

Immunological cross-reactivity of hemoglobins in the Diptera family *Chironomidae*

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Polyclonal human IgE antibodies of patients sensitized to one species of the Diptera family *Chironomidae* as well as polyclonal rabbit anti-*Chi t I* hyperimmune serum recognize antigenic sites present in nearly all of the 33 species of this insect family. Evolutionary distantly related genera usually show weaker antibody binding. According to the pattern of reactivity of rabbit anti-*Chi t I*-component III, expression of the epitopes of this molecule varies considerably in the genera *Chironomus* and *Glyptotendipes*; it appears to be almost totally absent in all other species. Of five monoclonal antibodies raised against *Chi t I*-component III, two recognize an epitope which is expressed in nearly all closely related species. Three monoclonal antibodies recognize epitopes which are expressed in only a few species of the same genus. Our results demonstrate the presence of common as well as species-specific epitopes in chironomid hemoglobins which behave as potent inhalant allergens.

Key words: *Chironomidae*; *Chi t I*; hemoglobins; monoclonal antibodies; B-cell epitopes.

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Chironomidae, known popularly as "non-biting midges" are a most abundant family of the insect order Diptera. The 5,000-10,000 species are distributed world-wide. They can appear in vast quantities in water-rich areas when the water contains a certain proportion of organic materials (1). However, high industrial pollution of the water diminishes their number. The small red larvae are aquatic. A high prevalence of allergic respiratory diseases has been reported in fancy-fish enthusiasts who use larvae of the species *C. thummi thummi* (*C. riparius*) as fish food (2, 3). Also inhabitants of areas where chironomid midges seasonally hatch in extraordinary numbers and aggregate in aerial swarms often suffer from immediate-type allergic reactions (5-10). Recently, our group showed that hemoglobins (*Chi t I*; 11, 12) are the predominant allergens in some of the species (13, 14). The

aim of this work was to broaden previous investigations, to identify common antigens and quantify hemoglobin-related immunological cross-reactivity between 33 different species. We used human IgE sera, rabbit antisera against *Chi t I* or against *Chi t I*-component III, and monoclonal antibodies raised with *Chi t I*-component III. Component III is a monomer and is one of 12 hemoglobins which comprise *Chi t I*. Furthermore, we intended to correlate immunological cross-reactivity with the evolutionary relationship between the species studied (Fig. 1).

PATIENTS AND METHODS

We prepared crude extracts of *Chironomidae* larvae and/or midges (imagines) from 33 species, collected from different areas or reared in the laboratory (14, 15).

