Expression, Purification, and Characterization of Inosine 5'-Monophosphate Dehydrogenase from *Borrelia burgdorferi*

(Received for publication, May 5, 1997, and in revised form, June 30, 1997)

Xun Zhou§, Marguerite Cahoon¶, Patricia Rosal, and Lizbeth Hedstrom

From the §Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254 and ¶Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, NIAID, National Institutes of Health, Hamilton, Montana 59840

Inosine 5'-monophosphate dehydrogenase (IMPDH) is the rate-limiting enzyme in *de novo* guanine nucleotide biosynthesis. IMPDH converts IMP to xanthosine 5'-monophosphate with concomitant conversion of NAD\(^+\) to NADH. All IMPDHs characterized to date contain a 130-residue “subdomain” that extends from an N-terminal loop of the \(\alpha/\beta\) barrel domain. The role of this subdomain is unknown. An IMPDH homolog has been cloned from *Borrelia burgdorferi*, the causative agent of Lyme disease (Margolis, N., Hogan, D., Tilly, K., and Rosa, P. A. (1994) *J. Bacteriol.* 176, 6427–6432). This homolog has replaced the subdomain with a 50-residue segment of unrelated sequence. We have expressed and characterized the *B. burgdorferi* IMPDH homolog. This protein has IMPDH activity, which unequivocally demonstrates that the subdomain is not required for catalytic activity. The monovalent cation and dinucleotide binding sites of *B. burgdorferi* IMPDH are significantly different from those of human IMPDH. Therefore, these sites are targets for the design of specific inhibitors for *B. burgdorferi* IMPDH. Such inhibitors might be new treatments for Lyme disease.

Inosine 5'-monophosphate dehydrogenase catalyzes the conversion of IMP to XMP\(^1\) with the concomitant reduction of NAD to NADH. This reaction is the rate-limiting step in guanine nucleotide biosynthesis, and is therefore a target for numerous chemotherapeutic agents (1). IMPDH inhibitors are used clinically in antiviral (ribavirin) and immunosuppressive therapies (mycophenolate mofetil and mizoribine) (2–4). In addition, IMPDH inhibitors have anti-tumor and antibiotic activity (5, 6). Mammalian and bacterial IMPDHs have significantly different kinetic properties and inhibitor sensitivities, which suggests that species-specific IMPDH inhibitors can be developed that will be useful in treating bacterial and parasitic infections (7–9). Indeed, many studies have shown that purine metabo-

\(^1\) This work was supported in part by a grant from the Lucille P. Markey Charitable Trust to Brandeis University and the Searle Scholar Program (to L. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This manuscript is Publication No. 1813 from the Department of Biochemistry, Brandeis University.

§ Howard Hughes Undergraduate Research Fellow. Present address: Health Sciences and Technology Program, Harvard Medicine School, 260 Longwood Ave., Boston, MA 02115.

¶ To whom correspondence should be addressed. Tel.: 617-736-2333; Fax: 617-736-2349.

The abbreviations used are: XMP, xanthosine 5'-monophosphate; IMPDH, inosine 5'-monophosphate dehydrogenase; CH\(_3\)-TAD, \(\beta\)-methylthiazole-4-carboxamide adenine dinucleotide; IPTG, isopropyl-1-thio-\(\beta\)-galactopyranoside; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis.
hermsii, which suggests a species-specific modification of IMPDH in Lyme disease spirochetes.

We have expressed the B. burgdorferi guaB homolog in E. coli. This protein has IMPDH activity, which demonstrates that the subdomain is not required for IMPDH activity. B. burgdorferi IMPDH has significantly different kinetic properties from human IMPDH. Therefore, B. burgdorferi IMPDH may be a target for the development of new treatments for Lyme disease.

EXPERIMENTAL PROCEDURES

Materials—IMP, NAD+, Trizma base, and dithiothreitol were purchased from Sigma. 5-Ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide 5'-monophosphate was the generous gift of Dr. Akira Matsuda (Hokkaido University). Oligonucleotides were obtained from the Brandeis Oligonucleotide Facility.

