Liganded and Unliganded Receptors Interact with Equal Affinity with the Membrane Complex of Periplasmic Permeases, a Subfamily of Traffic ATPases*

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Bacterial periplasmic permeases belong to a superfamily of transporters (traffic ATPases (1) or ABC proteins (2)) that comprises many prokaryotic and eukaryotic carriers, including the medically important cystic fibrosis transmembrane conductance regulator (CFTR) and the multidrug resistance P-glycoprotein (MDR) (3, 4). Because of the ease of manipulation, prokaryotic permeases are good model systems for understanding the eukaryotic carriers. Periplasmic permeases are composed of a soluble receptor (the periplasmic substrate-binding protein) and a membrane-bound complex, which translocates the substrate with concomitant ATP hydrolysis. Models for their mechanism of action generally depict the liganded receptor as interacting with high affinity with the membrane-bound complex and stimulating ATP hydrolysis by the ATP-binding subunits, which leads to the discharge and translocation of substrate; the unliganded receptor is then released because its affinity for the complex is reduced (reviewed in Ref. 3). The high-low switch in the affinity of the receptor for the membrane complex is central to such models. The histidine permease of Salmonella typhimurium (and Escherichia coli) is a well-characterized model system. It consists of the periplasmic receptor, the histidine-binding protein HisJ, and of the four subunits of the membrane-bound complex, HisQ/M/P, (with two integral membrane proteins, HisQ and HisM, and two identical ATP-binding subunits, HisP) (3). HisJ interacts with the membrane-bound complex, as shown indirectly by genetic analysis (5); chemical cross-linking experiments provided direct evidence for a specific interaction between HisJ and HisQ (6).

The structures of many periplasmic binding proteins have been resolved, and despite the fact that these proteins do not usually bear sequence homology, their structures are similar (7). The three-dimensional structure of liganded HisJ and of the homologous lysine-, arginine-, ornithine-binding protein, LAO, shows the existence of two globular domains delimiting a cleft containing the substrate-binding pocket (8, 9). The lobes are separate when the protein is unliganded (open conformation) and are positioned much closer to each other in the liganded form (closed conformation) in which the substrate is deeply buried. These receptors undergo a spontaneous conformational change in the absence of ligand, continuously "breathing" and assuming a closed empty form (10–12), as was demonstrated for HisJ in solution using conformation-specific monoclonal antibodies (10). This finding has raised the possibility that because the receptor in its closed empty form resembles the closed liganded form, it would compete in vivo with the liganded receptor for interaction with the membrane-bound complex. Such a situation would be disadvantageous because transport would be inhibited at low substrate concentrations, when there would be an excess of unliganded over liganded receptor in the periplasm. In addition, in the absence of substrate, the empty closed form interacting with the complex might stimulate a wasteful ATP hydrolysis. Therefore, the question of whether the unliganded receptor indeed interacts with the complex has to be addressed.

We previously observed that unliganded HisJ competes (effectively) with liganded HisJ in histidine transport assays using an in vitro reconstituted system (13). We suggested at the time that the unliganded protein must interact with the same site on the membrane-bound complex as liganded HisJ. Here we have undertaken a detailed study of the interaction between HisJ, both liganded and unliganded, and the membrane complex. We optimized the previously developed cross-linking

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1 The abbreviations used are: HisQ/M/P, membrane-bound complex; PLS, proteoliposomes; LAO, lysine-, arginine-, ornithine-binding protein; J → Q, cross-linked product between HisJ and HisQ; PAGE, polyacrylamide gel electrophoresis; Sulfo-SANPAH, sulfosucinimidyl 6-(4-azido-2-nitrophenyl)amino)hexanolate; MOPS, 4-morpholinopropanesulfonic acid.
methods (6) and introduced additional cross-linking reagents. We have also developed a novel approach to studying this interaction that utilizes the signaling properties of the receptor to induce ATP hydrolysis. We show that unliganded receptor indeed interacts with the membrane complex and does so as well as liganded receptor. Therefore, we challenge the conventional model and propose a different one in which the operation of the receptor is independent of a high-low switch in affinity. The model we propose is relevant for understanding the mechanism of action of several important eukaryotic membrane receptors that contain extracellular substrate-binding domains that are homologous to periplasmic receptors (14–16).