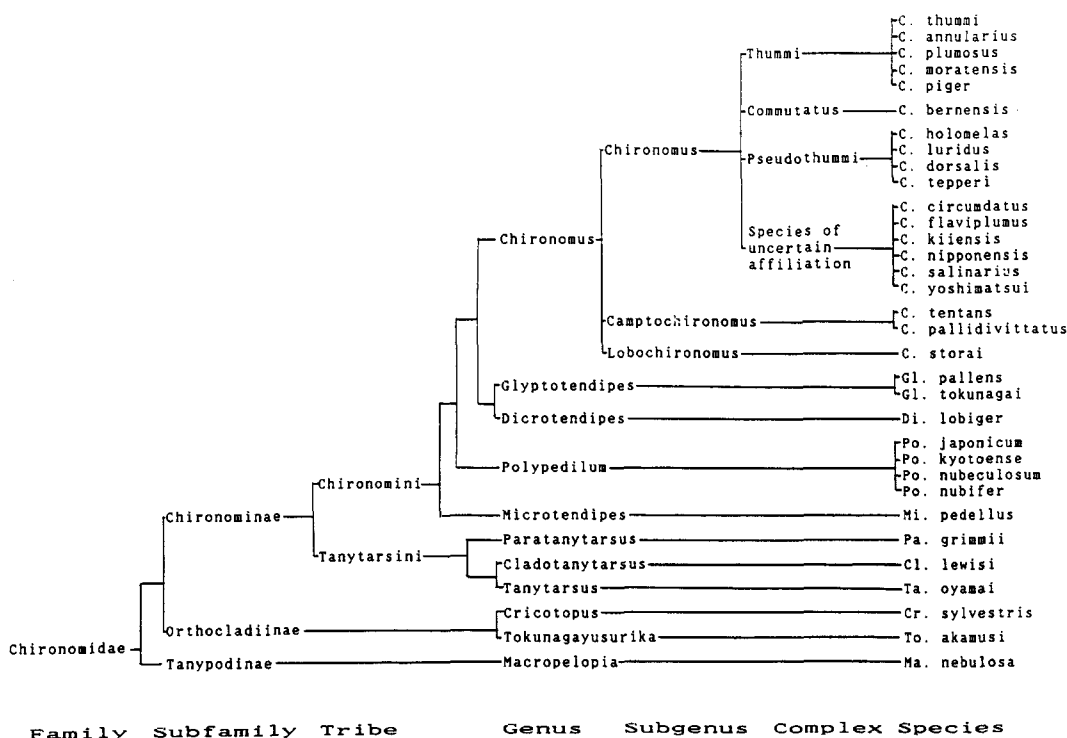


Fig. 1. Kladogenesis of the species of the Chironomidae family studied.

Patients' IgE pool serum "Aquarianer" was obtained with equal amounts of serum samples taken from five German asthmatic fish hobbyists who had been in contact with *C. thummi thummi* larvae for 2–12 years. Patients' IgE pool serum "USA" consisted of serum samples of three sensitized patients from Wisconsin, USA, who live near lakes and develop conjunctivitis, rhinitis and/or bronchial asthma when in contact with *C. plumosus* midges; in these cases contact with *C. plumosus* larvae components has also to be assumed. All these probands had positive results with *Chi t I* in skin tests and/or RAST investigations.

Rabbit antisera against *Chi t I* or *Chi t I*-component III as well as five monoclonal antibodies against *Chi t I*-component III were produced as previously described (16). mAbs H4B2H2 and H8G9D3 recognize the sequence 91–101 from *Chi t I*-component III (17).

All antibody measurements were done with solid phase-based radioimmunoassays. We

quantified patients' IgE antibodies by the RAST method. Rabbit IgE antibodies and mAbs were determined by radioimmunoassays using radiolabelled protein A and rabbit anti-mouse IgE, respectively (17).

Antibody levels are expressed as a percentage of those obtained with the appropriate immunogen; i.e. binding to *C. thummi thummi* larvae for "Aquarianer" pool serum, *C. plumosus* larvae for "USA" pool serum, *Chi t I* for rabbit antiserum against *Chi t I*, or to *Chi t I*-component III for rabbit antiserum against *Chi t I*-component III and mAbs. Values of more than 4% of the binding of the immunogenes are regarded as positive since with 10 negative control sera results were always < 4%.

RAST inhibition studies were performed using serum of a German fish-hobbyist with asthmatic symptoms due to sensitisation to *Chi t I*. The serum was preincubated overnight with *Chi t I*, with crude larval or midge extracts of the different chironomid species. As an unre-

lated species, we tested the wild silk moth *Antheraea pernyi* of the family *Saturniidae*. In these inhibition experiments amounts of larval or midge extracts were always 0.1 mg per tube (dissolved in 70 μ l 0.05 M phosphate - 0.15 M sodium chloride).

RESULTS

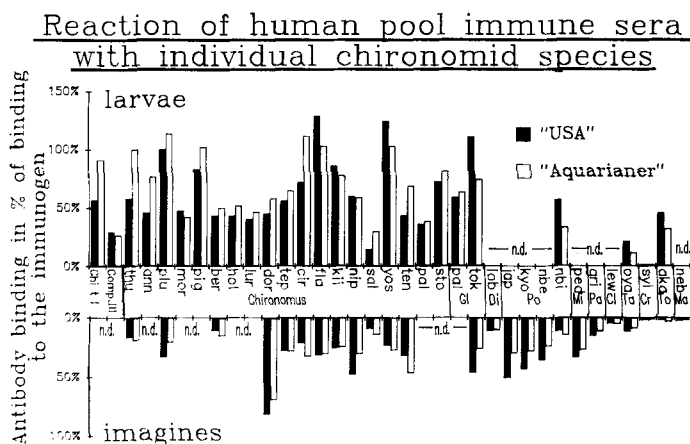
Both patients' pool sera show moderate to strong IgE reactivity with larval and midge extracts of all but one species (*C. salinarius*) of the genus *Chironomus* or *Glyptotendipes*. All other species give low or insignificant results (Fig. 2). IgE antibody binding of both human pool sera to the larval extracts or *C. thummi thummi* (defined for "Aquarianer" pool serum as 100%) is similar to antibody binding to *Chi t I*. This demonstrates that *Chi t I* is the predominant or exclusive allergen of *C. thummi thummi*. Interspecies cross-reactivity obtained with the "USA" pool serum is comparable to that of the "Aquarianer" pool serum. In all but one species (*C. dorsalis*), larval extracts produce higher IgE binding than midge extracts. This is also true