Construction of an Expression Plasmid for B. burgdorferi IMPDH—Plasmid p68, containing the guaB gene of B. burgdorferi B31, was previously isolated from a genomic library constructed in the vector ZAP II (Stratagene, La Jolla, CA) (15). PCR was used to insert convenient restriction sites at the beginning and end of the guaB gene. The following oligonucleotides were used (mutations are underlined, restriction sites are in bold lettering): TGA-CTC-ATA(A)-TGC-CAA-ATA-AGA-TAA-CAA-AAG-AAG-CTT-TTA-C, which inserts an NdeI site at the 5' end; TTT-TCT-GCA-GTT-TTA-TGT-TAT-GCT-AAA-AAC-ATC-CTG-AGG, which inserts a PstI site in the 3'-noncoding region. The guaB coding sequence was amplified with Vent DNA polymerase (New England Biolabs) using 30 cycles of the following protocol: 1-min denaturation at 92 °C, 1-min annealing at 45 °C, 4-min extension at 60 °C. The PCR reaction (100 μl) contained 200 μM deoxynucleoside triphosphates, 4 mM MgSO4, 2 ng of p68, 1.25 μM of each oligonucleotide, and 2 units of polymerase. The PCR product was digested with NdeI and PstI and ligated to the NdeI/PstI fragment of pTactac (19). The resulting construct is designated pB9. The guaB coding sequence of pB9 was completely sequenced using a PRISM Dyedex Terminator cycle sequencing kit (ABI) and an Applied Biosystems 373A DNA sequencer at the Brandeis DNA Facility. No unwanted mutations were introduced in the PCR reaction.

Expression and Purification of B. burgdorferi IMPDH—pB9 was transformed into E. coli strain H712, which contains a partial deletion of the guaB gene (20). An overnight culture of cells was diluted 200-fold into fresh LB broth containing 100 μg/ml ampicillin. After 1 h at 37 °C, 1 mM IPTG was added to induce expression of IMPDH. The cells were harvested after 13 h by centrifugation, resuspended in buffer A (50 mM Tris, pH 7.4, 1 mM dithiothreitol, 10% glycerol), and frozen at −20 °C. All of the following manipulations were performed at 4 °C. The cells were thawed and disrupted by sonication. Debris was removed by centrifugation at 12,000 × g for 25 min. The supernatant was applied to a Cibacron blue Sepharose column previously equilibrated in buffer A. IMPDH was eluted in a linear gradient of 0 to 2 M KCl in buffer A (IMPDH does not elute in the absence of KCl). Table I summarizes the purification. Protein concentration was measured using the Bio-Rad assay with IgG as a standard. Active sites were titrated with 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide 5'-monophosphate (9). N-terminal sequencing was performed by the Tufts Medical School Protein Sequencing Facility. Electrospray ionization mass spectroscopy was performed by the Harvard University Mass Spectrometry Laboratory.

Analytical Ultracentrifugation—Sedimentation equilibrium experiments were performed on a Beckman model XL-A analytical ultracentrifuge monitoring absorbance at 280 nm. The rotor speed was 9000 rpm and the temperature was 20 °C. Samples contained 0.2−1.5 mg/ml enzyme, 50 mM Tris, pH 7.5, and 1 mM dithiothreitol in the presence and absence of 0.1 M KCl. Data were fitted to the following equation, which describes the sedimentation of a single component system, using Origin Technical Graphics and Data Analysis (MicroCal):

\[
A = A_0 \exp\left(\frac{1}{2} M \omega^2 (r^2 - r_0^2) / 2RT\right)
\]

where \(A_0\) and \(A\) are the absorbance at the meniscus (\(r_0\)) and radius \(r\) respectively, \(M\) is the molecular weight, \(\omega\) is the partial specific volume of IMPDH, \(\rho\) is solvent density, \(\omega\) is the angular velocity, \(R\) is the gas constant, and \(T\) is temperature. The partial specific volume was 0.75 for B. burgdorferi IMPDH. Data were fit using the following equation, which describes the sedimentation of a single component system, using Origin Technical Graphics and Data Analysis (MicroCal):

\[
\nu = V_m [S] / [K_m + [S]]
\]

where \(V_m\) is the initial velocity, \(V_m\) is the maximal velocity, \(S\) is substrate, \(K_m\) is the Michaelis constant, and \(K_i\) and \(K_s\) are the intercept and slope inhibition constants, respectively.

