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Modulation of Erythrocyte Membrane Proteins by Membrane Cholesterol and Lipid Fluidity†

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ABSTRACT: Human erythrocyte membranes were enriched or depleted of cholesterol and effects on membrane proteins assessed with a membrane-impermeant sulfhydryl reagent, [^{35}S]glutathione–maleimide. Reaction of the probe with intact cells quantifies exofacial sulfhydryl groups and reaction with leaky ghost membranes permits quantification of endofacial sulfhydryl groups. The mean endofacial sulfhydryl titer of cholesterol-enriched membranes exceeded that of cholesterol-depleted membranes by approximately 45 nmol/mg of protein, or 64%. The corresponding exofacial titer of cholesterol-enriched cells was less than that of cholesterol-depleted cells by approximately 0.4 nmol/mg of protein, or 14%. Labeled membranes were examined by autoradiography of sodium dodecyl sulfate–polyacrylamide gel electropherograms to determine the labeling patterns of individual protein bands. Cholesterol enrichment enhanced the surface labeling of Coomassie brilliant blue stained bands 1, 2, 3, and 5, decreased the labeling of band 6, and did not change significantly that of band 4. The results demonstrate that changes in membrane cholesterol which influence lipid fluidity can alter the surface labeling of both intrinsic and extrinsic membrane proteins.

The fluidity of the lipids of biological membranes influences membrane proteins in several ways. Considerable evidence that the lateral and rotational movements of the proteins are affected has been reviewed (Edidin, 1974; Cherry, 1976). More recently, the results of fluorescence studies (Borochov & Shinitzky, 1976; Shinitzky & Rivnay, 1977) indicate that alteration of lipid fluidity also modulates the availability of protein substituents at the membrane surfaces. Thus, enrichment of human erythrocyte membranes in cholesterol, which decreases bulk lipid fluidity, enhanced the surface exposure of membrane proteins, and conversely, depletion in cholesterol decreased the surface exposure. A working hypothesis to explain these findings (Borochov & Shinitzky, 1976; Shinitzky & Rivnay, 1977) assumes that membrane proteins, as amphipathic molecules, are influenced strongly by their relative interactions with bulk membrane lipids and ambient water. Hence decreased lipid fluidity, which corresponds to enhanced lipid–lipid interactions, may result in decreased protein–lipid interactions and increased protein–water interactions. The proteins, or portions thereof, may thus be displaced toward the membrane surfaces.

To test the working hypothesis further and to quantify more precisely the effects of lipid fluidity on the surface availability of protein substituents, we have applied a membrane-impermeant sulfhydryl reagent, [^{35}S]glutathione–maleimide ([^{35}S]GSmal).[1] Sulfhydryl groups reactive at the exofacial (outer) and endofacial (inner) surfaces of human erythrocyte membranes are quantified by labeling separately intact cells and leaky ghost membranes, and mean values of 1.6 and 28 amol/cell, respectively, have been reported for the outer and inner surfaces (Abbott & Schachter, 1976). In the experiments described below, human erythrocytes were first treated to alter the membrane cholesterol to phospholipid molar ratio (C/PL) and the effects on the surface –SH titers and on the –SH groups of individual protein bands separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis were determined.

Materials and Methods

Sulfhydryl Reagent. The membrane-impermeant –SH reagent [^{35}S]glutathione–maleimide I was prepared and characterized as previously described (Abbott & Schachter, 1976).

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[1] Abbreviations used: C/PL, cholesterol to phospholipid molar ratio; GSmal, glutathione–maleimide; PBS, phosphate-buffered saline; $NaDodSO_4$, sodium dodecyl sulfate.

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Liposomes. Sonicated dispersions (liposomes) of egg lecithin (Lipid Products, England) and of egg lecithin plus cholesterol (Sigma Chemical Co.) in phosphate-buffered saline (PBS), pH 7.4, were prepared by sonication at 100 W for 1 h, at 0 °C, under N_2 with a Bronwill Biosonik III sonicator. Dispersions were centrifuged at 25 000*g* for 30 min to sediment undispersed material and used within less than 24 h.