for "USA" patients who are thought to be sensitized predominantly by midges instead of larvae. The lower antibody binding in midges is probably due to their lower hemoglobin content; in the adult midge, hemoglobins are progressively degraded (18).

Stronger cross-reactivities, especially between distantly related species were obtained with the rabbit antiserum against *Chi t I*, the only exceptions were *Cricotopus sylvestris* and *Macropelopia nebulosa* (Fig. 3).

The reaction pattern of the rabbit antiserum against *Chi t I*-component III demonstrates only moderate cross-reactivity between species of the genus *Chironomus* and *Glyptotendipes* (Fig. 3). The *Chi t I*-component III antiserum shows relatively low binding to *Chi t I*. Since component III represents less than 10% of *Chi t I* (11, 12) it can be expected that it does not include all antigenic sites recognized by this antiserum.

mAbs give quite different patterns of reactivity. mAbs H4B2H2 and H8G9D3, both recognising the sequence 91-101 of *Chi t I*-component III, bind to most examples of the genus *Chironomus* and *Glyptotendipes* to a similar degree; H8G9D3 shows much



Figs. 2-5. Screening of larvae and/or imagines of 33 species of the Diptera family *Chironomidae* by human (Fig. 2) and rabbit hyperimmune sera (Fig. 3) as well as by mAbs (Figs. 4-5).

Antibody levels are expressed as a percentage of those obtained with the corresponding immunogen. cpm obtained by the different immunogens: "Aquarianer" pool serum (50 μ l) 8929 cpm (40% of total; 42.4 RAST U/ml); "USA" pool serum (25 μ l) 16367 cpm (51.6% of total; 68.0 RAST U/ml); rabbit anti-*Chi t I* (0.025 μ l) 30727 cpm (35.5% of total); rabbit-anti *Chi t I*-component III (0.025 μ l) 24672 cpm (28.5% of total); mAb H4B2H2 (5 μ l) 28358 cpm (17.6% of total); mAb H8G9D3 (5 μ l) 29967 cpm (18.6% of total); mAb H2D6G10 (5 μ l) 23387 cpm (14.5% of total); mAb H7E10B8 (5 μ l) 18643 cpm (22.5% of total); mAb H8H1F9 (5 μ l) 17264 cpm (9.5% of total) (not shown).

Abbreviations used for different genera: see Fig. 1.

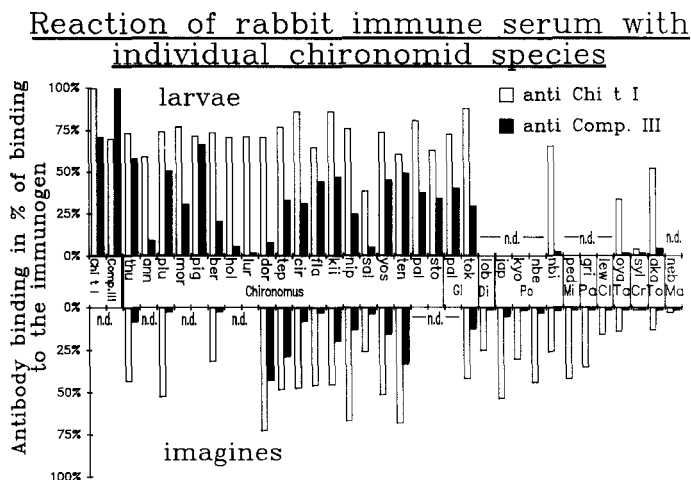


Fig. 3.

higher binding to *C. circumdatus*, *C. flaviplumus*, *C. kiiensis* and *C. yoshimatsui*, whereas H4B2H2 reacts more intensively with *C. nipponensis* and *C. tepperi* midge (Fig. 4).