RESULTS AND DISCUSSION

Expression and Purification of B. burgdorferi IMPDH—PCR was used to insert convenient restriction sites into the B. burgdorferi guaB gene. NdeI at the start site and PstI in the 3'-noncoding region, and the gene was cloned into pTactac (19). The guaB gene was sequenced to ensure that no mutations were introduced during PCR. This construct, pB9, was transformed into E. coli strain H712, which lacks endogenous IMPDH activity (20). H712 cells carrying pB9 could grow on minimal medium, while cells carrying the parent plasmid, pTactac, cannot grow (data not shown). This result demonstrates that pB9 can complement the IMPDH deficiency of B. burgdorferi.
H712 cells and indicates that pB9 expresses a functional IMPDH.

Recombinant *B. burgdorferi* IMPDH was purified from H712 cells carrying pB9; expression was induced with 1 mM IPTG. The purification was accomplished in two steps and high yield, using Cibacron blue Sepharose and IMP affinity resins (Table I). Purification of *B. burgdorferi* IMPDH closely resembled the purification of human type II and *E. coli* IMPDHs (9, 21), with the exception that 0.5 M KCl is required in addition to 500 μM IMP to elute the enzyme from the IMP resin. This observation suggests that the *B. burgdorferi* enzyme may have a different requirement for monovalent cations than other IMPDHs. The purified enzyme is >95% homogeneous as judged by SDS-PAGE (Fig. 1).

Characterization of *B. burgdorferi* IMPDH—The N-terminal sequence of purified IMPDH is Pro-Asn-Lys-Ile-Thr-Lys as determined by Edman degradation. This sequence corresponds to the predicted N-terminal sequence of *B. burgdorferi* IMPDH after removal of the first Met. SDS-PAGE analysis of purified IMPDH shows a single band at 44 kDa (Fig. 1), consistent with the molecular mass of 43,637 Da calculated from the deduced amino acid sequence. This molecular mass confirms that the IMPDH activity derives from the expression of *B. burgdorferi* guaB because IMPDHs typically have a molecular mass of ∼55,000 Da (22). A single species with a molecular mass of 43,660 Da is observed by electrospray ionization mass spectrometry (Fig. 2), which further confirms the identity of *B. burgdorferi* IMPDH.

Fig. 3 shows equilibrium sedimentation data for *B. burgdorferi* IMPDH. These data were fit to Equation 1, which describes the sedimentation of a single ideal species.

\[
S(t) = \frac{M}{M_c} \left[ 1 - \frac{1}{2} \left( 1 + \frac{M}{M_c} \right)^2 \right]^{1/2}
\]

where \( S(t) \) is the sedimentation coefficient, \( M \) is the molecular mass, and \( M_c \) is the critical mass for sedimentation.

Solid line, fit to a molecular mass \( M = 174,640 \) Da, corresponding to a tetramer; long dashes, fit to \( M = 87,320 \) Da, corresponding to a dimer; short dashes, fit to \( M = 43,660 \) Da, corresponding to a monomer.

Fig. 4. Steady state kinetics of *B. burgdorferi* IMPDH. A, plot of initial velocity against IMP concentration at fixed NAD = 500 μM. Data were fit to Equation 2, which describes Michaelis-Menten kinetics. B, plot of initial velocity against NAD concentration at fixed IMP = 980 μM. Data were fit to Equation 3, which describes substrate inhibition kinetics. 

\[
V = \frac{V_{	ext{max}} [S]^n}{K_M + [S]^n}
\]

where \( V \) is the initial velocity, \( V_{	ext{max}} \) is the maximum velocity, \( [S] \) is the substrate concentration, \( K_M \) is the Michaelis constant, and \( n \) is the Hill coefficient. 

