PMR STUDIES OF THE SUBSTRATE INDUCED CONFORMATIONAL CHANGE OF GLUTAMINE BINDING PROTEIN FROM *E. COLI*

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Received April 30, 1973

Summary

The substrate induced conformational change of glutamine binding protein isolated from *E. coli* has been studied by high resolution proton magnetic resonance spectroscopy. The addition of L-glutamine to a protein solution caused a marked change in the proton magnetic resonance spectrum. The chemical shifts of several resonances were considerably different for the free and complexed protein. The line width of the methyl protons decreased considerably with the addition of substrate indicating that the environment of a sizeable percentage of the methyl groups is different. The kinetics of binding as well as a possible mode of action of the binding proteins will be discussed.

The isolation of glutamine binding protein (Gln-BP) (1), as well as others (2), released from *E. coli* cells by the osmotic shock procedure of Neu and Heppel (3) has been described and experiments have been reported which implicate these proteins in the transport of amino acids, sugars, inorganic ions, and vitamins across cell membranes in gram negative bacteria cells (2,4). The exact mode of action of the binding proteins in the transport process has yet to be established (4-6).

Several studies, utilizing fluorescence, gel electrophoresis, optical rotary dispersion, circular dichroism, infrared spectroscopy, denaturants, and proton magnetic resonance (PMR) on the conformational properties of this class of proteins have been reported with conflicting results (1,7-10). Whereas minimal substrate induced conformational changes upon binding were reported utilizing optical and PMR experimental methods (1,7-9), the gel electrophoresis and binding data for galactose binding protein have shown two distinct conformational states (9-10). We have extended these studies

to Gln-BP utilizing high resolution PMR spectroscopy and have observed a marked conformational change in the protein upon substrate binding. A possible mechanism of action of the binding proteins in the transport process is discussed in light of these results.

EXPERIMENTAL

A mutant of *E. coli* strain 7 (GLNP-1) which can grow on glutamine as the sole carbon source and which was supplied by Dr. Leon A. Heppel was used for isolation of Gln-BP. The cells were grown in minimal medium supplimented with glucose. The Gln-BP was isolated from the osmotic shock fluid and assayed for glutamine binding activity by the previously published method (1). The binding protein was exchanged with D_2O (99.7%) from Merck, Sharpe & Dohme of Canada by repeated concentration utilizing ultrafiltration with an Amicon UM-10 membrane followed by dilution with D_2O. The L-glutamine (A grade) was purchased from Calbiochem. Unless otherwise noted, all samples were prepared in 0.05M NaCl, 0.01M potassium phosphate pD 7.1 buffer. The pD of solutions was determined by adding 0.4 pH unit (11) to the value read from a Radiometer Model 26 pH meter equipped with a Beckman 39030 combination electrode. The protein concentration was determined by the Lowry method (12).

The PMR spectra were obtained on the MPC-HF 250 MHz superconducting spectrometer (13). The signal-to-noise ratio was improved by the correlation spectroscopic method of Dadok *et al.* (14) utilizing a Sigma 5 computer. The probe temperature of the spectrometer was 31°C. Chemical shifts are reported in parts per million (ppm) from the residual HOD resonance in each sample. The HOD signal is -4.72 ppm downfield from the proton resonance of 2,2-dimethyl-2-silapentane-5-sulfonate. The negative sign of the chemical shifts indicates that the resonance is downfield from that of HOD and the positive sign indicates that the resonance is upfield from the HOD signal.