As opposed to this, mAb H2D8G10 recognizes structures only present in *C. thummi thummi*, *C. luridus*, *C. dorsalis* and to a weaker extent, in *C. piger*. mAb H7E10B8 and mAb H8H2F9 show almost identical reactivity patterns (data of

the latter not shown): in addition to *C. thummi thummi*, only *C. piger* and *C. tepperi* are significantly bound by these antibodies (Fig. 5).

Results of RAST inhibition (Table 1) with a single patient's serum in general reflect the degree of direct antibody binding in RAST (Fig. 2). Lower inhibition by midge extracts compared with larval extracts usually correlates with their lower antibody binding in the RAST.

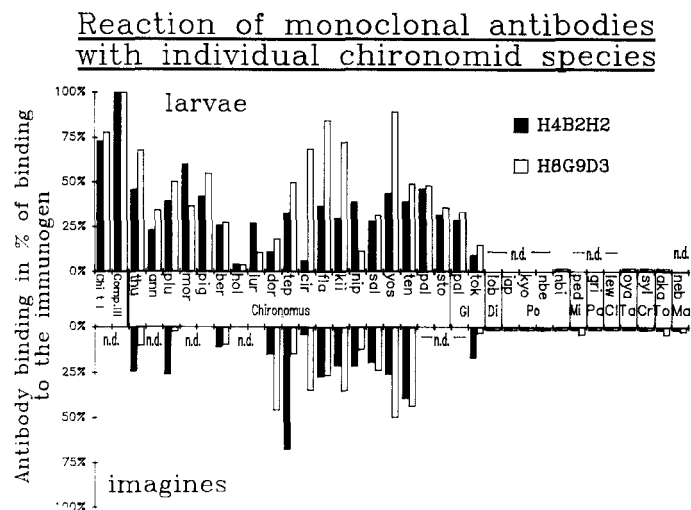


Fig. 4.

RAST inhibition seems to be more sensitive, since relatively strong cross-reactivity with some species distantly related to *C. thummi thummi* (showing low direct IgE binding) can be demonstrated, namely with *C. kiiensis*, *C. yoshimatsui*, *G. tokunagai* and *P. kyotoense*.

DISCUSSION

Our investigations include studies of mAbs, polyclonal human IgE and rabbit IgG antisera, as well as antibody inhibition experiments. The results clearly prove hemoglobin-related immunological cross-reactivity between larvae and midges of various species of *Chironomidae*. This cross-reactivity correlates to some extent with the evolutionary relationship of the investigated species which in turn is associated with the genomic and structural diversity of the hemoglobin molecules. By means of polyclonal human and rabbit antisera, even with distantly related species such as the Japanese species *Tokunagayusurika akamusi*, remarkable cross-reactivity can be demonstrated. As shown with mAbs, the continuous (or part of an assembled topographic) epitope 91-101 of *Chi t I*-component III is conserved in nearly all species of the genus *Chironomus* but not in the other genera. Conservation of this site is not surprising since X-ray analysis of component III indicates that posi-

tions 96 (glutamine), 97 (leucine) and 100 (phenylalanine) are involved in interatomic heme contacts (19). Further, after homologous alignment the amino acid residues glutamine and phenylalanine are present at the same positions in all individual *Chi t I*-components. This and the cross-reactivity indicate, even for phylogenetically distant chironomid species, an evolutionary conservation of the antigenic epitope 91-101 in identical or homologous sequences and superficial structures. On the other hand, epitopes recognized by mAbs H2D8G10, H7E10B8 and H8H2F9 are present in only a few species of the same genus. This result is not surprising since mAbs H7E10B8 and H8H2F9 show heteroclitic binding (16). Obviously, they bind to distinct sites which are only present in *Chi t I*-components III and IIIa and in hemoglobins from the species *C. luridus*, *C. dorsalis*, *C. piger* and/or *C. tepperi*.

These findings as well as broad IgE binding patterns from patients sensitized to individual chironomid species (e.g. to *C. thummi thummi*, *Cladotanytarsus lewisi* og *C. plumosus*; 13, 14) support our previous hypothesis that hemoglobins are the predominant antigens of this insect family for humans.

