles derived from oxyntic cells, of vesicles + sodium dodecyl sulfate (SDS) and of vesicles + MgATP (1 mM) (uncorrected).

amplitude of the band with the maximum near 223 nm was achieved; however, there was an enhancement of the second negative band which was blueshifted by about 2 nm; the crossover was also shifted to 198 nm and the peak at 192 nm shifted to 190 nm, but dampened in amplitude when compared to the peak resulting from the untreated sample.

Addition of MgATP caused a dramatic dampening of the 223-nm band as well as of the 192-nm band and the near disappearance of the 208-nm band. Furthermore, the 223-nm band was shifted toward the red by 1 nm and the crossover was shifted to 207 nm while the positive band was broadened and decreased 50%. The quite apparent modification of the CD pattern of the oxyntic cells upon addition of MgATP is interpreted as deriving in part from aggregation of the vesicles and modification of their size and shape. As observed for other membranous systems[11-13], these modifications, at least partially, arise from changes in absorption flattening and light scattering, which accompany changes in the state of aggregation. Corrections were therefore applied in order to compare the CD spectrum of the vesicles in the two different metabolic states. The pseudo reference state approach[11-13] was chosen and the corrected ellipticity was calculated by means of the following equation[13]:

$$\theta_{corr} = \frac{\theta_{susp}}{Q_A^2 \cdot Q_\sigma}$$

where Q_A is the absorption flattening quotient and Q_σ is the absorption obscuring quotient derived from light scattering effects[13]. The oxyntic cell vesicles treated with sodium dodecyl sulfate were chosen as the pseudo reference state since they were optically clear. However, the interaction of sodium dodecyl sulfate with the oxyntic cell vesicles was not characteristic of most other membrane systems, since no absorption increase is observed at 192 nm on dissolution in sodium dodecyl sulfate. Similarly there is no enhancement of the ellipticity at this wavelength upon treatment with sodium dodecyl sulfate. In the presence of ATP, however, these anomalies were not present. Other detergents, such as deoxycholate, or Triton X-100 also proved unsuitable for obtaining a pseudo reference state at 192 nm. It was felt, therefore, that a complete spectral correction for the ATP effect was not worthwhile since the sodium dodecyl sulfate-treated vesicles were a poor pseudo reference state at 192 nm and comparisons drawn between the two states at this wavelength would be inadequate. However, at 224 nm corrections can be applied, since here the corrections are almost entirely due to scatter.

The molar ellipticity at 224 nm improved, for the untreated vesicles from $-0.73 \cdot 10^4$ to $-0.9 \cdot 10^4$, whereas for the ATP-treated system the change was from $-0.38 \cdot 10^4$ to $-0.06 \cdot 10^4$.

The addition of MgAMP, as shown in Fig. 2, gave no significant change in the CD pattern of the vesicles. The data thus show that the changes observed are due to the nucleotide, rather than Mg^{2+}.

The data discussed here show that ATP induces significant changes in the overall conformation of the oxyntic cell vesicles suspension, these changes being in part due to alterations of the state of aggregation or the shape of vesicles. This aggregation causes changes in light scattering which can be corrected for at 224 nm, and even when these corrections are applied, the molar ellipticity

at 224 nm in the presence of ATP is considerably less than in its absence ($-0.9 \cdot 10^4$ compared to $-0.6 \cdot 10^4$). Moreover, when compared to most other

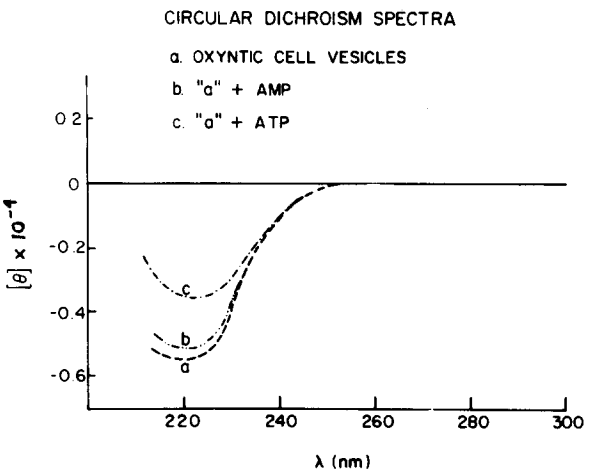

Fig. 2. CD spectrum of oxyntic cell vesicles, vesicles + MgATP (1 mM) and vesicles + MgAMP (1 mM) (uncorrected; the noise level was too high to carry the CD curve below 210 nm).

membrane systems[11-13] such as red blood cell membranes ($-2 \cdot 10^4$), mitochondria ($-1.7 \cdot 10^4$), plasma membranes ($-1.7 \cdot 10^4$) and sarcotubular vesicles ($-1.2 \cdot 10^4$), their ellipticity is low, but it is comparable to the ellipticity of axonal membranes ($-0.9 \cdot 10^4$)(L. Masotti, D.W. Urry and R. Leinas, unpublished results).

It may be suggested that as for the axonal membrane the low ellipticity at 224 nm reflects a considerable amount of disordered or of β-structure in the protein component of the membrane. One possible interpretation of the effect of ATP on oxyntic cell vesicles is a change in the protein conformation within the oxyntic cell vesicle, *e.g.* a decrease in the helical content and a corresponding increase in the disordered or β-structure.

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