Isolated Membranes. Erythrocyte leaky ghost membranes free of hemoglobin were prepared by the procedure of Dodge et al. (1963). Protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Alteration of Erythrocyte Membrane C/PL Ratio. Heparinized human blood drawn from normal subjects was used within 1 h. Erythrocytes and plasma were separated by centrifugation at 1000*g* and the cells washed three times with PBS. The plasma was inactivated by incubation at 56 °C for 30 min, clarified by centrifugation at 1000*g*, and used immediately. The erythrocyte membrane C/PL ratios were altered by a modification (Borochov & Shinitzky, 1976) of the method of Cooper et al. (1975). Cells were incubated in their inactivated plasma containing liposomes of one of the following three compositions: egg lecithin alone, to deplete membrane cholesterol; cholesterol/egg lecithin molar ratio of 0.95, to approximate the normal membrane cholesterol content; and cholesterol/lecithin ratios of 1.4–1.6, to enrich membrane cholesterol. Additional control erythrocyte suspensions were incubated similarly in the absence of liposomes. After incubation at 37 °C for 24 h (Borochov & Shinitzky, 1976), the cells were washed three times with PBS, pH 7.4, portions used to prepare leaky ghost membranes, and labeling experiments performed as described below. The membrane C/PL ratio was estimated in each instance by fluorescence polarization (Borochov & Shinitzky, 1976; Shinitzky & Inbar, 1976), using the fluorophor 1,6-diphenyl-1,3,5-hexatriene (Fluka, AG) and an empirical calibration curve relating the degree of fluorescence polarization to the C/PL ratio (Cooper et al., 1979).

Quantification of Sulfhydryl Groups. Exofacial –SH titers were determined as previously described (Abbott & Schachter, 1976) by incubating 0.2 mL of packed erythrocytes with 0.2 mL of freshly prepared 40 mM [^{35}S]GSmal, specific radioactivity approximately 300 cpm/nmol, in isotonic sodium phosphate of pH 6.7. After shaking at 37 °C for 2 h, the cells were washed three times with 10 mL of PBS and the membranes were isolated and dissolved in 0.5 mL of 2% sodium dodecyl sulfate containing 5 mM EDTA of pH 7.4 and 5 mM β-mercaptoethanol. Samples were heated at 100 °C for 3 min and subsequently aliquots were taken for estimation of protein content and radioactivity in a Beckman Model LS-230 spectrometer. To label quantitatively the endofacial plus exofacial surfaces, leaky ghost membranes prepared from treated erythrocytes were incubated with 40 mM [^{35}S]GSmal as described above, except that the specific radioactivity was approximately 100 cpm/nmol and the incubation proceeded for 1 h. (Where membranes were examined by autoradiography, as noted below, 2 mM [^{35}S]GSmal of specific radioactivity 10^3-10^4 cpm/nmol was used.) Membranes were then washed three times with 10 mL of 8 mM sodium phosphate of pH 7.4 and dissolved as indicated above. Although both surfaces of the membrane are labeled in this procedure, the resulting –SH titer is predominantly that of the endofacial surface, inasmuch as exofacial –SH comprises only 3–4% of the total surface –SH.