It should be mentioned that antigenic variability between the different species of the chironomid family is probably not only due to

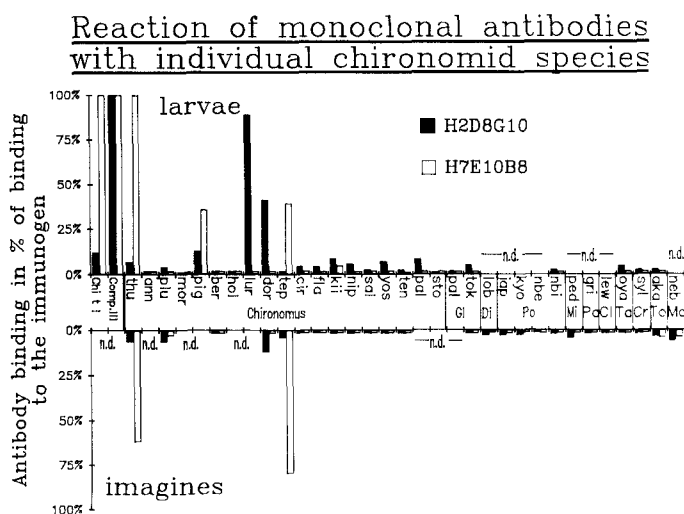


Fig. 5.

Table 1.

RAST inhibition studies using larval and midge extracts from different chironomid species and human IgE serum (German patient S.D.). Included are also some published data (13, 14) comparing results from different species

Species	% RAST inhibition by	
	larvae	midges
<i>C. thummi</i>	97	98
<i>C. annularius</i>	85	nd
<i>C. plumosus</i>	82	71
<i>C. moratensis</i>	97	nd
<i>C. piger</i>	99	nd
<i>C. bernensis</i>	86	nd
<i>C. holomelas</i>	97	nd
<i>C. luridus</i>	98	nd
<i>C. dorsalis</i>	nd	94
<i>C. tepperi</i>	77	nd
<i>C. circumdatus</i>	98	92
<i>C. flaviplumus</i>	97	nd
<i>C. kiensis</i>	98	89
<i>C. nipponensis</i>	99	nd
<i>C. salinarius</i>	70	55
<i>C. yoshimatsui</i>	99	86
<i>C. tentans</i>	69	nd
<i>C. pallidivittatus</i>	97	nd
<i>C. storai</i>	73	nd
<i>Gl. pallens</i>	64	nd
<i>Gl. tokunagai</i>	96	75
<i>Di. lobiger</i>	nd	69
<i>Po. japonicum</i>	nd	91
<i>Po. kyotoense</i>	nd	73
<i>Po. nubeculosum</i>	nd	89
<i>Po. nubifer</i>	88	35
<i>Mi. pedellus</i>	nd	92
<i>Pa. grimmii</i>	nd	72
<i>Cl. lewisi</i>	nd	19
<i>Ta. oyamai</i>	91	58
<i>Cr. sylvestris</i>	34	0
<i>To. akamusi</i>	42	35
<i>Ma. nebulosa</i>	nd	25

Solid phase allergen: *Chi t I*. Amount of human IgE serum used per individual tube: 30 µl. Without inhibition 4373 cpm were obtained (16.3% of total radioactivity; 8.1 RAST U/ml). nd = not done.

%

structural differences of the antigenic components. It may also be due to their variant concentrations, especially in midges. Among other factors, the degree and speed of hemoglobin degradation in the adult midge, which may differ from one species to another, could be of influence. However, in RAST inhibition studies at 0.1 mg/ml, differences in concentrations of antigenic components should not greatly affect the immunological cross-reactivity. We inter-

pret results of direct antibody measurement and especially the RAST inhibition experiments as evidence for the existence of antigenically relevant hemoglobin regions which are structurally and/or functionally identical even in distantly related species of the Diptera family *Chironomidae*.