Assays contained 46 nM enzyme, 100 mM KCl, 1 mM dithiothreitol, 50 mM Tris, pH 8.0, and were performed at 37 °C.
including a tetramer-octamer equilibrium. Such higher order aggregates have been observed in IMPDHs from other species (22, 23). No difference in sedimentation behavior was observed at different enzyme concentrations (0.2 to 1.5 mg/ml) or in the presence of 0.1 mM KCl. These results indicate that B. burgdorferi IMPDH is a tetramer like other IMPDHs (22, 24, 25) and may form higher order aggregates as also observed for other IMPDHs.

**Steady State Kinetic Parameters**—Initial velocity data were collected at varying concentrations of IMP (7 to 980 μM) and NAD (125 to 5000 μM). The initial velocity versus IMP plots at fixed concentrations of NAD follow Michaelis-Menten kinetics (Fig. 4A). In contrast, substrate inhibition is observed at high NAD concentrations (Fig. 4B). Such substrate inhibition is commonly observed in IMPDHs and suggests that product dissociation follows an ordered mechanism where NADH is the first product released. Steady state parameters were derived by first determining the apparent values of V\textsubscript{m} for the initial velocity versus IMP plots (as in Fig. 4A) and replotting these values against NAD concentration. These data were fit to Equation 3, which describes uncompetitive substrate inhibition: 

\[
V_m = \frac{k_{cat}}{K_m + [NAD]} 
\]

The value of \(k_{cat}\) (IMP) was derived by first determining the apparent values of \(V_m\) for the initial velocity versus NAD plots using Equation 3 (as in Fig. 4B) and replotting these values against IMP concentration. These data were fit to the Michaelis-Menten equation:

\[
V_m = \frac{V_{max} [NAD]}{K_m + [NAD]} 
\]

Monovalent Cation Dependence—B. burgdorferi IMPDH is a tetramer like other IMPDHs (22, 24, 25) and may form higher order aggregates as also observed for other IMPDHs. IMPDH is an ordered mechanism of action, mycophenolic acid inhibition of B. burgdorferi IMPDH with respect to both IMP and NAD (Table III). This observation suggests that B. burgdorferi IMPDH has an ordered mechanism of product release where NADH dissociates before hydrolysis of E-XMP\textsuperscript{e}, as observed with other IMPDHs (34). The \(K_i\) for mycophenolic acid inhibition of B. burgdorferi IMPDH is 10\textsuperscript{3}-fold greater than that for mammalian IMPDHs, as is typical of microbial IMPDHs (8, 7, 35). These results suggest that the dinucleotide site of microbial IMPDHs is a target for species-specific inhibitors.

**Mycophenolic acid** binds in the nicotinamide subsite of the dinucleotide site of IMPDH and prevents the hydrolysis of the covalent enzyme-XMP (E-XMP\textsuperscript{e}) intermediate (17, 32, 33). Consistent with this mechanism of action, mycophenolic acid is an uncompetitive inhibitor of B. burgdorferi IMPDH with respect to both IMP and NAD (Table III). This observation suggests that B. burgdorferi IMPDH has an ordered mechanism of product release where NADH dissociates before hydrolysis of E-XMP\textsuperscript{e}, as observed with other IMPDHs (34). The \(K_i\) for mycophenolic acid inhibition of B. burgdorferi IMPDH is 10\textsuperscript{3}-fold greater than that for mammalian IMPDHs, as is typical of microbial IMPDHs (8, 7, 35). These results suggest that the dinucleotide site of microbial IMPDHs is a target for species-specific inhibitors.

**CH\textsubscript{2}-TAD** is a nonhydrolyzable analog of the active metabolite of the anti-tumor drug tiazofurin. CH\textsubscript{2}-TAD is also an uncompetitive inhibitor of B. burgdorferi IMPDH with respect to both IMP and NAD. This observation suggests that CH\textsubscript{2}-TAD also binds to E-XMP\textsuperscript{e}. The \(K_i\) of CH\textsubscript{2}-TAD = 1.0 μM is approximately 20-fold greater than that of mammalian IMPDHs (36), further demonstrating a difference in the dinucleotide sites of microbial and mammalian IMPDHs.