**EXPERIMENTAL PROCEDURES**

Purification of Receptors and Removal of Bound Ligands—HisJ was purified to over 90% purity (as judged by SDS-PAGE) from strain GA363 (S. typhimurium LT2 carrying plasmid pA54, which produces the HisJ protein under the control of the Tac promoter). Bound ligand was removed from HisJ, and its absence was verified as described (17). Alternatively, bound ligand was removed as follows: i) the receptor was denatured by exposure to 6 m guanidine HCl and then renatured by extensive dialysis (18); ii) a dialysis bag containing 15 mg of receptor per ml was submerged in 500 ml of 5 m histidine in potassium acetate buffer, pH 5.0, with four changes; then histidine was removed as above. The removal of ligand was followed by visualizing either residual histidine or arginine with the respective reagents (19, 20); the removal of arginine was also followed by the loss of radioactive arginine added to the protein at the beginning of the treatment. Receptors were considered unliganded if they contained less than 5% ligand.

Cross-linking with Sulfo-SANPAH—Purified de-liganded receptors were derivatized with Sulfo-SANPAH and with formaldehyde were shown previously to yield a cross-linked product (10). To cross-link with Sulfo-SANPAH, the reaction was quenched by the addition of glycine to a final concentration of 1%, and the incubation continued at 37°C for 10 min, when the reaction was quenched by the addition of 3 m of 5 m ethanolamine. After 30 min at room temperature, the PLS were diluted with 10 m NaPi, pH 6.8, and harvested, and then analyzed as for in vivo experiments.

Cross-linking using Reconstituted PLS and Reagents with Different Arm Lengths—For cross-linking with Sulfo-SANPAH, the reaction contained 50 m of PLS from TA1889 (25) (prepared by dialysis) to 10 mg protein/ml in 50 m MOPS-K+ buffer, pH 7.0, briefly sonicated in a bath sonicator; 50 m of 50 m M derivatized HisJ in 17 m NaPi, pH 7.0, buffer; 25 m of 200 m NaPi, pH 7.0 buffer, and 1 m of 3 m histidine (or water). Irradiation was as described (6). Buffer (NaPi, 10 m, pH 6.8) was added to a final volume of 1.3 ml; the PLS were harvested by centrifugation (Beckman Optima TL ultracentrifuge) at 109,000 g for 90 min, resuspended in 50 m 10 m Tris/Cl, pH 6.8, and diluted with 100 ml of 1,5-difluoro-2,4-dinitrobenzene (DFDNB), disulfo-succinimidyl tartarate, dimethyl adipimidate dihydrochloride, and dimethyl sulfosuccinimidyl 2-(p-azidosalicyl-amido) ethyl-1,3-dithiopropionate (27). 20 m of PLS prepared as described above were mixed with 50 m of derivatized HisJ in 10 m NaPi, buffer, pH 7.0 (final HisJ concentration, 20 m), 50 m of 200 m NaPi,buffer, pH 8.0, and 1 m of 10 m histidine (or water). In all cases, products were analyzed as described for cross-linking in vivo (10).

Affinity Measured by Co-sedimentation—Membranes from heat-in-duced TA3662 (which carries the hisQ, hisM, and hisP genes under the temperature-sensitive control of the lambda P, promoter) and heat-induced TA3663 (in which the genes are carried in inverted orientation and therefore are not heat inducible) (28) were mixed in an Eppendorf tube with HisJ (5, 2.5, 1.25, or 0.63 m) in 50 m NaPi, buffer, pH 7.0, 1 m histidine, and 150 m NaCl in a total volume of 0.1 ml. After 30 min of incubation at room temperature, the samples were centrifuged at 45,000 rpm for 30 min, the supernatant was removed by aspiration, the pellet was rinsed gently with 1 m NaPi, buffer, and the tube was washed with a cotton swipe. The pellet after resolubilization was measured by SDS-PAGE and by immunoblotting with polyclonal anti-HisJ antibody using pure HisJ as standard (22). Because of the relatively poor affinity of HisJ for the complex, the amount of HisJ bound to membranes in the presence of HisQ/M/P is approximately double the amount bound to membranes lacking HisQ/M/P. Thus, to obtain an accurate estimate of the affinity value, data from three different experiments, each in triplicate, were averaged.