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REFERENCES

1. Sasa M. House dust mites and chironomid midges in Japan. In Miyamoto T (ed.): Int. Symposium mite and midge allergy, pp. 1-21, Tokyo, 1988.
2. Baur X, Dewair M, Fruhmman G, Aschauer H, Pfletschinger J, Braunitzer G. Hypersensitivity to chironomids (non-biting midges): localization of the antigenic determinants within certain polypeptide sequences of hemoglobins (erythrocrurins) of *Chironomus thummi thummi* (Diptera). *J Allergy Clin Immunol* 69, 66-76, 1982.
3. Baur X, Aschauer H, Mazur G, Dewair M, Prelicz H, Steigemann W. Structure, antigenic determinants of some clinically important insect allergens: chironomid hemoglobins. *Science* 233, 351-354, 1986.
4. Cranston PS. Allergens of non-biting midges (Diptera: Chironomidae): a systematic survey of chironomid hemoglobins. *Med Vet Entomol* 2, 117-127, 1988.
5. Kagen SL, Yunginger JW, Johnson R. Lake fly allergy: incidence of chironomid sensitivity in an atopic population. *J Allergy Clin Immunol* (abstract 315) 73, 187, 1984.
6. Eriksson NE, Ryden B, Jonsson P. Hypersensitivity to larvae of chironomids (non-biting midges). Cross sensitization with Crustaceans. *Allergy* 44, 309-315, 1989.
7. Igarashi T, Murakami G, Adachi Y, et al. Common occurrence in Toyama of bronchial asthma induced by chironomid midges. *Japan J Exp Med* 57, 1-9, 1987.
8. Ishii A, Matsuoka M, Uchida JY, et al. Chironomid midge and allergy around lake Kojama, Okayama. In Miyamoto T (ed.): Int. Symposium mite and midge allergy, pp. 318-337. Tokyo, 1988.
9. Ito K, Miyamoto T, Shibuya T, Kamei K, Mano K, Tanai T, Sasa M. Skin test and radioallergosorbent test with extracts of larval and adult midges of *Tokunogayusurika akamusi* Tokunaga (Diptera: Chironomidae) in asthmatic patients of the metropolitan area of Tokyo. *Ann Allergy* 57, 199-204, 1986.
10. Kino T, Chihara J, Fukuda K, Sasaki Y, Shogaki Y, Oshima S. Allergy to insects in Japan. High frequency of IgE antibody responses to insects (moth, butterfly,

- caddis fly and chironomid) in patients with bronchial asthma and immunochemical quantitation of the insect-related airborne particles smaller than 10 μm in diameter. *J Allergy Clin Immunol* 79, 857-866, 1987.
11. Goodman M, Braunitzer G, Kleinschmidt T, Aschauer H. The analysis of a protein polymorphism. Evolution of monomeric and homodimeric hemoglobins (erythrocruorins) of *Chironomus thummi thummi* (insecta diptera). *Hoppe Seyler's Z, Physiol Chem* 364, 205-217, 1983.
 12. Osmulski PA, Leyko W. Structure, function and physiological role of chironomus haemoglobin. *Comp Biochem Physiol* 85B, 701-722, 1986.
 13. Baur X, Mazur G. Structure of major chironomid allergens and epitopes: studies with allergen fragments, a synthetic peptide, monoclonal and human antibodies. In Miyamoto T (ed.): *Int. Symposium mite and midge allergy*, pp. 364-372. Tokyo, 1988.
 14. Baur X, Dewair M, Haegele K, Prelicz H, Scholl A, Tichy H. Common antigenic determinants of haemoglobin as basis of immunological cross-reactivity between chironomid species (Diptera, Chironomidae): studies with human and animal sera. *Clin Exp Immunol* 54, 599-607, 1983.
 15. Kawai K, Konishi K. Laboratory colonization and allergen analysis of some common Japanese chironomid species. In Miyamoto T. (ed.): *Int. Symposium mite and midge allergy*, pp. 243-260, Tokyo, 1988.
 16. Mazur G, Becker WM, Baur X. Epitope mapping of major insect allergens (chironomid hemoglobins) with monoclonal antibodies. *J Allergy Clin Immunol* 80, 876-883, 1987.
 17. Mazur G, Baur X, Modrow S, Becker WM. A common epitope on major allergens from non-biting midges (chironomidae). *Mol Immunol* 25, 1005-1010, 1988.
 18. Schin K, Poluhowich JJ, Gamo T, Laufer H. Degradation of haemoglobin in chironomus during metamorphosis. *J Insect Physiol* 20, 561-571, 1975.
 19. Steigemann W, Weber E. Structure of erythrocruorin in different ligand states refined at 1.4 Å resolution. *J Mol Biol* 127, 209-338, 1979.
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