Membrane Proteins. Membranes labeled with [^{35}S]glutathione-maleimide were dissolved with sodium dodecyl sulfate, the proteins separated by $NaDodSO_4$–polyacrylamide gel electrophoresis, and autoradiographs prepared from the gels as previously described (Abbott & Schachter, 1976). Autoradiographs were scanned in a Gilford densitometer and the area corresponding to each band was expressed as a fraction of the total area of the gel scan. This fractional area multiplied by the value for total incorporation of [^{35}S]GSmal yielded the number of –SH groups labeled in each protein band. In three experiments labeled membranes were extracted at low ionic strength to solubilize partially the extrinsic membrane proteins corresponding to Coomassie brilliant blue stained bands 1, 2, and 5 (Abbott & Schachter, 1976; Fairbanks et al., 1971; Steck & Yu, 1973). Approximately 1.0 mg of washed, labeled membranes was incubated in 1.0 mL of 0.1 mM sodium EDTA of pH 8.4, at 37 °C, with shaking, for 20 min. After centrifugation at 30 000*g* for 20 min at 5 °C, each supernatant solution and residual pellet were dissolved in sodium dodecyl sulfate and protein and ^{35}S estimated as described above. When examined by $NaDodSO_4$–polyacrylamide gel electrophoresis, the EDTA extracts contained bands 1, 2, and 5; the pellets contained the remaining proteins plus residual bands 1, 2, and 5 (Abbott & Schachter, 1976; Steck & Yu, 1973).

Table I: Membrane Surface Sulfhydryl Titers of Intact Erythrocytes and Isolated Ghost Membranes with Various Cholesterol/Phospholipid Molar Ratios[a]

prep labeled	membrane C/PL, rel to control[b] (%)	sulfhydryl titer,[c] mean (nmol/mg of protein)
intact erythrocytes	70	2.9
	100	3.0
	105	2.7
	125	2.5
isolated membranes	70	70
	100	98
	105	104
	125	115

[a] Each of six human blood samples was treated with liposomes to prepare cholesterol-depleted and -enriched membranes, and both the intact cells and the ghost membranes isolated from them were labeled with [^{35}S]GSmal (Materials and Methods). [b] Control cells (C/PL = 0.95) were incubated in the absence of liposomes. C/PL values of liposome-treated preparations were estimated from fluorescence polarization values, as described in Materials and Methods. [c] *P* values were estimated by paired *t* tests. For the intact erythrocytes, significant differences are between controls and C/PL = 125% (SE = 0.10, $P < 0.0025$) and between cholesterol-depleted (C/PL = 70%) and C/PL = 125% (SE = 0.16, $P < 0.05$). For the isolated membranes, significant differences are between C/PL = 70% and C/PL = 105% (SE = 10, $P < 0.01$), C/PL = 70% and C/PL = 125% (SE = 12, $P < 0.01$), and control and C/PL = 70% (SE = 5, $P < 0.0025$).

Results

Labeling Experiments. Liposome treatment of each of six samples of fresh human blood (Materials and Methods) altered the erythrocyte membrane C/PL ratio as previously described (Borochov & Shinitzky, 1976). As indicated in Table I the resulting C/PL values, expressed relative to control erythrocytes incubated in the absence of liposomes (C/PL = 0.95), were 70%, 105%, and 125%, respectively, for the cholesterol-depleted, essentially unchanged, and cholesterol-enriched preparations. The surface –SH titers obtained on labeling intact cells or the membranes isolated from them are also shown in Table I. The exofacial titer of the intact cells decreased only slightly, by approximately 0.4 nmol/mg of protein, or 14%, as the C/PL increased from 70% to 125% of the control value ($P < 0.05$).[2] In contrast, the surface –SH

Table II: EDTA Extraction of Erythrocyte Membranes Labeled with [^{35}S]Glutathione-Maleimide[a]

membrane C/PL, rel to control[b] (%)	protein extracted (%)	[^{35}S]GSmal extracted (%)	GSmal content (nmol)		sp incorp of GSmal (nmol/mg of protein)		
			extract	pellet	extract (*a*)	pellet (*b*)	(*b*)/(*a*)
70	16 ± 0.7	66 ± 4.5	45 ± 15	21 ± 2	228 ± 42	23 ± 2	0.10 ± 0.01
100	18 ± 0.7	68 ± 3.4	59 ± 14	27 ± 2	326 ± 60	33 ± 3	0.10 ± 0.01
105	21 ± 0.8	68 ± 1.7	56 ± 10	26 ± 3	246 ± 37	31 ± 3	0.13 ± 0.01
125	20 ± 1.0	62 ± 3.1	60 ± 7	36 ± 1	280 ± 36	42 ± 2	0.16 ± 0.03