Measurement of ATPase Activity—Various amounts of HisJ, in the presence or the absence of equivalent amounts of histidine, were added to reconstituted PLS (in 50 m MOPS-K+, pH 7.5; final protein concentration: 0.2–0.4 mg/ml) and incubated at 37°C for 5 min. ATP hydrolysis was initiated by the addition of ATP and MgSO4 (2 and 10 m, respectively); the removal of arginine with the respective reagents (19, 0.2, 0.05, and 0.15%. For cross-linking with sulfo-succinimidyl 2-(p-azidosalicyl-amido) ethyl-1,3-dithiopropionate (27), 20 m of PLS prepared as described above were mixed with 50 m of derivatized HisJ in 10 m NaPi, buffer, pH 7.0 (final HisJ concentration, 20 m), 50 m of 200 m NaPi, buffer, pH 8.0, and 1 m of 10 m histidine (or water). In all cases, products were analyzed as described for cross-linking in vivo (10).

RESULTS

Ligated and Unliganded HisJ Interact Equally Well with the Membrane Complex—Cross-linking with the light-activated heterobifunctional reagent Sulfo-SANPAH and with formaldehyde were shown previously to yield a cross-linked product between HisJ and HisQ, J–Q (6). Here we show that this method can be used to determine the affinity of liganded HisJ for the membrane complex by quantitating the amount of J–Q.
produced with increasing concentrations of Sulfo-SANPAH-
derivatized liganded HisJ. Fig. 1A shows that the reciprocal of the concentration of derivatized HisJ; and the apparent affinity of unliganded liganded HisJ was also determined using a co-sedimentation assay, which gave a value of 19 μM. This result indicates that derivatization does not affect the nature of the interaction between HisJ and the complex. It is also consistent with the value of 12–40 μM (depending on the calculation method used; see legend to Table I) calculated for unliganded liganded HisJ in experiments in which it had been used as a competitor for cross-linking (30).

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The apparent affinity of liganded liganded HisJ is also measured by a transport assay utilizing reconstituted PLS and was determined to be 8 μM,2 which is not significantly different from the above values. In summary, the various values obtained for unliganded liganded HisJ are essentially indistinguishable from that obtained directly for the Sulfo-SANPAH-derivatized protein. It can be concluded that: i) the cross-linked product results from a specific interaction between the derivatized form of liganded HisJ and a binding site(s) on HisQ; ii) the undervatized form of liganded HisJ competes for the same site(s); and iii) derivatization does not greatly perturb the structure of HisJ (6). Therefore, affinities can be determined either by directly measuring the level of cross-linking using the derivatized protein or by competition of cross-linking using undervatized protein.

Sulfo-SANPAH-derivatized unliganded HisJ was then examined for its interaction with HisQ/P, and it was also found to be cross-linked. Surprisingly, its apparent affinity is 6 μM, a value not significantly different from that obtained for liganded HisJ (Fig. 1B). This finding is consistent with puzzling results obtained previously, which showed that unliganded HisJ competes efficiently with liganded HisJ, thus inhibiting transport, in experiments using an in vitro reconstituted system (13, 30). The apparent Kᵢ values for unliganded HisJ are calculated to be 22 and 13 μM, respectively, which are very similar to those obtained by cross-linking of either liganded or unliganded derivatized HisJ. This also is evidence that cross-linking reflects a physiological interaction.

Because the finding that liganded and unliganded HisJ interact with similar affinities with the membrane complex was unexpected, it was important to determine whether this phenomenon is peculiar to HisJ. Fig. 1C and D shows that also derivatized LAO, unliganded and lysine-liganded, can be cross-linked with HisQ and have comparable apparent affinities (Kᵢ values of 12 and 22 μM, respectively). Unliganded and arginine-liganded derivatized LAO have also been shown to compete with derivatized histidine-liganded HisJ with apparent Kᵢ values of 8 and 11 μM, respectively (Fig. 1, E and F). Finally, liganded and unliganded LAO compete equally well with HisJ in a transport assay (30). Table I summarizes various combinations tested: all the Kₒ and Kᵢ values are essentially the same.

In conclusion, contrary to the assumptions made in all current models in which only the liganded form of the protein interacts efficiently with the membrane-bound complex, liganded and unliganded HisJ interact with the same apparent affinity with a site on the membrane, a site that is on the normal pathway leading to transport.

