Polymer Bulletin 2, 97–101 (1980)

Polymer Bulletin

Complex Formation Between the Biopolymer Glycogen and Histone

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ABSTRACT

On mixing the biopolymer glycogen with histone, partial complex formation takes place. Experimental absorption data of such mixtures in the ultraviolet agree with figures calculated on the basis of complex formation with an order of $10^7 M^{-1}$ for the association constant.

In the course of his studies (GEMANT, in print) concerning the enzymic oxidation of histone, a basic protein, the author found that certain compounds, EDTA and glycogen, a biopolymer of glucose, among others, inhibit the oxidation (GEMANT, under preparation). Essential in the present context is the question of the mechanism of inhibition. As for EDTA, the mechanism is probably chelation of the Fe present in the enzyme, peroxidase (LANIR and SCHEYTER, 1975). As for glycogen, such binding does not occur: the mechanism must be a different one. A cue to this was given by the following observation. When histone and glycogen are mixed, the UV absorption is less than that expected by the additivity of the constituents. Such a reduction could be caused by complex formation between the two constituents. This explanation is not unlikely. Apart from the ubiquitous occurrence of glycoproteins (HAUROWITZ, 1979), it is known, for instance, that polysaccharides act as antigens against immunoglobulins. Another instance is the polysaccharide containing O-antigen of symbionts that bind to lectins of legumes (DAZZO and BRILL, 1977). If, then, complex formation takes place in the present instance, it would explain its inhibitory action: glycogen could screen the histone against the attack of peroxidase.

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0170-0839/80/0002/0097/$01.00

The present report deals with a quantitative treatment of this complex formation; if agreement between measured data and theoretical calculation can be achieved, the view of complex formation in this case and of the assumed role of the complex in the mechanism of inhibition would be strengthened.

TABLE 1

Absorptions in the UV at 290 nm in 1-cm cuvette of histone and glycogene, singly, A_h and A_g, and in mixture, A, at molar concentrations, c_o, of each component. The ratio r is $A/(A_h + A_g)$.

10^5 c_o, M	A_h	A_g	A	r
0.212	0.118	0.117	0.138	0.59
0.425	0.144	0.149	0.196	0.67
0.850	0.177	0.217	0.300	0.76
1.70	0.262	0.348	0.510	0.84

Experimental data are given in Table 1. The histone used was a product of Sigma, Type II-A, from calf thymus, the glycogen also Sigma, Type III, from rabbit liver. In case the histone used was heterogeneous, average values are obtained, referring to an equivalent single species of histone. The first column of the table shows the initial molar concentrations, c_o, of both histone and glycogen, respective molecular weights being taken as 3×10^4 and 10^5 daltons. The second and third columns give the respective absorptions, A_h and A_g, at 290 nm wavelength in an 1-cm cuvette, when either is present alone; the fourth column is the absorption, A, when both components are present simultaneously. The fifth column shows the ratio $r=A/(A_h+A_g)$ which is a measure of the reduction observed, possibly due to complex formation.

Plotting absorptions *vs* concentrations on a bilogarithmic scale, approximately linear relations are obtained, permitting derivation of the following expressions. For histone we have:

$$A_h = 16.2\ c_h^{0.38} \qquad (1)$$

and for glycogen:

$$A_g = c_g^{0.51} \qquad (2)$$

Neither of the two compounds obeys Beer's law, which is to be expected, considering the many polar radicals present in both.

For the calculation of the equilibrium condition (MONTGOMERY and SWENSON) in a second order reaction we have:

$$K\, c_h\, c_g = c_c \qquad (3)$$

where K = association constant, and the c are molar concentrations of respectively histone, glycogen and complex. We have further:

$$c_o = c_h + c_c = c_g + c_c \qquad (4)$$

showing that in this case c_h and c_g have the same value. Eliminating c_h and c_g, one obtains:

$$c_c = 0.5\,(\,2\,c_o + K^{-1}\,) - 0.5\,[(\,2\,c_o + K^{-1}\,)^2 - 4\,c_o^{\,2}]\,0.5 \qquad (5)$$

The only arbitrary parameter is K. Its order of magnitude has been taken to be $10^7\ M^{-1}$, as giving satisfactory agreement between theory and experiment. Calculated values for equilibrium concentrations are shown in Table 2. The first column gives the initial concentrations, c_o, the same as listed in Table 1. The next two columns give $c_h = c_g$ and c_c.

Proceeding to the evaluation of absorptions, we have:

$$A = A_h + A_g + A_c \qquad (6)$$

The first two terms are calculated from equations (1) and (2), they are listed next in Table 2. As for A_c, we note that the polar groups of the two components are largely combined with each other in the complex, hence the latter probably obeys Beer's law in first approximation. Taking the value of A in the last row of Table 1, 0.510, and deducting the sum of $A_h = 0.092$ and $A_g = 0.085$, A_c becomes 0.333. From Table 2, c_c for this case is 1.575×10^{-5} M, hence we derive:

$$A_c = 21.1 \times 10^3\, c_c \qquad (7)$$

Table 2

Calculated values of equilibrium molar concentrations, $c_h = c_g$, c_c , of the three species present, the corresponding absorption terms, A_h , A_g , A_c , and the sum, A, of the three at four initial concentrations, c_o , the latter the same as in Table 1. Last column is the calculated ratio r.

10^5 c_o , M	10^5 c_h = 10^5 c_g , M	10^5 c_c , M	A_h	A_g	A_c	A	r
0.212	0.041	0.171	0.060	0.048	0.036	0.144	0.64
0.435	0.060	0.365	0.070	0.058	0.077	0.205	0.67
0.850	0.087	0.763	0.080	0.070	0.161	0.311	0.75
1.70	0.125	1.575	0.092	0.085	0.333	0.510 [a]	0.89

[a] This value was taken over identically from Table 1 for the evaluation of the extinction coefficient of the complex, 21.2 x 10^3 cm^{-1} M^{-1}.

the numerical factor being the molar extinction coefficient of the complex. This relation permits calculation of A_c for the three other concentrations, listed next in Table 2. The sum of the three terms gives the total absorption, also shown in Table 2.

In order to calculate the values of the ratio r, we need the absorptions obtaining in case no complex formation were to take place. These are calculated from the values of c_o, using equations (1) and (2) and dividing A by their sum. The values for r are shown in the last column of Table 2.

Comparing the calculated values for A and r with those found experimentally, Table 1, the agreement is seen to be satisfactory. We thus conclude that complex formation does take place when the biopolymer glycogen and the basic protein histone are simultaneously present in solution.

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Received November 9, 1979
Accepted November 15,1979