[a] Values are means ± SE for three ghost membrane preparations labeled with [^{35}S]GSmal as described in the text and extracted with 0.1 mM sodium EDTA (Materials and Methods). *P* values in text were calculated from paired *t* tests. [b] Control (C/PL = 0.95) membranes from erythrocytes incubated in the absence of liposomes.

titer of the isolated membranes, which is essentially the titer of the endofacial surface, increased by approximately 45 nmol/mg of protein, or 64% ($P < 0.01$), with the same increase in C/PL. The last observation confirms prior results of fluorescence studies (Borochov & Shinitzky, 1976; Shinitzky & Rivnay, 1977). Relative to the control preparations untreated with liposomes, the cholesterol-depleted membranes showed a reduction of 29% in the number of –SH groups labeled, whereas the cholesterol-enriched membranes showed an increase of 17%.

Extraction Experiments. To examine the effects of membrane cholesterol more specifically on extrinsic proteins, isolated membranes of different C/PL values were labeled with [^{35}S]GSmal as described above and subsequently extracted with 0.1 mM EDTA of pH 8.4. This procedure solubilizes partially the polypeptides of bands 1, 2, and 5 (Fairbanks et al., 1971; Abbott & Schachter, 1976), components which are located on the endofacial surface. Values for the protein and radioactivity recovered in the extracts as compared with the residual pellets are listed in Table II. EDTA extraction solubilized up to one-fifth of the membrane protein and the membrane C/PL value influenced the extent of solubilization. As the C/PL increased from 70% of the control value to 105%, the protein solubilized increased by 31% ($P < 0.001$). EDTA extraction solubilized 60–70% of the membrane radioactivity at all cholesterol levels. As the C/PL increased from 70% of the control value to 125%, the total radioactivity incorporated into both the EDTA extracts and the pellets increased; however, the relative increase in the pellets, 71%, exceeded that in the extracts, 30%. Table II also lists the values for the specific incorporation of [^{35}S]GSmal in nmol/mg of protein. These values are strikingly higher in the extracts as compared with the pellets, indicating that the extracted polypeptides are relatively enriched in cysteine residues reactive to GSmal. With increases in C/PL the specific incorporation into both the EDTA extracts and the pellets increased, but the relative increment was again greater in the pellets. The ratio of specific incorporation into pellet/extract rose progressively from 0.10 to 0.16 as membrane cholesterol increased.

Individual Protein Bands. The influence of membrane cholesterol on the labeling of individual protein bands was studied with four blood samples. Each sample was treated with liposomes to alter the C/PL values, isolated membranes were prepared and labeled with [^{35}S]GSmal, and the resulting preparations were examined by $NaDodSO_4$ polyacrylamide gel electrophoresis and autoradiography as described in Materials and Methods. Incorporation of the maleimide into the major membrane protein bands is illustrated in Figure 1. The values plotted in Figure 1A are in terms of nmol of GSmal incorporated into that quantity of each individual band contained in 1 mg of total membrane protein. The results demonstrate that bands 1 and 2 ("spectrin") are the predominant species labeled in the membrane, followed by bands 4 and 3. Variations in C/PL alter significantly the incorporation into individual bands and several patterns emerge. Increases in C/PL enhance the labeling of bands 1 and 2 ($P < 0.0125$), 3 ($P < 0.01$), and 5 ($P < 0.01$) but decrease the