The Mode of Interaction Is Different for Liganded and Unliganded Receptor—Despite the fact that the affinities of the two forms of the receptor for the membrane are very similar, it became clear that the character of the interaction is different. An early indication of this fact was that in any one experiment the level of J~Q produced in the presence of excess histidine was consistently 2-4 fold higher than in the absence of histidine (using either 15 or 50 μM HisJ) (data not shown). Thus,
liganded and unliganded HisJ have very similar affinity, but the conformation taken by the liganded protein appears to result in better access of the derivatized reactive group(s) on HisJ to the interacting group(s) on HisQ. The specific nature of the interaction was therefore studied by a completely different approach: the ability of the two conformations to send a signal for ATP hydrolysis. In addition, the effectiveness of a variety of chemical cross-linking reagents to yield J–Q was also analyzed.

It has been shown that liganded receptors send signals to the ATP-binding site (on the cytoplasmic face of the membrane-bound complex), activating it to hydrolyze ATP (25, 31, 32). Because this signal depends on the prior interaction between the receptor and the membrane-bound complex, such an activity could be used as an assay for the interaction. Investigations on the specific nature of the signal had demonstrated that also unliganded HisJ stimulates ATP hydrolysis, although at a lower level (in the case of the maltose receptor) (32). A more detailed analysis shows the relationship between various concentrations of liganded or unliganded HisJ and ATP hydrolysis in PLS reconstituted with HisQ/M/P; both forms stimulate ATP hydrolysis (Fig. 2). The possibility that the stimulation was due to HisJ being liganded because of contaminating histidine was carefully examined, because these proteins retain their ligands with well known tenacity (18). The HisJ preparations (over 90% purity) used were shown to contain less than 0.1 µmol of histidine/µmol of HisJ by thin layer chromatography (17). As an additional precaution, it was shown that after ligation of pure HisJ with radioactive l-histidine and repurification by HPLC, less than 0.01% of the radioactivity remained, i.e. less than 6 pmol of histidine/µmol of HisJ. The possible presence of histidine in reconstituted PLS was considered irrelevant because their preparation involves extensive dilutions steps which, by themselves, would drop the histidine concentration to less than 10 nM in the assay. Thus, histidine was determined to be less than 1 nM in solutions of 160 µM HisJ and less than 10 nM in the PLS. In addition, the ATPase activity is dependent on the concentration of HisJ, which excludes the possibility that histidine contaminates the PLS preparation. The stimulation can therefore be safely ascribed to unliganded HisJ. The apparent Kₚ values for ATPase stimulation are essentially the same for liganded and unliganded HisJ (11 and 5 µM, respectively). Although unliganded HisJ can take a closed empty conformation mimicking that of the closed liganded conformation, it has been estimated that the open empty form predominates (10). The fact that the Vₘₐₓ values in Fig. 2 are different (0.92 and 0.07 µmol/min/mg protein for liganded and unliganded HisJ, respectively) excludes the possibility that the form interacting and sending the signal is only a fraction of the added HisJ, because it mimics the closed liganded conformation. If the latter were the case, at sufficiently high concentration of unliganded HisJ, the Vₘₐₓ should be the same as for liganded HisJ. An important question is why unliganded receptor stimulates ATP hydrolysis. An attractive hypothesis is that unliganded receptor induces slow ATP hydrolysis to convert the membrane complex to a state of readiness for transport; such a state might possibly raise the affinity of the receptor for the ligand. Preliminary results suggest that this may indeed be the case.

Because different surface groups would be exposed in the two conformations, cross-linking reagents with a variety of arm lengths also could discriminate between the two types of interaction. The effect of liganding on cross-linking was first tested with formaldehyde. This reagent has been shown to be an effective cross-linking reagent in vivo (6). We first demonstrated that cross-linking does not occur artifically as a consequence of HisQ/M/P overproduction by the plasmid-borne genes, because it is formed normally in a strain that produces chromosomal levels of both HisJ and HisQ/M/P (it is absent in a strain lacking HisQ/M/P; data not shown). Fig. 3 (top inset, lanes 1 and 2) show that J–Q is formed efficiently in vivo only in the presence of histidine. Varying the l-histidine concentration shows that J–Q is not formed in the absence of histidine and increases in its presence until saturation is reached; a reciprocal plot of these data yields an apparent Kₐ of 56 nM (bottom inset). This value is similar to the Kₐ of HisJ for histidine binding (30 nM; Ref. 33), which indicates that the limiting step in the in vivo cross-linking reaction is the ligation of HisJ. Similar results were obtained when the fusion protein J–LAO (which behaves like LAO (34)), liganded with arginine or histidine, was used as the receptor (data not shown).