**Summary**—We have demonstrated that the guaB homolog of B. burgdorferi encodes IMPDH. This result demonstrates that the subdomain is not required for IMPDH activity. The function of this subdomain is unknown. In addition, we show that the monovalent cation and dinucleotide binding sites of B. burgdorferi IMPDH differ significantly from mammalian IMPDHs. Therefore these sites are targets for the design of species-specific inhibitors of IMPDH. Such inhibitors could be used to treat Lyme disease.

### Table II

**Monovalent cation dependence of B. burgdorferi IMPDH**

| Cation | \(V_m\) | \(K_m\) | \(K_a\) | \(K_s\) |
|--------|--------|--------|--------|--------|
| KCl\textsuperscript{a} | 1.0 | 25 ± 2 | 1400 ± 300 | n.a.\textsuperscript{b} |
| NH\textsubscript{4}Cl\textsuperscript{a} | 0.9 | 21 ± 3 | 500 ± 100 | n.a.\textsuperscript{b} |
| CsCl | 0.1 | 31 ± 5 | n.a. | n.a. \textsuperscript{c} |
| NaCl | n.a. | n.a. | 61 ± 12 | |
| LiCl | n.a. | n.a. | 51 ± 7 | |

\textsuperscript{a} Inhibition is observed at high concentrations of KCl and NH\textsubscript{4}Cl (>300 mM); the \(K_i\) is shown from the fit of the initial velocity data to Equation 2, which describes substrate inhibition.

\textsuperscript{b} n.a., not applicable.

\textsuperscript{c} No activity was observed in 1 mM NaCl (<1%) or 0.25 mM LiCl (<0.5%). Both Na\textsuperscript{+} and Li\textsuperscript{+} are competitive inhibitors with respect to K\textsuperscript{+}.

### Table III

**Inhibition of recombinant B. burgdorferi IMPDH**

| Inhibitor | Pattern type | \(K_m\) μM | Pattern type | \(K_m\) μM |
|-----------|--------------|-------------|--------------|-------------|
| XMP | C | 85 ± 10\textsuperscript{e} | n.a.\textsuperscript{b} | NC | 100 ± 30\textsuperscript{f} |
| GMP | C | 6.3 ± 0.9\textsuperscript{e} | n.a.\textsuperscript{b} | NC | 60 ± 20\textsuperscript{f} |
| Mycophenolic acid | UC | 7.9 ± 0.9\textsuperscript{d} | UC | n.a. | 6 ± 1\textsuperscript{f} |
| CH\textsubscript{2}-TAD | UC | 1.6 ± 0.2\textsuperscript{d} | UC | n.a. | 1.0 ± 0.1\textsuperscript{c} |

\textsuperscript{a} NAD fixed at 500 μM.

\textsuperscript{b} n.a., not applicable.

\textsuperscript{c} IMP was fixed at 100 μM.

\textsuperscript{d} NAD was fixed at 1.0 mM.