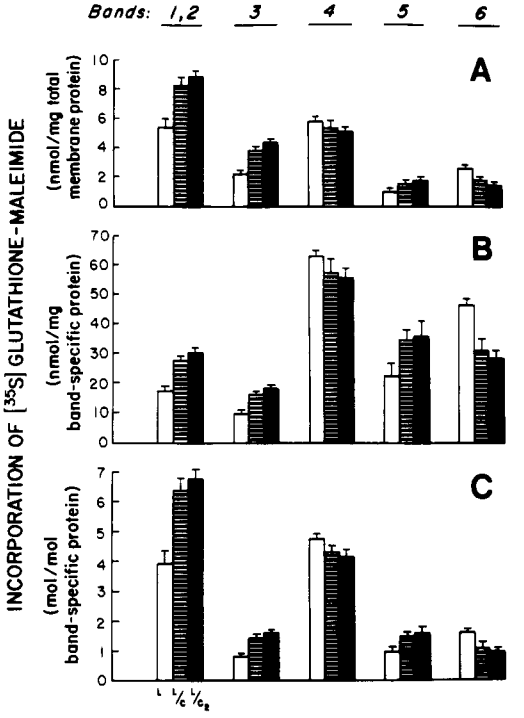

FIGURE 1: Incorporation of [^{35}S]glutathione-maleimide into erythrocyte membrane proteins as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Values plotted are means ± SE for four different samples of human blood. Intact erythrocytes were treated with liposomes to provide membranes with C/PL (relative to controls) of 70% (light bars, labeled L), 105% (striped bars, labeled L/C), and 125% (dark bars, labeled L/C_2). Ghost membranes were prepared, labeled with [^{35}S]GSmal and dissolved with sodium dodecyl sulfate. After separation of the proteins by polyacrylamide gel electrophoresis, the fractional distribution of the radioactivity in each band was estimated by autoradiography as described in the text. Band designations follow the conventional terminology (Steck, 1974). Band 4 includes 4.1 plus 4.2, which were not resolved sufficiently for separate quantification. Incorporation into each band is expressed per mg of total membrane protein (A), per mg of band-specific protein (B), or per mol of band-specific protein (C). *P* values obtained by paired *t* tests for differences between cholesterol-depleted (C/PL = 70% of control) and cholesterol-enriched (C/PL = 125% of control) preparations were <0.0125 for bands 1 and 2; <0.01 for bands 3 and 5; <0.02 for band 6; and >0.1 for band 4.

[2] The exofacial sulfhydryl titers in Table I do not exceed the values previously reported for untreated human erythrocytes, 1–2 amol/cell or 2.9–5.8 nmol/mg of membrane protein (Abbott & Schachter, 1976). Thus the incubations with or without liposomes in the present experiments did not increase the permeability of the intact erythrocyte membrane to the glutathione–maleimide probe. Increases in erythrocyte membrane permeability markedly increase the exofacial titer observed with glutathione–maleimide, inasmuch as exofacial sulfhydryl comprises only 3–4% of total membrane sulfhydryl.

labeling of band 6 ($P < 0.02$). Simultaneously, band 4 labeling is not changed significantly ($0.1 < P < 0.15$).

Incorporation into each band was also calculated in nmol per mg of band-specific protein, using published estimates of the fractional composition of erythrocyte membrane protein (Steck, 1974). This value reflects the frequency relative to other amino acids of cysteine residues labeled in the experiment. As shown in Figure 1B the rank order of values observed was band 4 > 5, 6 > 1, 2 > 3. Finally, incorporation was expressed per mol of band-specific protein using published estimates of the molecular weights (Steck, 1974). Figure 1C illustrates the rank order of these values: bands 1, 2 > 4 > 3, 5, 6. For bands 1 and 2 the increment in labeling between cholesterol-depleted and cholesterol-enriched membranes amounts to 2–3 –SH groups per molecule.

Discussion

The foregoing studies quantify –SH groups of the erythrocyte membrane under conditions demonstrated previously (Abbott & Schachter, 1976) to yield stoichiometric labeling of well-defined exofacial and endofacial populations. The overall –SH titers so observed increase considerably in cholesterol-enriched as compared with cholesterol-depleted membranes (Table I) and confirm the findings of two prior fluorescence studies. In the first report (Borochov & Shinitzky, 1976) erythrocyte membranes were labeled with the uncharged fluorescent –SH reagents *N*-dansylaziridine and *N*-(1-anilinonaphthalene-4)maleimide and the availability to exogenous quenchers was monitored. The second study (Shinitzky & Rivnay, 1977) quantified the endogenous tryptophan fluorescence of erythrocyte membrane proteins and determined the fraction of the fluorescence quenched by an impermeant collisional quencher, *N*-methylpicolinium perchlorate. As in the present investigation, increases in membrane C/PL enhanced considerably the aqueous availability of the protein substituents examined.