To exclude the possibility that the above results reflect a different behavior of the receptor in vivo and in vitro, an adaptation of the formaldehyde cross-linking method was developed to be used in vitro with reconstituted PLS. Fig. 3 (top inset, lanes 3 and 4) shows that also in reconstituted PLS J–Q is dependent on the presence of histidine; a Kₐ value of 30 µM was obtained by varying the concentration of histidine liganded HisJ (data not shown). A possible effect of the polymerization state of formaldehyde on the efficiency of cross-linking was excluded because autoclaving a 22% solution of formaldehyde in 0.1 M NaPi, pH 6.8 buffer (35) for 20 min before use had no effect. Thus, the difference between cross-linking with formal-

3 C. E. Liu, and G. F.-L. Ames, unpublished data.
4 A. Wolf, unpublished observations.
dehyde and Sulfo-SANPAH is not due to the particular assay system used. Rather, because formaldehyde is a small cross-linking molecule (36), it seems reasonable that it would detect a different kind of interaction than a larger molecule, such as Sulfo-SANPAH. On the basis of these results, we tested a number of cross-linking reagents with different arm lengths. Table II shows that unliganded HisJ can be cross-linked by reagents with an arm length of 18 Å, but not by formaldehyde and DFDNB, which have short arm lengths (2 and 3 Å, respectively). Reagents with intermediate arm lengths fail to cross-link, whether histidine is present or not. Therefore, the interaction between unliganded HisJ and HisQ can be distinguished from that with liganded HisJ also by the use of appropriate cross-linking reagents.

In conclusion, both cross-linking reagents and induction of ATP hydrolysis provide evidence that liganded and unliganded receptor are in different conformations while interacting with HisQ/M/P. The nature of these conformations will be discussed below.

**FIG. 3.** Cross-linking by formaldehyde and the effect of histidine. Direct and double (bottom inset) reciprocal plot of the extent of cross-linking versus \( \frac{1}{\text{histidine}} \) obtained by quantitation as described under “Experimental Procedures”. The ordinate is in arbitrary units. For this experiment the concentration of histidine during the assay was 2 min. Before resolution by SDS-PAGE, samples were concentrated to an absorbance at 650 nm of 1.0 by filtration. The time was 2 min. Before resolution by SDS-PAGE, samples were concentrated to an absorbance at 650 nm of 1.0 by filtration. The top inset shows immunoblots of in vivo (first two lanes) and in vitro (last two lanes) cross-linking experiments. Lanes 1 and 2, strain GA96 (ΔhisF 645 hisA1575 (deleting the hisQ, hisM, and hisP genes) and dhuA1, a promoter-up mutation in the histidine permease operon (47)), carrying pFA53 (48), which overproduces HisQ, HisM, and HisP under the temperature-sensitive control of the J promoter, in the absence and presence, respectively, of 1 mM \( \frac{1}{\text{histidine}} \); lanes 3 and 4, PLS in the absence and the presence, respectively, of 20 \( \mu M \) \( \frac{1}{\text{histidine}} \). Top and bottom arrows indicate the positions of J-Q and HisJ, respectively.

**TABLE II** Cross-linking by reagents with different arm lengths

| Crosslinker       | Arm length* | Relative amounts† | System          |
|-------------------|-------------|-------------------|-----------------|
|                   | + Histidine | − Histidine       |                 |
| Formaldehyde      | 2           | 100               | 5               |
| Formaldehyde      | 2           | 100               | 3               |
| DFDNB             | 3           | 100               | 5               |
| SulfosDST         | 6.4         | not detectable    | not detectable  |
| DMA               | 8.6         | not detectable    | not detectable  |
| DMS               | 11          | not detectable    | not detectable  |
| Glutaraldehyde    | 8           | not detectable    | not detectable  |
| Sulfo-SANPAH      | 18          | 100               | 32              |
| SASD              | 18          | 100               | 47              |