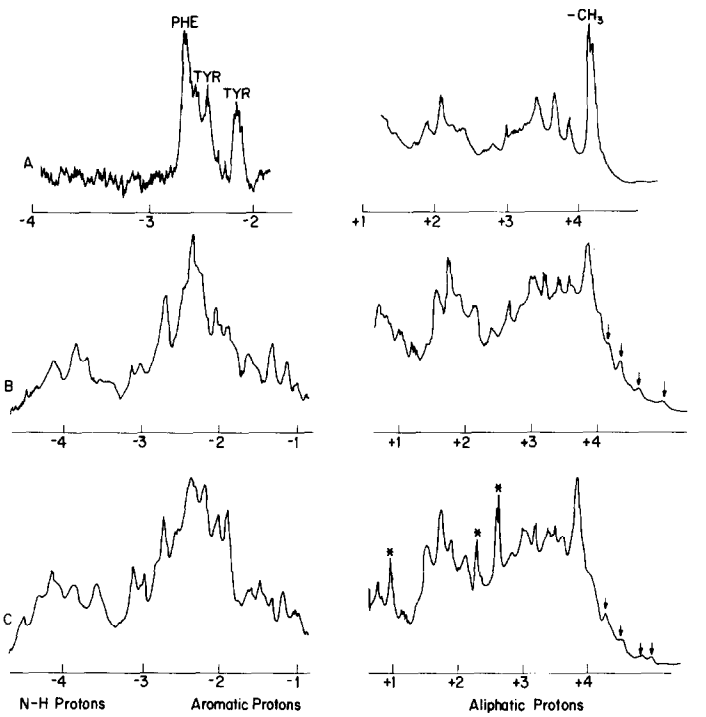

Fig. 1. The 250 MHz spectra of Gln-BP: A, $6x10^{-5}$M protein denatured with 7M d_4-urea; B, $2x10^{-4}$M protein in 0.05M NaCl, 0.01M potassium phosphate, pD 7.1 buffer; and C, $2x10^{-4}$M protein with 20:1 excess Gln in 0.05M NaCl, 0.01M potassium phosphate, pD 7.1 buffer. The aromatic region is the accumulation of 1000 scans and is shown at a higher attenuation than the aliphatic region for which only 200 scans were accumulated. The symbol (*) in spectrum C denotes resonances due to free Gln. The symbol (↓) in spectra B and C denotes ring-current shifted methyl groups. The position of the HOD resonance in A relative to B and C is different due to exchange with the urea.

RESULTS AND DISCUSSION

The 250 MHz PMR spectrum of Gln-BP which has been denatured by 7M d_4-urea is shown in Figure 1A. The resonances of the various protons are fairly sharp and each type of residue is magnetically equivalent, as would be expected for a random coil polypeptide chain (15). In the natural state, however, the linewidths are much broader and nonequivalence between various types of residues becomes apparent (Figure 1B). In addition to the nonexchangeable protons, a broad resonance ~4 ppm downfield from the HOD reso-

nance is observed in the region expected of N-H protons. Since these protons do not exchange with the deuterated solvent even after several weeks exposure, these protons are clearly in the interior of the protein and are not in contact with the solvent. Since the resonances of nonexchangeable protons both in the aromatic and aliphatic regions, are broad and exhibit considerable overlap, specific assignment of the resonances to specific residues was not possible at this time. The broad resonance at ∿3.8 ppm upfield from HOD can be attributed to a composite of the majority of the methyl groups. The resonances upfield from this peak can be assigned to methyl groups which are in the vicinity of aromatic residues and the upfield shift is due to the magnetic ring-current anisotropy of these groups (16).

The effect of the addition of Gln to a protein solution is shown in Figure 1C. Gross changes in the PMR spectrum can be observed over the entire spectral region. The most interesting feature of the spectrum is the marked sharpening of the methyl resonance at ∿+3.8 ppm. This line sharpening can be interpreted as a change of some of the methyl residues from a rigid, dipolar environment to a more mobile environment. Several of the ring current shifted methyl resonances are shifted upon the addition of Gln indicating that their position relative to the aromatic residues has changed. These results clearly indicate that magnetic environments of a large number of residues are different upon Gln binding and therefore, that the substrate induces a conformational change in Gln-BP. Since the spectrum of the binding protein and the linewidths of the excess Gln is unchanged over a range of protein-substrate ratio of 1:2 to 1:20, the kinetics of exchange is slow on the PMR time scale. The additional broadening of the free L-glutamine, as compared to that in the absence of protein, reflects the rate of chemical exchange of Gln between the free and bound states. In the limit of slow exchange, this leads to an additional broadening of the individual resonances by $\frac{1}{\pi}\frac{1}{\tau}$ where τ is the mean lifetime of the Gln in the free state (17). The additional broadening of ∿3Hz for the free Gln yields a pre-exchange lifetime of ∿0.1 second.

Although it has been reported that Gln-BP can be stored for several months at 3° and -90°C without loss of activity (1), we have observed severe line broadening in the PMR spectra, indicative of aggregation, when the sample was refrigerated at 3°C for several weeks or after dialysis against buffer to remove the Gln. Although the aggregation of the protein is probably due to the high concentration needed for PMR, extreme care must be exercised in the handling of this protein. The PMR spectrum was reproducible for several weeks if the sample was stored frozen at ∿-40°C and only these samples were used in this study.

The conformational change as observed by PMR for Gln-BP is consistent with the postulate of Boos and Gordon (10) that the change in electrophoretic mobility of galactose binding protein upon substrate binding indicates a structural change and perhaps a change in the surface charge. Care, however, must be exercised in comparing the structural properties of purified binding proteins with its *in vivo* transport function. If the binding protein is essentially free in the periplasmic space then one can speculate that upon Gln binding, the conformation of Gln-BP is sufficiently different that it can favorably interact with the lipid bilayer or bind to a membrane bound protein, similar to the P protein in the histidine transport system in *Salmonella typhimurium* (18). If, however, the binding protein is bound to the surface of the membrane, further studies on the effect of lipids on the conformational properties of binding proteins are required before meaningful conclusions can be drawn. These possible modes of action of binding proteins are currently under investigation.

Acknowledgement: This work is supported by research grants from the National Institutes of Health (GM-18698 and RR-00292) and from the National Science Foundation (GB-37096X).

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