In the present study the application of a membrane-impermeant reagent has shown further that the overall increase in surface –SH secondary to increased C/PL results more specifically from enhanced endofacial labeling of the major intrinsic (band 3) and extrinsic (bands 1, 2, and 5) membrane polypeptides. Moreover, under the same experimental conditions the endofacial labeling of band 6 is decreased and that of band 4 is not changed significantly. Hence these effects of membrane cholesterol must be interpreted in terms of the properties and disposition of specific erythrocyte membrane proteins. While the present information concerning these proteins does not permit the formulation of a rigorously detailed model, some qualitative interpretations are useful nonetheless. Considering that insertion of cholesterol molecules may lead to a more densely packed lipid leaflet (Dervichian, 1964; Van Deenen, 1965) and thereby to a lateral surface pressure (Bar et al., 1966) or force tending to displace included proteins,[3] it is reasonable to suggest that the net effect on a given membrane protein would depend on the individual force experienced and the resistance offered to displacement. The individual forces and resistances would in turn depend on a number of factors, including the asymmetric distribution of cholesterol molecules in the bilayer (Fisher, 1976), the shape, size, location, and composition of the polypeptide and aggregation or association with other proteins and membrane components. For example, models proposed for the organization of bands 1, 2, and 5 proteins, the spectrin–actin complex (Steck, 1974; Kirkpatrick, 1976; Marchesi & Furthmayr, 1976), suggest that they are arranged in a meshwork underlying the endofacial surface and interacting with membrane lipids, either directly or indirectly by association with intrinsic membrane proteins. Given the meshwork configuration, endofacial disposition and water solubility of these proteins, increased lateral pressure from within the bilayer would be predicted to displace them toward the cytosol, as observed in the present experiments (Table II and Figure 1).

The factors governing the responses of the other major polypeptides are less apparent. Bands 4 and 6 proteins are considered to be extrinsic, endofacial, and possibly associated with band 3 proteins (Steck, 1974; Carraway, 1975; Marchesi & Furthmayr, 1976). While it is noteworthy that band 6 (glyceraldehyde-3-phosphate dehydrogenase) is dissociated from the membrane by high ionic strength in contrast to the low ionic strength elution of the spectrin–actin complex, it is not clear why a superficial polypeptide-like band 6 becomes less accessible to the aqueous maleimide probe in response to membrane cholesterol or why band 4 proteins remain relatively unaffected. The displacement of the spectrin–actin complex may itself limit accessibility to band 6 or to portions of band 4, but this and other explanations, e.g., changes in interactions with band 3, remain speculative.

Band 3 polypeptides span the membrane (Steck, 1974; Boxer et al., 1974) and the observed effects of cholesterol are presumed to depend not only on the factors mentioned above but also on the asymmetric interaction of the polypeptides with the aqueous environment at both membrane surfaces. Increases in membrane cholesterol had a relatively greater effect on the intrinsic band 3 polypeptides than on the extrinsic band 1, 2, and 5 proteins. Comparison of cholesterol-depleted and -enriched membranes (Figure 1) indicates that the increment in labeling of band 3 was 100%, as compared with 63% and 65%, respectively, for bands 1 plus 2 and band 5. Similarly, the percent increase in labeling of extracted bands 1, 2, and 5 (Table II) was less than that of the unextracted membrane pellets containing intrinsic proteins.

The present results indicate that changes in erythrocyte membrane cholesterol and lipid fluidity alter the availability of protein sulfhydryl groups at the surfaces. It is reasonable to suggest that such effects on membrane proteins may influence membrane functions. Evidence that membrane receptors, enzymes, and transport proteins are affected by lipid fluidity has been reported (Papahadjopoulos et al., 1973; Shattil et al., 1975; Warren et al., 1975; Wiley & Cooper, 1975; Shinitzky & Inbar, 1976), and erythrocyte membrane cholesterol has been observed to alter mechanical viscoelastic properties (Cooper et al., 1975) believed to be associated with bands 1, 2, and 5 (Steck, 1974; Kirkpatrick, 1976).