*a The respective arm lengths have been obtained from the following references: formaldehyde (36); 1,5-difluoro-2,4-dinitrobenzene (DFDNB), disulfosuccinimidyl tartrate (SulfosDST), dimethyl adipimidate dihydrochloride (DMA), and dimethyl spermidate dihydrochloride (DMS) (Pierce Catalogue); glutaraldehyde (estimated from formula); Sulfo-SANPAH (30); and sulfosuccinimidyl 2-(p-azidosalicyl-amido) ethyl-1,3-dithiopropionate (SASD) (27).

† The extent of cross-linking obtained by quantitation as described under “Experimental Procedures” in the absence of histidine is expressed as a percentage of the amount obtained in the presence of 100 \( \mu M \) \( \frac{1}{\text{histidine}} \).
LAO protein, in the case of the glutamate receptor (15), capturing the substrate and signaling receptor occupancy to its membrane-embedded portion. The validity of this model depends on the ability of the substrate-binding domain to perform its function while being immobilized onto the membranous portion of the molecule. This notion is supported by our results indicating that this is likely the normal mode of action for periplasmic receptors. Indeed, in view of the findings and the model presented here, it is tempting to speculate that the binding and sensing/signaling elements belonging to bacterial permeases receptors have been adopted through evolution by eukaryotic receptors and incorporated into structures unrelated to traffic ATPases (in the above cases, an ion channel and a G protein-coupled receptor, respectively). Because soluble receptors have not yet been identified in eukaryotic traffic ATPases, it is possible that also in these systems a similar substrate-binding and signaling domain may have been incorporated into their membranous portion (at least in some cases). Understanding the mechanism of action of the periplasmic receptors is likely to yield useful information relative to the function of the eukaryotic systems.