\textsuperscript{e} IMP was fixed at 300 μM.
References

1. Weber, G. (1983) Cancer Res. 43, 3466–3492
2. Smith, R. A., and Kirkpatrick, W. (1980) in Developments in Antiviral Therapy (Collier, L. H., and Oxford, T., eds) pp. 133–156, Academic Press, London
3. Wu, J. C. (1994) Perspect. Drug Discov. Des. 2, 185–204
4. Sakaguchi, K. M., Tsujino, M., Yeshigaita, M., Mizuno, K., and Hayano, K. (1975) Cancer Res. 35, 1643–1648
5. Rohrs, R. (1982) Nucleosides Nucleotides I, 35–44
6. Florey, H. W., Gilliver, K., Jennings, M. A., and Sanders, A. G. (1946) Lancet I, 46–49
7. Hope, D. J., Azzolina, B. A., and Behrens, N. D. (1986) J. Biol. Chem. 261, 3863–3869
8. Verham, R., Meek, T. D., Hedstrom, L., and Wang, C. C. (1987) Mol. Biochem. Parasitol. 24, 1–12
9. Wang, W., Papov, Y. V., Minakawa, N., Matsuda, A., Biemann, K., and Hedstrom, L. (1996) Biochemistry 35, 95–101
10. McFarland, W. C., and Stocker, B. A. (1987) Microb. Pathog. 3, 129–141
11. Mahan, M. J., Slauch, J. M., and Mekalanos, J. J. (1993) Science 260, 686–688
12. Field, P. I., Swanson, R. V., Haidar, C. G., and Heffron, F. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5189–5193
13. Noreiga, F. R., Losonsky, G., Luderbaugh, C., Liao, F. M., Wang, J. Y., Levin, M. M. (1996) Infect. Immun. 64, 3055–3061
14. Burgdorfer, W., Barbour, A. G., Hayes, S. F., Benach, J. L., Grunwald, E., and Davis, J. P. (1982) Science 216, 1317–1319
15. Margolis, N., Hogan, D., Tilly, K., and Rosa, P. A. (1994) J. Bacteriol. 176, 6427–6432
16. Schwab, T. G., Piesman, J., Golde, W. T., Dolan, M. C., and Rosa, P. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2909–2913
17. Sintchak, M. D., Fleming, M. A., Futur, O., Raybuck, S. A., Chambers, S. P., Caron, P. R., Murcko, M., and Wilson, K. P. (1996) Cell 85, 921–930
18. Andrews, S., and Guest, J. (1988) Biochem. J. 255, 35–43
19. Muchmore, D. C., McIntosh, L. P., Russell, C. B., Anderson, D. E., and Dahlquest, F. W. (1989) Methods Enzymol. 177, 74–85
20. Nijkamp, H. J. J., and De Haan, P. G. (1967) Biochim. Biophys. Acta 145, 31–40
21. Farazi, T., Leichman, J., Harris, T., Cahoon, M., and Hedstrom, L. (1997) J. Biol. Chem. 272, 961–965
22. Gilbert, H., Lowe, C., and Drabble, W. (1979) Biochem. J. 183, 481–494
23. Heyde, E., and Morrison, J. (1976) Biochim. Biophys. Acta 429, 635–644
24. Carr, S. F., Papp, E., Wu, J. C., and Natsumeda, Y. (1993) J. Biol. Chem. 268, 27286–27290
25. Yamada, Y., Natsumeda, Y., and Weber, G. (1988) Biochemistry 27, 2193–2196
26. Heyde, E., Nagahshbanan, A., Venarz, M., and Morrison, J. (1976) Biochim. Biophys. Acta 429, 645–660
27. Xiang, B., Taylor, J. C., and Markham, G. D. (1996) J. Biol. Chem. 271, 1435–1440
28. Atkins, C., Shelp, B., and Storer, P. (1985) Arch. Biochem. Biophys. 236, 807–814
29. Wang, W., and Scrimgeour, K. G. (1973) Can. J. Biochem. 51, 1726–1731
30. Krishnaiah, K. (1975) Arch. Biochem. Biophys. 170, 567–575
31. Holmes, E., Pehlke, D., and Kelley, W. (1974) Biochim. Biophys. Acta 364, 209–217
32. Link, J. O., and Straub, K. (1996) J. Am. Chem. Soc. 118, 2091–2092
33. Hedstrom, L., and Wang, C. C. (1990) Biochemistry 29, 849–854
34. Wang, W., and Hedstrom, L. (1997) Biochemistry, in press
35. Franklin, T., and Cook, J. (1969) Biochem. J. 113, 515–524
36. Marquez, V., Tseng, C. K. H., Gebeheye, G., Cooney, D. A., Abluwalia, G. S., Kelley, J. A., Dalal, M., Fuller, R. W., Wilson, Y. A., and Johns, D. G. (1986) J. Med. Chem. 29, 1726–1731