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[3] Published data for monolayers of membrane lipid extracted from human erythrocytes (Bar et al., 1966) can be used to estimate the average change in lateral surface pressure in the lipid leaflets owing to a change in cholesterol content. We assume for the calculations that our liposome treatments altered only the cholesterol and not the phospholipid content and that reasonable approximations of the molecular areas of the phospholipids can be calculated without considering the intrinsic membrane proteins. Using the curves in Figure 2 of Bar et al. (1966) and their assigned value of 9 dynes/cm for the estimated surface pressure in untreated erythrocyte membranes, we calculate for our treated membranes with C/PL relative to the control of 70%, 105%, and 125%, respectively, surface pressures of 6, 11, and 14 dynes/cm. Relative to the untreated membranes, these surface pressures are 67%, 122%, and 156%, respectively, and could represent, therefore, considerable changes.

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Cooperative Binding to DNA of Catabolite Activator Protein of *Escherichia coli*†

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ABSTRACT: Binding parameters are reported for the interaction of *Escherichia coli* catabolite activator protein (CAP) with nonspecific DNAs (primarily native calf thymus DNA, which presumably does not contain specific functional sites for CAP). The main experimental techniques used were circular dichroism and a thermodynamically rigorous centrifugation method which allows quantitative analysis of the binding. It was found that in the absence of cyclic adenosine 3′,5′-monophosphate (cAMP) CAP binds cooperatively to double-helical DNAs, including poly[d(A-T)] and poly[d(I-C)]. The presence of cAMP eliminates the cooperativity, while the overall strength of the binding is increased. Circular dichroism spectra imply that the interaction of CAP with DNA causes a shift from the DNA B form to the C form; this occurs regardless of whether cAMP is present. Any conformational changes in the protein which may underlie the cooperative effect are not detectable by circular dichroism. It appears that CAP does *not* tend to melt double-helical DNA. The data show that a molecule of CAP covers 13 base pairs when bound to DNA. Values for other parameters of the cooperative interaction are reported for a range of ionic conditions (50–80 mM NaCl) at $T = 22$ °C. The intrinsic affinity of the protein for DNA, K, decreases at higher [NaCl], while the cooperativity parameter, ω (which measures the probability that two CAP molecules on DNA will be bound at adjacent sites), has a value of about 100, independent of ionic strength. The data indicate that approximately six ionic interactions are involved in the binding of a CAP molecule to DNA. Extrapolation of the $K\omega$ results to more physiological ionic conditions implies that in vivo some CAP molecules may be nonspecifically bound to the *E. coli* chromosome even in the absence of cAMP. At the higher cAMP levels at which CAP actively promotes transcription it seems likely that nonspecific DNA binding may play an in vivo role in modulating the action of CAP.

The catabolite activator protein (CAP)[1] of *Escherichia coli* stimulates transcription at certain catabolite-sensitive operons (Zubay et al., 1970; Emmer et al., 1970). Studies with both intact cells and cell-free systems indicate that CAP promotes mRNA synthesis only in the presence of relatively high levels

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[1] Abbreviations used: CAP, catabolite activator protein; cAMP, adenosine 3′,5′-monophosphate; cGMP, guanosine 3′,5′-monophosphate; 5′-AMP, adenosine 5′-monophosphate; PMSF, phenylmethanesulfonyl fluoride; PPO, 2,5-diphenyloxazole; Na_2EDTA, disodium ethylenediaminetetraacetate; Tris, tris(hydroxymethyl)aminomethane; DTT, dithiothreitol; $NaDodSO_4$, sodium dodecyl sulfate; GdnHCl, guanidine hydrochloride, CD, circular dichroism.

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