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REFERENCES

1. Ames, G. F.-L., Mimura, C., and Shiyamala, V. (1990) FEMS Microbiol. Rev. 75, 429–446
2. Hyde, S. C., Ensley, P., Hartshorn, M. J., Mimmack, M. M., Gileadi, U., Pearce, S. R., Gallagher, M. P., Gill, D. R., Hubbard, R. E., and Higgins, C. F. (1990) Nature 346, 362–365
3. Doige, C. A., and Ames, G. F.-L. (1993) Annu. Rev. Microbiol. 47, 291–319
4. Higgins, C. F. (1992) Annu. Rev. Cell Biol. 8, 67–113
5. Ames, G. F.-L., and Spudich, E. N. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 1877–1881
6. Prossnitz, E., Nikaido, K., Ulbrich, S. J., and Ames, G. F.-L. (1988) J. Biol. Chem. 263, 17917–17920
7. Quiocio, F. A. (1990) Philos. Trans. R. Soc. Lond-Biol. Sci. 326, 341–351
8. Oh, B.-H., Pandit, J., Kang, C.-H., Nikaido, K., Gokcen, S., Ames, G. F.-L., and Kim, S.-H. (1993) J. Biol. Chem. 268, 11348–11353
9. Oh, B.-H., Kang, C.-H., De Bondt, H., Kim, S.-H., Nikaido, K., Joshi, A., and Ames, G. F.-L. (1994) J. Biol. Chem. 269, 4135–4143
10. Wolf, A., Shaw, E. W., Nikaido, K., and Ames, G. F.-L. (1994) J. Biol. Chem. 269, 23051–23058
11. Walsinsley, A. R., Shaw, J., and Kelly, D. J. (1992) Biochemistry 31, 11175–11181
12. Flocco, M. M., and Mowbray, S. L. (1994) J. Biol. Chem. 269, 8931–8936
13. Prossnitz, E., Gee, A., and Ames, G. F.-L. (1989) J. Biol. Chem. 264, 5006–5014
14. O’Hara, P. J., Sheppard, P. O., Thøgersen, H., Venezia, D., Haldeman, B. A., McGlone, V., Houned, K. M., Thomsen, C., Gilbert, T. L., and Mulvihill, E. R. (1993) Neuron 11, 41–53
15. Stern-Bach, Y., Bitter, B., Hartley, M., Sheppard, P. O., O’Hara, P. J., and Heinemann, S. F. (1994) Neuron 13, 1345–1357
16. Brown, E. M., Gamba, G., Riccardi, D., Lombardi, M., Butters, R., Kifor, O., Sun, A., Hediger, M. A., Lytton, J., and Herbert, S. C. (1993) Neuron 13, 575–580
17. Nikaido, K., and Ames, G. F.-L. (1992) J. Biol. Chem. 267, 20706–20712
18. Miller, D. M. I., Olson, J. S., Pfugrath, J. W., and Quiocio, F. A. (1983) J. Biol. Chem. 258, 13665–13672
19. Ames, B. N., and Mitchell, H. K. (1952) J. Am. Chem. Soc. 74, 252–253
20. Easley, C. W., Zegers, B. J. M., and De Wijlder, M. (1969) Biochim. Biophys. Acta 175, 211–213
21. Kerppola, R. E., Shiyamala, V., Kleebb, P., and Ames, G. F.-L. (1991) J. Biol. Chem. 266, 9857–9865
22. Shiyamala, V., Baidhwal, V., Beall, E., and Ames, G. F.-L. (1991) J. Biol. Chem. 266, 18714–18719
23. Roth, S. R. (1970) Methods Enzymol. 23A, 3–25
24. Lannmiller, U. K. (1970) Nature 227, 680–685
25. Bishop, L., Aghayani, R. J., Ambabdak, S. V., Maloney, P. C., and Ames, G. F.-L. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 6953–6957
26. Huang, Z., Curtin, K. D., and Robbi, M. (1995) Science 267, 1119–1172
27. Ghinea, N., Eskensamy, M., Simonescu, M., and Simonescu, N. (1989) J. Biol. Chem. 264, 4755–4758
28. Hedenson, A., Weatheraw, R., and Ames, G. F.-L. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 7333–7337
29. Chifflet, S. (1988) Anal. Biochem. 169, 1–4
30. Prossnitz, E. (1989) In Vitro Reconstitution of the Histidine Transport System of Salmonella typhimurium. Ph.D. thesis, University of California, Berkeley
31. Petronilli, V., and Ames, G. F.-L. (1991) J. Biol. Chem. 266, 16923–16929
32. Davidson, A. L., Shuman, H. A., and Nikaido, H. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 2360–2364
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33. Ames, G. F.-L. (1986) Annu. Rev. Biochem. 55, 397-425
34. Speiser, D. M., and Ames, G. F.-L. (1991) J. Bacteriol. 173, 1444-1451
35. Boedtker, H. (1967) Biochemistry 6, 2718-2727
36. Jackson, V. (1978) Cell 15, 945-954
37. Sharff, A. J., Rodseth, L. E., Spurlino, J. C., and Quirocho, F. A. (1992) Biochemistry 31, 10657-10663
38. Shilton, B. H., and Mowbray, S. L. (1995) Protein Sci. 4, 1346-1355
39. Bohl, E., Shuman, H. A., and Boos, W. (1995) J. Theor. Biol. 172, 83-94
40. Hobot, J. A., Carlemalm, E., Villiger, W., and Kellenberger, E. (1984) J. Bacteriol. 160, 143-152
41. Brass, J. M., Higgins, C. F., Foley, M., Rugman, P. A., Birmingham, J., and Garland, P. B. (1986) J. Bacteriol. 165, 787-794
42. Stern, M. J. (1986) Regulation of the High Affinity Histidine Permease in Salmonella typhimurium. Ph.D. thesis, University of California, Berkeley
43. Silhavy, T. J., Szmelcman, S., Boos, W., and Schwartz, M. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 2120–2124
44. Willis, R. C., and Furlong, C. E. (1974) J. Biol. Chem. 249, 6926–6929
45. Cantor, C. R., and Schimmel, P. R. (1980) in Biophysical Chemistry, p. 943, W. H. Freeman & Co.
46. Ames, G. F.-L. (1964) Arch. Biochem. Biophys. 104, 1–18
47. Ames, G. F.-L., and Lever, J. E. (1972) J. Biol. Chem. 247, 4309–4316
48. Speiser, D. M. (1989) Mechanisms of Transport by the Histidine Permease of Salmonella typhimurium Which Do Not Involve Hist. Ph.D. thesis, University of California, Berkeley
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